Stabilization of tooth movement by administration of reveromycin A to osteoprotegerin-deficient knockout mice

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Introduction: In this study, mechanical stress in the form of tooth movement was applied to osteoprotegerin-deficient knockout mice, which served as an animal model for juvenile Paget’s disease. To compare and evaluate bone turnover and response of the surrounding bony tissue, we administered reveromycin A. We also investigated the ability of reveromycin A to control osteoclastic activity in juvenile Paget’s disease.

Methods: Eight-week-old male osteoprotegerin-deficient knockout and wild-type mice were injected with reveromycin A (15 mg/kg of body weight) intraperitoneally twice daily. An elastic module was inserted interproximally between the maxillary first and second molars.

Results: Administration of reveromycin A to osteoprotegerin-deficient knockout mice reduced tooth movement distances, increased bone volumes at the interradicular septum, decreased osteoclast counts, and reduced serum alkaline phosphatase and tartrate resistant acid phosphatase. Reveromycin A administration also caused a temporal shift in peak Runx2 staining in osteoprotegerin-deficient knockout mice so that the overall staining time course was similar to that observed for wild-type mice. Conclusions: Reveromycin A administration in osteoprotegerin-deficient knockout mice inhibited bone resorption and normalized bone formation. As a result, normal bone turnover was obtained. (Am J Orthod Dentofacial Orthop 2013;144:368-80)

During orthodontic treatment, tooth movement involves the resorption of alveolar bone by osteoclasts on the pressure side and its formation by osteoblasts on the tension side, leading to alterations of the surrounding tissues.1,2 Optimal tooth movement and bone healing, which depend on osteoclastic and osteoblastic activity, are important for successful orthodontic treatment.3 Osteoclasts are the only cells that participate in the breakdown and resorption of calcified bone tissue. The processes of osteoclast differentiation, maturation, and functioning are controlled by the receptor activator of NF-κB ligand (RANKL), which is expressed on the cell membranes of both osteoblasts and bone marrow stromal cells.4 Osteoclasts and their precursor cells express the receptor activator of NF-κB (RANK), which interacts with RANKL via intercellular contact, inducing differentiation into matured osteoclasts.5-7 Osteoprotegerin (OPG), produced by osteoblasts, is a member of the tumor necrosis factor receptor superfamily and a decoy receptor. OPG strongly inhibits the interaction between RANKL and RANK, suppressing osteoclast differentiation and functional expression as a decoy receptor for RANKL.8 In vivo, if OPG is overexpressed in mice, bone resorption is reduced, and the mice will develop severe osteopetrosis.9 On the other hand, OPG knockout mice, which recapitulate juvenile Paget’s disease (also known as idiopathic hyperphosphatase-mia), typically appear normal at birth, but as they...
grow, osteoclast activity is promoted. As a result, the adult mice have severe osteoporosis because bone resorption outpaces bone formation relative to wild-type mice.\(^\text{10,11}\) It is the lack of, or the mutation of, the gene encoding OPG (compound heterozygous or homozygous, inherited as an autosomal recessive trait) that causes juvenile Paget’s disease in humans.\(^\text{12,13}\) In this disease, patients can appear normal at birth, but the systemic clinical symptoms increase with age, leading to high levels of bone turnover.\(^\text{14}\) High levels of serum alkaline phosphatase are found in patients with juvenile Paget’s disease, and because OPG knockout mice show similarity to these patients, we believed that an investigation using OPG knockout mice will be helpful in understanding the bone turnover in juvenile Paget’s disease.\(^\text{13}\)

Certain drugs, such as bisphosphonates, are prescribed to treat bone diseases such as osteoporosis or Paget’s disease. Bisphosphonate reportedly binds rapidly and tightly to bone minerals, after which osteoclasts incorporate bisphosphonate during bone resorption and induce apoptosis of osteoclasts.\(^\text{15,16}\) Accordingly, the effect of the drug can continue for years after drug therapy has been stopped.\(^\text{1}\) However, there are occasions when not only tooth movement has been stopped but also the tooth must be moved again during orthodontic treatment, for which the use of bisphosphonate is considered unsuitable.

