Influence of romosozumab on micro-architecture and biomechanics of bone to cortical bone screws in a rabbit model of glucocorticoid-induced osteoporosis

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Directed by Professor Seong-Hwan Moon

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Ji-Won Kwon

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ABSTRACT

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Osteoporosis is a global health concern, and studies regarding improving bone mineral density (BMD) are needed. Romosozumab is a clinically approved anabolic drug for the treatment of osteoporosis. This study aimed to assess the osseointegration and biomechanical properties following the insertion of a cortical screw in a rabbit model of glucocorticoid-induced osteoporosis, to determine postoperative bone fusion in osteoporotic patients. The study used 55 New Zealand white rabbits to investigate the osseointegration and biomechanical properties following insertion of two 2.7 mm cortical screws into both iliac bones in a model of induced osteoporosis. Glucocorticoid (Predisol®) injection was performed to induce osteoporosis in rabbits categorized into 4 groups: Romosozumab, Parathyroid Hormone (PTH), a combination of PTH and denosumab, and untreated rabbits. Each group received a specific treatment for osteoporosis for 3 weeks, after which they were euthanized. Histomorphometric and biomechanical analyses were performed. Additionally, for biomechanical analysis, the pull-out strength of the cortical screw inserted into the iliac bone was determined using the load to displacement curve to determine the maximal load (N) when the cortical screw was displaced by 1 mm. Our results revealed distinct variations in histomorphometric parameters across the 4 groups. The group treated with romosozumab showed notable improvements, with bone-to-implant contact and bone fraction area occupancy increasing to 21.2 ± 18.1% and 56.9 ± 9.9%, respectively. The biomechanical properties for pull-out strength showed that anti-osteoporosis medications significantly influenced the resistance of the cortical bone screws during a 1-mm pull-out test compared to the control group. The romosozumab-treated group demonstrated the greatest improvement, with a pull-out strength of 275 ± 55 N. Romosozumab significantly strengthens bone micro-architecture and the biomechanical stability of the bone–implant interface. These findings imply that romosozumab may enhance the efficacy of bone implants in osteoporotic patients.

Key words: romosozumab, rabbit, osteoporosis, implant, glucocorticoid

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I. INTRODUCTION

Osteoporosis, a systemic skeletal disorder, leads to a gradual reduction in bone density and quantity. Primary osteoporosis, the major form including postmenopausal and age-related osteoporosis, is a worldwide public health issue[1]. The methods used to treat osteoporosis have greatly expanded, enabling clinicians to provide patients with individualized treatment plans[2-4]. However, stronger therapy regimens may be essential for individuals with extremely low bone mineral density and a new therapeutic objective is to develop osteoporosis medicines that raise bone mineralized density (BMD) T-scores to > 2.5 within 5 years.

Successful spine surgery implant treatment relies on achieving optimal primary stability, which is contingent upon having an adequate quantity and quality of bone. Primary stability is compromised in areas with low bone density, especially in patients with osteoporosis, increasing the risk of implant failure[5]. Consequently, the density of bone at the implant placement site is a pivotal factor closely associated with implant failure rates and primary stability. Patients with osteoporosis experience less favorable outcomes compared to those with healthy bone when undergoing implant treatments.

Romosozumab is a clinically approved strategy to enhance bone formation and increase bone quantity[6,7], functioning as an anabolic pharmacological agent for individuals with osteoporosis like intermittent administration of parathyroid hormone (PTH)[8]. Romosozumab is also known to exert clear anabolic effects on the remodeling of cancellous bone by boosting the number and activity of osteoblasts while reducing apoptosis and enhancing bone thickness not only in trabecular bone but also in cortical bone.

The therapeutic effectiveness of romosozumab, particularly for postoperative bone fusion

in osteoporotic patients who have undergone spine fusion surgery, has not yet been directly compared[9-11]. Furthermore, there are no clinical or animal studies comparing the degree of bone formation and internal fixation screw pull-out strength. We, therefore, aimed to investigate osseointegration and the biomechanical properties following insertion of a 2.7 mm cortical screw into both iliac bones in a rabbit model of glucocorticoid-induced osteoporosis.

II. MATERIALS AND METHODS

1. Animals and induction of osteoporosis

A total of 55 New Zealand white rabbits were included in this study (average weight 4.0 kg, 24 weeks-old, female, purchased from Avison Biomedical Research Center at Yonsei University College of Medicine, Seoul, South Korea). All protocols in this study were conducted in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals after approval by the Ethics Committee of Yonsei University college of medicine (IACUC approval no.: 2018-0266). Animals were acclimatized for 1 week, kept in standardized individual cages (60 cm x 60 cm x 50 cm) with sufficient supply of chow and water.

To apply the most appropriate induction of osteoporosis in a rabbit model, both ovariectomy (OVX) and glucocorticoid injection were considered. Among these methods, this study sought to determine which method would cause the lowest value of BMD compared to the that of the negative control group that did not receive any pretreatment. BMD was performed on the sacrificed rabbit's vertebral body and femur neck to quantify the baseline BMD value of the region of interest (ROI). BMD measurements were acquired using a dual-energy X-ray absorptiometry (DXA) system (Lunar Piximus 2; GE-Lunar, Madison, WI)[12]. The ROIs were the center of the vertebral body of the lower lumbar spine and the proximal femur metaphysis, respectively. ROI 1 and 2 were the proximal femur metaphysis, and ROIs 3 to 7 were the vertebral body of the lower lumbar spine, starting from the lowest segment (Figure 1).

A total of 15 rabbits were used to determine the method to induce osteoporosis, and were divided into the control group, ovariectomy induced osteoporosis group, and glucocorticoid-induced osteoporosis group, according to the presence or absence of induction of osteoporosis. The control group consisted of 3 rabbits that were not subjected to any adjustment. This group served as a reference to establish the baseline of normal BMD in the absence of osteoporosis. The bone density in the proximal femur metaphysis was determined by utilizing the minimum value derived from the DXA values of ROI 1 and ROI 2. As the standard to determine the bone density of the lower lumbar spine, the average values of ROI 3–7 were applied.

