Effect of parathyroid hormone (1-34) on early bone healing of sinus augmentation in healthy rabbit

Jisun Huh

Department of Dentistry
The Graduate School, Yonsei University

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Directed by Professor Kee-Deog Kim

A Doctoral Dissertation
submitted to the Department of Dentistry
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Ph.D. in Dental Science

Jisun Huh

June 2017

연세대학교
YONSEI UNIVERSITY

This certifies that the Doctoral Dissertation
of Jisun Huh is approved.

Thesis Supervisor : Kee-Deog Kim

Seong-Ho Choi

Hyung Jun Kim

Ui-Won Jung

Hyun Sil Kim

The Graduate School

Yonsei University

June 2017

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(Directed by Professor Kee-Deog Kim, D.D.S., M.S.D., Ph.D.)

Purpose: This study evaluated effect of administering intermittent parathyroid hormone [PTH (1-34), henceforth PTH] on early-stage bone healing of maxillary sinus augmentation and compared two different schedules of PTH administration in healthy rabbit.

Materials and Methods: Bovine bone mineral was grafted on sinuses of 25 female New Zealand white rabbits. The animals were randomly divided into three groups: **PTH** (*n* =10); PTH was injected subcutaneously 5 days a week for 2 weeks and killed at 2 weeks (*n*=5) or 4 weeks (*n*=5) postoperatively, **Saline** (*n*=10); saline was injected and killed as

same amount and schedule as the PTH group, **PTH_mod** (PTH modified interval, *n*=5); PTH was injected 2 days a week for 4 weeks and killed at 4 weeks postoperatively. The dosage of PTH was 10 μg/kg/day. Radiographic and histomorphometric analyses were performed.

Result: The new bone area (NBA) did not differ significantly among the saline, PTH and PTH_mod groups. The NBA in the PTH group in the total augmented area and in the demarcated window, center, and Schneiderian membrane regions increased significantly from 2 to 4 weeks. The number of osteoclasts decreased significantly from 2 to 4 weeks in the saline and PTH groups, with no difference between the two groups. The PTH_mod group had larger number of osteoclasts than the saline and PTH groups in the Schneiderian membrane region. Osteocalcin was expressed in the PTH and PTH_mod groups and not in the saline group.

Conclusion: Intermittent PTH might not stimulate new bone formation in healthy rabbits during the first 4 weeks of healing. In the PTH group, the consistency of NBA was increased at 4 weeks.

Keywords: teriparatide, parathyroid hormone, sinus augmentation, rabbit sinus model

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I. Introduction

Teriparatide, a portion of recombinant human parathyroid hormone [PTH (1-34), henceforth PTH], is an osteoanabolic agent that was approved for osteoporosis treatment by the United States Food and Drug Administration in 2002[1,2]. It was reported first in 1923 that parathyroid extract stimulated new bone formation contrary to the original function, calcium release from bone tissue [3], and PTH is known to have osteocatabolic effect when infused continuously and osteoanabolic effect when administrated intermittently [4]. PTH stimulates new bone formation by increasing the

number of osteoblasts and decreasing the apoptosis of osteoblasts when it is administrated intermittently[5]. PTH has uniqueness in being a real anabolic agent, while osteoporosis medicaments that have been used are antiresorptive agents[6]. The anabolic effect has been applied in diverse applications beyond osteoporosis treatment, and many animal and clinical studies have found that it decreased the fracture risk, stimulated fracture healing, increased the bone density, and exerted other positive effects[7-9]. In the dental field, it was reported that PTH stimulated periodontal regeneration[10], implant healing[11], healing of medication-related osteonecrosis of the jaw[12,13], and orthodontic movement[14].

Several studies have investigated the effects of PTH on bone graft. The early trials involved spinal fusion surgery, with PTH showing positive effects on the survival of allografts and autografts[15-17]. In other animal studies, PTH stimulated distraction osteogenesis in rabbits[18] and allograft integration in calvarial bone defects in mice[19]. Animal studies related to dental effects found that PTH stimulated healing after bone graft on extraction sockets[20] and calvarial bone autografts on the mandible in rats[21]. However, studies of the effect of PTH on bone grafts are still deficient, and PTH has not been evaluated previously in sinus augmentation.

Many studies using PTH have shown that it is effective when injected intermittently[16,22]. In human, the protocol for osteoporosis treatment is daily self-injection of 20 μg on thigh or abdomen within 24 months[23,24]. In animal studies, the dosage and injection interval were vary from 5 to 80 μg/kg/day and from 2 days a week to daily

injection. The common protocol is 10 to 40 μg/kg/day dosage and 5 days a week or daily injection[22,25-27].

Daily self-injection may be very stressful to patients. Instead of daily injection, once-weekly efficacy research has been recently tried in Japan and it has been reported that it is effective on decreasing fracture risk and increasing bone density[28-30]. Also in animal studies 2 or 3 days a week injection showed positive effect[11, 21, 31, 32]. If once or twice a week injection schedule is as equally effective as 5 days a week or daily injection, we could reduce the stress from daily self-infection and side effects like nausea, hypercalcemia, hyperuricemia and osteosarcoma[33].

Maxillary sinus augmentation via a lateral window approach is a widely performed operation for vertical bone deficiency due to maxillary sinus pneumatization. It is the grafting procedure of bone substitute material into maxillary sinus in order to obtain adequate bone height for placement of dental implants. It has the limitation of a long healing period of about 6 months[34]. If PTH stimulates the bone healing of sinus augmentation, total treatment period of the implant restoration for posterior maxillary dentition with lack of bone height could be reduced and so the masticatory function of these patients could be restored earlier.

The rabbit sinus model is a common animal model used in studies of sinus augmentation. The air pressure in the maxillary sinus is similar and the Schneiderian membrane lining is the same in rabbits and humans, and rabbits are both easy to care for and inexpensive[35,36]. The metabolic rate is three to four times faster in rabbits than in

humans, and the effects on bone healing can be confirmed within weeks[37]. Several studies using this model have shown differences between control and experimental groups at healing periods of 2 and 4 weeks[38-40].

Based on previous studies that were mentioned above, it is expected that intermittent PTH could stimulate the bone regeneration after maxillary sinus augmentation. Especially several studies performed in maxillofacial region of rabbit or rat showed that PTH increased jaw mineral density in rabbit [26], increased volume of calvarial autograft on mandible in rat [21], and increased bone fill of graft on extraction socket in rat [20]. These might support our hypothesis.

The present study is the first trial to investigate PTH on sinus augmentation and evaluated the effect of intermittent PTH administration on early bone healing of sinus augmentation using healthy rabbit. And two different intervals of PTH administration were compared.

II. Materials and Methods

1. Animal

Twenty-five female New Zealand white rabbits weighing 2.8–3.2 kg were used. Animals were cared under standard laboratory conditions with free access to water and a standard laboratory pellet diet. The selection, management, and preparation of animal followed the protocol of the Institutional Animal Care and Use Committee of Yonsei Medical Center, Seoul, Korea (the ethics approval number was 2012-0224).

2. Experimental materials

PTH

PTH (Forsteo®, Eli Lilly, Houten, Netherlands) was used to stimulate bone healing. Each 80 μl of injection solution contained 20 μg of PTH. Based on previous studies, PTH was applied at a dose of 10 μg/kg, with the amount injected calculated according to the concentration of PTH and the animal's weight.