Recently, the drug reveromycin A was developed to selectively suppress osteoclast activity. Reveromycin A is an acidic substance produced by actinomyces.\(^\text{17}\) It is not absorbed by regular cells but is selectively absorbed by osteoclasts actively secreting acid and dissolving bone. It has been reported that reveromycin A selectively controls apoptosis of activated osteoclasts, has a short half-life, and inhibits bone resorption both in vitro and in vivo.\(^\text{18,19}\)

In this study, mechanical stress in the form of tooth movement was applied to OPG knockout mice serving as an animal model for juvenile Paget’s disease. Although bone tissue undergoes modeling or remodeling by osteoclasts and osteoblasts during tooth movement, the relationships between osteoclasts, osteoblasts, and bone metabolism are not fully understood. Therefore, observation of what occurs to the bony tissues surrounding the teeth during tooth movement can be an effective method for helping to understand the mechanism of bone turnover. The goal of the study was to assess the effects of reveromycin A on the surrounding bony tissue during tooth movement. We examined alveolar bone remodeling in OPG knockout and wild-type mice during tooth movement to test our hypothesis that reveromycin A administration can inhibit bone resorption, decrease tooth movement, and lead to normal bone turnover.

**MATERIAL AND METHODS**

Eight-week-old male OPG knockout (n = 40) and wild-type (C57BL/6J; n = 40) mice were used as the experimental and control animals, respectively. The mice were randomly divided into 2 groups: without reveromycin A for control (n = 20) and with reveromycin A (n = 20). These groups were further divided into 5 subgroups based on the duration of elastic insertion: 0 hours, 2 hours, 12 hours, 1 day, and 3 days (n = 4 in each group). The mice were purchased from Clea Japan (Osaka, Japan) and housed at the animal experimentation laboratory in the School of Dentistry, Aichi-Gakuin University in Nagoya, Japan. The mice were genotyped by polymerase chain reaction analysis following the methods of Mizuno et al.\(^\text{11}\) Room temperature and humidity were maintained at 22°C ± 2°C and 50% ± 10%, respectively. A 12-hour light and dark cycle was used. The mice were given free access to solid food (CE-2; Clea Japan) and tap water. All procedures involving the care and use of laboratory animals in this study were performed in accordance with the guidelines for animal experiments at the School of Dentistry at Aichi-Gakuin University and approved by its animal care committee. In this study, reveromycin A (reveromycin A 3 sodium) was used as the experimental drug. Based on the methods of Watanabe et al.\(^\text{20,21}\) and Shoji et al.,\(^\text{22}\) the mice were anesthetized using intraperitoneal pentobarbital. Orthodontic elastic (3M Unitek, Tokyo, Japan) was inserted between the maxillary left first and second molars using 2 tweezers to induce experimental tooth movement of the first molar (Fig 1, A). After insertion of the elastic, excess elastic was cut on the buccal and lingual sides.

Reveromycin A (15 mg/kg of weight) was administered intraperitoneally twice a day starting 3 days before using the Waldo method in the experimental groups of mice, according to the method of Woo et al.\(^\text{18}\) (Fig 1, B). An identical volume of physiologic saline solution was administered to the control group.

Two hours, 12 hours, 1 day, and 3 days after elastic insertion, images of the maxillary left alveolar bones and molars were obtained with a microcomputed tomography scanner (SMX-225CT-SV2; Shimazu, Kyoto, Japan). The images were analyzed with TRI/3D–BON software (Ratoc System Engineering, Tokyo, Japan) to determine how much each tooth had moved. Images were rotated 360° for observation and adjusted to ensure that the occlusal view with the narrowest gap between the first and second molars was observed, based on the methods of Shoji et al.\(^\text{22}\) A sagittal view was used for observing the height of the alveolar crest of the right first molar’s interradicular septum, the roots of the first...
molar, and the surrounding alveolar bone. In addition, the bone density of trabecular bone in the first molar’s interradicular septum was measured at the baseline and at 3 days after elastic insertion.