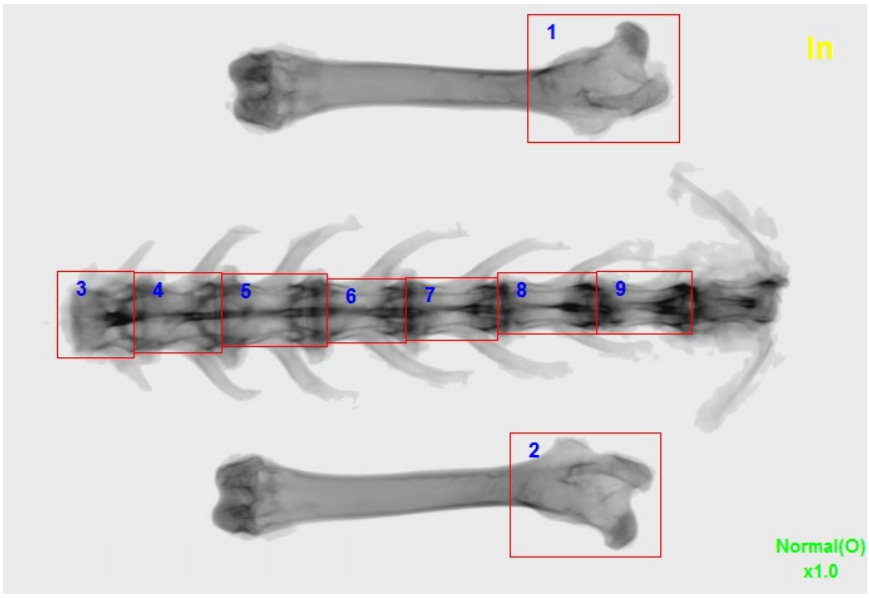

Figure 1. A BMD measurements using a dual-energy X-ray absorptiometry (DXA) system. Region of interest (ROI) 1 and 2 refer to the proximal femur metaphysis, and ROIs 3 to 7 refer to the lower lumbar spine.

2. Bilateral ovariectomy

The OVX-induced osteoporosis group used the method reported in a previous study,[13-15] performed on a total of 3 rabbits. Under general anesthesia, the rabbits were injected with Buprenorphine SR 0.15 mg/kg, intubated, and maintained using isoflurane 1.5–3% with oxygen. All rabbits underwent bilateral OVX. A midline incision was made distally from the umbilicus for 4–5 cm. The linea alba and peritoneal tissue below were incised, thereby protecting the intestines. The ovaries were then localized, and the ovarian vessels were ligated. The ovaries were removed with their ligamentous attachment on the uterine horn (Figure 2). The linea alba and abdominal muscles were then closed, followed by the skin, using an absorbable suture (Vicryl 3.0; ETHICON Inc., Somerville, NJ). Animals were then observed twice a day until incision healed. Rabbits that had bilateral ovariectomy were grown for around 17 weeks, following which they were sacrificed and the BMD was measured.

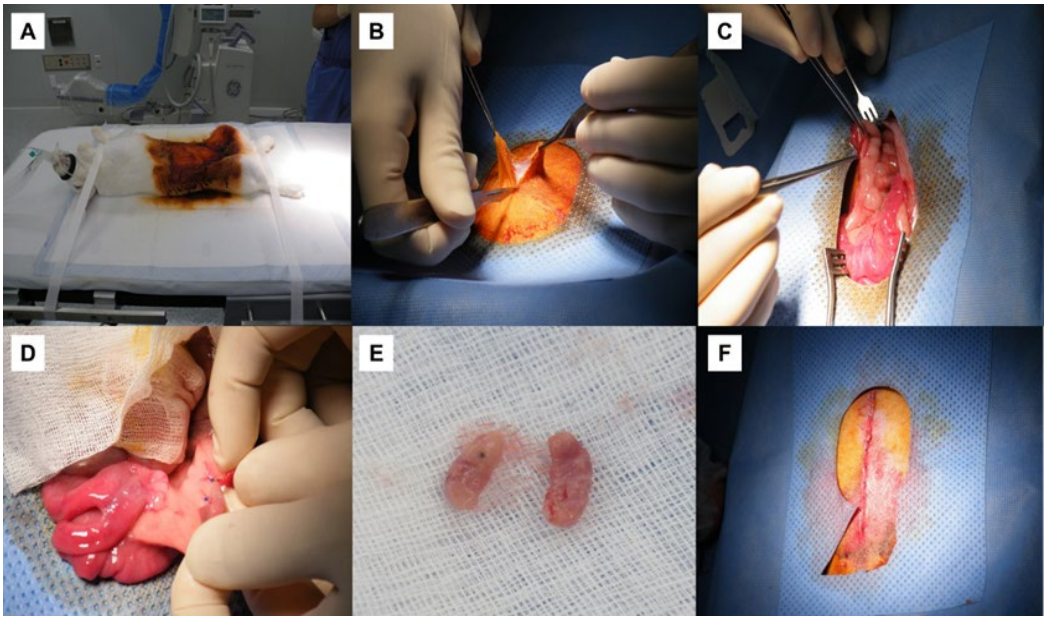

Figure 2. In vivo bilateral ovariectomy in a rabbit model. (A) A rabbit with lithotomy position on the operation table. (B) An incision is made in the abdominal midline. (C) Exposure the ovary by dissection along the uterus. (D) Ligation was performed on the ovarian artery to prevent bleeding. (E) Remove both ovaries. (F) Suture the inner muscle and outer skin using vicryl 4-0 and nylon 4-0.

3. Glucocorticoid injection

Induction of osteoporosis was performed through intramuscular injection of methylprednisolone succinate sodium (Predisol®; ReYon Inc., Seoul, Korea). The glucocorticoid dose and duration of administration were divided into 2 groups: one receiving 4 mg/kg and the other receiving 8 mg/kg, both provided weekly. The administration period was further divided into 2 time points to measure BMD: after 3 weeks of administration and after 7 weeks of administration. Consequently, a group of 3 rabbits received a dosage of 4 mg/kg once a week for a duration of 3 weeks; another group received the same dosage once a week for a duration of 7 weeks; and the last group of 3 rabbits received a dosage of 8 mg/kg for a duration of 7 weeks. After administering the drugs, the rabbits were euthanized and the ROIs were measured on the femur neck and lumbar spine body.

