The Correlation between Postmenopausal Osteoporosis and Inflammatory Periodontitis Regarding Bone Loss in Experimental Models

Megumi KOBAYASHI\textsuperscript{1)}, Chiho MATSUMOTO\textsuperscript{2)}, Michiko HIRATA\textsuperscript{1)}, Tsukasa TOMINARI\textsuperscript{2)}, Masaki INADA\textsuperscript{1)}, and Chisato MIYURA\textsuperscript{1,2)}

\textsuperscript{1)}Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, 2–24–16 Nakamachi, Koganei, Tokyo 184-8588, Japan
\textsuperscript{2)}Cooperative Major in Advanced Health Science, Tokyo University of Agriculture and Technology, 2–24–16 Nakamachi, Koganei, Tokyo 184-8588, Japan

Abstract: We have invented a mouse model of periodontitis associated with alveolar bone loss induced by lipopolysaccharide. Ovariectomized (OVX) animals are widely used as a model for osteoporosis due to estrogen deficiency. To define the relationship between periodontitis and osteoporosis, we examined the influence of estrogen deficiency on the mouse alveolar bone mass. In OVX mice, bone loss was detected not only in the femur, but also in the alveolar bone, indicating that estrogen deficiency could induce resorption in alveolar bone. In experiments using a combination of osteoporosis and periodontitis models, OVX significantly enhanced the alveolar bone loss in the model of periodontitis. Therefore, postmenopausal osteoporosis may enhance the risk of periodontitis associated with inflammatory alveolar bone resorption.

Key words: inflammatory bone resorption, osteoporosis, periodontal disease

Bone remodeling is regulated by the balance between osteoclastic bone resorption and new bone formation by osteoblasts. During bone resorption, all bone-resorbing factors, including interleukin (IL)-1, IL-6, and TNF\textalpha, induce the receptor activator of NF-\kappaB ligand (RANKL) expression on the surface of osteoblasts. Bone-resorbing cytokines, such as IL-1, are known to induce prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) production by osteoblasts \cite{13, 17}. For PGE\textsubscript{2} synthesis, two types of cyclooxygenase (COX), COX-1 and COX-2, are expressed in osteoblasts, and COX-2 is markedly induced by inflammatory stimuli. During bone resorption associated with inflammation, PGE\textsubscript{2} is critical for RANKL-dependent osteoclast formation.

Periodontal diseases are infectious diseases that develop as a result of bacterial accumulation on the tooth surface in the junction of the gingiva and the tooth. Mixed Gram-negative anaerobic bacteria are considered to be pathogenic to the periodontal tissues, and are involved in the development and progression of periodontitis \cite{4, 11}. Periodontitis is a bone destructive disease, and lipopolysaccharide (LPS) is a known pathogen-associated molecule involved in periodontitis. We have recently reported the development of an original mouse model of periodontitis that is associated with the resorption of alveolar bone induced by LPS injection into the lower gingiva, and successfully detected alveolar bone loss induced by LPS \cite{5}. In this model of periodontitis, we have found that LPS induced the loss of alveolar bone in wild-type mice, but not in membrane-bound PGE synthase-1 (mPGES-1; Ptges)-deficient mice, suggesting that mPGES-1-dependent PGE\textsubscript{2} production is essential for LPS-induced periodontal bone resorption \cite{5}.

Osteoporosis is the most common bone disease in

\textcopyright 2012 Japanese Association for Laboratory Animal Science
humans. Postmenopausal females lose bone mass due to a decrease in ovarian estrogen and an increase in bone resorption. Ovariectomized (OVX) animals are widely used as a model of osteoporosis due to estrogen deficiency. OVX mice exhibit severe bone loss in the femur, and inflammatory cytokines such as IL-1, IL-6, and PGE$_2$ may be involved in the mechanism of bone loss due to estrogen deficiency [7–9, 14]. These cytokines are also involved in the inflammatory pathogenesis of periodontitis [11]. However, the relationship between osteoporosis and periodontitis is still unclear. In the present study, we examined the influence of estrogen deficiency on alveolar bone mass using both periodontitis and osteoporosis models. We herein show that postmenopausal osteoporosis may act as a risk factor for periodontitis associated with inflammatory loss of mandibular alveolar bone.