On the day of elastic insertion and at 2 and 12 hours, 1 day, and 3 days later (Fig 1, B), the mice were killed, and maxillary bone was removed. The bone samples were fixed in 10% neutral buffered formalin. The samples were decalcified for 4 weeks using 10% EDTA (pH 7.2) at 4°C, embedded in paraffin, and then cut into 5-μm horizontal sections. The occlusal third of the region between the root furcation and the apex was examined (Fig 1, A). Next, hematoxylin eosin staining, tartrate resistant acid phosphatase (TRAP) staining with an acid phosphatase leukocyte kit (Sigma-Aldrich, St Louis, Mo), and Runx2 immunostaining were

Fig 1. A, Diagram showing insertion of the orthodontic elastic between the maxillary left first molar (M1) and second molar (M2), and the plane at which the histologic specimen was obtained (horizontal line); – orthodontic elastic; — histologically observed area. B, Time course of the experiment, showing the reveromycin A (RMA) administration schedule. C, Schematic diagram of a horizontal section through the roots of M1 (at the plane shown in A); the alveolar bone volume (BV) in the area defined by the 3 roots of the M1, as a proportion of total volume (TV), was determined histopathologically; M1M, M1 mesial root; M1DP, M1 distopalatal root; M1DB, M1 distobuccal root. D, Section stained for Runx2, showing both positively and negatively stained areas.
performed. The stained sections of the periodontal tissues were examined with a light microscope. The bone volume in the interradicular septum was measured in accordance with the method of Sprogar et al.\textsuperscript{23} (Fig 1, C). Osteoclast counts (osteoclast number/bone surface) were measured for the TRAP-stained sections of the distal surface of the alveolar bone of the maxillary first molar distopalatal root. To evaluate local osteoblast activity, Runx2 immunohistologic staining was conducted using an Envision+ kit (Dako Cytomation, Kyoto, Japan) with antirabbit Runx2 (M-70, Runx2: 1/1000; Santa Cruz Biotechnology, Santa Cruz, Calif). Following the method of Shoji et al.,\textsuperscript{22} saturation in the immunohistologic staining was evaluated as follows.

1. After converting the immunostained tissue sections to a gray-scale image with Photoshop (Adobe Systems, San Jose, Calif) (Fig 1, D), the saturation ratios of positive to negative stained areas of the periodontal ligament were expressed at one of 256 gradation levels.

2. Ratios of positive to negative stained areas of the periodontal ligament were also calculated with a point-hit method using grids according to the methods of Kimmel and Jee\textsuperscript{24} and Shoji et al.\textsuperscript{22}

3. Osteoblastic surface areas (osteoblast surface/bone surface) were examined for sections stained with hematoxylin and eosin of the alveolar bone from the distal surface of the palatal root to obtain the bone-formation parameters.

For the measurement of serum markers of bone turnover, blood samples from the OPG knockout and wild-type mice were collected under diethyl ether anesthesia. A commercial alkaline phosphatase kit was used to measure the alkaline phosphatase serum levels (Liquitech ALP; Roche Diagnostic K.K., Tokyo, Japan). The level of TRAP in blood was measured using an enzyme-linked immunosorbet assay (ELISA) kit (Immunodiagnostic Systems, Scottsdale, Ariz).

**Statistical analysis**

All data are presented as means and standard deviations, and statistical analyses were carried out by 1-way analysis of variance (Tukey multiple comparison test). All statistical analyses were performed with Prism software (version 5; GraphPad Software, San Diego, Calif), and values of $P < 0.05$ were considered to be significant.