4. Implant procedure

The osteoporosis induction approach that resulted in the least significant decrease in BMD value, as compared to the control group, was identified through a preliminary study on osteoporosis induction. All rabbits participating in the subsequent study had osteoporosis induced using the prescribed methods of either OVX or glucocorticoid injection. Following the induction of osteoporosis, 2 implants were surgically placed into the iliac bones of the rabbit. All procedures were performed under general anesthesia with an intravenous injection of ketamine (40 mg/kg) and xylazine (6 mg/kg). After draping with povidone betadine in the usual orthopedic manner, 2–3 cm incisions were made to the posterior superior iliac spine of both iliac bones. After soft tissue dissection, the periosteum was exposed. Implant socket preparation (tapering) was performed using a 2.3-mm round drill, taking care not to breech or deviate from the path surrounded within cortex of the iliac bone. A full threaded cortical bone screw made of titanium alloy, Ti6Al4, (2.7 mm diameter, 16 mm length, non-locking type, CO-27XX, Acu-Sinch®, Acumed, TX) was inserted. Afterwards, meticulous suturing was performed on the subcutaneous and skin layer using 4-0 absorbable sutures (Figure 3). Then, after implantation, rabbits with osteoporosis were subgrouped according to anti-osteoporosis treatment. Detailed grouping was conducted using intermittent administration of parathyroid hormone, a group using a combination of PTH and denosumab, and finally, a group using romosozumab.

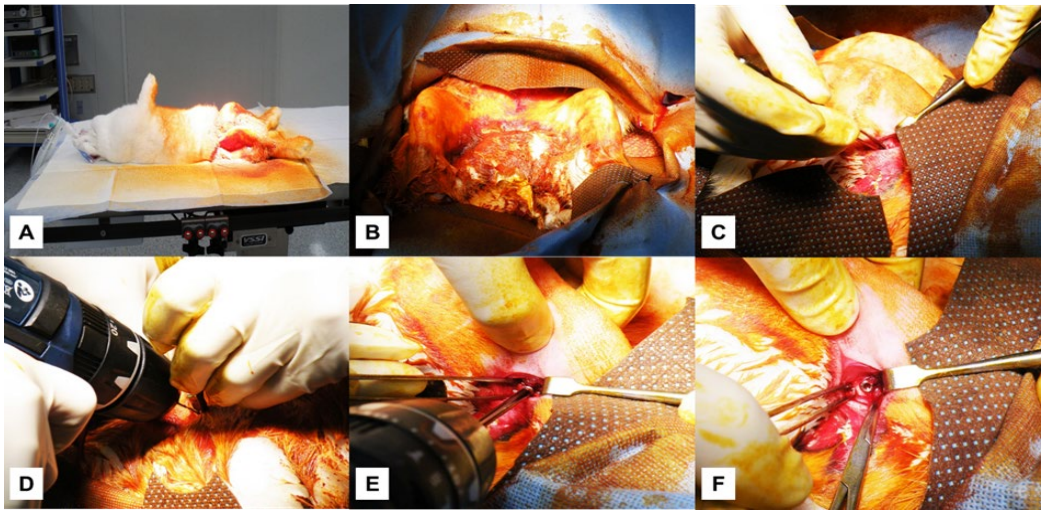

Figure 3. In vivo bony manipulation to insertion of 2.3mm cortical screw on both iliac bone. (A) A rabbit with lithotomy position on the operation table. (B) Draping was performed with povidone betadine. (C) Skin incision was made 2-3 cm sized based on the posterior superior iliac spine (PSIS). (D) After exposing the PSIS, tapering was performed with a 2.3mm sized round drill bit. (E) A 2.7mm cortical bone screw was inserted. (F) Insert the head of screw all the way until it contacts the cortex of iliac bone as much as possible.

5. Anti-osteoporosis medication after implantation

Anti-osteoporosis medication was administered from the day after the implant procedure. The treatment groups were divided into 3: PTH, PTH + denosumab, and romosozumab. Treatment was carried out for a total of 3 weeks. Denosumab (Prolia®) was administered once, subcutaneously, with a dose regiment of 1 mg/kg. Since the average weight of one rabbit was 4 kg, 432 μl of normal saline was mixed in 67 μl (1 ampoule contains 60 mg) and injected subcutaneously around the buttock area of the rabbit at a total volume of 0.5 ml. Intermittent parathyroid hormone (PTH, Forsteo®) was administered at 0.33 μg/kg and administered daily, subcutaneously[16]. Since 1 pen of the product contained 600 μg/2.4 ml, the dose regimen was quantified as 1.4 μg, mixed with normal saline, and administered in a total of 0.5 ml daily for 3 weeks. Lastly, romosozumab (Evenity®) was administered subcutaneously, twice a week (Monday and Thursday), quantified as 25 mg/kg. All rabbits that had completed treatment for osteoporosis were housed in standardized individual cages and provided with sufficient access to chow and water.

6. Histological inspections and histomorphometric analyses

For histological inspection of the bone to cortical bone screw interface, the harvested hemipelvis embedded with the screw was extracted separately after 3-weeks of osteoporosis treatment. Hemipelvis blocks were stored in 4% formaldehyde phosphate buffer until preparation for 2 weeks. The blocks were cut into 2 fragments precisely to observe the bone to screw interface from the center of the diameter of the inserted cortical screw along the long axis of the screw thread (Jig making and cutting, GENOSS CO., LTD, Suwon, Korea). Dehydration was performed on longitudinally cleaved fragments with hydrochloride solution. Then, these were sectioned into paraffin-embedded, 5 μm thickness slices and stained with hematoxylin and eosin (H&E) (Figure 4). The remaining sections were stained with Goldner's trichrome and made into slides. Histological inspection and histomorphometric analysis was performed using a high-precision, light microscope at 2,000 μm magnification (BZ-9000, Keyence, Japan) and image analysis software (Fiji-win64, National Institutes of Health, Bethesda, MD). The histomorphometric assessment of the examined sections was carried out using 2 histomorphometric parameters—bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO)[17,18]. BIC denotes the proportion of an implant's surface that is in direct contact with bone along the whole implant's length. BAFO considers the total microscopic field occupied by the mineralized bone matrix between the threads, calculated as a percentage by subtracting the bone surface area from the overall field area between the threads. The stained sectioned slides were scanned with image analysis software (Pannoramic 250 Flash III, 3D HISTECH, Hungary)

to acquire a total of 4 images per screw based on the screw thread portion (Figure 5).

