Synergistic effect of hyperbaric oxygen therapy with parathyroid hormone [1-34] on calvarial bone graft in irradiated rat

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Synergistic effect of hyperbaric oxygen therapy with parathyroid hormone [1-34] on calvarial bone graft in irradiated rat

Directed by Professor Wonse Park

A Dissertation Thesis

Submitted to the Department of Dentistry

and the Graduate School of Yonsei University

in partial fulfillment of the requirements for the degree of

Doctor of Philosophy in Dental Science

Kyung-Mi Park

June 2018

This certifies that the Doctoral dissertation

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저의 가장 큰 버팀목이자 후원자인 박호균 아버님, 김정숙 어머님, 언니와 동생 그리고 성희에게 감사를 전합니다. 자주 연락도 드리지 못 하는 철없는 조카의 박사 졸업 순간을 저보다 더 애타게 기다리셨던 윤종국 이모부님, 김영숙 이모님께도 감사 드립니다. 더 바르고 훌륭하게 살아야 함을 늘 가슴 뜨겁게 느끼게 해주시는 저의 조부모님이신 김화섭 옹, 최순이 여사님, 박용문 옹, 故윤종수 여사님께 가득한 존경과 사랑을 드리며 이 작은 결실의 기쁨을 나누고자 합니다.

연구원이자 대학원생으로서 지내온 지난 세월 동안 나를 돌볼 시간도 없이 앞만 보며 열심히 달려 이 순간을 맞이하게 되었습니다. 참으로 셀 수 조차 없는 긴 시간 동안 늘 곁에서 힘이 되어 주시고, 사랑과 관심을 보내주신 모든 분들께 다시 한 번 감사를 전합니다. 마지막으로, 이 논문의 진짜 주인공으로써 소중한 연구 결과를 저에게 안겨주기 위해 짧은 생을 살다 떠난 쥐돌이들 에게 이 논문을 바칩니다.

2018 년 6 월

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ABSTRACT

Synergistic effect of hyperbaric oxygen therapy with parathyroid hormone [1-34] on calvarial bone graft in irradiated rat

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(Directed by Professor Wonse Park, D.D.S., M.S.D., Ph.D.)

Purpose

To determine the synergistic effect of parathyroid hormone [1-34] in combination with hyperbaric oxygen on bone graft in rat calvarial bone defect model under impaired osteogenic condition.

Materials and Methods

Twenty four rats were divided into 3 groups. Localized radiation with a single 12 Gy dose was administered to the calvarial. 4 weeks after radiation, calvarial circular defects were created in the parietal bones. All defects were

filled with biphasic calcium phosphate. After grafting, parathyroid hormone was injected subcutaneously and hyperbaric oxygen therapy was administered. At 6 weeks after the bone graft, the rats were sacrificed and specimens were harvested.

Results

Histomorphometric evaluation showed the percent new bone area was higher in the PTH and PTH/HBO groups than in the Control group. Micro computed tomographic evaluation showed bone volume of new bone volume was higher PTH group than Control group. Bone surface in new bone volume was higher PTH/HBO group than Control group. In new bone volume, bone surface density was higher in the order of Control, PTH and PTH/HBO groups; all group was significant difference (P<0.017).

Conclusions

Within the limitations of this study, our data indicate that parathyroid hormone with hyperbaric oxygen may reverse the impairment of bone healing by irradiation.

Key Words : animal model, calvarial defect, bone graft, bone regeneration, parathyroid hormone, hyperbaric oxygen therapy

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Ⅰ. INTRODUCTION

Osteoradionecrosis (ORN) is a representative complication in patients who receive a high dose of radiation and most commonly affects the lower jaw bone. Patients undergoing radiation therapy have tissue destruction and chronic non-healing wounds because bone marrow, collagen, periosteum, and endothelial cells are damaged, leading to tissue hypoxia.[1] Similar loss and severe fibrosis of remodeling elements occurs at the periosteum as well as blood vessels, and consequently the regenerative ability of irradiated tissue

gradually decreases.[2] As a result, the failure rate of bone grafts in irradiated bone was reported to be 2 to 3 times greater than that in non-irradiated bone.[3]

Medication-related osteonecrosis of the jaw (MRONJ, new nomenclature replacing bisphosphonate-related osteonecrosis of the jaw [BRONJ]) is one of the major complications after tooth extraction in patients using bisphosphonates and recent studies have focused more on the treatment of MRONJ than ORN because the incidence of MRONJ is significantly increasing and its treatment is more complicated than that for ORN.[4] In such cases, although bone graft materials can be used to rebuild the surgically removed necrotic bone, great care should be taken because the curative capacity of bone tissue that underwent ORN or MRONJ is very low compared to that of normal tissue.[5] Many treatment options have been considered to overcome these issues, and it was previously suggested that non-surgical maxillary treatments such as hyperbaric oxygen (HBO) and parathyroid hormone (PTH) can be used for the treatment of bone diseases in which osteogenic potential is severely impaired[5,6] as well as in cases where a poor prognosis is expected even after use of bone graft materials.[7-12]

PTH is a major endocrine regulator of calcium and phosphorus homeostasis and has demonstrated a clear anabolic effect on cancellous bone by enhancing the activity of osteoblasts and inhibiting bone resorption, leading to a net bone gain.[13] Because of this property, PTH has become an effective treatment modality for osteoporosis, either alone or in combination

with antiresorptive agents such as bisphosphonates. An animal model study showed enhanced cure of fracture in a model with surgical osteotomy and significantly increased bone formation at the alveolar crest of the mandible in aged ovariectomized rats.[14] Moreover, clinical trials also revealed that PTH increases bone mineral density and reduces the fracture risk in females with postmenopausal osteoporosis.[15]

HBO therapy is known to be an effective adjunctive therapy for chronic osteomyelitis and ORN and can also be used for the treatment of MRONJ.[16,17] Although how HBO affects the cure of MRONJ is not fully clarified, this treatment modality has been proven to be successful in increasing the incorporation rate of grafted bone[18] and the dental implant success rate[19] in the irradiated mandible by increasing the oxygen in blood followed by suppression of the formation of osteoclasts, thereby reducing bone resorption.[20]

To the best of the authors' knowledge, HBO and PTH have been explored independently for their effect on new bone regeneration; however, there have been no *in vivo* studies replicating impaired osteogenic conditions to evaluate their possible synergistic effect, which would optimize new bone regeneration. This may be attributable to the difficulty in developing such translational animal models simulating all of the complex interdependent processes implicated in human disease.[21] In fact, previous studies reporting the occurrence of MRONJ-like features in animal models had limited success

rates and highly variable reproducibility.[22] The irradiated rat model has the advantages of easy manipulation and development of a standardized model for the assessment of a specific treatment modality without the need to consider serious systemic damage[18]. Therefore, the irradiated rat model is preferred when a standardized animal model that accurately simulates an impaired osteogenic condition is required.[23]

The aim of this study was to determine the influence of PTH in combination with HBO on regeneration after bone graft in rat calvarial bone defects model under impaired osteogenic conditions induced by irradiation.