Deproteinized bovine bone mineral

Deproteinized bovine bone mineral (Bio-Oss®, Geistlich Pharma, Wolhusen, Switzerland) was used as a bone substitute material for maxillary sinus augmentation. The particle size was 250-1000 μm and 0.15 g was grafted per sinus.

3. Experiment design

In the PTH group (*n*=10), PTH was injected 5 days a week for 2 weeks, for a total of nine times (except on the operation day). The dosage of PTH was 10 μg/kg/day. The animals were killed at 2 weeks (*n*=5) or 4 weeks (*n* =5) postoperatively. Animals in the saline group (*n*=10) were injected with the same amount of saline on the same schedule as in the PTH group, and they were also killed at 2 weeks (*n*=5) or 4 weeks (*n*=5) postoperatively. For the PTH_mod group (PTH modified interval group, *n*=5), same does of PTH was injected 2 days a week for 4 weeks, total 8 times and killed at 4 weeks postoperatively (**Figure 1**).

4. Surgical procedure

The overall surgical procedure used in this study followed that used by Choi et al.[35]. A mixture of ketamine hydrochloride (Ketalar®, Yuhan, Seoul, Korea) and xylazine (Rompun®, Bayer Korea, Seoul, Korea) was injected intramuscularly for general anesthesia. After shaving the nasal bone area and preparing the skin surface with povidone iodine, 2% lidocaine (LidocaineHCl®, Huons, Seoul, Korea) was injected for local anesthesia. A linear incision was made along the sagittal midline on the nasal bone, and then a full-thickness flap was elevated bilaterally. On the lining bony plate over the maxillary sinus area at a position decided in the previous study[35], a circular bony window with a diameter of 6 mm was made using a trephine bur (3i Implant Innovation, Palm

Beach Gardens, FL, USA) under saline irrigation while taking great care not to perforate the Schneiderian membrane (**Figure 2**). The excised bony disc was removed, and then the Schneiderian membrane was elevated carefully and 0.15g of deproteinized bovine bone mineral was grafted. The flap was replaced and sutured layer by layer with 4-0 absorbable glyconate monofilament (Monosyn®, B-Braun, Aesculap, Center Valley, PA, USA). The stitches were removed 7 days postoperatively.

5. Radiographic analysis: microcomputed tomography (microCT)

Block sections that included the maxillary sinus and surrounding tissue were excised and fixed in 10% formalin solution for 10 days. Microcomputed tomography (microCT) images of the fixed specimens were obtained using high-resolution microCT (Skyscan 1173, Bruker, Kontich, Belgium) at a resolution of 35 μm (achieved using 130 kV and 60 μA). The images were stored in Digital Imaging Communication in Medicine format (**Figure 3**). A region of interest (ROI) that included all grafted material and newly formed tissue was selected, and the total augmented volume was measured. A grayscale threshold range of 58~255 was applied for the total bone volume (TBV) and 72~255 for bone substitute material within each ROI.

The following parameters were measured in ROI:

- Total bone volume (TBV, mm^3)
- Grafted bone substitute material volume (GBV, mm^3)
- New bone volume (NBV, mm^3) = TBV – GBV

- Fibrovascular tissue volume (FVV, mm^3) = TAA - TBV

6. Histomorphometric analysis

After microCT scanning, the block specimens were decalcified using a decalcification solution (Rapid Cal Immuno, BBC Biochemical, Mt Vernon, WA, USA), embedded in paraffin, and sectioned serially at 5 μm in the coronal direction. The two center-most sections were selected: one was stained with Masson's trichrome and the other was stained with hematoxylin-eosin. Images were obtained with the aid of a light microscope (Leica DM 2500, Leica Microsystems, Wetzlar, Germany, and Virtual Microscope VS120, Olympus Corporation, Tokyo, Japan), and they were analyzed histomorphometrically by a masked experienced observer (K.P.) using PC-based image-processing software (Adobe Photoshop CS4, Adobe Systems, San Jose, CA, USA).

The boundary of the total augmented area (TAA) was traced in slides stained with Masson's trichrome. Square window, center, and Schneiderian membrane regions (1 mm×1 mm) were selected within the TAA in each slide (**Figure 4**). The following parameters were measured in the TAA, window, center, and Schneiderian membrane regions:

- New bone area (NBA, mm^2)
- Grafted bone substitute area (GBA, mm^2)
- Fibrovascular tissue area (FVA, mm^2)

In slides stained with hematoxylin-eosin, five square regions (also 1 mm×1 mm) were selected in each animal (**Figure 5**). The osteoclasts (which are multinucleated giant cells)

were counted within the five regions, and the total number of osteoclasts in the five regions in each slide was obtained.

7. Immunohistochemistry

One specimen among each group and each healing period was randomly selected for osteocalcin (OCN) immunohistochemistry. Selected block sections embedded in paraffin were cut into 5 μm thickness and mounted on slides. The specimens were treated with xylene 3 times for 15 minutes each and then ethanol 2 times for 5 minutes each. 0.25% trypsin solution for 30 minutes, H_2O_2 solution for 20 minutes, and 5% bovine serum albumin for 10 minutes were applied sequentially. The sections were allowed to react with 1^{st} and 2^{nd} antibody (1^{st} antibody: Anti-osteocalcin antibody [OC4-30], Abcam, Cambridge, UK; 2^{nd} antibody: anti-mouse ig, Vector, CA, USA) for 30 minutes each and were stained with DAB (3,3'-diaminobenzidine) and hematoxylin. The images were obtained by same method with other histologic sections.

8. Statistics

Sample size was calculated based on three previous studies in rabbits. Mashiba et al. reported that the bone mineral density in tibia was 0.25±0.004 g/cm^2 in saline vehicle injected group and 0.27±0.009 g/cm^2 in PTH injected group [41]. Calculated sample size

from these data under 80% of power level and alpha level 0.5 was three per group. The other two studies were performed in rabbit model of osteoporosis. Almagro et al.'s study of tibial implant represented that bone area % in peri implant trabecular bone of PTH and vehicle injected group were 19.0±6.4 and 11.6±3.7, respectively [27]. Bone density in mandible reported by Bellido et al. was 0.231±0.004 g/cm^2 in PTH injected group and 0.224±0.003 g/cm^2 in saline injected group [26]. Sample size calculated from these two studies under same power and alpha levels was five per group.

Data were analyzed using SPSS (version 23.0, IBM Corporation, Armonk, NY, USA). The Mann-Whitney test was used to compare each parameter between the saline and PTH groups, and to compare between 2 and 4 weeks of healing in the saline and PTH groups. The Kruskal–Wallis test was performed to compare the saline, PTH, and PTH_mod groups at 4 weeks and when its result represented statistical significance, the Mann-Whitney test was applied to confirm which pairs of the three groups had statistically significant differences. The Friedman test and the Wilcoxon test were used to assess differences in NBA values between the window, center, and Schneiderian membrane regions within each group. The cutoff for statistical significance was set at $p<0.05$, with Bonferroni correction being applied.

III. Results

1. Clinical findings

The Schneiderian membrane was perforated in four sinuses during the operations: two in the saline group and two in the PTH group. All of these perforations were minor (smaller than 1 mm) and three of them healed well. However, one animal in the saline group suffered from maxillary sinusitis during the healing period, and severe pus was observed in the sinus when the animal was killed at 2 weeks. This animal was therefore excluded from the analysis.