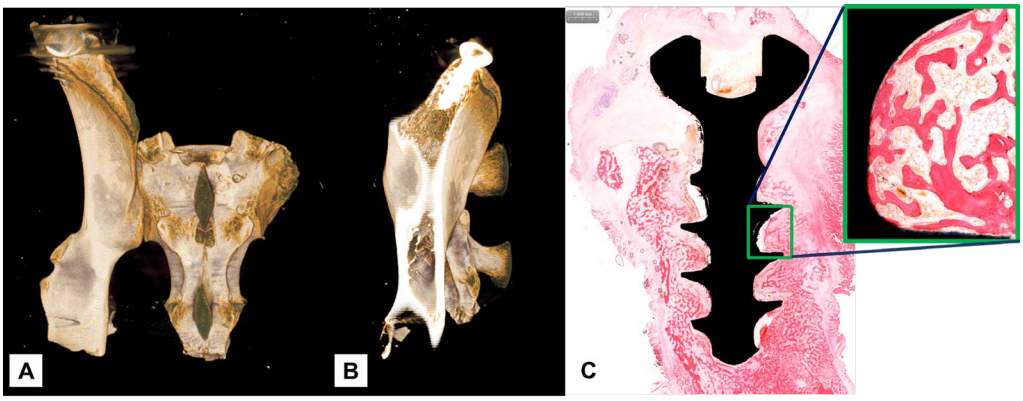

Figure 4. Photograph (A and B) showing micro-CT evaluation of implanted hemipelvis blocks after storing in 4% formaldehyde phosphate. (C) Histological evaluation of the implanted hemipelvis blocks. As a definition of ROI, the area is designated based on the top and bottom ends of the screw thread. ROI, region of interest.

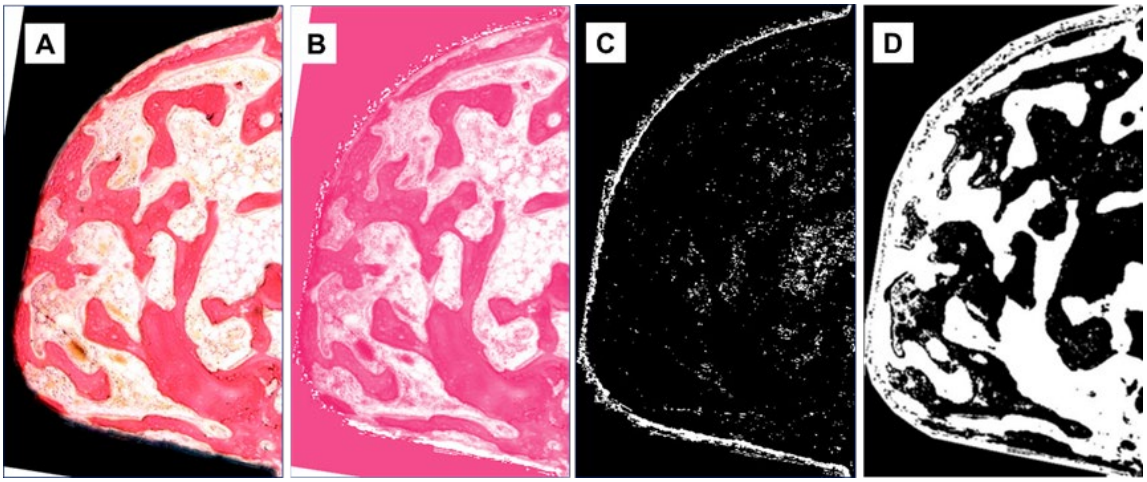

Figure 5. Histomorphometric evaluation of ROI using image analysis software. Hematoxylin and eosin stain for (A) and color deconvolution for (B). Black and white inverted image (C) to measure bone to implant contact (BIC) and black and white inverted image (D) to measure bone area fraction occupancy (BAFO).

7. Mechanical testing

For biomechanical analysis, the harvested hemipelvis was extracted separately on postoperative day 21, and stored surrounded by saline soaked gauze and sealed in aseptic plastic containers at 18~20°C until mechanical testing. The pull-out strength of the cortical screw inserted into the iliac bone was determined using the load to displacement curve to determine the maximal load (N) when the cortical screw was displaced by 1 mm. To facilitate alignment of the excised hemipelvis and the inserted cortical screw, the distractor was aligned parallel to the axis of the screw shank and tip of the screw and mounted on the Acumen® 3 electrodynamic test machine (MTS Systems Co, Eden Prairie, MN). To minimize the movement of the hemipelvis while pulling the screw for maximal load, the area around the mounted specimen was reinforced with resin, with 6 degrees of freedom of constraint, which allows control over each degree of freedom across 3 translational and 3 rotational axes; the pull-out external pressure was applied at a rate of 1 mm/min. Finally, the load was recorded according to the load to displacement curve (Figure 6).

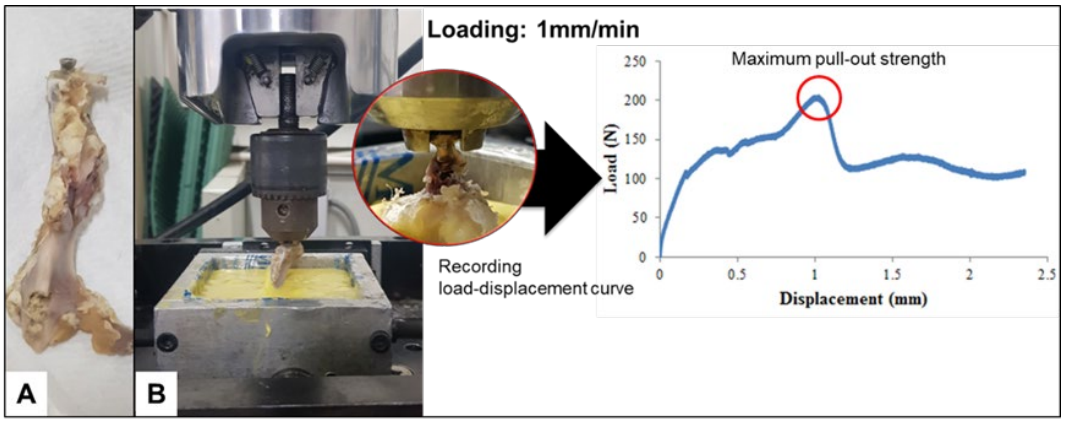

Figure 6. Hemipelvis mounted on MTS machine and cortical screw inserted. (A) Photograph of the hemipelvis with the cortical screw inserted (B) The MTS machine was aligned and attached according to the cortical screw alignment, and the maximal load (N) required to pull out at a speed of 1 mm per minute was measured.