Ⅱ. MATERIALS & METHODS

2.1. Animals

Twenty four male Sprague-Dawley rats weighing 300 g were used in this study. All animal selection, preparation, and surgical protocols were conducted according to Association for Assessment and Accreditation of Laboratory Animal Care international (AAALAC) guidelines and approved by the Institutional Animal Care and Use Committee, Yonsei Medical Center, Seoul, Korea (Approval no. 2013-0292). The animal laboratory was set to 22℃ and 50% humidity with a 12 hours light-dark cycle for the experiment. Two rats were kept in each cage.

2.2. Experimental design

The rats were divided into three groups: Control, PTH, and PTH/HBO (each n=8). All animals were irradiated at the calvarial, and calvarial bone was grafted after 4 weeks. Starting the day after surgery, PTH was injected into rats in the PTH and PHT/HBO groups for 2 weeks. The PTH/HBO group additionally received HBO therapy for 1 week postoperatively (Figure 1) (Table 1). Defect was filled with bone graft materials (OSTEON II Collagen; Dentium, Suwon, Korea). Considering decreased osteogenic ability due to irradiation, a calvarial defect with outer diameter of 5.0 mm was used.

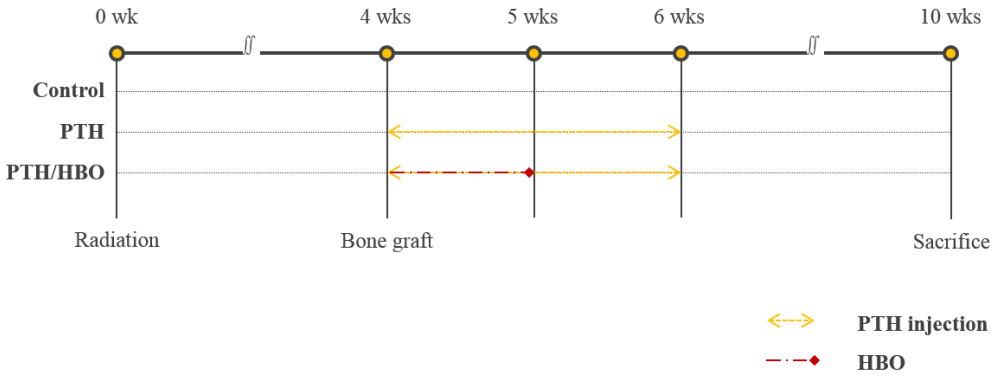

Figure 1. Experimental design protocol

	Radiation	Bone graft	PTH	HBO
Control	O	O	-	-
PTH	O	O	O	-
PTH/HBO	O	O	O	O

Table 1. Experimental design

2.3. Experimental procedure

All animals were irradiated according to the experimental conditions under general anesthesia (Figure 2). Anesthesia consisted of an intraperitoneal injection of combined Zoletil (tiletamine and zolazepam, 50 mg/ml, 0.6 ml/kg body mass; Virbac lab. Carros, France) and Rompun (zylazine, 23.32 mg/ml, 0.4 ml/kg body mass; Bayer, Leverkusen, Germany). The rats received a single, 12.0 Gy localized radiation dose[24] to the calvarial area at 300 kV/12.5 mA using an X-RAD 320 (Precision X-Ray, North Branford, CT, USA) and a field size of 2 × 2cm (Figure 4).[24] Four weeks after commencement of irradiation, additional infiltration anesthesia (2% lidocaine hydrochloride with 1:80,000 epinephrine) was administered at the surgical sites. A straight incision was made along the sagittal midline and a full-thickness flap including skin and periosteum was raised, exposing calvarial bone. Under copious saline irrigation, standardized and circular defects were made on both sides of the sagittal suture using a trephine bur with a 5.0 mm outer diameter (Figure 3). Biphasic calcium phosphate (BCP) synthetic bone graft materials (OSTEON II Collagen; Dentium, Suwon, Korea) were grafted and quantifiably covered with collagen membrane (HA Collagen Membrane; Dentium, Suwon, Korea). The periosteum was repositioned over the defects on each side and the periosteum/skin flaps were sutured using 4-0 polyglactin 910 suture material (Vicryl® , Ethicon, Somerville, NJ, US). To decrease post-surgical infection and pain, meloxicam (1 mg/kg, once a day for 5 days;

metacam®, Boehringer Ingelheim, Rhein, Germany) and enfloxacin (10 mg/kg/day, once a day for 5 days, baytril®, Bayer, Germany) were subcutaneously administered. The day after the surgery, PTH[1-34] (30 μg/kg/day for 3 days weekly, Forsteo; Eli Lilly, Houten, the Netherlands) was subcutaneously injected into animals in the PTH group and PTH/HBO group. The PTH/HBO group additionally received HBO treatment (2.4 ATA/day, for 6 days) from the day after surgery. HBO involved pressurization to 2.4 ATA over 15 min, maintenance of pressure for 60 min, and depressurization for 15 min.[21] A customized HBO chamber for the experiment was made in the laboratory. At 6 weeks after surgery, all the subjects were sacrificed with perfusion after general anesthesia. After removing the skin, organs were extracted and fixed with 10% formalin.

