Intermittent Administration of Parathyroid Hormone Ameliorates Periapical Lesions in Mice

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Abstract

Introduction: Intermittent administration of parathyroid hormone (PTH) promotes oral osseous wound healing and protects against ligature-induced alveolar bone loss. However, its therapeutic value on periapical periodontitis is unknown. The goal of this study was to determine the effect of intermittent PTH administration on the progression of periapical periodontitis. **Methods:** Seven lymphotoxin alpha–deficient mice received pulp exposures of mandibular first and second molars. Exposed pulp in the right mandible was covered with plaque-contaminated fibrin, whereas exposed pulp in the left mandible was left open. After 4 weeks, the periapical tissues were examined to determine the effect of plaque-contaminated fibrin to induce periapical lesions. Fourteen mice received pulp exposure covered with plaque-contaminated fibrin. PTH (40 μg/kg/d) was administered intermittently to half of the mice for 3 weeks beginning 1 week after pulp exposure. The remaining half received saline injections as the vehicle control. At sacrifice, mandibles and tibiae were harvested and processed for histologic examination. Evaluation of neutrophils and blood vessels was performed after staining with immunofluorescence, and periradicular bone was histomorphometrically analyzed. **Results:** The exposed pulp covered with plaque-contaminated fibrin resulted in significantly larger periapical lesions compared with the control. Intermittent PTH administration reduced the size of periapical lesions significantly. Significantly less neutrophil infiltration around the root apex was found in PTH-treated animals compared with the control. **Conclusions:** PTH treatment suppressed periapical inflammation by reducing neutrophil infiltration and protected against tissue destruction by periapical periodontitis. *(J Endod 2015;41:646–651)*

Key Words

Lymphotoxin alpha, neutrophil infiltration, parathyroid hormone, periapical periodontitis

The parathyroid hormone (PTH) increases bone mass when administered intermittently. Teriparatide, a recombinant form of human PTH (1–34), is used for the treatment of osteoporosis. Although the mechanisms for the bone anabolic action of intermittent PTH are unclear, it is known to activate both osteoblasts and osteoclasts in favor of osteoblastic bone formation over osteoclastic resorption, resulting in an increase in total bone mass (1). PTH is effective in promoting fracture healing (2, 3). Such an enhanced osseous healing by PTH is seen in craniofacial bones as well where PTH administration promotes bone fill and growth in tooth extraction sockets (4, 5). Barros et al (6) reported that intermittent PTH administration protected ligature-induced bone loss in a rat model of periodontitis. In that study, the numbers of inflammatory cells were significantly reduced in the PTH-treated group compared with the control. Likewise, we have discovered that intermittent PTH administration promotes tooth extraction socket healing in rodents (7, 8). PTH-promoted tooth extraction wound healing was accompanied with decreased inflammatory cell infiltration. These findings imply that intermittent PTH administration may have protective effects against inflammation-induced bone loss in the oral cavity.

Periapical periodontitis is an inflammatory disease resulting from root canal infections by mainly anaerobic microorganisms (9–11). Such microorganisms, their endotoxins and enzymes together, stimulate periapical tissues and alert the host defense mechanism (12, 13). Neutrophils play a crucial role in orchestrating the host defense system starting from the early phase of periapical infection. Neutrophils are phagocytes that fight against microorganisms but also cause host tissue breakdown because of their release of proinflammatory cytokines, chemokines, and proteinases (14, 15). Therefore, suppression of the intensity of inflammatory responses in periapical tissues, which is orchestrated by neutrophils, would lead to reduced damage in the host tissues. In this study, we hypothesized that the intermittent administration of PTH protects against tissue damage caused by apical periodontitis. Using lymphotoxin alpha (LTA)-deficient mice, which exhibit defects in secondary lymphoid structures and therefore in adaptive immune responses (16), periapical periodontitis was induced, and the effect of PTH administration on the disease progression of periapical periodontitis was determined.

Materials and Methods

Experimental Design

A breeding pair of mice homozygous mutant for LTA (B6.129S2-Lta^{tm1Dch}/J) was obtained from Jackson Laboratory (Bar Harbor, ME). Twenty-one offspring at the age of 8 weeks were used. Seven mice were subjected to pulp exposure of the mandibular molars to assess the effect of plaque contamination and confirm the development of