8. Statistical analysis

The results are presented as mean ± standard deviation by SPSS program, version 23. Statistical analysis of the data was performed using A two-tailed Student's t-test and the Mann–Whitney U test. An analysis of variance with Tukey post hoc test was also used. Statistically significant differences were defined as $P < 0.05$.

III. RESULTS

1. Glucocorticoid induced osteoporosis

The glucocorticoid-induced osteoporosis group had the lowest DXA values between ROI 1 and ROI 2, which were expected to indicate the bone density of the proximal femur metaphysis. The average DXA values of ROIs 3 to 7, which represent the lower lumbar spine, also showed a lower BMD reduction in the glucocorticoid induction group than in the ovariectomy group. This outcome influenced the decision to utilize glucocorticoids as the preferred method to induce osteoporosis. The specific regimen of Predisol® administered not only induced osteoporosis but did so effectively, suggesting that the dosage of 8 mg/kg, given weekly for 3 weeks, is optimal to model osteoporosis in rabbits (Figure 7 & Table 1).

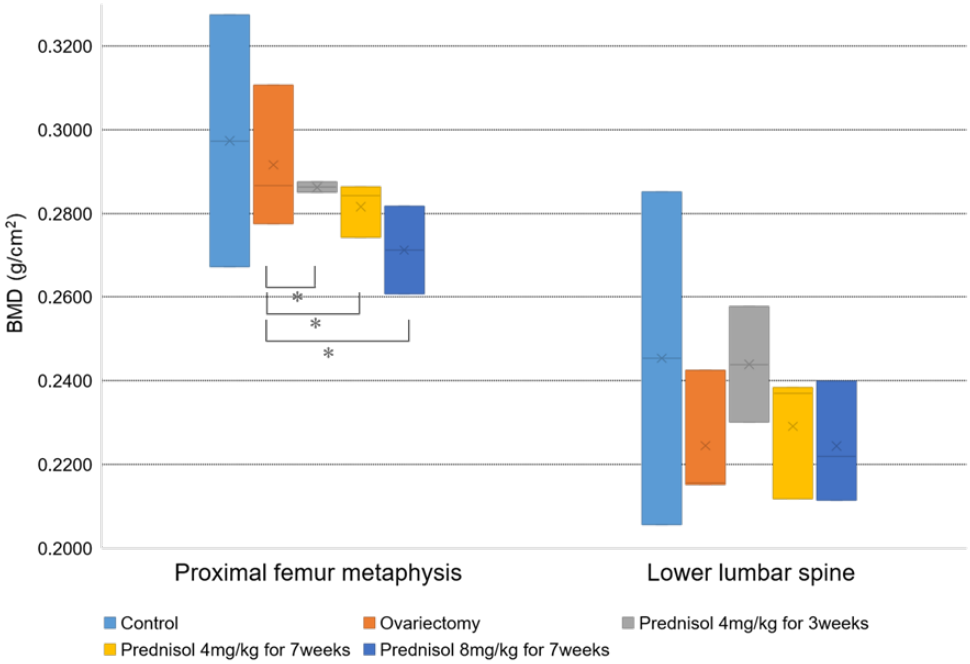

Figure 7. Results on BMD after osteoporosis induction. Box plots of interquartile range (IQR), range, and median BMD (g/cm2). *Statistically significant difference with $P < 0.05$.

Table 1. Results of the BMD

	Rabbit	BMD (g/cm^2)	
		Proximal femur metaphysis	Lower lumbar spine
Control group	#1	0.3274	0.2851
	#2	0.2672	0.2057
	#3	0.2973	0.2454
Mean ± SD		0.2973 ± 0.0301	0.2454 ± 0.0397
Ovariectomy	#4	0.3106	0.2425
	#5	0.2775	0.1888
	#6	0.2867	0.2157
Mean ± SD		0.2916 ± 0.0171	0.2244 ± 0.0156
Predisol 4mg/kg for 3weeks	#7	0.2874	0.2577
	#8	0.2850	0.2301
	#9	0.2862	0.2439
Mean ± SD		0.2862 ± 0.0012	0.2439 ± 0.0138
Predisol 4mg/kg for 7weeks	#10	0.2842	0.2370
	#11	0.2862	0.1918
	#12	0.2743	0.2384
Mean ± SD		0.2816 ± 0.0064	0.2291 ± 0.0150
Predisol 8mg/kg for 7weeks	#13	0.2817	0.2398
	#14	0.2608	0.2532
	#15	0.2713	0.1335
Mean ± SD		0.2713 ± 0.0105	0.2244 ± 0.0143

BMD, bone mineral density; SD, standard deviation

2. Histomorphology and histomorphometry

Sections of the hemipelvis, with a total of 50 implants in 25 rabbits, were stained and analyzed (H&E for 25, Goldner's trichrome stain for the remaining 25). All threads of the inserted implants were attached to the trabecular bone. In general inspection of the junction between the cortical bone screw and the implant, defect areas were frequently observed in the group that had induced osteoporosis and did not receive any treatment. Along the defect margin, an abundance of newly formed bone was observed, accompanied by few unresorbed scaffold remnants. Furthermore, in the center of the defect, osteons containing osteocytes and blood vessels were observed, suggesting active formation of neo-bone. Compared to the group treated with PTH and combined PTH + denosumab, abundant new collagen fibers and a completely normal architecture of natural bone with osteocytes and blood vessels was observed in the group treated with romosozumab. Additionally, the

establishment of a completely normal architecture of natural bone and blood vessels was clearly observed (Figures 8 & 9). Robust osseointegration, indicating successful implant integration in osteoporotic conditions, focused on 2 crucial parameters: BIC and BAFO. The control group, without any treatment, served as a baseline with lower BIC and BAFO values, at 41.7 ± 10.2% and 8.8 ± 6.6%, respectively, reflecting typical osteoporotic bone characteristics. In contrast, the group treated with PTH exhibited notable improvements, with BIC and BAFO increasing to 12.9 ± 4.7% and 52.1 ± 14.1%, respectively. The group treated with combined PTH and denosumab yielded slightly higher values of 13.4 ± 3.7% and 62.5 ± 7.1%, respectively. However, the group treated with romosozumab demonstrated the most significant improvements, achieving the highest BIC and BAFO values of 21.2 ± 18.1% and 56.9 ± 9.9%, respectively (Figure 10, 11).