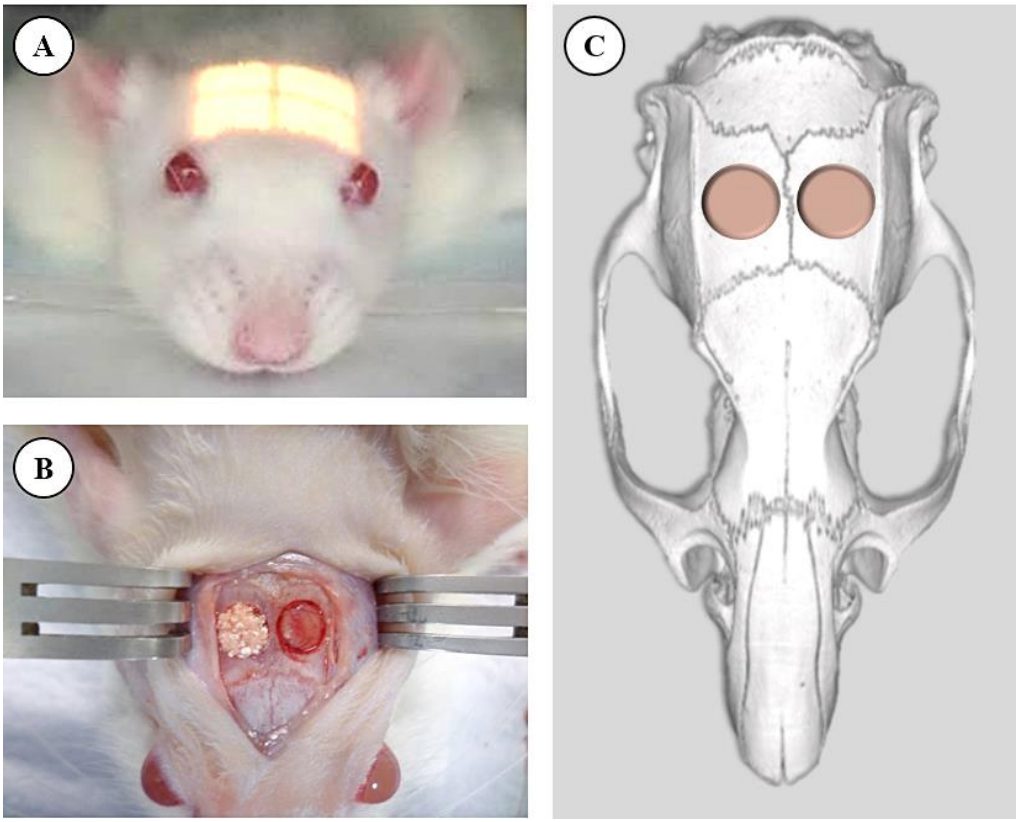

Figure 2. Local irradiation and surgical procedure

Ⓐ Field size of 2x2 cm (see light window); Ⓑ Bone graft; Ⓒ Anatomical location

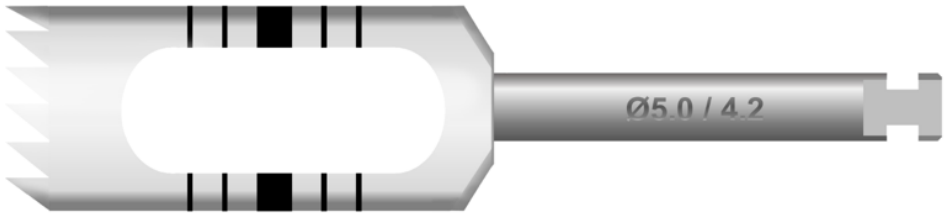

Figure 3. Trephine bur

Figure 4. Local irradiation procedures

Ⓐ Ⓑ Verifying radiation fields using an external beam simulator; Ⓑ Local radiation irradiation equipment

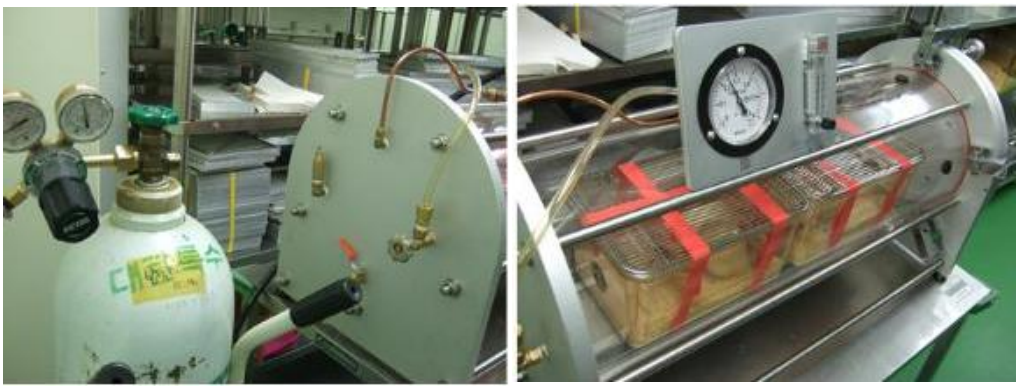

Figure 5. Experimental hyperbaric oxygen chamber

2.4. Micro computed tomographic (micro-CT) analysis

Three-dimensional (3D) images were taken for each sample using high-resolution micro-CT (Skyscan1173, Skyscan, Konitch, Belgium) at 100 kVp, 100 μA, a 8.17 μm pixel size, and the images were reconstructed and analyzed via CTAn (Skyscan, Aartselaar, Belgium). The photographed samples were graphically categorized into residual materials (gray scale: 135-255) and new bone (gray scale: 90-135), then total volume (TV; mm^3), bone volume (BV; mm^3), bone surface (BS; mm^2) and bone surface density (BS/TV; mm^2/mm^3) were analyzed accordingly (Figure 6, 7) (Table 2).

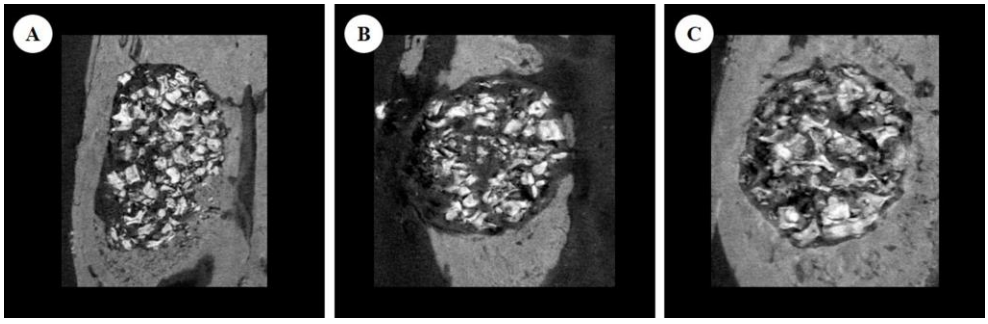

Figure 6. Horizontal plane view of micro-CT analysis in surgical site

Ⓐ Control group; Ⓑ PTH group; Ⓒ PTH/HBO group

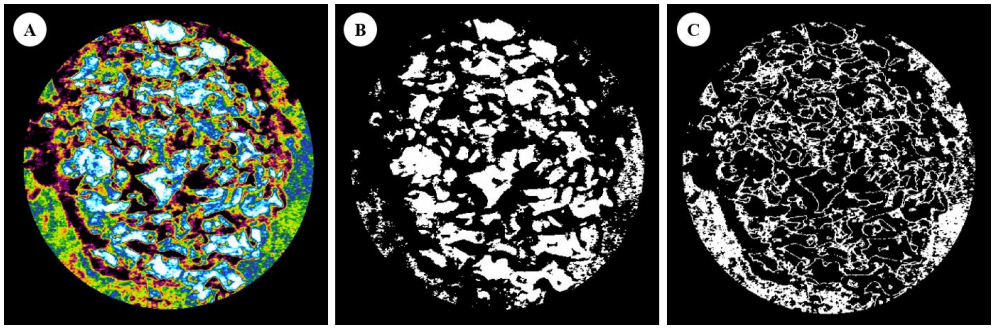

Figure 7. Gray scale for micro-CT analysis in surgical site

Ⓐ total bone (90-255); Ⓑ residual materials (135-255); Ⓒ new bone (90-135)

Abbreviation	Variable	Description	unit
BV	bone volume	Volume of the region segmented as bone	mm^3
BS	bone surface	Surface of the region segmented as bone	mm^2
BS/TV	bone surface density	Ratio of the segmented bone surface to the total volume of the region of interest	mm^2/mm^3

Table 2. Definition and description of three-dimensional outcomes for bone microarchitecture

2.5. Histological and histomorphometric analysis

The samples were decalcified with 5% HCl and embedded in paraffin. The paraffin blocks were serially sliced in a 5 μm coronal plane. The sectioned specimens were dyed with hematoxylin and eosin (H&E) staining and observed with light microscopy (BX50, Olympus Co., Tokyo, Japan). Image analysis was conducted using image software (Adobe Photoshop CS4, Mountain View, CA, USA) and the pixel number was digitized with ScopeEyE 3.6 (TOMORO, Samkyung Co., Seoul, Korea). Stained slide were measured by a single researcher in the blind state. The total augmented area (TAA), residual material area (RMA), new bone area (NBA), connective tissue area (CTA). Percent new bone area (%NBA; NBA/TAA*100), percent residual material area (%RMA; RMA/TAA*100), and percent connective tissue area (%CTA; CTA/TAA*100) were measured (Figure 8). Additionally, the number of osteoclasts and blood vessels was recorded (Table 3).

