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Original Article

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Romosozumab Enhances Implant Stability in Glucocorticoid-Induced Osteoporotic Bone: A Rabbit Model Study

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Objective: Implant fixation in osteoporotic bone presents substantial challenges due to reduced bone mass and compromised microarchitecture. This study investigated whether romosozumab, a sclerostin inhibitor, improves osseointegration and mechanical stability of cancellous bone screws in glucocorticoid-induced osteoporosis.
Methods: Fifty-five New Zealand white rabbits were enrolled. Osteoporosis was induced via either bilateral ovariectomy or weekly intramuscular glucocorticoid injections (4–8 mg/kg). Based on bone mineral density results, glucocorticoid injection was selected for experimental induction. Rabbits were divided into 5 groups: control, untreated osteoporosis, parathyroid hormone (PTH), PTH combined with denosumab, and romosozumab. Cancellous bone screws (4.0-mm diameter, titanium alloy) were bilaterally inserted into the iliac bones. Antiosteoporosis treatments were administered for 3-week postimplantation. Histomorphometric evaluation of bone-to-implant contact (BIC) and bone area fraction occupancy (BAFO) was performed using nondecalcified sectioning and Goldner trichrome staining. Biomechanical pull-out testing measured resistance at 1-mm displacement using a standardized setup on the MTS system.
Results: The romosozumab-treated group exhibited superior outcomes. BIC reached 21.2% ± 18.1%, and BAFO was 56.9% ± 9.9%. Pull-out strength significantly increased to 275 ± 55 N in the romosozumab group, outperforming PTH (184 ± 61 N), PTH+denosumab (202 ± 23 N), and untreated osteoporosis (120 ± 33 N). Enhanced collagen structure and neobone formation were observed histologically around implants.
Conclusion: Romosozumab significantly enhances cancellous bone screw fixation strength and osseointegration in glucocorticoid-induced osteoporotic bone. These findings suggest its clinical potential as an adjuvant therapy in improving spinal implant outcomes in osteoporotic patients.

Keywords: Glucocorticoid, Implant, Osteoporosis, Romosozumab, Rabbit

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.[2-5] However, stronger therapy regimens may be essential for individuals with extremely low bone mineral density (BMD) and a new therapeutic objective is to develop osteoporosis medicines that raise 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.[6,7] 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,[8,9] functioning as an anabolic pharmacological agent for individuals with osteoporosis like intermittent administration of parathyroid hormone (PTH).[10] 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.[11-13] Furthermore, there are no clinical or animal studies comparing the degree of bone formation and internal fixation screw pull-out strength. Glucocorticoids inhibit osteoblast differentiation and function, induce osteocyte apoptosis, and prolong osteoclast lifespan, consequently leading to a rapid decline in bone mass and deterioration of trabecular architecture. In the context of orthopedic and spine surgery, the impact of glucocorticoid-induced osteoporosis on implant stability is of particular clinical relevance. Long-term glucocorticoid therapy is a well-known risk factor for impaired bone healing and implant failure, because it compromises both osseointegration and mechanical fixation.[14] Furthermore, while postmenopausal osteoporosis primarily affects trabecular bone, glucocorticoid-induced osteoporosis significantly weakens both trabecular and cortical bone, which is highly relevant for assessing cancellous bone screw fixation. Given that our study aimed to evaluate biomechanical and osseointegration properties of cancellous bone screws in an osteoporotic setting, a glucocorticoid-induced osteoporosis model was deemed more appropriate. Additionally, in preclinical animal models, postmenopausal osteoporosis is typically induced via ovariectomy (OVX). However, the bone-density reduction following OVX alone is often insufficient to replicate the severity of osteoporosis seen in humans, particularly in the short term.[15] In contrast, glucocorticoid administration reliably produces a significant reduction in BMD within a short timeframe, making it a practical and efficient model for evaluating implant stability and osseointegration.[16]

Therefore, we aimed to investigate osseointegration and the biomechanical properties following insertion of a 4.0-mm cancellous screw into both iliac bones in a rabbit model of glucocorticoid-induced osteoporosis.