Four-week-old and 6-week-old ddy strain mice were obtained from Japan SLC (Shizuoka, Japan). To measure the bone-resorbing activity in organ culture of the mandibular alveolar bone, mandibular alveolar bone specimens were collected from the mouse molar region under a microscope and cultured for 24 h in BGM containing 1 mg/ml BSA. After 24 h, alveolar bone was transferred to new medium, with or without LPS, and cultured for 5 days. The bone-resorbing activity was determined by measuring the concentration of calcium in the conditioned medium [13].

As a model for experimental periodontitis, LPS (25 µg) was dissolved in 50 µl of PBS and injected into the outside of the mouse lower gingiva on days 0, 2, and 4. As a control, PBS was injected into the lower gingiva at each time point. The mandibular alveolar bone was collected from the mouse molar region 7 days after the first injection [5]. After extraction of the tooth, the bone mineral density (BMD) of the mandibular alveolar bone was measured by dual x-ray absorptiometry (DXA; model DCS-600R; Aloka, Tokyo, Japan). The BMD was calculated by dividing the bone mineral content of the measured area by the total area.

In the model for osteoporosis, 4-week-old female mice were either subjected to a sham operation or OVX and were fed laboratory chow containing 1.12% calcium and 1.07% phosphorus. After 4 weeks, the BMD of the femurs and alveolar bone were measured by DXA. In some experiments, after 3 weeks, the sham-operated or OVX mice were treated with LPS or PBS to elicit experimental periodontitis. All procedures were performed in accordance with the institutional guidelines and permission for animal research.

In the model for periodontitis, mandibular alveolar bone was collected from the mouse lower mandible (Fig. 1A) and cultured with or without LPS to detect bone resorption, as measured by the calcium level in the medium. We then detected the increase in bone resorbing activity in the LPS-treated alveolar bone in vitro (Fig. 1B), and PGE$_2$ production was markedly elevated in the conditioned medium of the organ cultures of alveolar

---

**Fig. 1.** The in vitro and in vivo experiment models for periodontitis. (A) Mandibular alveolar bone was collected from the molar region of a 6-week-old mouse under a microscope. (B) The collected mandibular alveolar bones were cultured for 2 h in BGM containing 1 mg/ml BSA. After 24 h, the alveolar bone was transferred to new medium and cultured for 5 days with or without LPS (10 µg/ml). The bone-resorbing activity was measured by evaluating the calcium present in the medium. The data are expressed as the means ± SEM of 4 independent wells. (C) The level of PGE$_2$ was measured by EIA using the conditioned medium of organ cultures of alveolar bone shown in (B). The data are expressed as the means ± SEM of 3 independent wells. (D) As a model of in vivo experimental periodontitis, LPS (25 µg/mouse) was injected into the lower gingiva of a 6-week-old mouse on days 0, 2, and 4. As a control, PBS was injected into the lower gingiva at each time point. The mandibular alveolar bone was collected 7 days after the first injection, and the BMD of the respective mandibular alveolar bone was measured. The data are expressed as the means ± SEM of 4 mice. * and **: There is a significant difference between the two groups (P<0.01 vs the control and P<0.001 vs the control, respectively).
osteoporosis and periodontal disease

In in vivo experiments, LPS was injected into the gingiva of the lower mandible, and the alveolar bone was collected from the mouse molar region, as shown in Fig. 1A, on day 7 for measurement of BMD by DXA. LPS administration induced a significant decrease in the BMD of the mandibular alveolar bone in mice (Fig. 1D).

To determine the influence of estrogen deficiency on the alveolar bone mass in vivo, 4-week-old female mice were either subjected to a sham operation or O VX, and the BMDs of femurs and alveolar bones were measured. At 4 weeks after the operation, the uterine weight was markedly decreased in O VX mice, indicating estrogen deficiency in these mice (Fig. 2A). The BMD of the femurs was decreased at the distal metaphysis in O VX mice due to increased bone resorption (Fig. 2B). When we measured the BMD of the mandibular alveolar bone by DXA after extraction of the tooth, the BMD was significantly reduced in the O VX mice (Fig. 2C). These data clearly indicate that bone loss could be detected not only in the femur, but also in the alveolar bone, and that the bone loss was related to the state of estrogen deficiency.