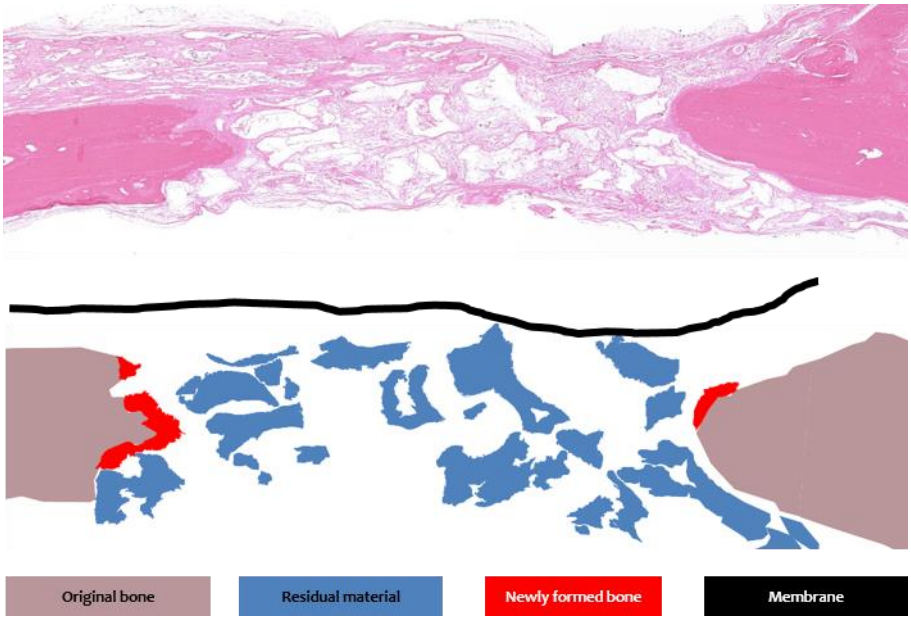

Figure 8. Schematic diagram depicting the parameter used in histologic/ histomorphometric analysis

Abbreviation	Variable	Description	unit
% NBA	Percent New Bone Area	Percent of new bone area to the total area of the region of interest	%
% RMA	Percent Residual Materials Area	Percent of residual materials area to the total area of the region of interest	%
% CTA	Percent Connective Tissue Area	Percent of connective tissue area to the total area of the region of interest	%
% OCN	Percent Osteoclasts Number	Percent of osteoclasts number to the total area of the region of interest	%
% BVN	Percent Blood Vessels Number	Percent of blood vessels number to the total area of the region of interest	%

Table 3. Definition and description of parameter for histomorphometry

2.6. Statistical analysis

The statistical analysis was performed using a commercially available software program (SPSS 20.0, SPSS Inc., Chicago, IL, USA). Data obtained in each group are expressed as mean and standard deviations. Kruskal-Wallis analysis based on ranks and the post-hoc Mann-Whitney U test were used to assess the differences among groups. A *P*-value less than 0.017 was considered statistically significant. Test of three were performed to correct the significance level (Significance level = 5% / number of tests; 0.017 = 5% / 3). No sample size calculation was performed due to the exploratory nature of this study.

Ⅲ. RESULTS

3.1. Clinical observation

No signs of inflammation and adverse effects were identified in all Control and experimental groups apart from one rat in the Control group (Figure 9).

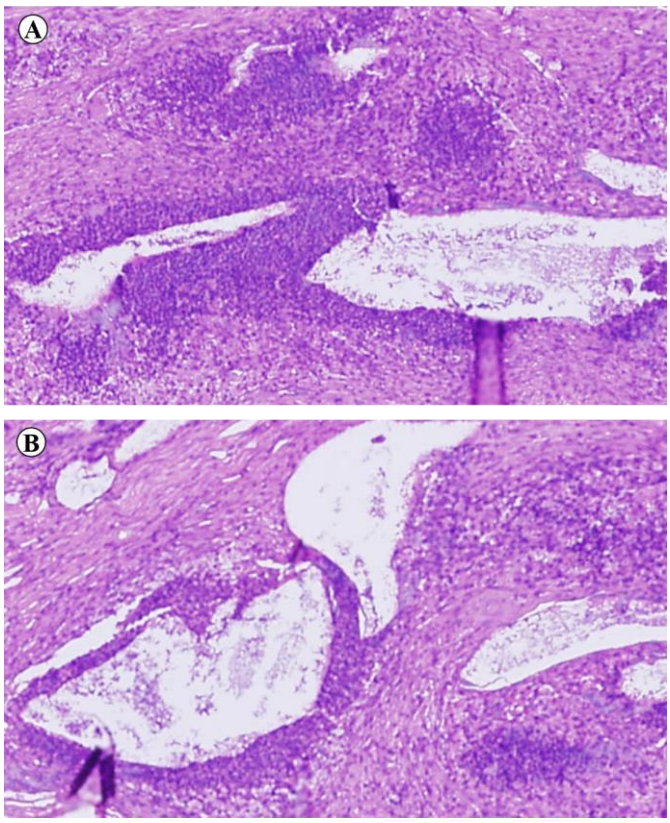

Figure 9. Inflammation observed around graft material on calvarial defect center

3.2. Micro computed tomographic (micro-CT) analysis

In BV of new bone, PTH group showed significantly higher values than Control group. In BS of new bone, PTH/HBO group showed significantly higher than Control group. In new bone, BS/TV was higher in the order of Control, PTH and PTH/HBO group. Also, there was a significant difference in all groups (Table 4, 5) (Figure 10, 11, 12).

In BS of residual material, Control group showed significantly lower than PTH/HBO group. In BS/TV of residual material, Control group showed significantly lower than PTH/HBO group (Table 4, 5) (Figure 10, 11, 12).

		Control	PTH	PTH/HBO
New Bone	BV	2.06 ± 0.54 *	2.79 ± 0.25 *	2.73 ± 0.43
	BS	229.77 ± 70.33 †	283.30 ± 22.25	327.66 ± 55.75 †
	BS/TV	17.55 ± 4.13 *†	23.46 ± 1.17 *‡	26.59 ± 5.46 †‡
Residual Materials	BV	2.50 ± 1.39	3.09 ± 0.40	3.78 ± 0.85
	BS	103.62 ± 39.36 †	127.61 ± 11.67	158.72 ± 33.59 †
	BS/TV	7.87 ± 2.45 †	10.56 ± 0.48	12.86 ± 2.89 †

Table 4. Volumetric analysis in micro-CT (Mean ± SD) ($P < 0.017$)

BV, bone volume (mm^3); BS, bone surface (mm^2); BS/TV, bone surface density (mm^2/mm^3)

***** Significant differences compared the Control and PTH group

† Significant differences compared the Control and PTH/HBO group

‡ Significant differences compared the PTH and PTH/HBO group

P value		Control – PTH	Control – PTH/HBO	PTH - PTH/HBO
New Bone	BV	0.010 *	0.038	0.442
	BS	0.130	0.010 *	0.038
	BS/TV	0.010 *	0.003 *	0.010 *
Residual Materials	BV	0.505	0.083	0.065
	BS	0.279	0.015 *	0.038
	BS/TV	0.083	0.007 *	0.021

Table 5. Statistical result of micro-CT data

Kruskal-Wallis and posthoc Mann-Whitney U test. Asterisk (*) ($P<0.017$) means statistically significant.