MATERIALS AND METHODS

1. Animals and Induction of Osteoporosis

All methods were carried out in accordance with relevant guidelines and regulations. All protocols in this study were conducted in accordance with the ARRIVE guidelines for reporting animal research. This study was approved by the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and by the Ethics Committee of Yonsei University college of medicine (IACUC approval No. 2018-0266).

In total, 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, Korea). Animals were acclimatized for 1 week, kept in standardized individual cages (60 cm × 60 cm × 50 cm) with sufficient supply of chow and water. To apply the most appropriate induction of osteoporosis in a rabbit model, both 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, USA).[17] 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. A total of 15 rabbits were used to determine the method to induce osteoporosis, and were divided into the control group, OVX-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 (Fig. 1).

2. Bilateral OVX

The OVX-induced osteoporosis group used the method reported in a previous study,[16,18,19] performed on a total of 3 rab-

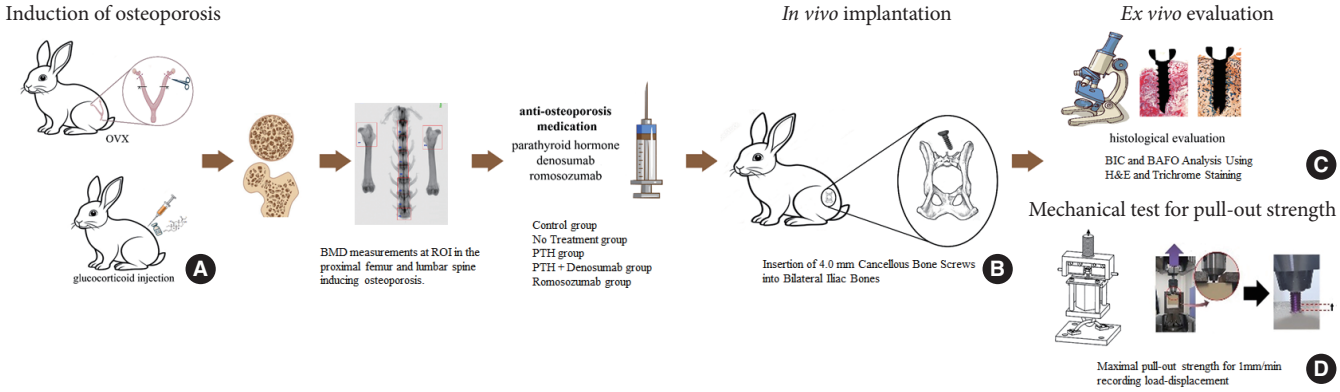

Fig. 1. Study overview. Induction of osteoporosis (A), *in vivo* implantation (B), *ex vivo* evaluation (C), and mechanical testing (D). OVX, ovariectomy; BMD, bone mineral density; ROI, region of interest; PTH, parathyroid hormone; BIC, bone-to-implant contact; BAFO, bone area fraction occupancy; H&E, hematoxylin and eosin.

bits. 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. The linea alba and abdominal muscles were then closed, followed by the skin, using an absorbable suture (Vicryl 3.0; ETHICON Inc., USA). Animals were then observed twice a day until incision healed. Rabbits that had bilateral OVX were grown for around 17 weeks, following which they were euthanized, and the BMD was measured.

3. Glucocorticoid Injection

Induction of osteoporosis was performed through intramuscular injection of methylprednisolone succinate sodium (Prednisol, ReYon Inc., 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. The iliac bone was selected as the implantation site because it contains a substantial amount of cortical bone, providing structural stability during insertion; however, cancellous screws—designed for trabecular bone—were ultimately used to ensure more consistent sectioning and histomorphometric analysis at the bone-implant interface. Additionally, the iliac bone provides a favorable surgical approach, allowing for consistent and reproducible screw insertion.