2. Radiographic analysis: microCT

Volumetric analysis results for microCT are displayed on **Table 1**. GBV, NBV and FVV did not differ significantly between the saline and PTH groups at 2 weeks. NBV and FVV did not differ significantly among the three groups at 4 weeks. GBV values have significant difference among the three groups ($p=0.008$) but the following Mann-Whitney test did not reveal significant differences between any pairs of them. TBV values represented significant difference among the three groups [saline group, 14.03±12.6 mm^3 (mean±SD), PTH group, 151.7±13.7 mm^3, PTH_mod group, 119.6±14.6 mm^3, $p=0.016$] by Kruskal-Wallis test followed by Bonferroni correction which result showed TBV of

the PTH_mod group was significantly lesser than the PTH group (p=0.016) at 4 weeks (**Figure 6**).

3. Histological findings

At 2 weeks, both the saline and PTH groups showed newly formed bone along the lateral wall in addition to the existing bone present mainly in the window area. New bone formation started from the original bone surface and incorporated bone substitute particles. In some animals, thin new bone surrounding bone substitute particles was observed near the Schneiderian membrane.

At 4 weeks, the PTH_mod group demonstrated different healing pattern from the saline and PTH groups. In the saline and PTH groups, all of the animals except for one in the saline group exhibited progressive new bone formation. The new bone was evenly distributed in the entire window and also within the middle region, extending from lateral to center, and was advanced compared to the situation at 2 weeks. Newly formed bone, bone substitute material, and existing bone were densely interconnected as if they had originally been in the same body. On the other hand, animals belonged to the PTH_mod group revealed variant degree of new bone formation. In two of them, newly formed bone was hardly detected and osteoclasts were observed at high frequency. One animal of the PTH_mod group showed advanced new bone formation filling all of the augmented area. The other two animals represented as same amount of new bone as the other groups.

4. Histomorphometric analysis

The results of histomorphometric analysis in TAA are presented in **Table 2**. NBA, GBA, and FVA did not differ between the PTH and saline groups at 2 weeks. NBA was larger at 4 weeks than at 2 weeks in the PTH group (p=0.009), but did not differ significantly between these two time points in the saline group (p=0.142). At 4 weeks, NBA, GBA, and FVA did not differ among the saline, PTH, and PTH_mod groups. The variation of NBA among individual animals was smaller in the PTH group than in the saline and PTH_mod groups (**Figure 7**).

Figure 8 shows the NBA values in the window, center, and Schneiderian membrane regions. At 2 weeks, NBA was larger in the saline group than in the PTH group in the Schneiderian membrane region being statistically significant [saline group, 0.047±0.051 mm^2; PTH group, 0.004±0.006 mm^2; p=0.046]. NBA values in the window, center, and Schneiderian membrane regions were significantly larger at 4 weeks than at 2 weeks in the PTH groups, but did not differ significantly between these two time points in the saline group. When comparing between different regions, there were no significant differences at either 2 or 4 weeks in all of three groups.

The number of osteoclasts decreased significantly from 2 to 4 weeks in both the saline and PTH groups [saline group: 2 weeks, 153.8±36.7, 4 weeks, 79.0±28.2; p=0.027; PTH group: 2 weeks, 181.0±48.6, 4 weeks, 81.0±22.3; p=0.009] (**Figure 9**). The PTH_mod groups showed larger number of osteoclasts than the saline and PTH groups in the Schneiderian membrane region [saline group, 16.6±6.7; PTH group, 12.6±1.5; PTH_mod

group, 27.2±3.8; p=0.018] (**Figure 10**).

5. Immunohistochemistry

OCN was expressed around existing bone of window margin in the PTH and PTH_mod groups. The saline group didn't represent OCN expression (**Figure 11**).

IV. Discussion

This study evaluated the effect of administering intermittent PTH on early bone healing of sinus augmentation in a healthy rabbit sinus model. The amount of new bone formation and number of osteoclasts did not differ between the PTH and saline groups at both 2 and 4 weeks. NBA increased significantly from 2 to 4 weeks in the PTH group, with this group characterized by relatively small variations in NBA values among the individual animals. The PTH_mod group had lesser TBV than the PTH group and more osteoclasts than the other two groups in the Schneiderian membrane region. OCN was expressed in PTH injected groups.

The dose and injection interval of PTH used in the present study followed those in previous studies. Studies involving rabbits have used PTH doses of 6~40 μg/kg and injection schedules varying from three times a week to daily. The most common values were 10 μg/kg and five days a week or daily administration[27,41,42], and so we used a dose of 10 μg/kg and a schedule of five days a week. One animal study reported intermittent PTH of twice a week accelerated the fracture healing[32]. Therefore we chose the other schedule of twice a week.

MicroCT analysis was used for confirming the proper retention of bone substitute material within the maxillary sinus. The mineralization of the newly formed bone was partly observed in some histologic section and the new bone was almost under mineralized state, which could not be detected by microCT. Same level of TMV in the

saline and PTH groups at 2 and 4 weeks means that the bone substitute material was retained well through the healing period up to 4 weeks. The quantity of the newly formed bone was measured in Masson's trichrome stained histologic section. Special method for distinguishing between the new and preexisting bone was not necessary because the bone substitute material was grafted into maxillary sinus, which is empty space without preexisting bone. NBA values were measured in 2 dimensional histologic sections so the total augmented area could vary according to the shape of sinus and total augmented volume. Percentage of NBA is the proportion of NBA to TAA in the histologic section so it is more reliable value.

The numbers of osteoclasts were counted to verify the stimulation of bone remodeling by PTH. The extension of the bone remodeling space as determined by the number and activity of osteoclasts is important for confirming an osteoanabolic effect of PTH, in addition to increasing the number of osteoblasts[43]. Osteoclasts are multinucleated giant cells that are easily countable, in contrast to osteoblasts having shapes similar to fibroblasts at the early stage of new bone formation. The osteoclast population was consistent with the NBA values.

OCN is an abundant component of bone extracellular matrix and is thought as the marker of bone formation and bone remodeling[44]. In the present study, immunohistochemistry for OCN was performed to evaluate whether PTH stimulated bone formation. There are many articles reported that intermittent PTH increased OCN with increasing bone mineral density and stimulating graft healing[45,46]. In this study, OCN was

expressed only in the groups injected PTH but it was not related with increased NBA. OCN expression may be related with the significant increase of NBA from 2 to 4 weeks and condensed values of NBA at 4 weeks in the PTH group. OCN expression in the PTH_mod group indicates that the same event could happen later healing stage after 4 weeks.

Data on the osteoanabolic effects of intermittent PTH in animal studies are accumulating[4]. When reviewing studies limited to rabbits, most studies have found positive effects of intermittent PTH on implant osteogenesis[11,25,27] and distraction osteogenesis[18,47]. A search for studies involving other animals and other sites did not reveal results of intermittent PTH administration not enhancing bone healing.

Several factors could be considered when explaining no difference of NBA and the osteoclast population between the saline and PTH groups. The first factor is that young and healthy animals were used in this study. In healthy animals, the normal regulation system for maintaining homeostasis is working well and any temporary increase in the PTH level such as induced by the administration of PTH could be cancelled by the homeostasis system. PTH is an osteoanabolic agent for osteoporosis patients[1,2], and it may affect sinus augmentation in individuals who have diseases that compromised bone.

The second factor is that PTH is one of the systemic factors related to bone metabolism. Other systemic factors are estrogen, progesterone, and cortisol, and there are also several local factors[48]. The differentiation and activation of osteoblasts and osteoclasts are regulated by interactions between these factors, and so the effect of PTH on bone could be offset by the action of other related factors.