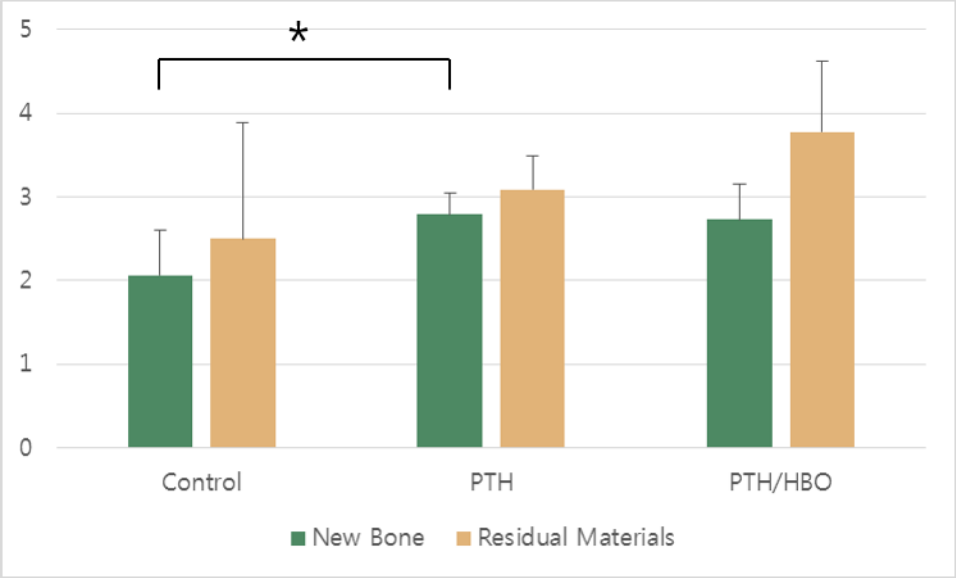

Figure 10. Bone volume (BV) in micro-CT (unit : mm^3)

***** Significant differences compared the Control and PTH group

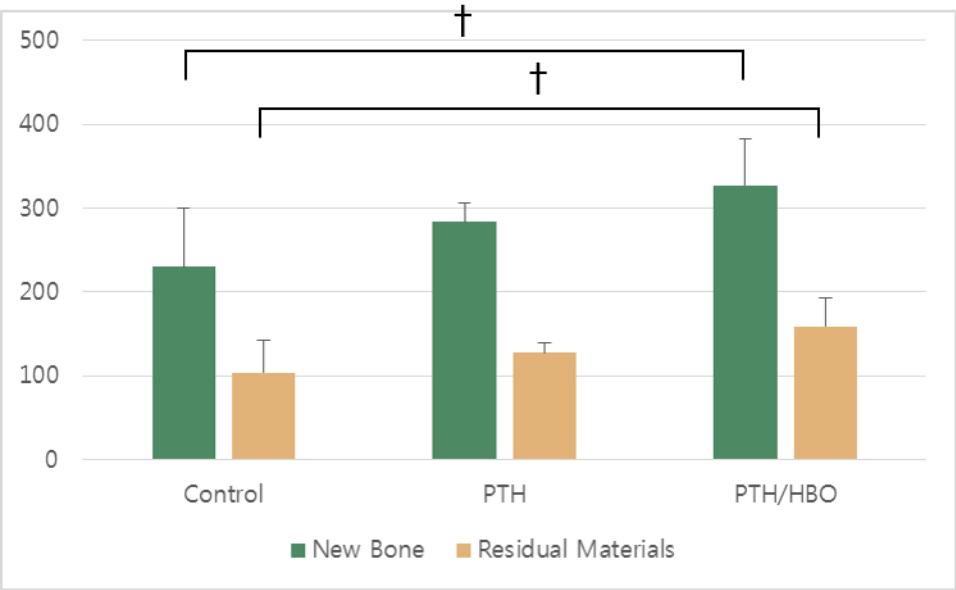

Figure 11. Bone surface (BS) in micro-CT (unit : mm^2)

† Significant differences compared the Control and PTH/HBO group

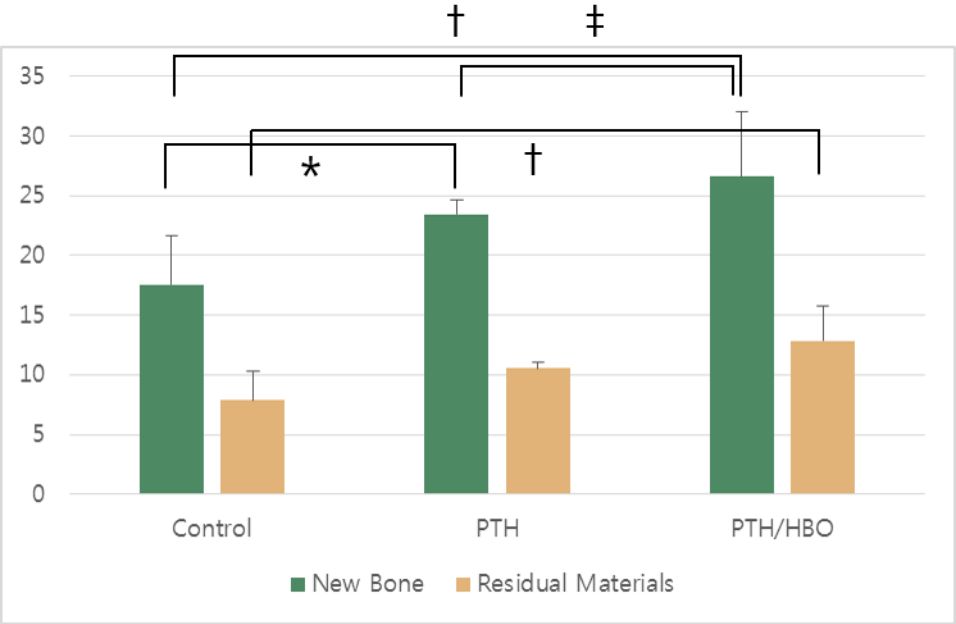

Figure 12. Bone surface density (BS/TV) in micro-CT (unit : mm^2/mm^3)

***** Significant differences compared the Control and PTH group

† Significant differences compared the Control and PTH/HBO group

‡ Significant differences compared the PTH and PTH/HBO group

3.3. Histological analysis

New bone formation was identifiable in the defect margin areas. Especially, in the PTH/HBO group, an island-shaped immature woven bone area was created near the defect margin. Although resorption of existing bone was generally observed in each individual, it was particularly apparent in the PTH group. In the central defect region, immature woven bone was observed surrounding the graft materials. In the PTH/HBO group, massive immature woven bone was observed enveloping the graft bone. In particular, the creation of string-shaped long new bone was identified and it appeared that the mature bone and immature woven bone were combined (Figure 13). Osteoclasts were usually observed near the graft material and were mostly observed in the PTH group (Figure 14).