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- to 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 cancellous screw made of titanium alloy (Ti-6Al-7Nb), 4.0 mm in diameter and 12 mm in length, with a Hexalobular T15 socket and non-locking design (DePuy Synthes, Loughbeg, Ringaskiddy, Co., Ireland), was inserted. Afterwards, meticulous suturing was performed on the subcutaneous and skin layer using 4-0 absorbable sutures. Then, after implantation, rabbits with osteoporosis

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were subgrouped according to antiosteoporosis treatment. Detailed grouping was conducted using intermittent administration of PTH, a group using a combination of PTH and denosumab, and finally, a group using romosozumab.

5. Antiosteoporosis Medication After Implantation

Antiosteoporosis medication was administered from the day after the implant procedure. The treatment groups were divided into three: PTH (Forsteo, Sandoz, a division of Novartis, Switzerland), PTH+denosumab, and romosozumab. Treatment was carried out for a total of 3 weeks. Denosumab (Prolia, Amgen, USA) 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 PTH was administered at 0.33 μg/kg and administered daily, subcutaneously.[20] 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, Amgen, USA) 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. On the 21st day postsurgery, rabbits were euthanized under general anesthesia induced by intramuscular injection of alfaxan (1 mg/kg), xylazine (2 mg/kg), and azaperone (2 mg/kg), followed by an intravenous overdose of potassium chloride.

6. Histological Inspections and Histomorphometric Analyses

For histological inspection of the bone to cancellous 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 cancellous bone screw along the long axis of the screw thread (Jig making and cutting, GENOSS CO., LTD, Korea). To clarify the histological preparation process, implants were not removed after longitudinal cutting. Specimens were embedded in resin and sectioned using a diamond saw to preserve the bone-implant interface. This nondecalcified histology approach allowed for the accurate assessment of bone-implant contact (BIC) and bone area fraction occupancy (BAFO) without compromising structural integrity. 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). The remaining sections were stained with Goldner trichrome and made into slides. Histological inspection and histomorphometric analysis were 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, USA). The histomorphometric assessment of the examined sections was carried out using 2 histomorphometric parameters—BIC and BAFO.[21,22] 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.

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°C–20°C until mechanical testing. The pull-out strength of the cancellous bone screw inserted into the iliac bone was determined using the load to displacement curve to determine the maximal load (N) when the cancellous bone screw was displaced by 1 mm. To facilitate alignment of the excised hemipelvis and the inserted cancellous bone 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., USA). 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.

8. Statistical Analysis

The results are presented as mean ± standard deviation by IBM SPSS Statistics ver. 23.0 (IBM Co., USA). Statistical analysis of the data was performed using A 2-tailed Student t-test and