**RESULTS**

There was no significant difference in tooth movement at 1 day after elastic insertion in any group. However, there was a statistically significant increase in tooth movement in the OPG knockout mice without the reveromycin A after 3 days. This group also showed significantly greater movement than did the wild-type mice without the reveromycin A after 3 days (Fig 2). On the other hand, the OPG knockout mice given the reveromycin A showed significantly less tooth movement than did the OPG knockout mice without the reveromycin A after 3 days. Finally, after 3 days, there was no significant difference between the OPG knockout mice given the reveromycin A and wild-type mice without the reveromycin A (Fig 2).

The alveolar crest of the interradicular septum was observed, and bone density was measured 3 days after elastic insertion using microcomputed tomography scanning. An elastomer predisposes to periodontal inflammation and periodontal bone loss, in addition to subjecting the teeth to mechanical forces. In the wild-type mice without the reveromycin A, there were few changes in the density of the trabecular bone at the first molar interradicular septum and in the alveolar bone between the first and second molars. However, there was considerable resorption of alveolar bone at the first molar furcation and the distopalatal root in the OPG knockout mice without the reveromycin A. The alveolar bone between the first and second molars also exhibited considerable resorption. The bone density of the first molar interradicular septum was significantly lower in the OPG knockout mice without the reveromycin A than in the wild-type mice without the reveromycin A. Bone resorption at the first molar furcation, distopalatal root, and alveolar bone between the first and second molars of the OPG knockout mice given the reveromycin A was suppressed relative to the OPG knockout mice without the reveromycin A. The decrease in bone density at the first molar interradicular septum was significantly lower in the OPG knockout mice given the reveromycin A relative to the OPG knockout mice without the reveromycin A (Fig 3).

The histologic findings in the periodontal tissue were as follows. Before elastic insertion, the alveolar bone trabeculae around the root were arranged in a looser mesh in the OPG knockout mice without the reveromycin A than in the wild-type mice without the reveromycin A. At 1 and 3 days, there was widening on the tension side (bone formation side) and narrowing on the compression side (bone resorption side) of the periodontal ligament relative to the specimens before elastic insertion in the wild-type mice without the reveromycin A. Although there was a small amount of alveolar bone resorption, no significant changes in the bone trabeculae were observed. After 3 days, there were considerable changes in the bone trabeculae and a significant bone volume decrease in the interradicular septum in the OPG knockout mice without the
Reveromycin A. Interradicular septum bone resorption observed in the OPG knockout mice without the reveromycin A was suppressed in the OPG knockout mice given the reveromycin A; no considerable changes in the bone trabeculae were observed, indicating no change of volume in the alveolar bone (Fig 4, A and B). Moreover, the OPG knockout mice without the reveromycin A showed a significantly higher number of osteoclasts than did the wild-type mice without the reveromycin A at 3 days (Fig 4, C). The number of osteoclasts was significantly lower in the wild-type mice given the reveromycin A relative to the wild-type mice without the reveromycin A. In the OPG knockout mice groups, the mice given the reveromycin A showed significantly fewer osteoclasts than did the mice without the reveromycin A (Fig 4, C).

In the OPG knockout mice without the reveromycin A, expression of Runx2 on the tension side of the periodontal ligament was considerably greater than that of the wild-type mice without the reveromycin A at 2 hours, but it had decreased considerably at 12 hours. There was a further decrease in Runx2 expression at 1 day. Peak Runx2 expression occurred between 1 and 12 hours in the wild-type mice without the reveromycin A and at 2 hours in the OPG knockout mice without the reveromycin A (Fig 5, A and B). In both Fig 2. A, Representative microcomputed tomography images 3 days after elastic insertion (occlusal view); WT, wild type mice; KO, OPG knockout mice; RMA+, reveromycin A administered mice; RMA−, reveromycin A nonadministered mice. B, Mean distance (mm) between M1 and M2 at 2 and 12 hours, and 1 and 3 days after elastic insertion. Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; n.s., not significant; *P < 0.05.
groups, Runx2 positivity was first observed on the tension side of the periodontal ligament and then on the compressed side, spreading over the entire ligament. At all times, the areas surrounding the periodontal ligament tended to stain more widely for Runx2 in the OPG knockout mice without the reveromycin A than in the wild-type mice without the reveromycin A. There was a significant difference in the area stained between the 2 groups, with Runx2 staining at 1 day (Fig 5, A and C). In the OPG knockout mice given the reveromycin A, the expression of Runx2 was strongly expressed on the tension side of the periodontal ligament 2 hours after elastic insertion. At 12 hours in the OPG knockout mice given the reveromycin A, the expression of Runx2 became stronger relative to the OPG knockout mice without the reveromycin A. The peak in the OPG knockout mice without the reveromycin A was at 2 hours, but after administration of reveromycin A, the expression peak was at 12 hours. Although there was a difference in the strength of the expression, there was a similarity in the transition of the Runx2 expression of the OPG knockout mice given the reveromycin A and the wild-type mice without the reveromycin A (Fig 5, A and B). The area of the OPG knockout mice