The third factor is the site specificity of PTH. Qi et al. reported that lumbar vertebra represented total restoration of lost cancellous bone and only marginal restoration was observed in proximal tibia in ovariectomized rat[49]. Several studies showed that the responsiveness to PTH varies according to skeletal sites in human[50,51]. Most studies in rabbit were performed in tibia or lumbar spine and PTH have not been evaluated previously in maxillary sinus.

Last possible explanation is that the penetration rate into sinus mucosa varies according to medicines. It is supported by a study showed that amoxicillin and clavulanic acid had different penetration rate into sinus mucosa[52]. There was no study that revealed the level of systemically administrated PTH in sinus mucosa. The results of the present study can make it possible to speculate low penetration rate of PTH into sinus mucosa. Jung et al. reported that locally applied PTH stimulated bone regeneration on peri-implant bone defect in dog and calvarial bone defect in rabbit[53,54]. Local application of PTH can be alternative to systemic administration on sinus augmentation.

However, meaningful results were obtained in the PTH group, with a significant increase in new bone formation from 2 to 4 weeks and with smaller deviations in NBA values among the individual animals. Also OCN wax expressed in PTH injected groups but not in the saline group. These results indicate that PTH could enhance bone formation after the early healing period and also increase the consistency of bone healing.

This study was performed in young healthy rabbits, and so it was not possible to confirm whether intermittent PTH exerts on osteoanabolic effect in individuals with

diseases that compromised bone. This could be evaluated in further studies involving aged animals with osteoporosis induced by ovariectomy and longer healing periods.

Within the limitations of this study, it is concluded that intermittent PTH might not stimulate new bone formation in healthy rabbits for up to 4 weeks of healing. The clinical relevance of this study is that PTH could increase the predictability of the sinus augmentation procedure, although greater new bone formation might not be expected if PTH is used as a supplement treatment in sinus augmentation in healthy individuals.

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Table 1. Volumetric analysis (mm^3)

	2 weeks				4 weeks			
	NBV	GBV	FVV	TAV	NBV	GBV	FVV	TAV
Saline group								
Mean (S.D.)	40.4 (4.9)	96.9 (12.0)	159.4 (29.2)	296.8 (33.2)	41.1 (4.5)	99.2 (12.4)	141.7 (15.2)	282.0 (15.6)
Median (Q1, Q3)	40.3 (35.9, 45.1)	95.5 (86.5, 95.5)	159.6 (132.8, 185.9)	295.3 (265.4, 329.6)	41.9 (36.6, 45.2)	97.7 (89.1, 110.1)	145.3 (127.7, 153.9)	285.9 (266.0, 296.1)
PTH group								
Mean (S.D.)	44.2 (6.8)	102.8 (6.8)	164.2 (14.7)	311.2 (22.2)	41.4 (4.5)	110.3 (10.7)	138.3 (25.1)	290.0 (30.5)
Median (Q1, Q3)	42.4 (44.2, 49.4)	104.9 (96.8, 107.8)	157.7 (151.7, 179.9)	298.4 (293.9, 334.9)	42.2 (36.7, 45.7)	111.2 (100.2, 120.1)	125.3 (118.9, 164.2)	293.1 (259.4, 319.1)
PTH_mod group								
Mean (S.D.)			NA		36.1 (4.1)	83.5 (12.6)	180.6 (27.8)	300.3 (17.2)
Median (Q1, Q3)					36.1 (32.5, 39.9)	77.6 (73.1, 96.8)	182.5 (155.6, 204.7)	306.3 (283.1, 314.3)
						p=0.038*		

NBV : new bone volume, GBV : grafted bone substitute volume, FVV : fibrovacular tissue volume, TAV : total augmented volume

* Significant different among the three groups

Table 2. Histomorphometric analysis (mm^2)

	2 weeks				4 weeks			
	NBA	GBA	FVA	TAA	NBA	GBA	FVA	TAA
Saline group								
Mean (S.D.)	1.10 (0.21)	8.30 (1.00)	9.64 (1.88)	19.04 (2.49)	2.74 (1.34)	7.38 (1.24)	7.78 (1.41)	17.90 (3.10)
Median (Q1, Q3)	1.16 (0.87, 1.25)	7.81 (7.79, 9.30)	10.46 (7.69, 10.78)	19.80 (16.50, 20.82)	2.73 (1.63, 3.85)	7.38 (6.07, 8.68)	8.31 (6.29, 9.01)	17.21 (15.03, 21.11)
PTH group								
Mean (S.D.)	1.18 (0.34)	6.76 (0.96)	8.35 (1.64)	16.29 (2.42)	2.85 (0.69)	7.71 (1.28)	8.97 (0.45)	19.53 (2.26)
Median (Q1, Q3)	1.05 (0.89, 1.54)*	6.22 (6.13, 7.67)	8.86 (6.67, 9.78)	17.34 (13.71, 18.35)	2.46 (2.34, 3.56)*	7.80 (6.48, 8.89)	9.01 (8.55, 9.67)	19.01 (17.46, 21.82)
PTH_mod group								
Mean (S.D.)	NA				1.99 (1.55)	7.66 (1.42)	9.02 (1.62)	18.67 (3.23)
Median (Q1, Q3)					2.75 (0.35, 3.25)	8.56 (6.24, 8.88)	8.61 (7.81, 10.43)	18.27 (15.57, 21.96)

NBA : new bone area, GBA : grafted bone (Bio-Oss) area, FVA : fibrovacular tissue area, TAA : total augmented area

*Significantly different between 2 weeks and 4 weeks in the PTH group (p=0.009)

Figure Legends

Figure 1. Experimental design. ✓: saline injection, ✔: PTH 10 μg/kg injection

Figure 2. Full thickness flap was elevated and windows of 6 mm diameter were formed bilaterally. The Schneiderian membrane was seen within bony window (left). Deproteinized bovine bone mineral was grafted (right). Only left maxillary sinus was used for this study.

Figure 3. Microcomputed tomography analysis. Graysclae images (upper row) and color images (lower row). Axial, coronal, and sagittal view (left to right). In the color images, yellow-green and purple represent bone substitute material and newly formed bone, respectively.

Figure 4. Masson's trichrome stained slide with 3 squares at the window, center and Schneiderian membrane region. The particles stained with light purple color are grafted bone substitute material and new bone was stained as blue color. W : window region, C : center region, M : the Schneiderian membrane region, NB : new bone, GB : grafted bone substitute

Figure 5. Hematoxylin-eosin stained slide with 5 selected regions for osteoclasts counting (left) and magnified image of the center region (right). Multinucleated cells indicated by black arrowheads are osteoclasts

Figure 6. Total bone volume (mm^3) in total augmented region by microCT analysis. At 2

weeks there is no significant difference between the saline and PTH group. At 4 weeks significant difference was shown among the three groups by the Kruskal-Wallis test (p=0.016) and * means significant difference between the PTH and PTH_mod group by Bonferroni correction (p=0.016)

Figure 7. Percentage of new bone area to total augmented area

* Significant increase from 2 to 4 weeks in the PTH group (p=0.009)

Figure 8. New bone area (mm^2) within 1 mm^2 squares of the window, center and Schneiderian membrane regions

* Significantly increase from 2 to 4 weeks in the PTH group (p=0.009, 0.024 and 0.015 in the window, center and Schneiderian membrane region, respectively)

† Significantly different between the saline and PTH groups (p=0.046)