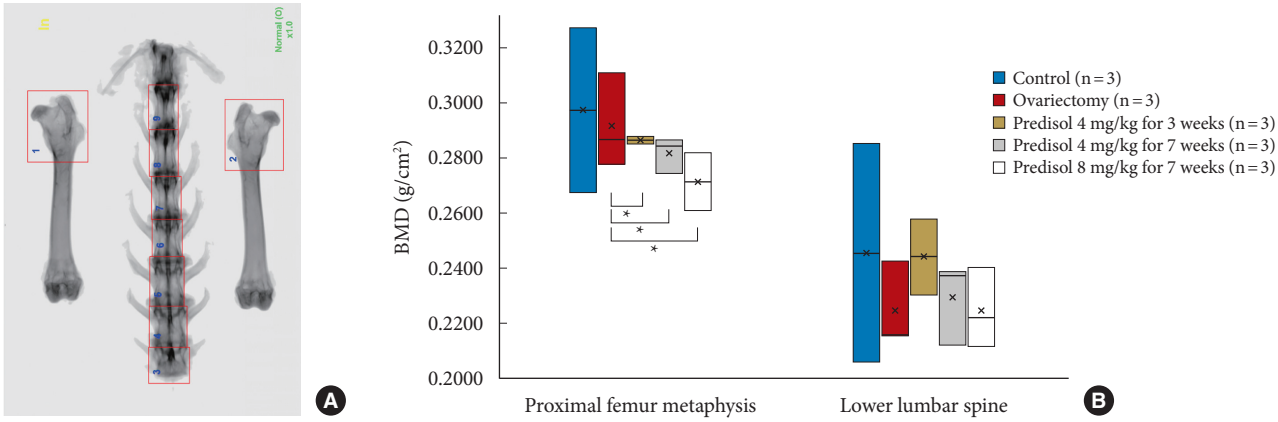

Fig. 2. (A) BMD measurements using a dual-energy x-ray absorptiometry system. ROIs 1 and 2 refer to the proximal femur metaphysis, and ROIs 3 to 7 refer to the lower lumbar spine. (B) Results on BMD after osteoporosis induction. Box plots of interquartile range, range, and median BMD (g/cm^2). N refers to the number of rabbits used for each group. Selected pairwise comparisons were conducted as indicated by asterisks. BMD, bone mineral density; ROI, region of interest. *Statistically significant difference with $p < 0.05$.

the Mann-Whitney U-test. Given the exploratory nature of this study, a formal power analysis was not conducted. Instead, we selected a sample size that balanced feasibility, ethical considerations, and meaningful data acquisition. An analysis of variance with Tukey *post hoc* test was also used. Statistically significant differences were defined as $p < 0.05$.

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 OVX 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 (Fig. 2).

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 trichrome stain for the remaining 25). All threads of the inserted implants were attached to the trabecular bone. In the general inspection of the junction between the cancellous bone screw and the trabecular bone, 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 neobone. 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 (Figs. 3 and 4). 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 in BIC, reaching 21.2% ± 18.1%. Meanwhile, the highest BAFO value was observed in the PTH-denosumab group (58.4% ± 10.2%), followed by the romosozumab group (56.9% ± 9.9%) (Fig. 5).

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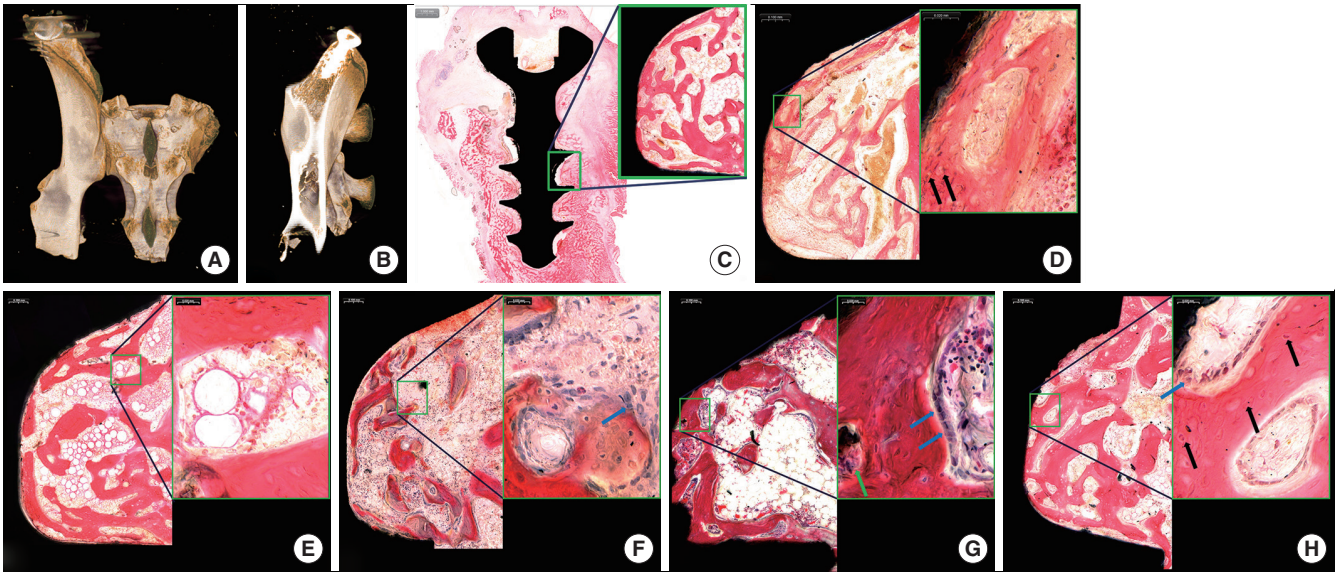

