Preexisting Periapical Inflammatory Condition Exacerbates Tooth Extraction–induced Bisphosphonate-related Osteonecrosis of the Jaw Lesions in Mice

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Abstract

Introduction: Surgical interventions such as tooth extraction increase the chances of developing osteonecrosis of the jaw in patients receiving bisphosphonates (BPs) for the treatment of bone-related diseases. Tooth extraction is often performed to eliminate preexisting pathological inflammatory conditions that make the tooth unsalvageable; however, the role of such conditions on bisphosphonate-related osteonecrosis of the jaw (BRONJ) development after tooth extraction is not clearly defined. Here, we examined the effects of periapical periodontitis on tooth extraction–induced BRONJ development in mice. **Methods:** Periapical periodontitis was induced by exposing the pulp of the maxillary first molar for 3 weeks in C57/BL6 mice that were intravenously administered with BPs. The same tooth was extracted, and after an 3 additional weeks, the mice were harvested for histologic, histomorphometric, and histochemical staining analyses. **Results:** Pulp exposure induced periapical radiolucency as shown by increased inflammatory cells, tartrate-resistant acid phosphatase–positive osteoclasts, and bone resorption. When BPs were administered, pulp exposure did not induce apical bone resorption despite the presence of inflammatory cells and tartrate-resistant acid phosphatase–positive osteoclasts. Although tooth extraction alone induced BRONJ lesions, pulp exposure further increased tooth extraction-induced BRONJ development as shown by the presence of more bone necrosis. **Conclusions:** Our study demonstrates that a preexisting pathological inflammatory condition such as periapical periodontitis is a predisposing factor that may exacerbate BRONJ development after tooth extraction. Our study further provides a clinical implication wherein periapical periodontitis should be controlled before performing tooth extraction in BP users in order to reduce the risk of developing BRONJ. *(J Endod 2016;■:1–6)*

Key Words

Bisphosphonate, bisphosphonate-related osteonecrosis of the jaw, inflammation, periapical periodontitis, tooth extraction

Significance

Our study provides a clinical implication wherein periapical periodontitis should be controlled before performing tooth extraction in BP users in order to reduce the risk of developing BRONJ.

Bisphosphonate-related osteonecrosis of the jaw (BRONJ) is a devastating side effect that predominantly occurs in patients who are undergoing or have undergone therapy with bisphosphonates (BPs) (1). Clinical presentation includes exposed or "probable" bone that persists more than 8 weeks without a history of radiation exposure (2). Increasing lines of evidence support a notion that such clinical presentations also occur in users of other drugs such as antiresorptive or antiangiogenic medications and hence have been redefined as medication-related osteonecrosis of the jaw (3). Because there are currently no reproducible and predictable therapeutic modalities to cure BRONJ, it imposes significant problems in both medical and dental communities.

Dentoalveolar trauma such as tooth extraction is one of the major known risk factors for BRONJ development. Studies showed that more than 50% of patients having osteonecrosis of the jaw (ONJ) experienced a history of having tooth extraction (4, 5). Also, tooth extraction was reported to increase the risk of developing ONJ by 33-fold (3, 5). Interestingly, but intuitively, the vast majority of tooth extraction procedures are performed to resolve prior existing pathological lesions such as periodontal or periapical diseases that make the tooth unsalvageable. Such observation implies that these preexisting pathological problems may already have predisposed the affected areas to BRONJ development even before tooth extraction.

Bacterial infection and inflammation are almost always found universally in ONJ lesions (6), and periodontal and periapical diseases are associated with bacterial infection and host inflammation (7, 8). Indeed, these pathological inflammatory conditions

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have been known to be the precipitating factors for ONJ (3, 9, 10). Periapical diseases are typically initiated by bacterial penetration through the infected pulp followed by local inflammation and bone destruction at the root apex. Nonetheless, the extent to which periapical diseases affect ONJ development after dentoalveolar trauma remains to be elucidated.

Previously, we have established the mouse model for BRONJ and showed that tooth extraction alone induces ONJ (11). Here, we hypothesize that preexisting inflammatory conditions exacerbate BRONJ development after tooth extraction. To test this, we established a mouse model for periapical periodontitis by performing pulp exposure and examined the effects of prolonged periapical endodontic lesion in ONJ development after tooth extraction.

Materials and Methods

Animals

C57BL/6 mice (6-week-old females) were purchased from the Jackson Laboratory (Bar Harbor, ME) and kept in a pathogen-free vivarium in the Division of Laboratory Animal Medicine, University of California Los Angeles, Los Angeles, CA. All experimental protocols were approved by institutional guidelines from the Chancellor's Animal Research Committee (#2011-062).

The Mouse Model of a Periapical Lesion

Mice were anesthetized with ketamine/xylazine (100 and 5 mg/kg body weight, respectively) by intraperitoneal injection. Pulp exposure was performed on the left maxillary first molar using a high-speed ¼ round bur on a portable dental unit (Aseptico Inc, Woodinville, WA) under 10× magnification of an endodontic microscope (BM-LED Stereo Microscope; MEIJI Techno, Saitama, Japan). Exposed teeth were left open to the oral environment without any coverage. The right maxillary first molar was used as a control without pulp exposure. Mice were sacrificed, and maxillae were harvested at 1, 3, 7, 21, and 42 days after pulp exposure ($n = 3$).