Figure 9. Total numbers of osteoclasts in the five regions

* Significantly decreased from 2 to 4 weeks

Figure 10. The number of osteoclasts in the Schneiderian membrane regions

* The PTH_mod group has significantly larger value than the saline and PTH groups

Figure 11. Immunohistochemistry result for osteocalcin. White arrowheads indicate osteocalcin expression

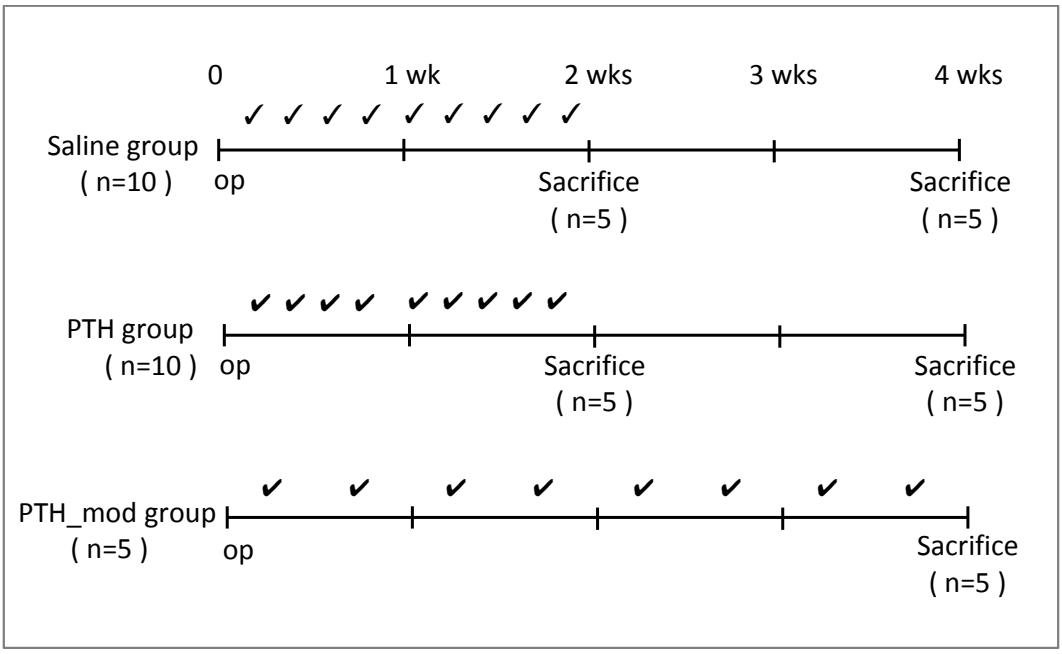
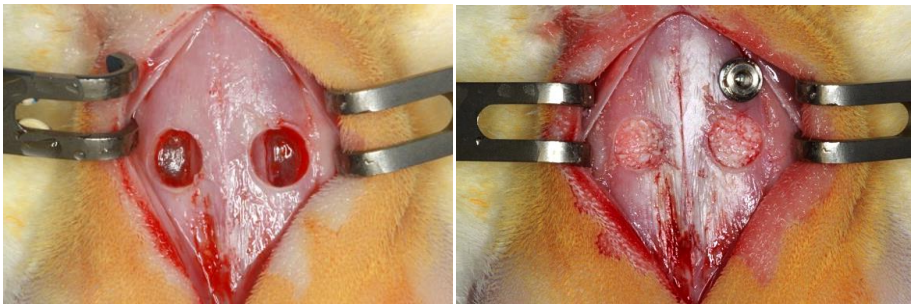


Figure 1



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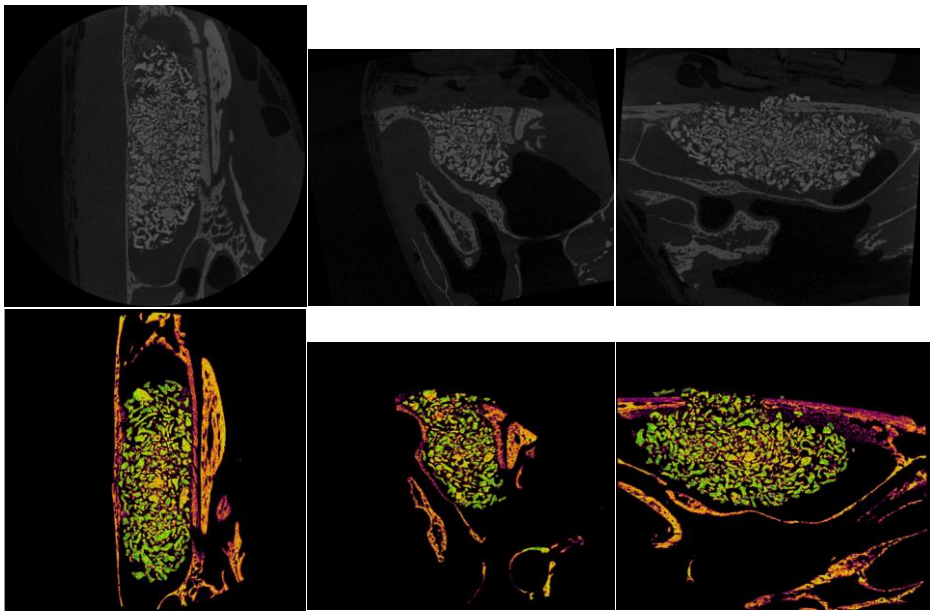

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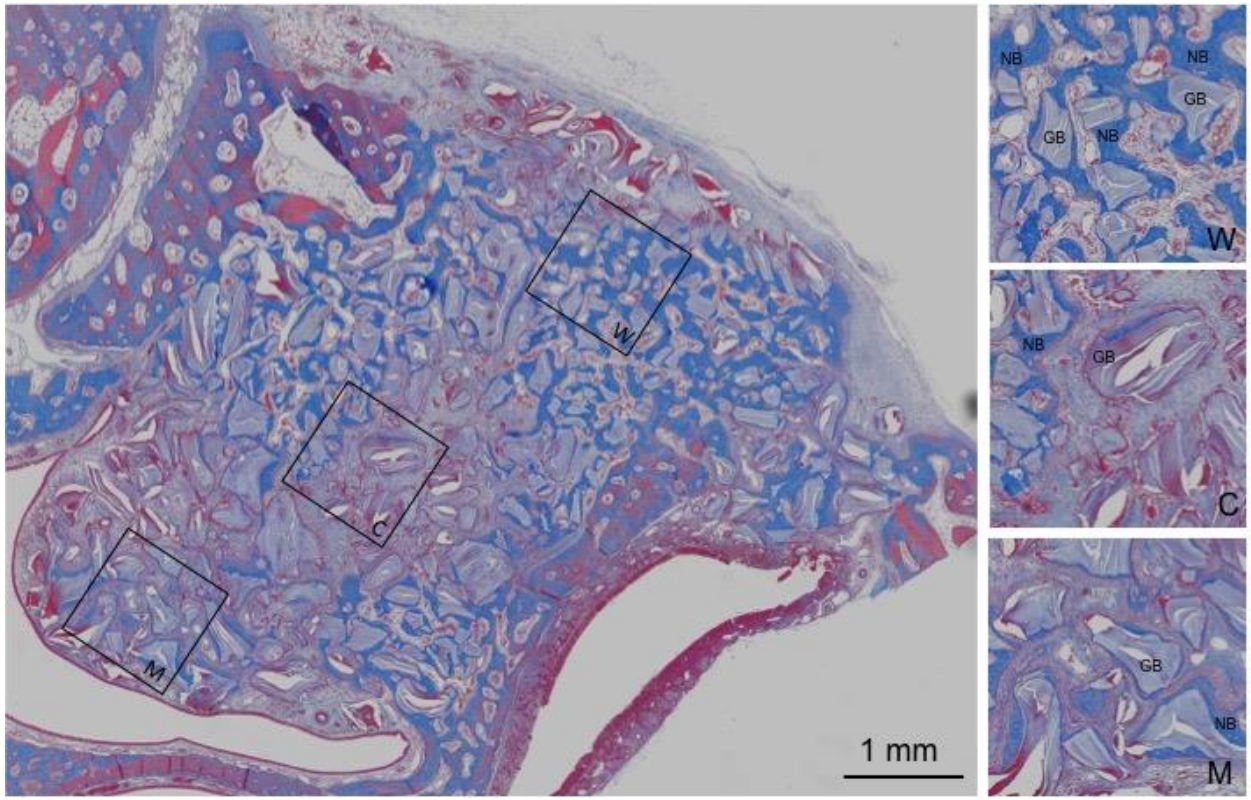