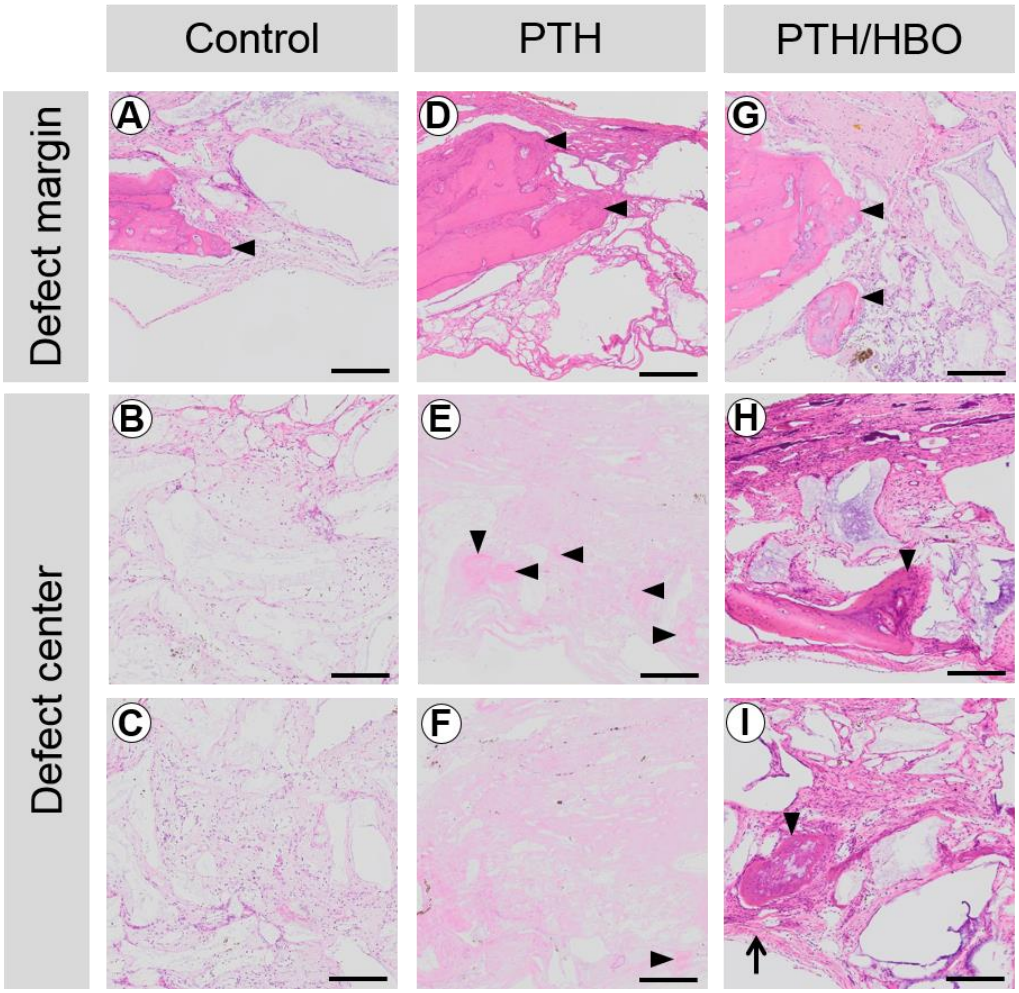

Figure 13. Histological analysis (x200; Scale bar = 200μm)

Arrowheads, immature woven bone; arrow, mature bone. Ⓐ-Ⓒ Control group; Ⓓ-Ⓕ PTH group; Ⓖ-Ⓘ PTH/HBO group

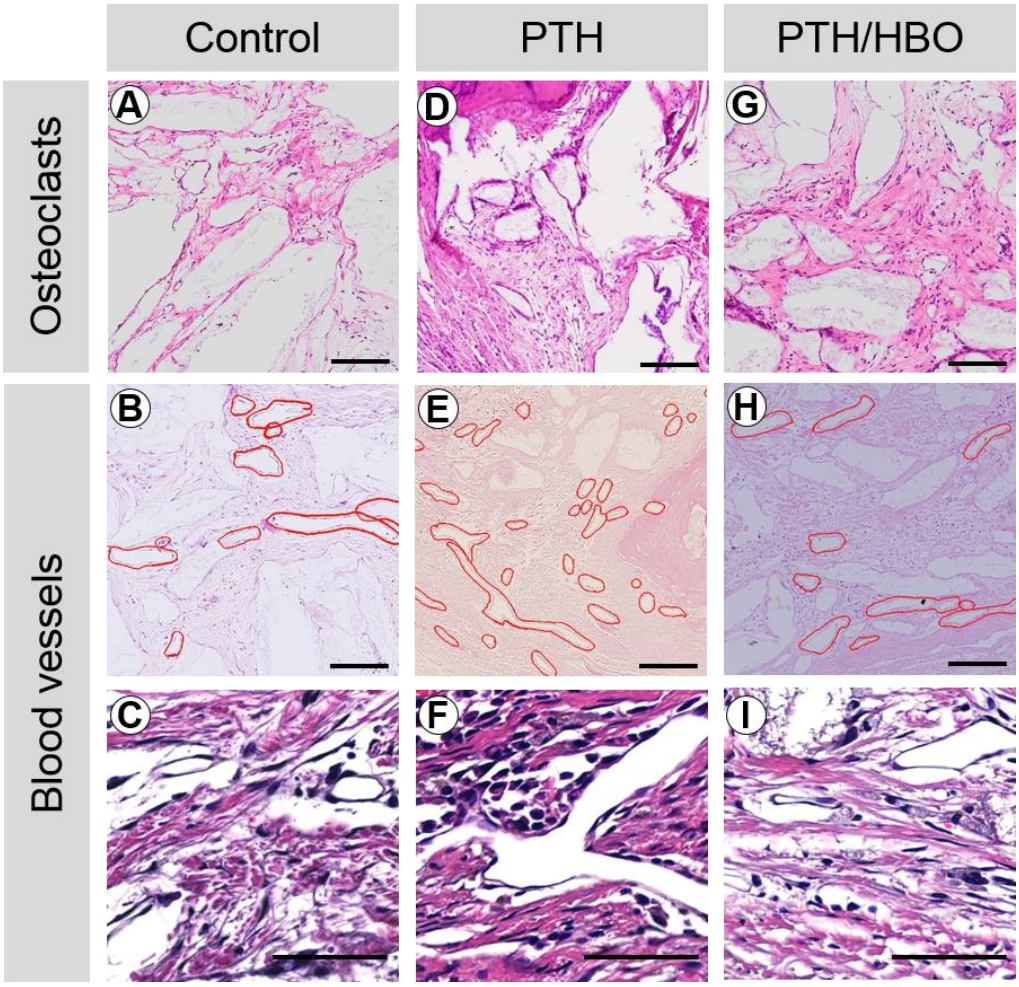

Figure 14. Osteoclasts and blood vessels

Ⓐ-Ⓒ Control group; Ⓓ-Ⓕ PTH group; Ⓖ-Ⓘ PTH/HBO group

ⒶⒷⒹⒺⒼⒽ x200; Scale bar = 200μm

ⒸⒻⒾ x1000; Scale bar = 10μm

3.4. Histomorphometric analysis

%NBA was significantly higher in the PTH group than in the Control group. Additionally, %NBA was significantly increased in the PTH/HBO group compared with the PTH group; however, there was no significant difference between the Control and PTH/HBO group (Table 6, 7) (Figure 15).

