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Effect of Intermittent Parathyroid Hormone Administration on the Microstructure of Jaw Bone in the Ovariectomized Rats

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Purpose: Parathyroid hormone (PTH) therapy has drawn attention, as an alternative to anti-resorptive drugs since PTH accelerates bone density by anabolic action. The purpose of this study was to identify the effect of intermittent PTH administration on jaw bones of rat undergone bilateral ovariectomy.

Materials and Methods: Nine female Sprague-Dawley rats were divided into three groups. PTH group was ovariectomized (OVX) to induce osteoporosis and PTH 30 μg/kg was administered 1 week after the surgery. In OVX group, ovariectomy was performed and only vehicle was administered by subcutaneous injection 3 times per week. Control group was subjected to sham surgery. The animals were sacrificed 8 weeks after the surgery and specimens were obtained from ilium and upper and lower jaw bones. Histological investigation was carried out by using an optical microscope and micro-computed tomography was taken to examine structural property changes in each bone sample.

Result: In the ilium, the bone volume ratio (bone volume/total volume, BV/TV) of PTH, OVX and control groups was 53.75%±7.57%, 50.61%±12.89%, 76.20%±5.92% (P=0.061) and bone mineral density (BMD) was 1.12±0.09, 0.88±0.48, 1.38±0.07 g/cm^3 (P=0.061). In the mandible, BV/TV of PTH, OVX and control groups was 64.60%±12.17%, 58.26%±9.63%, 67.54%±14.74% (P=0.670) and BMD was 1.21±0.17, 1.19±0.13, 1.27±0.18 g/cm^3 (P=0.587). In the maxilla, BV/TV of PTH, OVX and control groups was 61.19%±8.92%, 52.50%±11.22%, 64.60%±12.17% (P=0.430) and BMD was 1.20±0.11, 1.11±0.16, 1.21±0.17 g/cm^3 (P=0.561). No statistically significant difference was found in any variables in all groups. Histological observation revealed that the ilium in OVX group demonstrated sparsely formed trabecular bones compared with other groups. However, upper and lower trabecular bones did not present significant differences.

Conclusion: Intermittent administration of PTH appears to affect the microstructure of rat jaw bones, but statistical significance was not found. However, the measurements in this study partly implicated the possible anabolic effect of PTH *in vivo*.

Key Words: Bone density; Osteoporosis; Ovariectomy; Parathyroid hormone

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Introduction

Due to the rise in life expectancy of human beings, the percentage of elderly patients visiting dental clinic has dramatically increased[1]. Osteoporosis, one of the senile diseases, is characterized by bone volume reduction, micro-architectural deterioration and skeletal fragility causing an increased risk of bone fractures in vertebrae, femur, wrist, and others[2-4].

Estrogen deficiency after menopause accounts for osteopenia, osteoporosis and bone loss in humans and experimental animals[5-7] and numerous studies suggested that systematic osteoporosis and oral bone loss as well as tooth loss are at positive correlation and occurrence of osteoporosis is increasing every year[7].

Various experimental animal models including rabbits, monkeys and rats have been used for the relevant research and among those, a useful experimental model for postmenopausal osteoporosis research has been provided by bilaterally ovariectomized (OVX) rats which acquire trabecular bone loss[2,4,7-9]. Patients with osteoporosis have been taking various types of therapeutic agent including bisphosphonates, calcium, hormones, etc. Among these therapeutic agents, several clinical studies on effectiveness of parathyroid hormone (PTH) therapy in osteoporosis indicated that systemic application of PTH induces new bone formation on fractured sites as well as increasing new bone strength[10-14].

PTH is known to maintain calcium homeostasis systemically; however, it has been reported that through anabolic action, it induces acceleration of damaged oral bone healing, latent therapeutic effects on local bony defect and formation of bone around dental implant sites with evident bony defects[5,9,15-18].

Teriparatide, recombinant human PTH (1-34) is the first therapeutic drug approved by U.S. Food and Drug Administration and has widely been used as a therapeutic agent for increase in bone strength and prevention of bone fracture[14].