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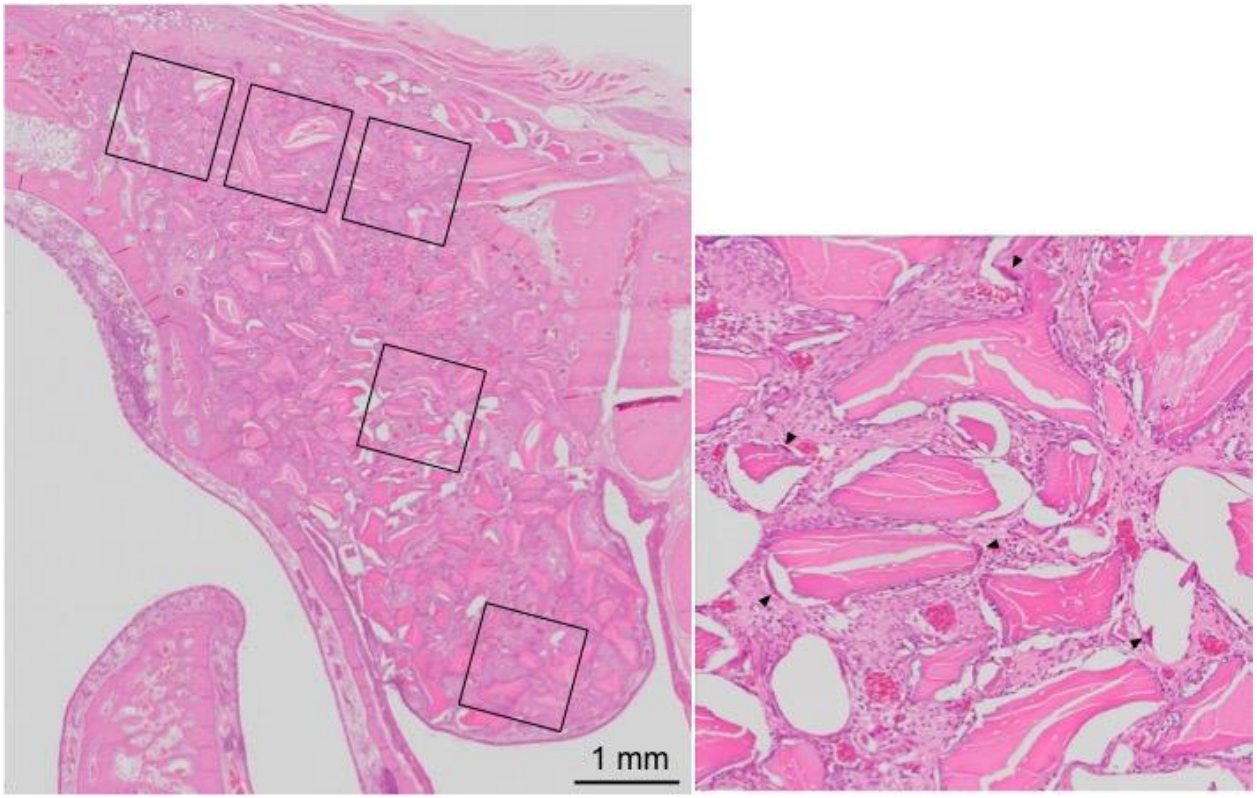