%RMA decreased in the order of Control, PTH and PTH/HBO groups. %OCN increased in the order of Control, PTH/HBO and PTH group. %BVN was the most abundant in the PTH group, and less in the PTH/HBO group (Table 6, 7) (Figure 15, 16).

	Control	PTH	PTH/HBO
%NBA	2.10 ± 1.34 *	6.92 ± 2.80 *†	10.07 ± 8.09 †
%RMA	32.07 ± 9.09	24.71 ± 14.00	21.74 ± 6.76
%CTA	65.84 ± 9.33	64.50 ± 19.00	68.19 ± 8.52
%OCN	0.35 ± 0.29	1.10 ± 1.06	0.55 ± 0.45
%BVN	2.71 ± 1.70	4.26 ± 1.45	2.61 ± 1.22

Table 6. Histomorphometric analysis (Mean ± SD) (*P* < 0.017)

%NBV, percent new bone area; %RMA, percent residual materials area; %CTA, percent connective tissue area; %OCN, percent osteoclasts number; %BVN, percent blood vessels number.

***** Significant differences compared the Control and PTH group

† Significant differences compared the PTH and PTH/HBO group

P value	Control – PTH	Control – PTH/HBO	PTH – PTH/HBO
%NBA	0.001 *	0.000 *	0.442
%RMA	0.195	0.028	0.878
%CTA	0.645	0.505	0.959
%OCN	0.050	0.279	0.645
%BVN	0.105	0.959	0.028

Table 7. Statistical result of histomorphometric data. Kruskal-Wallis and posthoc Mann-Whitney U test. Asterisk (*) (P<0.017) means statistically significant.

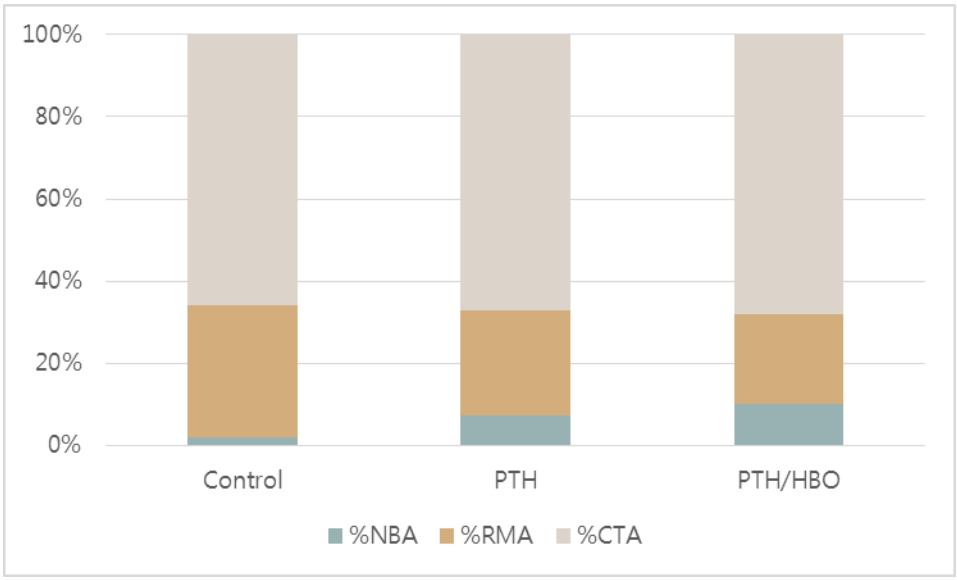

Figure 15. Histomorphometric analysis (%NBA, %RMA, %CTA)

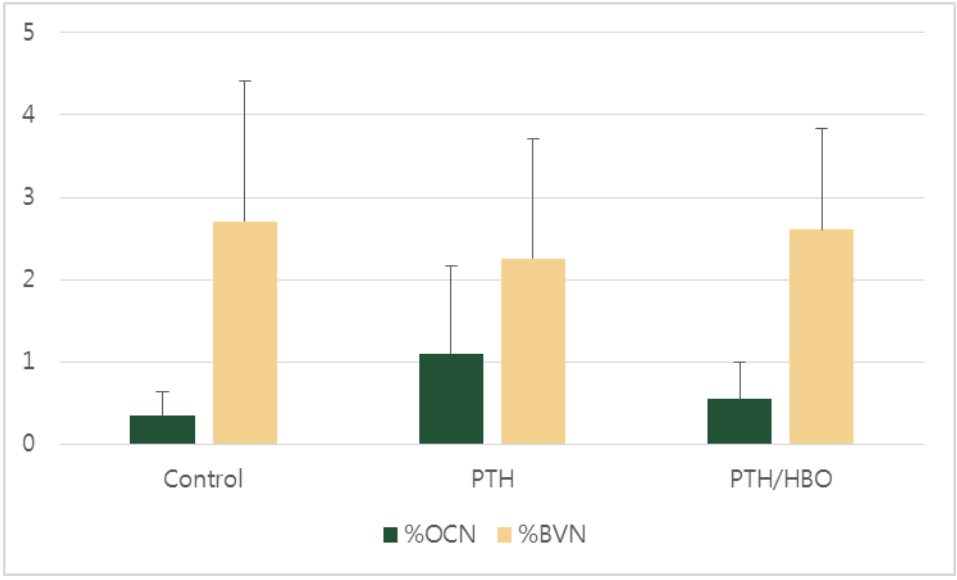

Figure 16. Histomorphometric analysis (%OCN, %BVN)

Ⅳ. DISCUSSION

In this study, we evaluated the synergistic effect of PTH in combination with HBO, which were previously reported to be effective for osteogenesis and bone regeneration after bone graft treatment in an irradiation-induced osteogenic condition. To date, animal models developed to investigate the complex processes involved in the pathogenesis of bone-related diseases such as ORN and MRONJ have had limited success because simulating the conditions that result in the development of the representative clinical lesions found in humans is extremely difficult and cannot be accurately achieved during the surgical procedure. However, with the increasing incidence of MRONJ, the importance of evaluating treatment modalities that can be used for its treatment is proportionally increasing and establishment of a standardized animal model with high reproducibility and reliability is required to evaluate specific treatments. In this study, the synergistic effect of PTH and HBO on bone regeneration was evaluated by histomorphometric and micro-CT analysis when bone graft was performed after calvarial defect formation in an easy and reproducible irradiated rat model simulated by impaired osteogenic condition.