The BRONJ Mouse Model with Pulp Exposure and Tooth Extraction

The BRONJ mouse model with pulp exposure and tooth extraction was performed as described previously with modifications (11). Briefly, a total of 20 mice were divided into 2 groups ($n = 10$); 1 group was intravenously administered with vehicle (Veh) solution (0.9% NaCl saline) and the other group with 125 mg/kg Zometa (ZOL; Novartis Oncology, East Hanover, NJ). Under general anesthesia, periapical periodontitis was induced by exposing the pulp of the maxillary first molar for 3 weeks, and the same tooth was extracted. The mice were allowed to heal for an additional 3 weeks during which the maxilla and femur were harvested from each mouse for further analysis.

Tissue Preparation

The maxillae were fixed with 4% paraformaldehyde in phosphate-buffered saline (pH = 7.4) at 4°C overnight and stored in 70% ethanol solution. Fixed tissues were subjected to micro–computed tomographic (μCT) scanning; after which, maxillae were decalcified with 5% EDTA and 4% sucrose in phosphate-buffered saline (pH = 7.4) for 2 weeks at 4°C. The decalcification solution was changed daily for 3 weeks, and decalcified tissues were sent to the University of California Los Angeles Translational Procurement Core Laboratory for paraffin embedding.

μCT Scan and 3-dimensional Volumetric Analysis

μCT scanning (Scanco μCT 40; Scanco Medical, Brüttisellen, Switzerland) of the maxilla and femur was performed using a voxel size of 20 μm^3 and a 0.5-mm aluminum filter at 55 kVp and 145 μA with an integration time of 200 milliseconds using a cylindrical tube (field of view/diameter: 20.48 mm). The resolution was set to medium (1024 × 1024 × 148 pixels). Two-dimensional images were reconstructed using μCT v6.1 software (Scanco Medical, Brüttisellen, Switzerland), and 3-dimensional images were obtained using CTvol software (Bruker microCT, Kontich, Belgium).

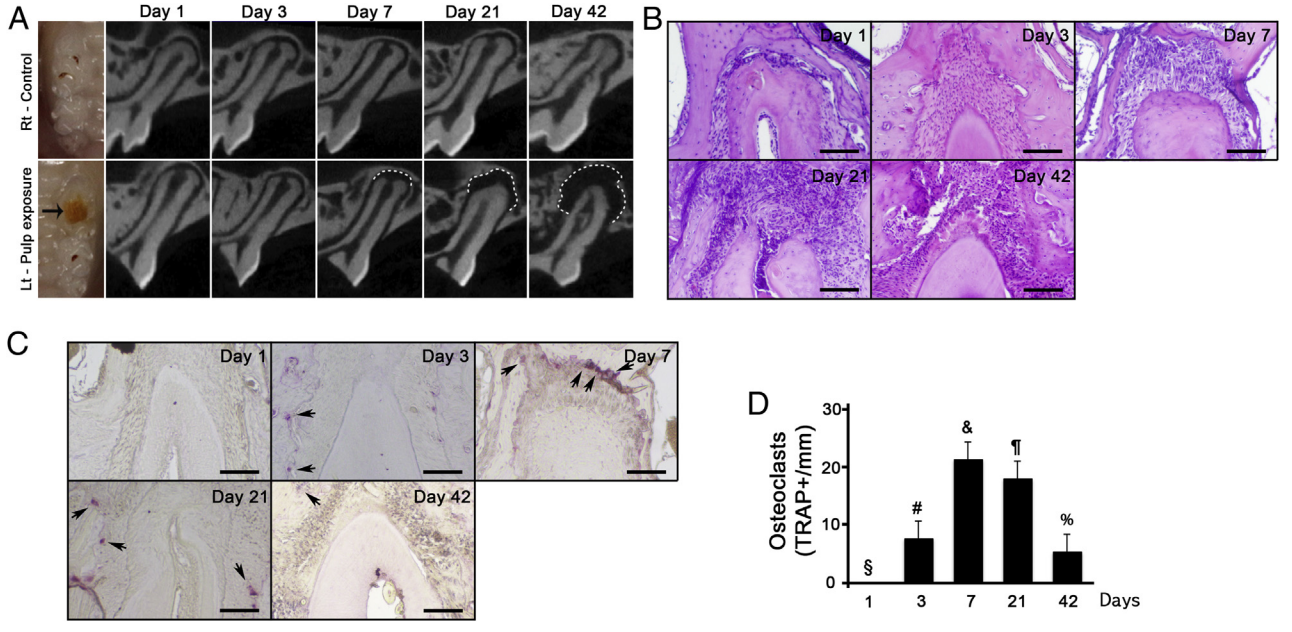

Figure 1. Pulp exposure induces PARL with increased inflammatory cells and activated osteoclasts in mice. Pulp was exposed using a round bur on the first molar in mice (*left panels*). The exposed pulp was left as is for 1, 3, 7, 21, and 42 days. (*A*) μCT scans of the distopalatal root. (*B*) Hematoxylin-eosin staining at the apex. (*C*) TRAP staining at the apex. *Arrows* indicate TRAP+ osteoclasts. (*D*) Quantification of TRAP+ osteoclasts around the apex of the root ($P < .05$). Different symbols are significantly different by analysis of variance and the Tukey post hoc test ($P < .05$). Scale bars = 100 μm.