Generally, endogenous PTH acts as the first regulator for calcium and phosphorus metabolism in bone and kidney and increases blood calcium level by promoting bone resorption. However, the effects of PTH on bone appeared to have opposite actions depending on continuous or intermittent exposure[19,20]. In most cases, chronic exposure to PTH reduces bone volume but in contrast, intermittent administeration of PTH activates more osteoblasts than osteoclasts which reesults in new bone formation and increased bone density[17,20-22]. Therefore, it has been shown that intermittent PTH administeration greatly assists in prevention and healing of osteoporotic fractures[14,23]. In spite of ample preclinical animal experiements regarding the effectiveness of PTH therapy in osteoporosis in iliums and fibulas, researches on alveolar bone in jaw bones have been scarce[8,9,24]. Hence, this study was conducted to investigate the effects of PTH on bone quality and structure in jaw bones in osteoporosis induced rats that were bilaterally OVX to simulate estrogen deficiency by histological and radiographical comparisons in illiums, upper and lower jaw bones between groups with and without intermittent PTH administration.

Materials and Methods

1. Experimental Animal

Nine female Sprague-Dawley rats (Nara Biotech., Pyeongtaek, Korea) weighing an average of 200 g and 8 weeks of age were selected and divided into three groups. Group one (PTH) was subjected to ovariectomy with PTH administration after the surgery, group two (OVX) was subjected to ovariectomy only and group three (control) was the control group.

The experimental animals were housed with a light/dark cycle of 12 hr/12 hr and fed a standard laboratory diet and sterile water *ad libitum* with

controlled temperature at 25°C±1°C. The study was proceeded after approval by the committee of ethic for animal experimentation (HFA 13-002).

2. Induction of Osteoporosis by Ovariectomy

The experimental animals were acclimatized for a week prior to use and underwent inhalation anesthesia with 3% isoflurane (JW Medical, Seoul, Korea). Two groups were subjected to bilateral ovariectomy whereas control group was subjected to sham operation only. All rats were given intramuscular injection of gentamycin (5 mg/kg; Kukje Pharm. Co., Seongnam, Korea) as pre and post operational antibiotic and ketoprofen (5 mg/kg; Bukwang Pharm. Co., Seoul, Korea) as anti-inflammatory.

3. Administration of Parathyroid Hormone

One week after ovariectomy, vehicle was made with 2% bovine serum albumin (0.1 M Tris-HCl, pH 7.5) and 1-34 synthetic PTH (R&D Systems Inc., Minneapolis, MN, USA) was administered subcutaneously in dorsal area of the rats to PTH group (30 μg/kg, 3 days weekly) whereas vehicle of the same amount was administered subcutaneously to OVX and control groups.

4. Histological Observation

Eight weeks after ovariectomy, experimental animals were sacrificed using CO_2 and samples were collected from ilium and upper and lower jaw bones. The collected samples were demineralized with 10% EDTA for approximately 30 days then dehydrated in ethanol and embedded in paraffin. Sections were made in mesio-distal direction of tooth in 5 μm thickness and stained with H&E then histological observation was carried out using an optical microscope.

5. Micro-Computed Tomography Analysis

The collected bone samples were fixed with 10% neutrally buffered formalin (Fischer Scientific Co., Columbus, OH, USA) and micro-computed tomography (SkyScan1173; SkyScan, Kontich, Belgium) was taken for evaluation. Images were acquired using 10 μm resolution, 130 kV voltage, 60 μA current and 250 ms exposure time. The volume of interest (VOI) was set to alveolar bones between roots of upper and lower first molars and heads of femur (Fig. 1). The manufacture's reconstruction program was used for evaluation of three-dimensional images and the settings used were 10 μm pixel size, 0.2° angular step and 40% beam hardening.

Furthermore, Skyscan computed tomography-analyser program was used to measure total volume (TV), bone volume (BV), bone volume ratio (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp) and bone mineral density (BMD) in VOI.

6. Statistical Analysis

All results were expressed as the mean±standard deviation and comparisons among each variable

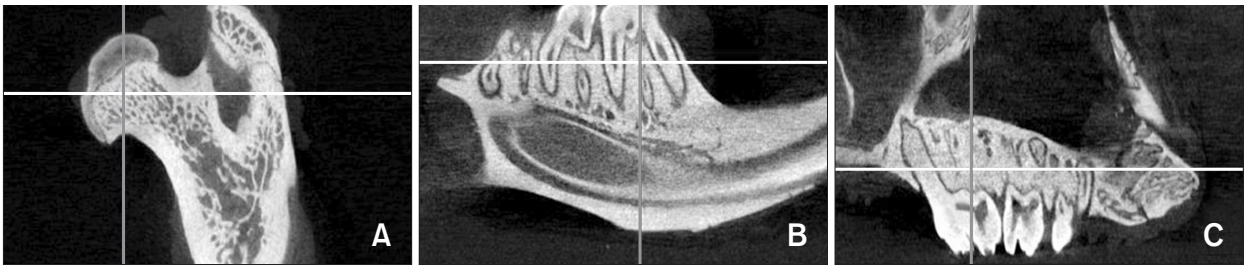

Fig. 1. Volume of interest for three-dimensional micro-computed tomographic analysis of head of femur (A), in the alveolar bone between the roots of the first molar in mandible (B) and in maxilla (C).