Fig 3. A. Representative microfocus x-ray computed tomography images of the alveolar crest of the interradicular septum and the alveolar bone between M1 and M2 3 days after elastic insertion (sagittal microcomputed tomography images); WT, wild-type mice; KO; OPG knockout mice; RMA+, reveromycin A administered mice; RMA−, reveromycin A nonadministered mice. B. Bone density of the interradicular septum (BV/TV). Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; n.s., not significant; **P < 0.01; *** P < 0.001.
given the reveromycin A showed a reaction exclusively at the tension side by 1 day after elastic placement. The transition of the expression of the OPG knockout mice given the reveromycin A was similar to that of the wild-type mice without the reveromycin A in terms of the area that reacted to Runx2 (Fig 5, A and C).

At 3 days, the bone formation parameter (osteoblast surface areas) of the OPG knockout mice without the reveromycin A showed a significantly higher value than that of the wild-type mice without the reveromycin A. In the OPG knockout mice, the bone formation parameter of the group that received the reveromycin A was

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Fig 4. A, Histologic photomicrographs of the bone structure surrounding the M1 roots (hematoxylin and eosin). Black arrow, marked bone loss; white arrow, bone structure maintained; D, day; WT, wild-type mice; KO, OPG knockout mice; RMA+, reveromycin A administered mice; RMA−, reveromycin A nonadministered mice. B, Representative bone volume (BV/TV). Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; *P < 0.05; ***P < 0.001. C, Representative osteoclast count (number/mm) 3 days after elastic insertion. Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; *P < 0.05; **P < 0.01.
Fig 5. A. Histologic photomicrographs of the M1 distopalatal root, immunohistochemically stained for Runx2. WT, wild-type mice; KO, OPG knockout mice; RMA+, reveromycin A administered mice; RMA−, reveromycin A nonadministered mice.

B. For Runx2 immunostaining, density of negative-staining area relative to density of positive-staining area. Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; **P < 0.01; ***P < 0.001.

C. For Runx2 immunostaining, negative-staining area relative to density of positive-staining area. Data are presented as means and standard deviations. Asterisks indicate statistically significant differences between the groups; **P < 0.01.
significantly lower than that of the group without the reveromycin A (Fig 6).

We measured the serum bone turnover markers. The OPG knockout mice without the reveromycin A showed significantly higher values of blood TRAP and serum alkaline phosphatase levels than did the wild-type mice without the reveromycin A because blood TRAP and serum alkaline phosphatase levels were significantly reduced by reveromycin A administration (Fig 7).