To examine the relationship between periodontitis and osteoporosis, we performed experiments using combined O VX and periodontitis models. In this experiment, 4-week-old female mice were either subjected to a sham operation or O VX, and LPS or PBS was injected into the gingiva in the lower mandible in some mice from each group 3 weeks after the operation (Fig. 3A). Four
weeks after the operation, the BMD of the mandibular alveolar bone was measured by DXA. In this experiment, OVX significantly enhanced the loss of alveolar bone mass in the group with LPS-induced periodontitis (Fig. 3B), suggesting that osteoporosis due to estrogen deficiency may act as a risk factor for the pathogenesis of periodontal disease and is associated with increased inflammatory bone loss of the mandibular alveolar bone.

In healthy periodontal tissues, the roots of the teeth embed into individual sockets in the alveolar bone. However, the roots of the teeth are exposed by the degradation of alveolar bone due to inflammation, and the loss of teeth is the end-point of periodontal disease. In the traditional model for periodontitis, a cotton ligature is placed around lower molars in the mandible [1]. The induction period for the model requires more than 30 days for the promotion of inflammation and periodontal destruction, and the pathological condition is occasionally unstable among experimental animals. We have developed a rapid experimental model of periodontitis that requires only 7 days for detection of a decrease in alveolar bone mass in all animals [5]. In the model, local treatment with indomethacin, a nonsteroidal anti-inflammatory drug (NSAID), in the lower mandible prevented LPS-induced alveolar bone loss in vivo (Inada M. and Miyaura C., unpublished data). In the present study, LPS induced marked production of PGE_2 in organ cultures of mouse alveolar bone (Fig. 1C). We have also reported that LPS induced the loss of alveolar bone in wild-type mice, but not in mPGES-1-deficient mice, suggesting that PGE_2 production is essential for LPS-induced periodontal bone resorption [5]. Previous studies have shown that high expression of COX-2 could be detected in the gingiva from patients with periodontitis [18]. Therefore, our experimental model for periodontitis induces PGE-dependent alveolar bone resorption associated with inflammation and is available to evaluate new drug compounds for periodontitis in human patients.

In patients with postmenopausal osteoporosis and concomitant periodontal disease, the progression of alveolar bone loss may be enhanced by systemic estrogen deficiency. In clinical studies, alveolar bone density decreased more rapidly in postmenopausal women compared with the normal control [15], and estrogen replacement prevented bone loss not only in the spine, but also in the alveolar bone [2, 6, 16], indicating that the bone tissue in the jaws is also affected by estrogen deficiency. In contrast, Lundström et al. [12] have reported that the hip BMD was not related to alveolar bone loss in postmenopausal females. Therefore, the relationship between osteoporosis and periodontitis is not clear in human patients. In another study of 38 postmenopausal females, the loss of alveolar bone mass was significantly associated with the initial spine BMD [15]. In addition, other studies have shown that the decreased BMD was associated with the number of lost teeth in females and that estrogen treatment decreases the risk of tooth loss [3, 10]. These data suggest that there is a positive correlation between periodontitis and postmenopausal osteoporosis in regard to bone loss in patients, but it is difficult to prove direct correlation in clinical studies.

In the present study using the mouse models for periodontitis and osteoporosis, we clearly showed that the alveolar bone mass was reduced by OVX and that estrogen deficiency significantly enhanced the loss of alveolar bone in LPS-induced periodontitis. We suggest that systemic postmenopausal osteoporosis may act as a risk factor for the pathogenesis of periodontal disease with inflammatory bone loss of mandibular alveolar bone. Therefore, the anti-bone resorptive drugs used for postmenopausal osteoporosis may also be effective for periodontitis accompanied by local alveolar bone loss. The animal models for periodontitis and osteoporosis will be useful for evaluating novel approaches to the prevention and treatment of various diseases, especially those affecting the bones and teeth.

References

8. Kawaguchi, H., Pilbeam, C.C., Vargas, S.J., Morse, E.E.,