Figure 5

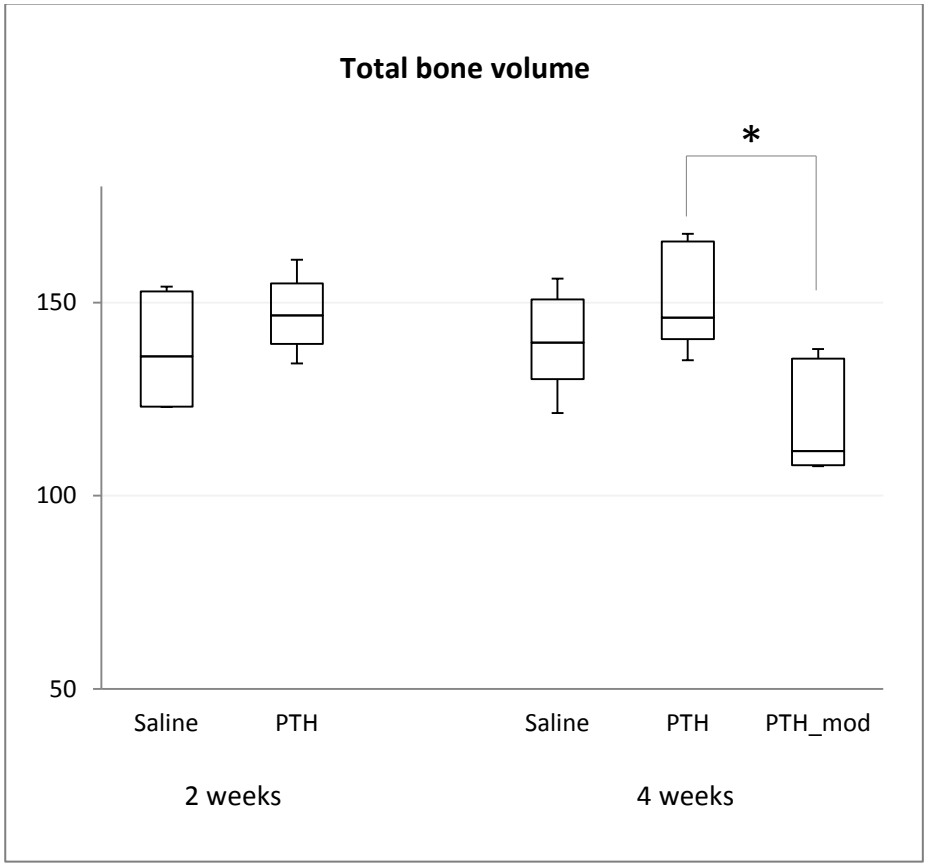

Figure 6

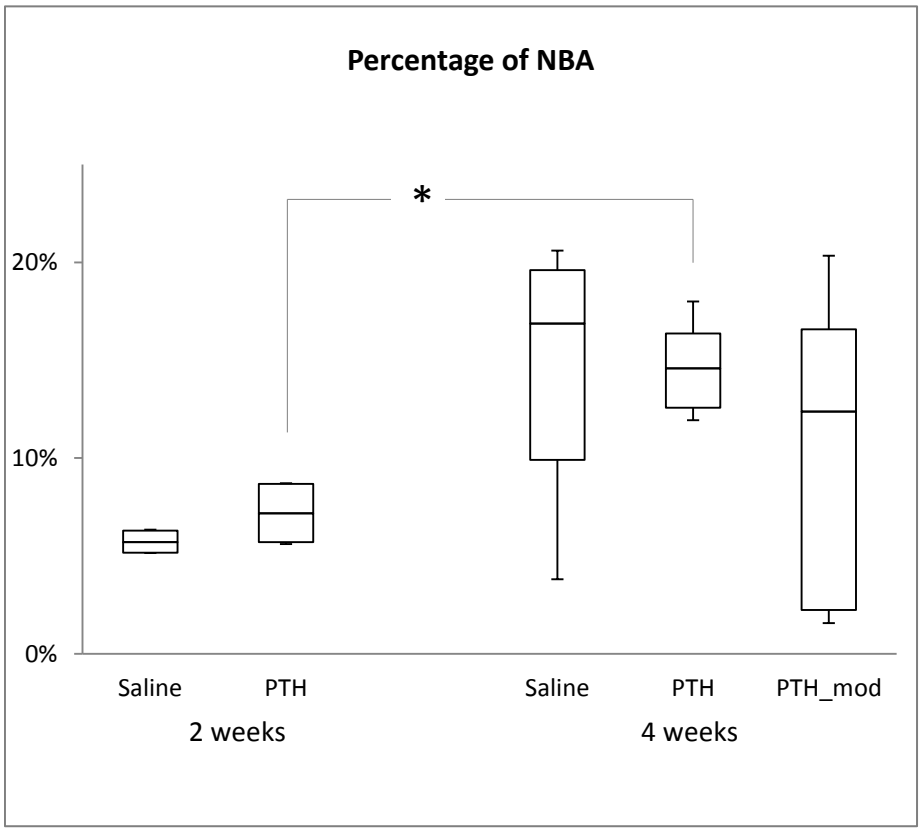

Figure 7

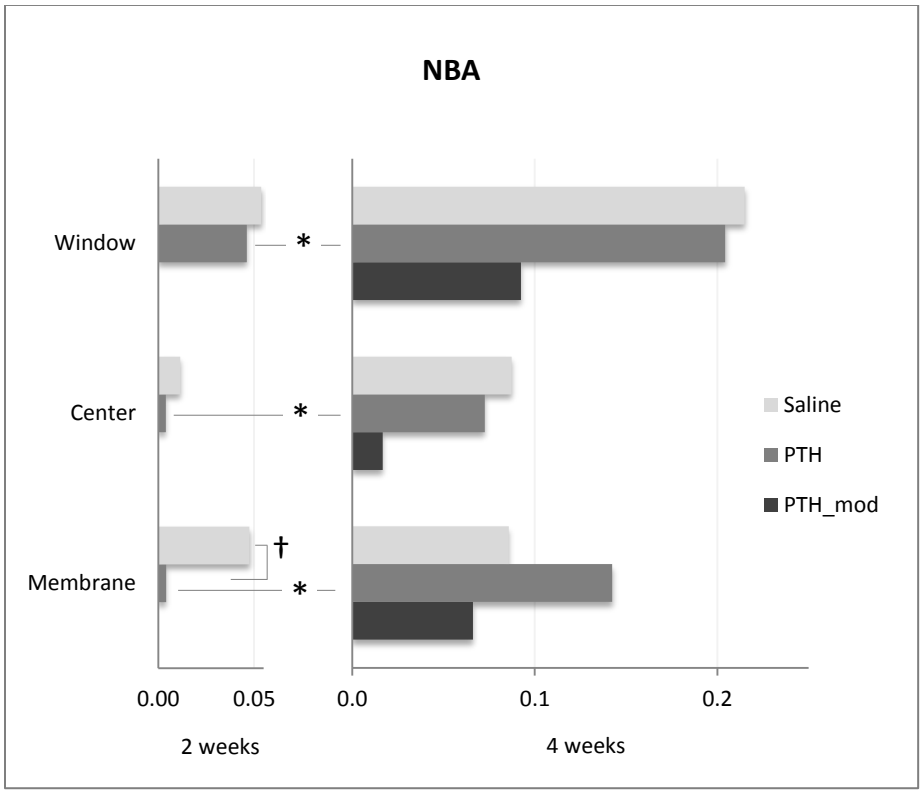

Figure 8

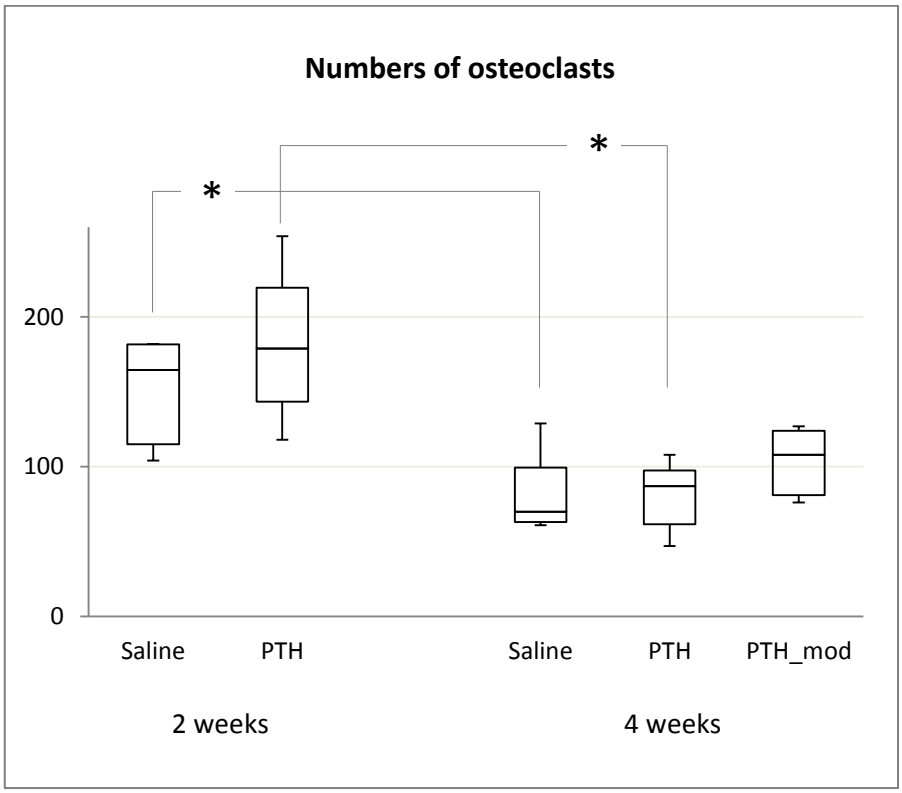

Figure 9

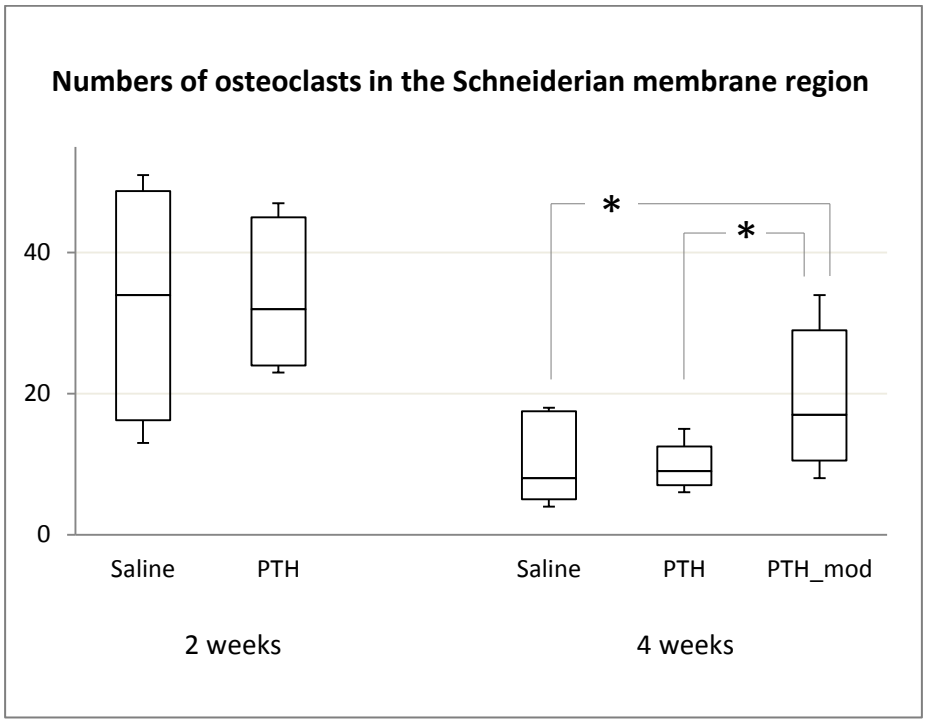

Figure 10

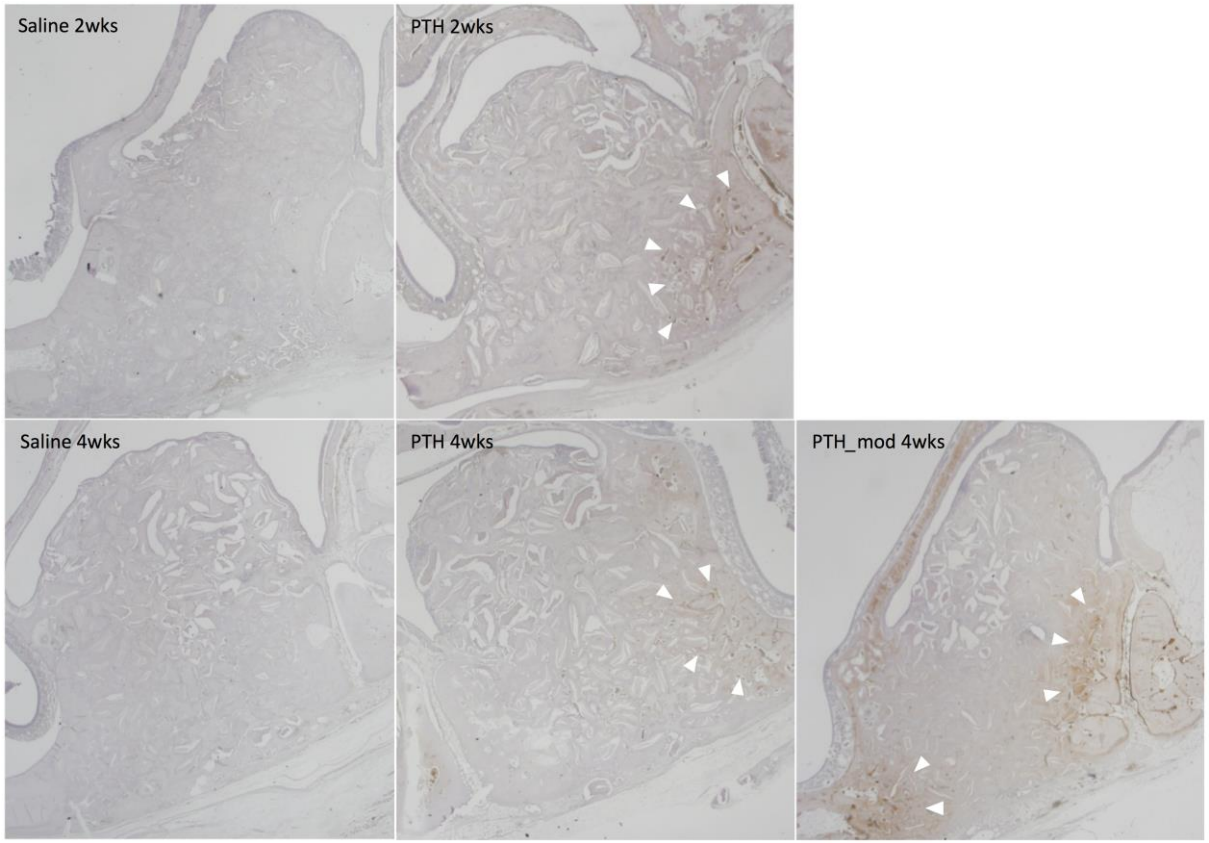

Figure 11

국문요약

부갑상선 호르몬 (1-34)가 건강한 토끼에서 상악동 증강술 후 초기 골 치유에 미치는 영향

〈 지도교수 김기덕 〉

연세대학교 대학원 치의학과

허 지 선

목적: 본 연구는 상악동 증강술에 대한 부갑상선 호르몬 (1-34) [이하 PTH]의 골 치유 촉진 효과를 구명하고자 한 첫 연구로서 건강한 토끼를 이용하여 PTH가 상악동 증강술 후 초기 골 치유에 미치는 효과 및 그 주입 일정에 따른 차이를 알아보고자 하였다.

재료 및 방법: 뉴질랜드 흰 토끼 암컷 25 마리의 상악동에 탈단백 우골 무기질을 이식한 후 무작위로 3 군으로 나누었다. PTH 군 (n=10)과 saline 군(n=10)은 군에 따라 PTH 10