**DISCUSSION**

Several rare inherited osteolytic disorders have been described that show phenotypic overlap with Paget’s disease of bone, including familial expansile osteolysis, early-onset familial Paget’s disease of bone, and expansile skeletal hyperphosphatasia. These are related disorders caused by mutations affecting the TNFRSF11A gene, which encodes the receptor activator of RANK. The mutations result in failure of normal processing of RANK and osteoclast activation. It has been recognized that orthodontic treatment in this state of enhanced RANK activity can lead to premature tooth loss from aggressive bone resorption. On the other hand, recent studies have shown that juvenile Paget’s disease is related to the lack or the mutation of a gene encoding OPG (TNFRSF11B). In addition, it was reported that high levels of serum alkaline phosphatase—the
quintessential biochemical marker of juvenile Paget's disease—were found. In other studies, high levels of soluble RANKL were detected in patients with juvenile Paget's disease.\textsuperscript{12,27} In the knockout mice having a gene deletion of OPG as a decoy receptor of RANKL, osteoclast formation was elevated. Severe osteoporosis occurs from bone resorption. In those mice, bone density was normal at birth, but bone loss was clearly observed from the evaluation of the trabeculae at the age of 1 week and from the cortical bone at the age of 4 weeks. The cortical bone had been replaced by cancellous bone.\textsuperscript{10,11} Serum alkaline phosphatase activity was promoted in the OPG knockout mice, and high levels of soluble RANKL were observed.\textsuperscript{28} From these results, Whyte et al\textsuperscript{12} reported that the findings for juvenile Paget's disease are quite similar to those for OPG knockout mice; in turn, OPG knockout mice exhibit the pathology of juvenile Paget's disease. The use of recombinant OPG to treat juvenile Paget's disease has also been reported.\textsuperscript{29} When we consider OPG and RANKL, as well as physiologic, pathologic, and pharmacologic regulators of bone resorption, RANKL inhibition via denosumab, a fully human neutralizing monoclonal antibody to RANKL, might prove to be superior for juvenile Paget's disease.\textsuperscript{30} However, currently, bisphosphonate is used as the drug of choice for its therapeutic effects on the inhibition of osteoclast activity, reduction of blood alkaline phosphatase activity, normalization of bone turnover, and improvement in bone density.\textsuperscript{14,31-33} Bisphosphonate is analogous to synthetic pyrophosphoric acid, directly acting on osteoclasts to inhibit bone resorption.\textsuperscript{34-36} Both inhibit the entry of blood vessels, prevent mast cell layer apoptosis, reduce cartilage breakdown, and increase production of cartilage matrix.\textsuperscript{37-39}

Some researchers have reported the effects of bisphosphonate administration on OPG knockout mice. Nakamura et al\textsuperscript{28} found an increase of the reduced femoral trabeculae in OPG knockout mice from the administration of a bisphosphonate (risedronate). Tabuchi et al\textsuperscript{40} grafted crude bone morphogenetic protein into OPG knockout mice to evaluate its ability to stimulate new bone formation. They reported that the new bone volume increased after bisphosphonate administration, so that high bone turnover was normalized. Kimura et al\textsuperscript{19} showed that the mandibular ramus of OPG knockout mice was short, with similar clinical findings in juvenile Paget's disease.\textsuperscript{41} Growth of the mandibular ramus was normalized by bisphosphonate administration. Additionally, in a study by Shoji et al,\textsuperscript{22} inhibition of resorption of alveolar bone, reduction of tooth movement, and normalization of bone metabolism by the action of osteoblast activity after bisphosphonate administration were observed. Bisphosphonate might perfectly adjust the accelerated bone turnover if the ideal dose could be used in patients with juvenile Paget's disease.\textsuperscript{42} As can be seen, bisphosphonate has produced helpful effects. Some studies have reported that although administration of bisphosphonate improved bone density, the bone tended to become hard and brittle, leading to adverse effects such as small cracks and fractures.\textsuperscript{43-45} Moreover, maxillary and mandibular necrosis at tooth extraction sites was reported as an adverse effect of bisphosphonates.\textsuperscript{46-48}

Because long-term application of bisphosphonate from infancy or early childhood might increase the risk of the adverse effects, we focused on reveromycin A, an alternative to bisphosphonate. Reveromycin A, an
acids. In a similar study, Shoji et al. reported administration of bisphosphonate to OPG knockout mice. After identifying YRS1/YOR1 encoding ABC transporters specific to acidic substances, as well as ILS1 encoding tRNA synthase as genes rendering resistance, researchers found ILS1 to be resistant to the 660th aspartic acid. As a result, reveromycin A is an effective substance for the treatment of bone metastasis, causing apoptosis of osteoclasts. In addition, in ovariectomized mice or mice fed with calcium-deficient food, reveromycin A administration showed therapeutic effects for osteoporosis. However, reveromycin A has not been used in studies of osteoporosis with high levels of bone turnover or in OPG knockout mice as a model for juvenile Paget’s disease.