Fig. 3. Histological analysis of screw-bone interface. (A and B) Micro-CT images of implanted hemipelvis. (C) H&E staining (×8), with higher magnification (×120, ×480) in (D–H): (D) control, (E) no treatment, (F) PTH, (G) PTH+denosumab, (H) romosozumab. PTH, parathyroid hormone; CT, computed tomography; H&E, hematoxylin and eosin. Blue arrow: osteoblasts; green arrow: osteoclasts; black arrow: newly formed osteocytes.

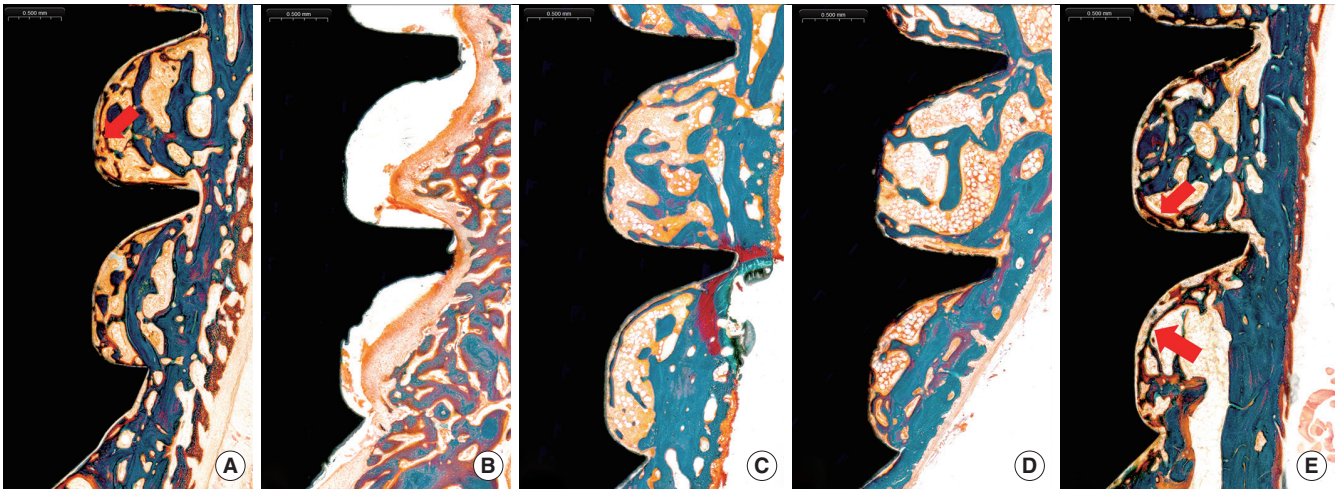

Fig. 4. Masson trichrome staining of screw-bone interface. (A) Control, (B) no treatment, (C) PTH, (D) PTH+denosumab, (E) romosozumab. PTH, parathyroid hormone. Red arrows indicate collagenous tissue adjacent to implant without abnormal immune response.

3. Biomechanical Properties for Pull-Out Strength

In total, 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). Antiosteoporosis medications considerably influenced the resistance of the cancellous 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 antiosteoporosis therapy was statistically significantly different ($p < 0.05$) (Fig. 6).

