Osseous Characteristics of Mice Lacking Cannabinoid Receptor 2 after Pulp Exposure

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Abstract

Introduction: Endogenous cannabinoid compounds are involved in many physiological processes, including bone metabolism. Cannabinoid receptor 2 (CB2) plays a role in modulating bone density, but published research results are conflicting. Furthermore, the specific role of CB2 in inflammation-induced bone resorption and craniofacial bone density has not been reported. The objective of this study was to assess the role of CB2 in dental pulp exposure–induced periapical bone loss and mandibular bone density. **Methods:** Adult female wild-type (WT) and CB2 homozygous knockout (KO) mice were used. Pulp exposures were created unilaterally in the mandibular first molars, and the pulp was left exposed to the oral cavity to induce periapical lesion formation. Mandibles were harvested 26 days after pulp exposure. Mandibular bone mineral density and periapical lesion volume were assessed using micro–computed tomographic imaging. **Results:** Periapical lesion volume measured on the mesial root of the pulp-exposed first molar was significantly less in CB2 KO than WT mice ($P < .05$). No significant difference was detected between KO and WT mice in the size of the PDL space measured on the mesial root of the contralateral intact first molar. CB2 KO mice exhibited greater mandibular bone density than WT mice ($P < .05$). **Conclusions:** CB2 plays a role in mandibular bone metabolism. Increased bone density in CB2 KO mice may contribute to the smaller periapical lesion size observed after pulp exposure in KO compared with WT mice. Additional experiments are needed to further elucidate the function of CB2 and clinical implications of cannabinoids on bone and periapical pathosis. *(J Endod 2015;41:853–857)*

Key Words

Bone density, cannabinoid, cannabinoid receptor 2, micro–computed tomographic imaging, periapical lesion, pulp exposure

Endogenous cannabinoid compounds (endocannabinoids) are involved in many physiological processes, including pain, appetite, immune responses, and bone metabolism. Cannabinoid signaling involves endocannabinoids, cannabinoid receptors, and enzymes that synthesize and break down endocannabinoids. Three main cannabinoid receptors have been identified: cannabinoid receptor 1 (CB1), cannabinoid receptor 2 (CB2), and the G protein–coupled receptor 55 (GPR55). CB2 agonists are of therapeutic interest because they are analgesic but lack psychoactive effects (1). CB2 is also of therapeutic interest because it plays a role in regulating bone density (2–8). CB2 is expressed in many monocyte-derived cells, including circulating macrophages, microglia, and dendritic cells, as well as osteoblasts, osteoclasts, and osteocytes (1, 2).

Although CB2 has been shown to play a role in modulating bone density, published results from both *in vivo* and *in vitro* research are conflicting (3, 4, 6). Furthermore, few studies have investigated the bones of the craniofacial region. The specific role of CB2 in inflammation-induced bone resorption and craniofacial bone density has not been reported. Bone metabolism plays a critical role in endodontics in the development and healing of apical periodontitis. Thus, identifying the molecular events that regulate bone resorption and apposition will provide insight into the mechanisms regulating the development and healing of apical periodontitis.

There is a great body of literature on the effects of cigarette smoking on periodontal disease, periodontal treatment, and dental implants (9–12). Furthermore, the effect of cigarette smoking on apical periodontitis and endodontic outcomes has been reported in the endodontic literature (13–16). In contrast, there remains no published research on the effects of cannabinoids and cannabis on endodontic treatment, apical periodontitis, and/or endodontic outcomes. Marijuana is the most commonly used illegal drug in the United States (17). With the increase in medicinal marijuana use and the legalization of recreational marijuana in the states of Colorado and Washington, it is likely that patients will be more forthcoming in reporting their use of marijuana. Thus, investigations into the effects of the cannabinoid system, and both endogenous and exogenous compounds that affect this system, on apical periodontitis and bone metabolism are warranted.

The objective of this study was to assess the role of CB2 in mandibular bone metabolism using a CB2 knockout (KO) mouse model and an established model of inflammation-induced periapical bone resorption. The role of CB2 in dental pulp exposure–induced periapical bone loss and mandibular bone density was assessed. Two null hypotheses were tested:

1. There is no difference in the size of pulp exposure–induced periapical lesions between wild-type (WT) and CB2 KO mice.
2. There is no difference in mandibular bone mineral density between WT and CB2 KO mice.

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0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2015.01.030

Methods

Animals

All protocols were approved by the University of Washington Institutional Animal Care and Use Committee. Adult female mice were used for all experiments. WT (C57BL/6J, n = 10) and CB2 homozygous KO mice (B6.129P2-*Cnr2*^{tm1Dgen}/J, n = 10) were obtained from The Jackson Laboratory (Bar Harbor, ME; stock numbers: 000664 and 005786, respectively). Experiments were started when mice were 8 to 10 weeks old. Mice were housed in a room with a 12-hour dark/12-hour light cycle.

