

Technical Note

Establishment of Experimental Periapical Inflammatory Lesions in Mice

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Abstract: Although studies on the formation of apical periodontitis have somehow been carried out but detailed cellular dynamics remain unclear. We recently established an experiment that could easily be performed using ddY mice. First, under general anesthesia using isoflurane inhalation, the coronal portion of the maxillary first molar was penetrated using a round bur and drill with water irrigation causing pulp exposure until the root apex. Micro computed tomography (R_mCT) was taken over time during observation. Four weeks later, R_mCT confirmed the presence of a radiolucent image at the apex of the tooth, which was then removed for histological examination. The results showed that granulation tissue with fibrosis had gradually formed at the periphery of the abscess. The present method confirmed the effectiveness of the experimental mode to examine the formation of chronic inflammatory lesions at the root apex.

Key words: Inflammatory apical periodontitis, Experimental model, Mouse, Micro-CT, Histopathology

Introduction

Apical periodontitis is a disease characterized by tissue destruction at the root apex of tooth said to be a host immune response due to bacterial infection¹⁻⁴⁾. The involvement of cytokines produced during inflammation has been mentioned in studies involving bone lesions^{5,6)}. Various factors inducing inflammatory response, osteoclast activation, etc., in bone destruction have been reported. However, extensive cellular dynamics, growth, infiltration and the mechanism of formation are still unclear.

Therefore, we attempted to establish a convenient experiment in animal model using ddY mouse, which can be verified. The method also focused on the histopathological findings of chronic inflammatory apical lesion including the immunohistochemical findings.

Materials and Methods

Five 6-week-old ddY male mice were used in the experiment. The mice were acclimatized to the environment for 2 weeks before the start of the experiment. The mice were housed in a breeding

room with 12-hour cycle of day and night and controlled temperature throughout the study period. The animals were given adequate food and water and the pain induced to the experimental animals was considerably reduced.

Pre-operative R_mCT was taken in which the animals were placed under general anesthesia by isoflurane inhalation and then fixed on the plate. Tincture of iodine was used to disinfect the left maxillary first molar. Using one-half round bur (Maillefer, Ballaiques) and drill, the coronal portion of the pulp was exposed and allowed to penetrate until the apex. The opening was confirmed using a size 20 file. After pulp exposure, post-operative R_mCT was done to confirm the treatment. Then after, observation with R_mCT was done 2 and 4 weeks post-operatively to confirm the presence a radiolucent lesion at the root apex. When confirmed, the bone was excised as a whole. The specimens were fixed in 10% neutral buffered formalin solution, demineralized in 10% EDTA, dehydrated in increasing series of alcohol in a routine manner and embedded in paraffin. Sections of 4 mm were made from the paraffin blocks. Specimens were then subjected to hematoxylin-eosin staining and observed under a light microscope.

For immunohistochemistry, some samples were stained with CD31 antibody (Monoclonal mouse anti-Human CD31, clone JC70A; 1/50). Pre-treatment was carried out in autoclave at 121°C for 10 min and Dako staining Kit was used.

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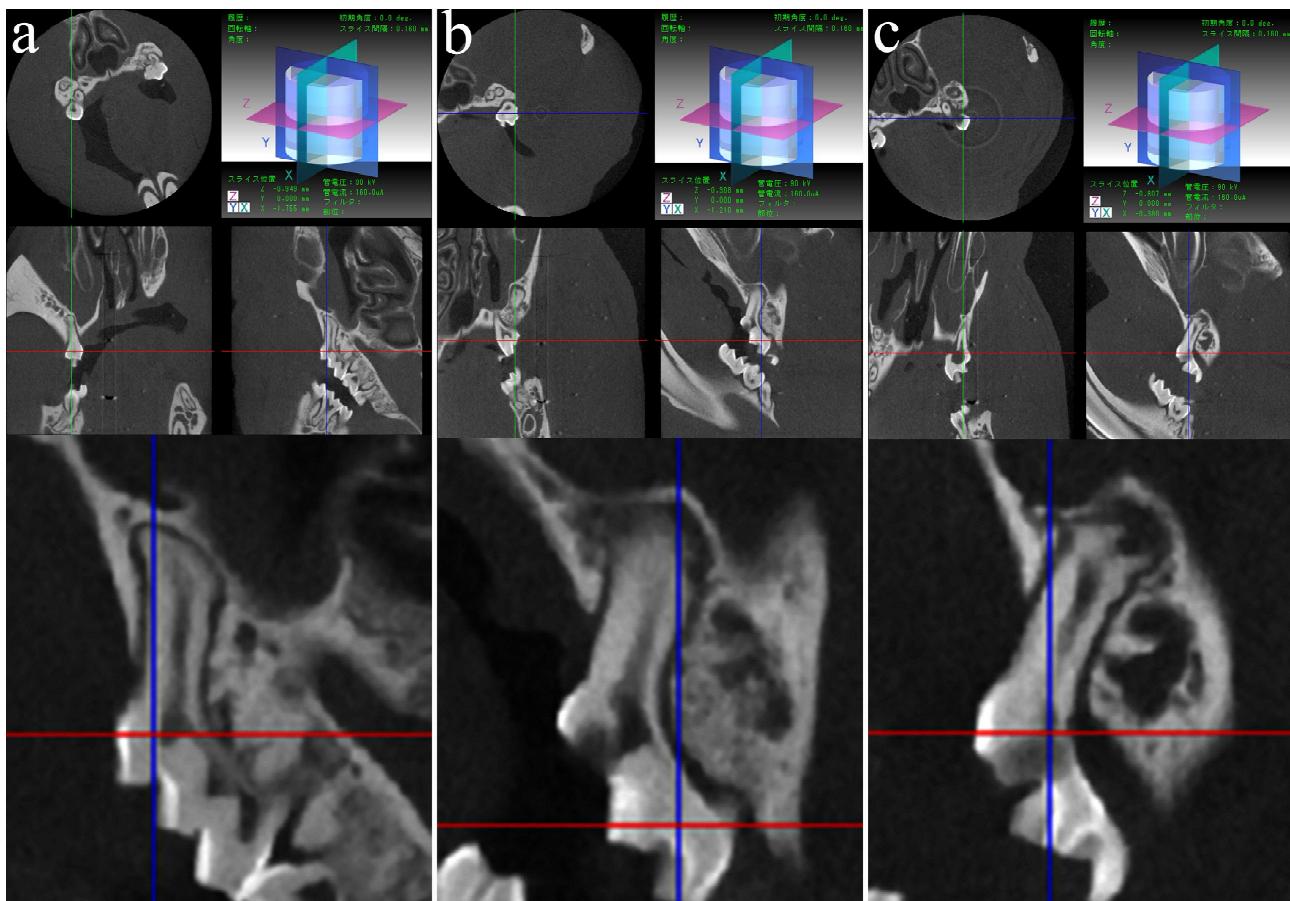