in different groups were done using the Kruskal-Walis test. Post-hoc Mann-Whitney analyses were conducted for all significant parameters. All statistical significance was set to $P<0.05$. All analyses were performed with a commercial statistical program (IBM SPSS version 20.0; IBM Co., Armonk, NY, USA).

Result

1. Histological Opinion

The effects of PTH administration were observed with an optical microscope at 12.5 times magnification around alveolar bones between roots of upper and lower first molars. PTH, OVX and control groups were all evident that alveolar bones were presented in compact lamellar bone patterns and new bone formation occurred. However, in the articular head of ilium of OVX group demonstrated more sparsely formed trabecular bones compared with PTH group. Nevertheless, there wasn't a remarkable difference of trabecular bone between tooth roots in upper and lower jaw bones (Fig. 2~4).

2. Micro-Computed Tomography Taking and Evaluation

In the ilium samples, values of BV, BV/TV, Tb.Th, Tb.N and BMD were lower in OVX group

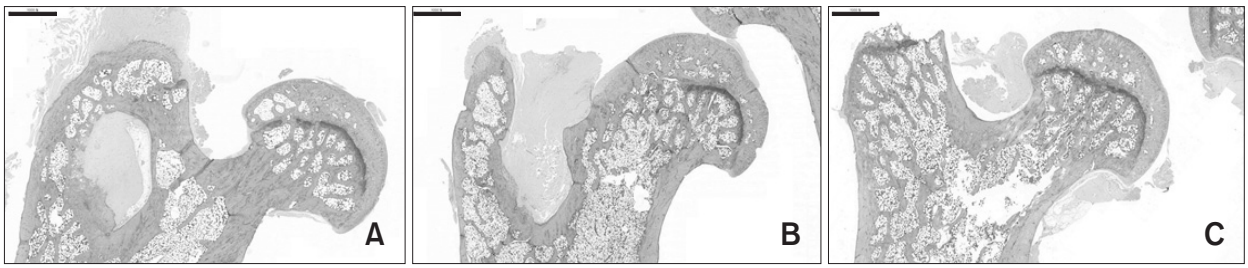

Fig. 2. Representative histological view of head of femur of parathyroid administered group (A), ovariectomized only group (B), and control group (C) (H&E staining, ×20; scale bars=1,000 μm).

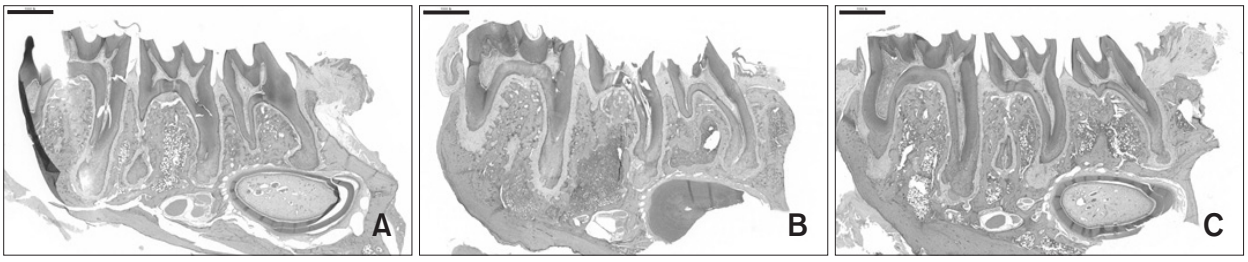

Fig. 3. Representative histological view of alveolar bone between the roots of first molar in mandible of parathyroid administered group (A), ovariectomized only group (B), and control group (C) (H&E staining, ×20; scale bars=1,000 μm).