μg/kg, 혹은 동량의 생리식염수를 2 주간 주 5 회 피하 주사하고 술 후 2 주와 4 주째에 다섯 마리씩 희생하였으며, PTH_mod 군 (n=5)은 PTH 10 μg/kg을 4 주간 주 2 회 피하 주사하고 술 후 4 주째에 희생하였다. 방사선학적 및 조직계측학적 분석을 시행하였고 비모수적 방법으로 통계 처리하였다.

결과: PTH 군과 saline 군은 new bone area (NBA) 및 파골세포 수에서 차이를 보이지 않았다. PTH 군은 2 주에서 4 주 사이에 유의한 NBA 증가를 보였으며 4 주째에 개체간 변이가 적은 고른 NBA 분포를 보였다. PTH_mod 군은 Schneiderian membrane 영역에서 다른 두 군에 비해 더 많은 파골세포 수를 보였으며 PTH 군에 비해 더 적은 total bone volume을 보였다. Osteocalcin은 PTH 군과 PTH_mod 군에서만 발현되었다.

결론: 건강한 토끼에서 상악동 증강술 후 부갑상선 호르몬 (1-34)의 간헐적 투여 시 4 주간의 초기 치유 기간 내에서는 신생골 형성 촉진 효과를 규명할 수 없었으나 PTH 군에서 치유 일관성이 향상되었다.

핵심되는 말: teriparatide, 부갑상선 호르몬, 상악동 증강술, 토끼 상악동 모델