Tanaka et al. examined alveolar bone remodeling in OPG knockout mice during continuous and long-term tooth movement using a nickel-titanium closed-coil spring. They reported that reveromycin A normalizes bone metabolism and loss of alveolar bone during continuous tooth movement, similar to our results, and they mainly studied the effects of long-term changes. In contrast, we focused on the early-stage response using orthodontic elastic with reveromycin A administration, and the duration of tooth movement was 3 days in our experiment. As a result, at the time of application of mechanical stress (tooth movement), reveromycin A locally inhibited bone resorption and reduced the amount of tooth movement, while maintaining trabeculae. In addition, blood TRAP and alkaline phosphatase measurements were higher in the OPG knockout mice without reveromycin A than in the mice that received it. In the study by Kimura et al., the levels of blood TRAP and alkaline phosphatase were lower after administration of bisphosphonate to OPG knockout mice. In a similar study, Shoji et al. reported that bone resorption was inhibited and tooth movement decreased after the administration of bisphosphonate. Those studies indicated that reveromycin A has effects similar to those of bisphosphonate, promoting systemic and local inhibition of osteoclastic activity and normalization of bone metabolism by influencing osteoblasts. Systemically high levels of bone turnover with dispersed trabeculae and low bone density were also seen. Our results indicate the possibility of verifying these findings.

Reveromycin A might be effective in the treatment of juvenile Paget’s disease as well as for the metastasis of bone cancers and for osteoporosis because of its similar effects to bisphosphonate. In addition, reveromycin A is reported to be rapidly metabolized without concentrating in the bone matrix, unlike bisphosphonate. Recent research on bisphosphonate-related osteonecrosis of the jaw showed that bisphosphonate is long acting and useful in long-term therapy. Yet adverse dental side effects from bisphosphonate have also been reported, including decreased tooth movement and impaired bone healing, reportedly interfering with orthodontic results. Therefore, the rapid rate of metabolism and the short half-life of reveromycin A are considered to be advantageous in minimizing adverse side effects in diseases requiring long-term therapy and orthodontics. For example, when a tooth needs to be moved, reveromycin A administration can be stopped and then started again when sufficient movement has been achieved. However, further studies are necessary to fully understand how to control tooth movement pharmacologically. We expect that the future of dentistry will include the expansion of reveromycin A applications and improvements in methods of administration, such as ointments or direct injections.

In this study, by inhibiting the promoted osteoclastic activity of OPG knockout mice with reveromycin A, we inhibited excessive osteoblast activity. It is suggested that osteoblast activity was inhibited because some negative factors from osteoclasts to osteoblasts functioned after inhibition of the osteoclast activity. Although an effect of osteoblasts on osteoclasts has been demonstrated in previous studies, the effects of osteoclasts on osteoblasts remain unclear. The mechanism and factors also have not been determined. Shoji et al. suggested the possibility of a signal transmission from osteoclasts to osteoblasts using bisphosphonate. In contrast, Im et al. reported that although bisphosphonate has effects on osteoblasts, reveromycin A selectively acts on activated osteoclasts without affecting osteoblasts. Further studies are necessary to understand the relationship of osteoclasts and osteoblasts to bone metabolism. We believe that the identification of signal transmissions and their potentially negative mechanisms would contribute to the understanding and treatment of metabolic bone diseases.

CONCLUSIONS

In this study, the trabeculae of OPG knockout mice were dispersed and the bone density was low, showing...
similarities to juvenile Paget's disease. Application of mechanical stress (tooth movement) caused severe bone resorption. Reveromycin A administration in OPG knockout mice inhibited bone resorption and normalized bone formation.

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