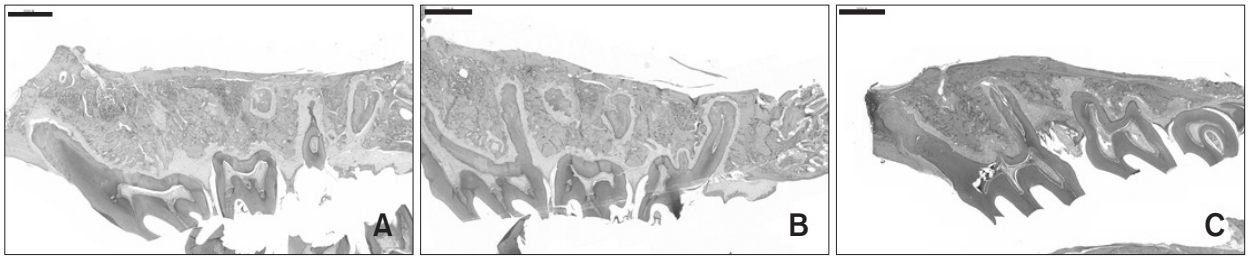

Fig. 4. Representative histological view of alveolar bone between the roots of first molar in maxilla of parathyroid administered group (A), ovariectomized only group (B), and control group (C) (H&E staining, ×20; scale bar=1,000 μm).

Table 1. Three-dimensional microstructural parameters of the head of femur of ovariectomized rat

	PTH group	OVX group	Control group	P-value
Total volume (mm^3)	0.27±0.03	0.26±0.01	0.26±0.01	0.733
Bone volume (mm^3)	0.15±0.01	0.13±0.03	0.20±0.02	0.061
Bone volume ratio (%)	53.75±7.57	50.61±12.89	76.20±5.92	0.061
Trabecular thickness (mm)	0.05±0.00	0.05±0.01	0.06±0.01	0.066
Trabecular number ($1/mm^3$)	10.32±1.67	6.97±5.72	11.93±1.10	0.329
Trabecular separation (mm)	0.06±0.01	0.11±0.09	0.04±0.01	0.177
Bone mineral density (g/cm^3)	1.12±0.09	0.88±0.48	1.38±0.07	0.061

PTH group: intermittent parathyroid hormone administered group, OVX group: ovariectomized only group, Control group: sham operated group.

Values are presented as mean±standard deviation.

Table 2. Three-dimensional microstructural parameters of the mandibular alveolar bone of ovariectomized rat

	PTH group	OVX group	Control group	P-value
Total volume (mm^3)	0.27±0.03	0.27±0.03	0.26±0.01	0.733
Bone volume (mm^3)	0.17±0.03	0.15±0.01	0.18±0.04	0.733
Bone volume ratio (%)	64.60±12.17	58.26±9.63	67.54±14.74	0.670
Trabecular thickness (mm)	0.06±0.00	0.05±0.00	0.06±0.01	0.113
Trabecular number ($1/mm^3$)	11.20±1.83	10.96±1.71	10.78±0.91	0.957
Trabecular separation (mm)	0.06±0.03	0.06±0.01	0.06±0.02	0.957
Bone mineral density (g/cm^3)	1.21±0.17	1.19±0.13	1.27±0.18	0.587

PTH group: intermittent parathyroid hormone administered group, OVX group: ovariectomized only group, Control group: sham operated group.

Values are presented as mean±standard deviation.

Table 3. Three-dimensional microstructural parameters of the maxillary alveolar bone of ovariectomized rat

	PTH group	OVX group	Control group	P-value
Total volume (mm^3)	0.26±0.01	0.27±0.03	0.27±0.03	0.875
Bone volume (mm^3)	0.16±0.02	0.14±0.02	0.17±0.03	0.491
Bone volume ratio (%)	61.19±8.92	52.50±11.22	64.60±12.17	0.430
Trabecular thickness (mm)	0.06±0.01	0.05±0.00	0.06±0.00	0.252
Trabecular number ($1/mm^3$)	11.04±1.23	9.77±1.67	11.20±1.83	0.430
Trabecular separation (mm)	0.06±0.01	0.06±0.02	0.06±0.03	0.875
Bone mineral density (g/cm^3)	1.20±0.11	1.11±0.16	1.21±0.17	0.561

PTH group: intermittent parathyroid hormone administered group, OVX group: ovariectomized only group, Control group: sham operated group.

Values are presented as mean±standard deviation.

than PTH and control groups and a large Tb.Sp value confirmed induction of osteoporosis (Table 1). Also, in the lower jaw bone samples, values of BV, BV/TV, Tb.Th, Tb.N and BMD were lower in OVX group than PTH and control groups (Table 2). Furthermore, the same results were obtained from the upper jaw bone samples (Table 3), but there was no statistically significant difference.