In the previous study by Chang et al.,[18] although enhanced angiogenesis was identified in the HBO group, the authors concluded that a healing time of 4 weeks might not be sufficient to confirm the treatment modality employed in

the rat model. This was supported by Hu et al.,[25] who showed that a healing time of 6 weeks in a rabbit model, which corresponds to a healing period of 8 weeks in a rat, was required to reveal the effects of a specific treatment modality. As the process of new bone formation in rats cannot be completed within 4 weeks after bone graft, in the present study a healing period of 6 weeks was implemented to observe the synergistic effect of PTH in combination with HBO on the regeneration of a calvarial bone defect in a rat model under impaired osteogenic conditions.

From the histological point of view, new bone formation was mainly observed around the defect margin of all groups, while an island form of immature woven bone was distinguishably identified in the PTH/HBO group. Interestingly, in the PTH and PTH/HBO groups, more immature woven bone seemed to be confined around the grafted materials, forming a long and large new bone consisting of mature compact bone and immature woven bone. This result is consistent with previous studies reporting that HBO enhanced wound healing.[21,22] In addition, our results also showed that intermittent PTH administration is effective in accelerating cancellous bone remodeling, thereby shortening the treatment period after bone grafting.[26]

In the histomorphometric analysis, %CTA was not different among the groups however the PTH and PTH/HBO groups showed increased %NBA compared with the Control group. In particular, the PTH/HBO group showed significantly higher %NBA than the PTH group and %RMA in PTH/HBO was

smaller than that of the PTH group, indicating that significant active new bone formation occurred in the PTH/HBO group. These results are supported by many previous studies and are mainly attributable to increased vascular endothelial growth factor (VEGF) expression followed by increased bone marrow.[27,28] The osteoclasts number and blood vessels number were highest in the PTH group and lowest in the Control group. Both PTH and HBO increase the levels of osteoblasts and decrease those of osteoclasts to promote bone formation.[22,29,30] Osteoclasts play a role in promoting angiogenic factor secretion (MMP-9 pathway) to promote neovascularization.[19,31] Therefore, the greater number of blood vessels and osteoclasts in the PTH group compared with the PTH/HBO group may indicate that combinational use of PTH and HBO excessively over inhibited osteoclast activity, reducing blood vessel formation by blocking MMP-9 pathway compared with PTH alone; this is supported by the proportional relationship between the number of osteoclasts and the number of blood vessels. However, the reduction of blood vessel number in the PTH/HBO group did not lead to a reduction of new bone formation because of the specific effect of HBO on BCP use. HBO enhanced rapid absorption of BCP and improved new bone formation ability.[18,27,28] These results suggest that concurrent use of PTH and HBO may lead to better new bone formation and absorption of residual material compared with PTH alone. HBO was reported to increase replacement of marrow at defects grafted with BCP.[28] Also, HBO resulted in a decrease of the bone mineral content of

the grafted materials,[27] speeding up the union of grafted bone with existing bone.[32] Plain defects without BCP are expected to have reduced bone formation or no significant difference, and further study is needed to test this.

Because histology and histomorphometry only give two-dimensional results, qualitative evaluation is limited. Recently, it became possible to evaluate the quality of the experimental site through various parameters of micro-CT.[2,6,33] Therefore, it was important to select parameters that were meaningful to this study among the various parameters. BV is the volume segmented as bone, and can analyze the volume of new bone and residual implant within the region of interest. BS is the surface of the region segmented as bone, and is a parameter that can measure the bone surface of new bone and residual implant in the region of interest. BS/TV is the ratio of the segmented bone surface to the segmented bone volume, and is used as a parameter for specific bone surface.[33] In this study, bone quality of the new bone was best for the PTH/HBO group for when qualitative evaluation of the experimental site was performed through micro-CT. In addition, the quality of the residual material was best in the Control group. This is consistent with the histomorphometric analysis, showing not only an increase in the quality of new bone but also adequate absorption of residual materials when PTH and HBO were used together. It has already been shown in many animal and clinical studies that HBO is effective in bone regeneration not only in the bone of normal condition but also in the impaired osteogenic condistion.[1,18]

Therefore, we did not compare the HBO only group in this study. However, since there are not many studies evaluating the effect of HBO according to the duration of treatment, our team is currently working on further studies.

The clinical significance of this study is that bone grafting can shorten bone healing time and provide a positive prognosis in patients with reduced bone turnover or hormone control function. However, the limitation of this study is that it is difficult to compare bone healing patterns at various time points. In addition, it is difficult to compare the results of PTH and HBO treatment with time. Nevertheless, it is easy to evaluate certain forms of treatment without severe systemic impairment. Furthermore, to reproduce impaired osteogenic condition the synergistic effect of PTH and HBO was evaluated using the irradiated model, which is a more standardized animal model and yielded meaningful results.

Ⅴ. CONCLUSION

We could confirm the quantitative and qualitative synergistic effect of concurrent treatment with PTH and HBO on new bone formation after bone graft in an impaired osteogenic condition induced by irradiation.

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국문요약

방사선 조사 된 백서 두개골 골 이식에 고압산소 치료와 부갑상선호르몬 투여의 시너지 효과

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목적

본 연구의 목적은 손상된 골 형성 조건의 백서 두경부 결손 모델에서 골 이식 후 부갑상선호르몬과 고압산소치료를 함께 시행 하였을 때 골재생의 효과를 평가하는 것이다.

재료 및 방법

24 마리의 웅성 백서를 3 그룹으로 나누었고, 12 Gy 단일 선량을 가진 국소 방사선이 두경부에 조사되었다. 방사선 조사 4 주 후 방사선이 조사되었던 부위에 골 결함을 생성하였고, 모든 결함은 bisphasic

calcium phosphate (BCP)로 채워졌다. 골 이식 후 부갑상선 호르몬을 피하투여 하였고, 같은 시기에 고압산소치료를 시행하였다. 골 이식 6 주 후, 동물을 희생시키고 시편을 채득하였다.

결과

조직형태학적 평가에서 PTH 및 PTH/HBO 군에서 신생골의 양이 대조군보다 높았다. 방사선학적 분석 결과 신생골은 대조군보다 PTH 군이 높았다. 신생골의 골 표면적은 대조군보다 PTH/HBO 군이 높게 나타났다. 신생골의 골 표면적 밀도는 대조군, PTH 군, PTH/HBO 군 순으로 높았다; 모든 군간에 유의한 차이가 있었다 ($P<0.017$).

결론

본 연구의 한계 내에서, 방사선 조사로 인해 손상된 골에서 부갑상선호르몬과 고압산소치료를 결합하여 시행하였을 때 골 치유에 시너지 효과를 나타낸다는 것을 확인하였다.

핵심되는 말: 부갑상선호르몬; 고압산소치료; 동물 모델; 두경부 결손; 골이식; 골재생