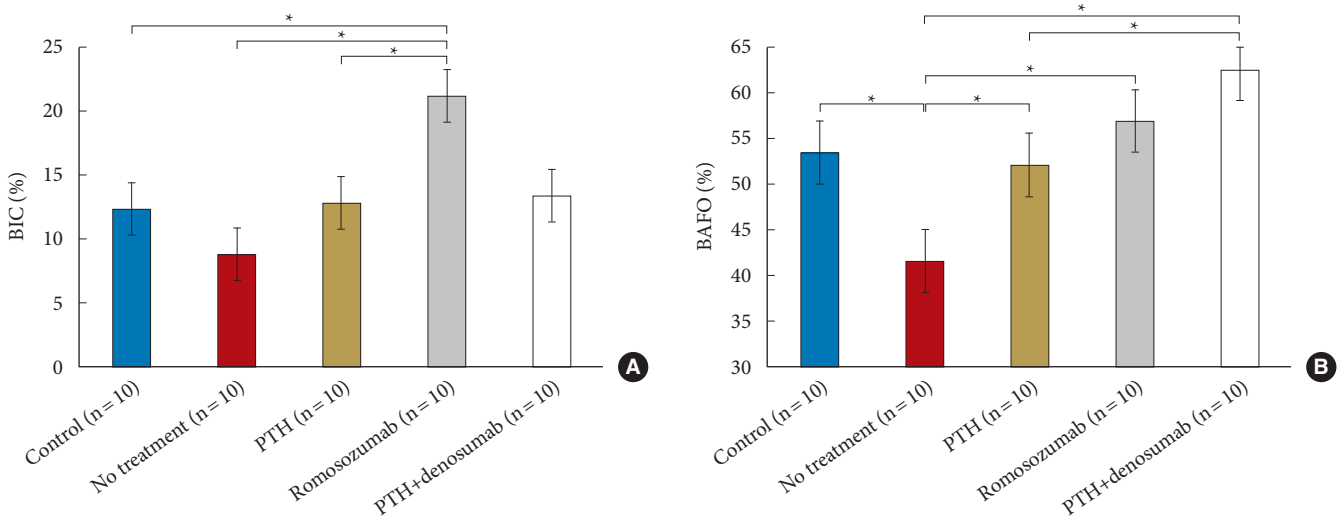

Fig. 5. (A) Percentage bone-to-implant contact (BIC). (B) Percentage of bone area fraction occupancy (BAFO). PTH, parathyroid hormone. Data were shown as mean ± standard deviation. N represents the number of hemipelvis samples analyzed per group. Selected pairwise comparisons were conducted as indicated by asterisks. *Statistically significant difference with $p < 0.05$.