Discussion

Estrogen deficiency after menopause causes osteoporotic changes in jaw bones such as thinning of alveolar bones and this phenomenon accelerates destruction of alveolar bones and tooth loss especially in middle aged women suffering from periodontal diseases[2,4,6-8].

In addition, estrogen deficiency alters bone density around already placed dental implants, and reduces the degree of osseointegration and is shown to decline overall biomechanical abilities[25]. This implies osteoporotic patients with severe trabecular bone loss are expected to be clinically benefited from intermittent PTH administration when placing dental implants by increasing bone density around the implants and helps with maintenance.

Accordingly, this study design was based on hypothesis of intermittent administration of PTH, which is an effective therapeutic agent for osteoporotic fracture prevention, would have the same effect on jaw bones in osteoporotic rats[5,16,26]. This research is a preparation stage for further research on expected changes by administration of PTH after surgery in surrounding bones of the patients with osteoporosis who are subjected to dental implant placement[1,6,25,27].

However, the previous studies mostly focused on iliums and fibulas of rats[8,9,24] which are limited in number. Therefore, this study was designed to examine intermittent administration of PTH causing expected changes in bone structure of jaw bones that is in the field of dentistry.

The histological opinion suggested that sparsely formed trabecular bones in OVX group compared with PTH and control groups meant bone loss was induced by OVX and administration of PTH stimulated reformation of bone. However, newly formed bones were observed in alveolar bones of upper and lower jaw bones in both OVX and PTH groups and there wasn't a huge morphological difference in trabecular bones which leads to consideration of an embryological difference in upper and lower jaw bones for bone formation process[13,20].

It was anticipated that similar to the rabbit's jaw bones, intermittent administration of PTH after OVX causing almost full recovery of bone density[8] would occur in rat's jaw bones at similar level prior to OVX but the result didn't match. According to the micro-computed tomography evaluation, in the ilium samples, osteoporotic changes after ovariectomy were clearly different among PTH, control and OVX groups although there weren't statistically significant differences. Nonetheless, Tb.Th, Tb.N and Tb.Sp values which indicate microstructure of trabecular bones weren't different in PTH and OVX groups in the jaw bones. This can be inferred as different anatomical structures between ilium and jaw bones in which constant exposure to masticatory force occurs[8,24].

Additionally, difference in BMD values can be expected due to bone formation causing increase in BV and mineral deposition by administration of PTH after progression of bone loss by OVX. The histological opinion revealed bone destruction in the ilium after OVX and reformation occurred after PTH administration whereas same phenomenon didn't occur clearly in the upper and lower jaw bones suggested that like in previous studies, every bone doesn't exhibit constant changes in bone density and quality[7,8,24]. The statistical meaning of this study is not considered to be at confident level because it was an animal experimental study with limited numbers of animal not an individual follow-up study.

Therefore, obtaining a statistically increased confident level can be possible by conducting a follow-up study in individuals or by increasing the number of units in the same condition for long term administration of PTH over 8 weeks of period. If additional research could confirm that intermittent PTH administration therapy increases bone volumes of cortical and cancellous bones

in osteoporotic jaw bone, hereafter, it is expected to get increased success rate of osseointegration after placement of dental implants within alveolar bone and better maintenance of the implants in osteoporotic patients[1,23,28]. Although osteoporosis has effects on alveolar bone surroundings, the response of intermittent PTH administration allows the prediction of increase in osseointegration after dental implant placement in those patients suffering from osteoporosis[1,6,23,27,28].

In this study, PTH was administered by subcutaneous injection. Later on, additional experiments required on local injection or intermittent oral administration to find out research results. In addition, research on the dosage and frequency of administration for effective PTH therapy are needed as well as side effects on long-term administration.

In contrast to the rarity of human cases, the risk of osteosarcoma in rat models has been reported[29,30]. Therefore, the possibility of any pathologic consequence of extension of PTH administration period needs to be confirmed. Furthermore, any complications related to the use of PTH have to be monitored as well.

Conclusion

According to the above results, it can be speculated that OVX may cause osteoporotic changes in rat's jaw bone and intermittent administration of PTH appears to cause overall improvement of bone quality as well as having effects on microstructure of osteoporotic jaw bones. Therefore, intermittent administration of PTH is expected to exert positive effects in various fields of dentistry including orthodontic, periodontic and implant placement and maintenance in elderly patients.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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