Pulp Exposure Surgery

Mice were anesthetized by intraperitoneal injection of ketamine/xylazine/acepromazine (KXA; 117 mg/kg ketamine, 7.2 mg/kg xylazine, 3 mg/kg acepromazine). Pulp chambers of the mandibular first molars were exposed unilaterally under magnification using a ¼ round bur. A #08 endodontic file was used to verify the pulp exposure and allow contamination of the pulp with oral microorganisms. The pulp was left exposed to the oral cavity to induce periapical lesion formation. Using this model, periapical lesions are detectable by 2 weeks after the pulp exposure surgery (18, 19).

Sample Collection

Twenty-six days after pulp exposure surgery, mice were anesthetized with KXA and euthanized by transcardial perfusion with 4% paraformaldehyde. The mandibles were dissected and post-fixed in 4% paraformaldehyde overnight at 4°C and then stored in phosphate-buffered saline at 4°C.

Micro–computed Tomographic Imaging

Micro–computed tomographic scans were performed at the Small Animal Tomographic Analysis (SANTA) facility located at the Seattle Children's Research Institute, Seattle, WA, using a SkyScan 1076 instrument (SkyScan, Antwerp, Belgium). Scans were performed at an isotropic resolution of 17.63 μm using the following settings: 65 kV, 150 μA, 1.0-mm aluminum filter, 460-millisecond exposure, rotation step of 0.7°, 180° scan, and 3 frame averaging. Raw data were reconstructed using NRecon V1.6.0 software (SkyScan) and the data resliced in the coronal plane to simplify subsequent delineation of regions of interest. The 3-dimensional rendered images of each data set were generated using Drishti V2 Volume Exploration software (http://sf.anu.edu.au/Vizlab/drishti).

Analysis of Periapical Lesion Size

Using CTan software (SkyScan), a polygonal region of interest was chosen to outline the space between the apical portion of the mesial root and the alveolar bone (20). The volume of the periapical lesion or periodontal ligament (PDL) was determined from the area of the space within 20 consecutive sections with the first slice starting at the bottom of the tooth socket (Fig. 1*A* and *B*). An example of the volume measured in each case is visually represented by rendering the volume of interest with the entire mandibular volume but using different render settings (ie, transfer functions). Volumes were calculated for the periapical lesion on the pulp-exposed side and the PDL on the nonexposed side for all 20 specimens, with the nonexposed sides used as controls. All measurements were taken blind to the genotype of the specimen.

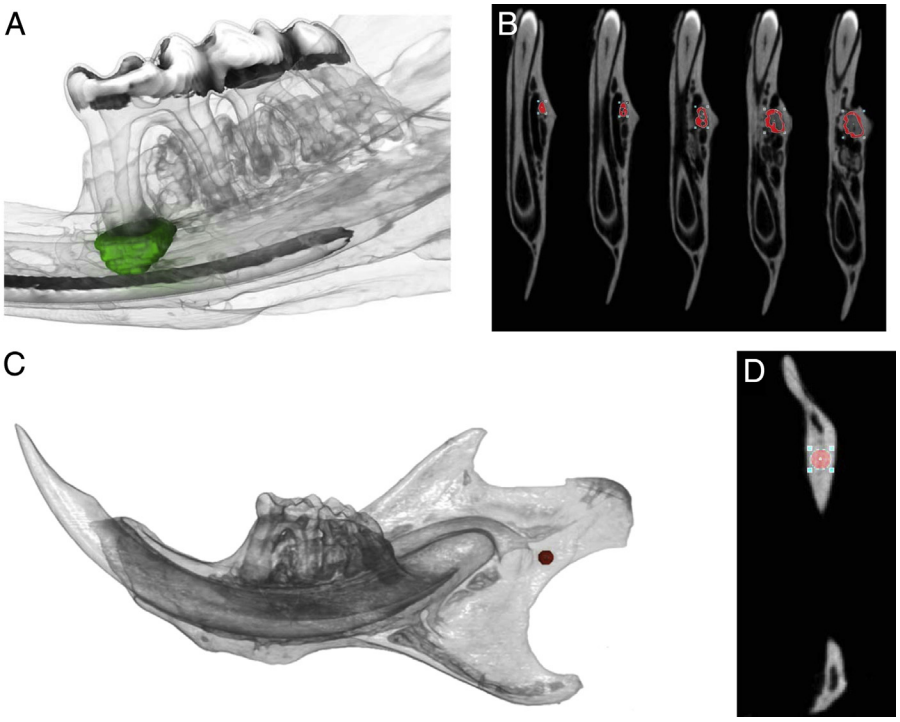

Figure 1. Images of a mandibular first molar with pulp exposure obtained by micro–computed tomographic imaging. (*A*) Three-dimensional rendered image. The *green* area represents a periapical volume of interest consistent with a periapical lesion. (*B*) Slices in the axial plane. The *red* areas represent examples of the region of interest used to calculate periapical lesion volume. Every fifth slice from the first slice starting at the bottom of the tooth socket is shown (slices #1, 6, 11, 16, and 20). (*C*) Three-dimensional rendered image. The *red* area represents a sphere in the mandibular ramus where bone density was measured. (*D*) A slice in the coronal plane. The *red* area represents an example of a region of interest used to calculate bone density.