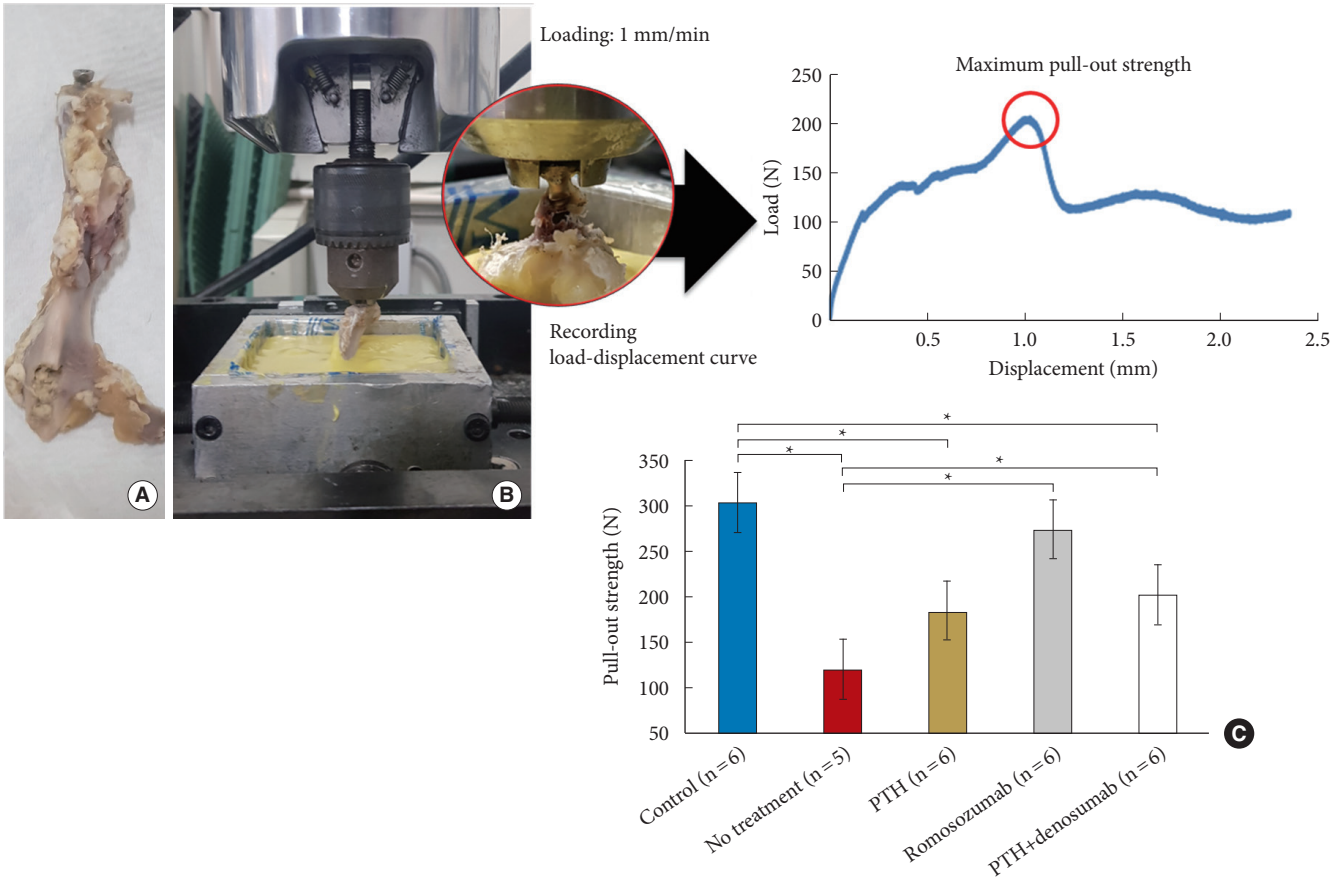

Fig. 6. Mechanical testing of screw pull-out strength. (A and B) MTS testing setup aligned with screw trajectory. (C) Pull-out strength results (N) at 1 mm/min. Data were shown mean ± standard deviation. N represents the number of hemipelvis samples analyzed per group. Selected pairwise comparisons were conducted as indicated by asterisks. PTH, parathyroid hormone. *Statistically significant difference with $p < 0.05$.

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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 antiosteoporosis medications significantly influenced the resistance of the cancellous 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 microarchitecture 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 Maríe,[23] in research on osteoporosis in rabbit models, this can be broadly classified through 2 mechanisms, including increasing bone resorption through OVX 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.[24] 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 OVX,[15,16,23] 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.[15] Ohtori et al.[25] 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%). Ide et al.[26] compared the effects of combined administration of teriparatide and denosumab in combination therapy after spinal fusion. 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.[27,28] 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.[29] 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 (MBBF) and greater filling of the bone space. Additionally, while romosozumab demonstrated favorable outcomes across multiple parameters—including the highest pull-out strength and BIC—it did not exhibit the highest value in every metric. Specifically, BAFO was slightly higher in the PTH+denosumab group. This highlights a key limitation of the current study: although composite trends favored romosozumab, no single treatment demonstrated universal superiority across all histomorphometric and biomechanical measures. Therefore, interpretations regarding treatment efficacy should be made with caution, and further studies with larger sample sizes and extended observation periods are needed to confirm these findings.