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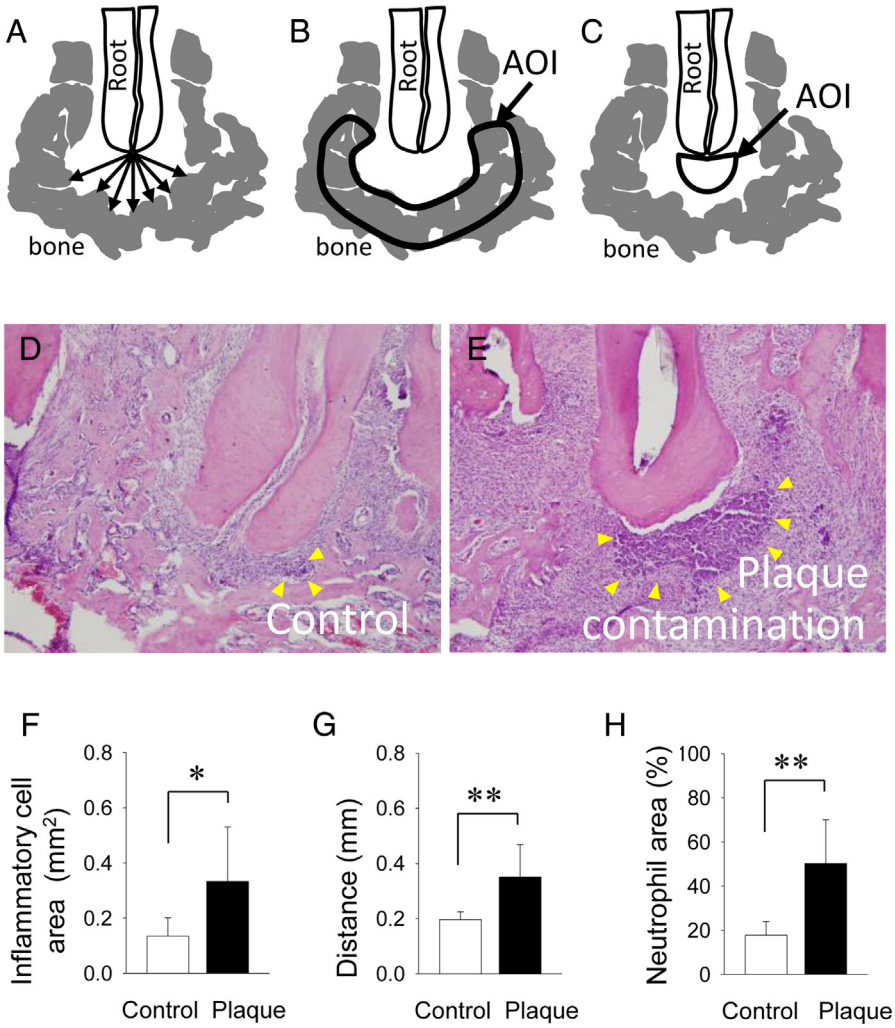

Figure 1. Exposed pulp covered with plaque-contaminated fibrin induced large periapical lesions. (*A*) The distance between the root apex and the surface of periradicular bone was measured at 7 points, and the results were averaged. The average was used as a representative distance to estimate the size of the lesion. (*B*) The bone tissue within 0.4 mm of the bone surface was defined as the AOI for the measurements of the numbers of bone fragments, bone area/tissue area, and Oc.N/BS. (*C*) The periapical soft tissue area in a semicircle with a radius of 0.6 mm was defined as the AOI for the assessment of inflammatory cells and neutrophils. (*D* and *E*) Representative photomicrographs of hematoxylin-eosin–stained sections of periapical lesions (original magnification, ×100). *Arrowheads* indicate inflammatory cell infiltration. (*F*) Tissue areas occupied by inflammatory cells were measured. A significantly larger inflammatory cell area was noted in the plaque-contaminated fibrin group than control. (*G*) The size of the periapical lesions was assessed by measuring the distance between the apex and the bone surface. Significantly larger periapical lesions were found in the plaque-contaminated fibrin group than control. (*H*) Ly6G(+) cells, which represent neutrophils, were assessed next to the apex. Significantly more neutrophils were observed in the plaque-contaminated fibrin group than the control (n = 7/group; paired t test; $^*P < .05$, $^{**}P < .01$).

periapical lesions (Supplemental Figure S1). Fourteen mice were subjected to pulp exposure with plaque contamination to induce periapical lesions. Subsequently, daily injections of either PTH or saline were performed for 3 weeks to evaluate the therapeutic value of PTH treatment on periapical lesions (Supplemental Figure S2). The experimental protocol was approved, and all animals were treated in accordance with the guidelines of the University Committee on Use and Care of Animals.

Mouse Model of Periapical Lesions

Mice were subjected to ligature placement (5/0 Silk) around each of the maxillary second molars 5 days before pulp exposure (17). On the day of pulp exposure, the ligatures were removed, and 1 ligature was placed in a tube with 1.0 mL saline and vortexed while the other was either used as a backup or discarded. Half of the plaque/saline-mixed solution (0.5 mL) was transferred to another tube and centrifuged. The pelleted plaque was mixed with 10 μL fibrinogen solution (Sigma-Aldrich, St Louis, MO) and placed on ice until use. The pulp exposure of the mandibular first and second molars were conducted with a dental handpiece and 1/4 round bur in 7 mice. The exposed pulp in the left mandible was left open to the oral environment. The exposed dental pulp in the right mandible was covered with 2 μL plaque/fibrinogen mixture followed by 5 μL thrombin (Sigma-Aldrich). Mice were euthanized 4 weeks after pulp exposure to assess the effect of plaque-contaminated fibrin on the development of periapical lesions.

Injections and Euthanasia

Fourteen mice received pulp exposure covered with plaque-contaminated fibrin as described previously to induce periapical lesions. Daily subcutaneous injections of either PTH (1–34) (40 μg/kg/d; Bachem, Torrance, CA) or an equivalent volume of 0.9% saline