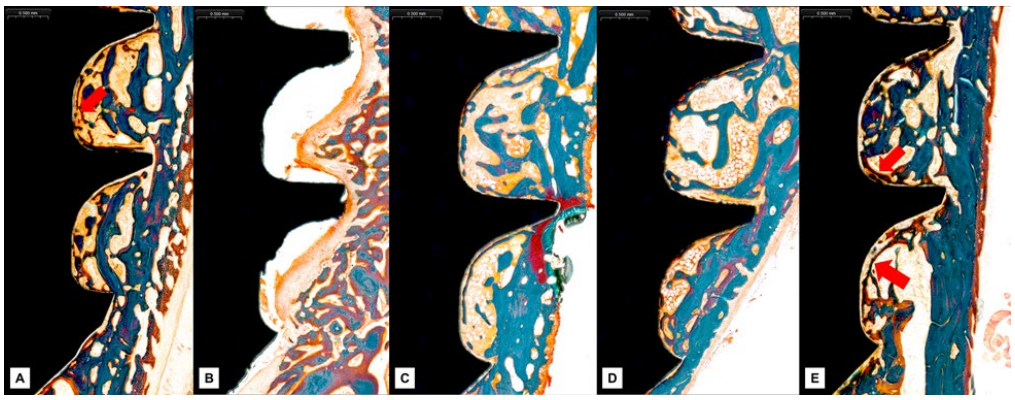

Figure 8. Masson's trichrome stain (x16). (A) Control group, (B) No treatment group, (C) PTH treatment group, (D) PTH + Denosumab treatment group, and (E) Romosozumab treatment group. The implant in the group treated with romosozumab was surrounded by a thin fibrous capsule (red arrow) without abnormal immune reaction, showing close contact with highly collagenous scaffolds.

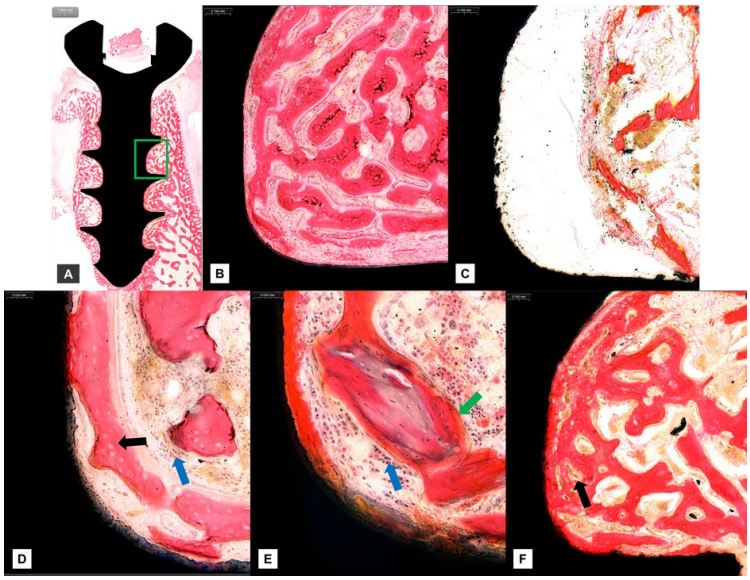

Figure 9. Hematoxylin and eosin stain. The boxed area in (A) (×8) was depicted at higher magnification in (B), (C), (D), (E), and (F) (×120). (B) Control group, (C) No treatment group, (D) PTH treatment group, (E) PTH + denosumab treatment group, and (E) Romosozumab treatment group. The defect area of the bone to implant is observed (B). Newly-formed bone and lots of residual scaffolds joined with osteoblast (blue arrow) and osteoclast (green arrow) are observed (D) and (E).

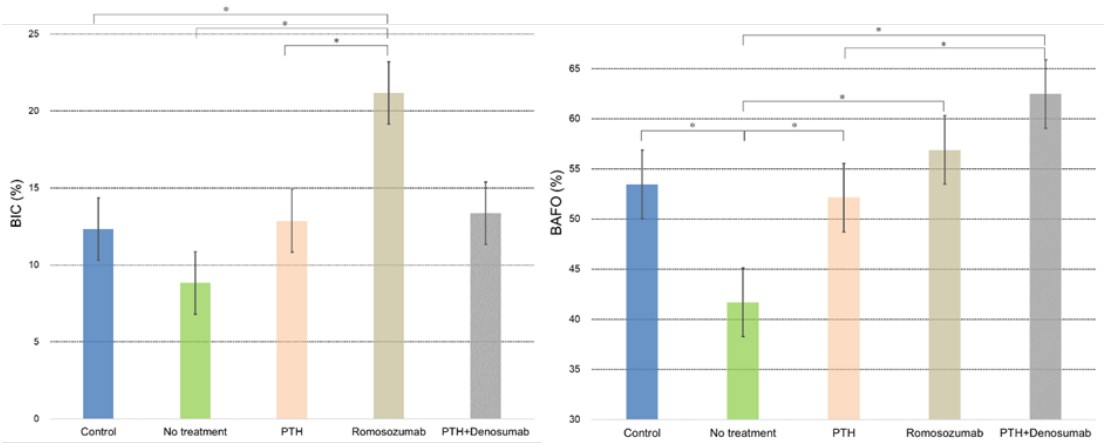

Figure 10. Percentage bone-to-implant contact and bone area fraction occupancy. BIC, bone-to-implant contact. BAFO, bone area fraction occupancy. Data were shown mean ± standard deviation. *Statistically significant difference with $P < 0.05$.

3. Biomechanical property for pull-out strength

A total of 29 implants were inserted in 15 rabbits, with each rabbit receiving 1–2 implants (from each test group, in both the right and left iliac bone). Anti-osteoporosis medications considerably influenced the resistance of the cortical bone screws during a 1 mm pull-out test compared to the group without osteoporosis treatment. The romosozumab group outperformed the other groups in the biomechanical measurement of maximum pull-out strength. The control group, without therapy, had an average pull-out strength of 120 ± 33 N. The group treated with PTH showed enhanced resistance, with a pull-out strength of 184 ± 61 N. In comparison, PTH plus denosumab combination increased pull-out strength to 202 ± 23 N. The romosozumab-treated group demonstrated the greatest improvement, with a pull-out strength of 275 ± 55 N. The biomechanical response to anti-osteoporosis therapy was statistically significantly different ($p < 0.05$) (Figure 11).