One distinguishing characteristic of this study is that Goldner 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 cancellous bone strength induced by romosozumab is related to cortical thickness but not to cortical porosity.[30] 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. MBBF is not just generally impeded by osteoclast inhibitors, and MBBF may contribute to BMD gains throughout a long-term course of antiresorptive therapy.[31-33] 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 with-

in cancellous bone.[34]

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 BMD and lowering fracture incidence.[35] 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.[36,37] Preclinical research has shown that antisclerostin treatment works in various animal models of glucocorticoid-induced osteoporosis.[32,38,39] For example, investigations in SOST KO mice, ovariectomized rats, and cynomolgus monkeys revealed significant improvements in BMD,[40] bone volume, and bone strength, indicating improved bone quality that would favor implant integration and stability.[41] 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.[6,17,27] This is consistent with the rapid and significant increase in BMD observed in clinical trials with romosozumab, such as the FRAME[8] and ARCH studies,[42] where it has shown efficacy in reducing fracture risks and increasing bone density.

Our study has several limitations. First, in designing this animal model, one of the major challenges was identifying a suitable anatomical site for screw placement that would allow for consistent trajectory, minimize surgical complications, and enable reliable histological and biomechanical analyses. To overcome these technical limitations, we selected the iliac bone as the implantation site due to its relatively wider surface area and accessibility. Iliac screws are clinically relevant, particularly in spinopelvic fixation procedures, and the iliac crest provides sufficient bone stock to ensure a reproducible screw trajectory. However, we acknowledge that the iliac bone is not a load-bearing site like the vertebral body, and its mechanical and biological properties differ from those of the spine. As such, the biomechanical environment of the screw-bone interface in this model does not fully recapitulate the physiological loading conditions experienced by spinal implants. Further studies utilizing larger animal models with vertebral instrumentation and load-bearing assessments will be necessary to confirm the translational relevance of these findings. Second, the relatively short duration of treatment and observation (3 weeks) represents an important limitation of our study. While this time frame was sufficient to assess early-stage osseointegration and mechanical fixation—particularly relevant in osteoporotic settings prone to early failure—it does not allow for evaluation of long-term remodeling dynamics or implant longevity. Future studies with extended follow-up periods will be needed to determine the durability of these early anabolic effects and their implications for sustained implant stability. Third, although this study's findings provide valuable insights into the effects of romosozumab, PTH, and denosumab on bone-implant integration in glucocorticoid-induced osteoporosis, the underlying molecular mechanisms remain to be fully elucidated. Future studies incorporating immunohistochemical staining for osteogenic (e.g., RUNX2, OCN, ALP) and osteoclastic (e.g., TRAP) markers would provide a deeper mechanistic understanding of how these treatments influence bone remodeling, osseointegration, and implant stability. Furthermore, such analyses would help clarify whether the observed improvements in BIC and BAFO are primarily driven by enhanced osteoblast activity, suppressed osteoclast function, or a combination of both processes. Fourth, another limitation is the absence of a priori power analysis to determine sample size. As this was an exploratory preclinical study, group sizes were based on prior literature and feasibility within the constraints of an animal model. However, we acknowledge that the lack of formal statistical justification may limit the interpretability of some comparisons, and future studies should incorporate power calculations to optimize experimental design. Fifth limitation of this study is the lack of systemic evaluation, including BMD measurements and monitoring for potential adverse effects of romosozumab. While our primary focus was on local bone-implant interactions, future studies should incorporate systemic assessments and extended observation periods to better evaluate the translational safety and efficacy of anabolic therapies in osteoporotic conditions.

CONCLUSION

In this preclinical study, we demonstrated that romosozumab significantly enhances both bone microarchitecture and the

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mechanical stability of bone-implant interfaces in a glucocorticoid-induced osteoporosis rabbit model. These findings support the translational relevance of romosozumab for improving surgical outcomes in patients with compromised bone integrity.

NOTES

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