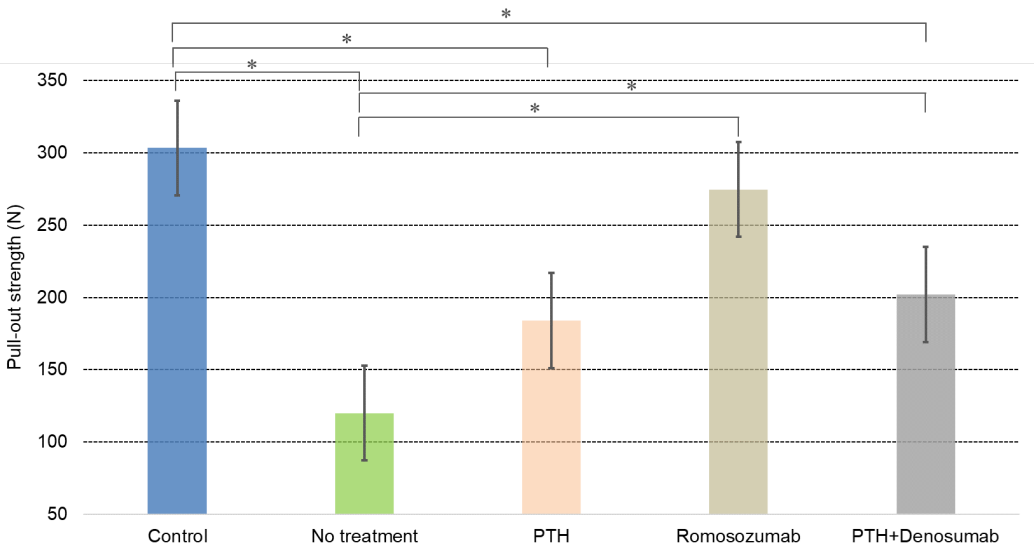

Figure 11. Results of maximum pull-out strength for 1mm pulling out of cortical screw. Data were shown mean ± standard deviation. *Statistically significant difference with $P < 0.05$.

IV. DISCUSSION

Our results revealed distinct variations in histomorphometric parameters across the 4 groups. The group treated with romosozumab showed notable improvements, with increased BIC and BAFO. Pull-out strength results showed that anti-osteoporosis medications significantly influenced the resistance of the cortical bone screws during a 1-mm pull-out test compared to the control group. The romosozumab-treated group demonstrated the greatest improvement. Romosozumab significantly strengthens bone micro-architecture and the biomechanical stability of the bone–implant interface, implying that romosozumab might significantly enhance the efficacy of bone implants in osteoporotic patients.

There are practical limitations in inducing primary osteoporosis in rabbits under the same conditions as humans. According to a report by María et al., in research on osteoporosis in rabbit models, this can be broadly classified through 2 mechanisms[19], including increasing bone resorption through ovariectomy and reducing bone formation through glucocorticoid administration. However, applications in animal models cannot mimic all osteoporosis induction in humans. Therefore, in reality, there is no choice but to conduct experiments based on the decrease in BMD, which is the standard to diagnose osteoporosis[20].

This study attempted to conduct osteoporosis induction, which results in the lowest bone density, as a preliminary experiment in rabbits of the same age, gender, and species. Previous studies were conducted 17 weeks after ovariectomy[14,19,21], showing that it was not possible to induce a significant decrease in bone density compared to the control group, especially in the proximal femur metaphysis. Even when BMD tests were performed to adjust the ROI, the results were the same; induction through glucocorticoid administration led to a clear decrease in BMD. Clinically, when spinal fusion surgery is performed on a patient with osteoporosis, anabolic agents can be administered after surgery[21]. Ohtori et al. administered teriparatide and risedronate as osteoporosis drugs 2 months before and 8 months after surgery in a group of patients undergoing posterolateral lumbar fusion surgery and compared the fusion rate between the 2 groups (Teriparatide 82% vs risedronate 68%)[22]. Manabu et al. compared the effects of combined administration of teriparatide and denosumab in combination therapy after spinal fusion[23]. The combination group had a higher bone union rate at 1 year after surgery compared to the teriparatide alone group (PTH + Denosumab 82% vs PTH 36%). Therefore, since the induction of osteoporosis by glucocorticoid administration applied in this study is based on a mechanism that inhibits bone formation, it can be considered suitable to examine the purchase strength of the instruments and bone in spine fusion surgery.

Osseointegration refers to the process in which the healthy bone and the implant come into

close contact with one another on a microscopic level[24,25]. A broader definition considers the apposition of new bone and the presence of connective tissue that is in direct contact with the implant. This study hypothesized that a comparable modeling process occurred in the bone-to-implant interface, resulting in an augment of both the average BIC volume and the proportion of BAFO following the administration of an anabolic agent such as PTH or romosozumab[26]. As a result, there were favorable histologic findings with a notable increase in the presence of dense trabecular bone structures surrounding the implant socket. Conversely, in the group with no treatment, there were few trabecular bone structures surrounding the implant, and there was evidence of tunneling resorption. The results of histomorphometric findings showed that anabolic agents enable osseointegration through modeling-based bone formation and greater filling of the bone space. In addition, the cellularity of osteoblastic cells lining the interface was significantly higher in the group treated with romosozumab compared to the group treated with PTH and PTH + denosumab. One distinguishing characteristic of this study is that Goldner's trichrome stain revealed abundant osseo-collagenous fibrous tissue that appeared to encircle the bone to the implant border in the group treated with romosozumab. These fibrous bands may facilitate osseointegration by functioning as a scaffold. The improvement in cortical bone strength induced by romosozumab is related to cortical thickness but not to cortical porosity[27]. Our study did not perform histomorphometric analysis focusing on cortical bone. In this study, cancellous bone in touch with the implant tended to reduce cancellous porosity. Modeling-based bone formation (MBBF) is not just generally impeded by osteoclast inhibitors, and MBBF may contribute to BMD gains throughout a long-term course of antiresorptive therapy[28-30]. Bone formation might be increased by the dual action of romosozumab on bone turnover, which may have reduced the extent and depth of eroded surfaces within cancellous bone[31].

Romosozumab, a monoclonal antibody that inhibits sclerostin, has emerged with a dual mode of action, promoting bone growth while reducing bone resorption. This method, which uses the Wnt pathway, is critical to reverse the deteriorating architecture of osteoporotic bone, increasing bone mineral density (BMD) and lowering fracture incidence[32]. This pharmacological profile of romosozumab is consistent with the needs to enhance implant stability in osteoporotic conditions where impaired bone quality is a significant concern[33,34]. Pre-clinical research has shown that anti-sclerostin treatment works in a variety of animal models[31,35]. For example, investigations in SOST KO mice, ovariectomized rats, and cynomolgus monkeys revealed significant improvements in BMD[36], bone volume, and bone strength, indicating improved bone quality that would favor implant integration and stability[37]. These animal model findings provide an adequate basis to investigate the potential of romosozumab to enhance the biomechanical

environment for implants in osteoporotic conditions. Furthermore, human clinical trials with romosozumab demonstrate positive outcomes. Phase 1 and 2 trials have demonstrated significant increases in bone formation markers and decreases in bone resorption markers, as well as significant increases in BMD at important sites including the lumbar spine and whole hip. These findings demonstrate the ability of romosozumab to generate a more favorable biomechanical environment for bone implants, which is critical in osteoporotic patients at high risk of fractures. Higher BMD is associated with stronger bone–implant interfaces, which is likely to increase the maximal pull-out strength[5,12,24]. This is consistent with the rapid and significant increase in BMD observed in clinical trials with romosozumab, such as the FRAME[6] and ARCH studies[38], where it has shown efficacy in reducing fracture risks and increasing bone density.

Our study has several limitations. The iliac crest studied is not weight-bearing and has a greater turnover rate than the long bone metaphysis. The cortical bone in this area is thinner than the long bones and cannot withstand as much stress as axial bones. The iliac crest's mechanical environment differs from the vertebrae and femoral neck, which are usually compromised by osteoporosis. The biomechanical reactions of these locations are critical for osteoporotic orthopedic implant stability. Changes in bone turnover rates might influence how bone remodels in response to disease and treatment. Romosozumab promotes bone formation and resorption. Therefore, the rabbit's iliac crest high turnover area might not accurately reflect the drug's effects in human bones with slower turnover rates. The iliac crest implant site could restrict the generalizability of the findings to clinical settings, where implants are frequently placed in places that experience distinct mechanical loads. Additionally, while our study provides valuable insights into the effects of romosozumab on bone micro-architecture and biomechanics, these anatomical and physiological differences may limit its clinical application to osteoporosis. Prudent interpretation of the results and more research utilizing substitute animal models or human clinical studies should be conducted to validate the findings in more typical clinical circumstances.

V. CONCLUSION

Romosozumab significantly improves bone micro-architecture and the biomechanical stability of bone implants, as indicated by the enhanced pull-out strength in a rabbit osteoporosis model. These results suggest that romosozumab could be highly beneficial for bone–implant success in osteoporotic conditions.

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ABSTRACT(IN KOREAN)

글루코코르티코이드 유발 골다공증의 토끼 모델에서 피질골 나사에 대한 뼈의 미세 구조 및 생체 역학에 대한 로모소주맙의 영향

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권 지 원

골다공증은 전 세계적인 건강 문제로 부각되고 있으며, 골밀도를 높이는 효율적인 치료법의 필요성이 강조되고 있다. 골다공증 치료를 위해 비교적 최근 임상적으로 승인된 약물인 로모소주맙에 대한 생체 내 연구를 수행하는 것이 매우 필요하나, 임상적인 연구를 통해서만 로모소주맙이 소주골과 피질골 리모델링 모두에 영향을 미친다고 알려져 있다. 이 연구는 글루코코르티코이드 유발 골다공증의 토끼 모델에 피질 나사 삽입 후 골유착 및 생체역학적 특성을 평가하여 골다공증 환자의 수술 후 뼈 유합에 대한 임상 적용의 우수성에 대해 규명해보고 한다. 이 연구에서는 골다공증이 유발된 토끼 모델에서 양쪽 장골에 2개의 2.7mm 피질 나사를 삽입한 후 골유착 및 생체역학적 특성을 조사하기 위해 55마리의 뉴질랜드 흰 토끼를 사용하였다. 8mg/kg 용량의 글루코코르티코이드(Predisol®) 주사를 매주 3주 동안 투여한 결과 토끼의 골다공증이 유발된 것으로 확인한 후, Romosozumab, 부갑상선 호르몬(PTH), PTH와 Denosumab의 조합, 치료되지 않은 토끼의 네

그룹으로 분류하였다. 각 그룹은 3주 동안 골다공증에 대한 특정 치료를 받은 후 안락사하였다. 뼈와 임플란트 표면을 H&E, Goldner's trichrome 염색을 사용하여 조직형태학적 분석으로 검사하였다. 또한, 생체역학적 분석을 위해 장골에 삽입된 피질나사의 인발강도를 하중 대 변위 곡선을 이용하여 피질나사가 1 mm 변위되었을 때의 최대 하중(N)을 측정하였다. 토끼의 뼈-임플란트 상호작용에 대한 골다공증 약물의 효과로 대조군 대비 BIC(Bone to Implant Contact) 및 BAFO(Bone Fraction Area Occupancy) 값을 비교하였다. 로모소주맙 치료군은 BIC와 BAFO 값이 각각 21.2% ± 18.1% and 56.9% ± 9.9%로 가장 우수한 소견을 가리켰다. 풀아웃 강도에 대한 생체역학적 특성은 골다공증 약물이 대조군에 비해 모든 그룹에서 피질골 나사의 저항에 유의미한 영향을 미치는 것으로 나타났다. 그 중에서도 로모소주맙 치료군은 당김 강도가 275 ± 55 N으로 가장 높은 수치를 보였다. 이번 연구를 통해 로모소주맙이 뼈의 미세구조와 뼈와 임플란트 계면의 생체역학적 안정성을 크게 강화한다는 것을 확인했다. 이러한 발견은 로모소주맙이 골다공증 환자의 뼈 임플란트의 효능을 향상시키는 데 중요할 수 있다는 의의를 가질 수 있다.

핵심되는 말: 로모소주맙, 토끼, 골다공증, 임플란트, 글루코코르티코이드