Figure 1. R_mCT image of each period (Enlarged image below). a: The presence of the radiolucent image reminiscent of inflammatory apical lesion was not observed. b: There is loss of tooth enamel crown and dentin underneath; the root canal is also irregular. c: Radiolucent image is formed continuously at the root apex, the apex protruded inside the lesion.

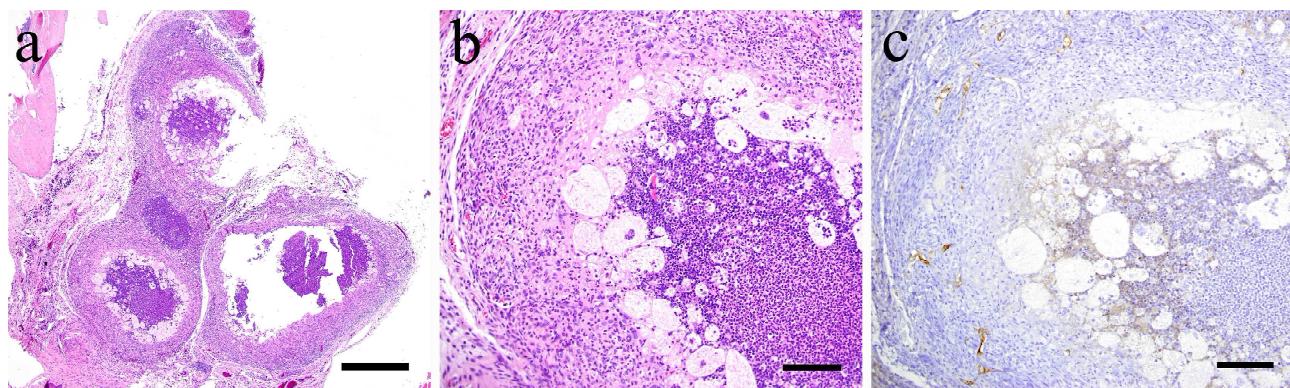


Figure 2. Histopathological photograph. a. Abscess formed within the granulation tissue (scale bar: 0.5 mm); b. A high power view showing many macrophages at the periphery of the abscess surrounded by granulation tissue (scale bar: 100 μ m); c. CD-31 positive cells are present at the periphery of the granulation tissue (scale bar: 100 μ m).

This study was approved by the Matsumoto Dental University Ethical Committee of Animal Experiment (Number # 202-12).

Results

R_mCT observation

Pre-operative R_mCT revealed normal pulp cavity of the maxillary first molar specifically the mesial root being the subject

of the experiment. The root canal of the tooth is normal having an approximately equal width of dentin on both sides of the root canal. The periodontal membrane is relatively wide but its width is uniform; apical radiolucency suggestive of inflammatory apical lesion was not observed (Fig. 1a).

After pulp exposure of the coronal portion and penetration until the root apex of the first molar, size 20 file was used to

confirm the patency of root canal in R-mCT image; the opening toward the center of pulp cavity could be clearly observed (Fig. 1b). Loss of dentin and its immediate enamel crown is evident. The width of the lumen of root canal lumen became irregular clearly indicating the effect of the instrument.

Two weeks later, R_mCT revealed a small radiolucency at the root apex, which increased in size after 4 weeks. The external part is opened from the central part of the crown slightly induced by caries. The radiolucent image appeared at the apex of the mesial root. The radiolucent image continued to grow in the apical portion. Inside the radiolucency, a portion of the root apex could be seen protruding. The radiolucency was relatively uniform from R_mCT image. In addition, radiopaque line at the border of the radiolucent image was detected (Fig. 1c).

Histopathological examination

Histopathological examination of the tissue removed at the apex of the maxillary first molar 4 weeks after surgery, revealed the presence of three abscesses surrounded by granulation tissue (Fig. 2a). At high magnification, the center is filled with neutrophils, lipids and pus. Furthermore, numerous macrophages in the same area where pus is growing slowly were noted. The abscess is surrounded by a young granulation tissue, which gradually became fibrotic. Macrophages and fibroblasts were also observed in the same area (Fig. 2b). The macrophages are large with light and granular cytoplasm hence, are also called foam cells.

Immunohistochemical staining with CD31 showed positive reaction in the center of the lesion although capillaries positive to CD31 were hardly seen. Cells positive to CD31 were confined in granulation tissue in the area of fibrosis relatively around the abscess (Fig. 2c).

Discussion

Periapical lesion is a disease caused by bacterial infection characterized by destruction of the apical periodontal tissues by osteoclasts coupled with expanding inflammation. Cytokines produced during inflammation are said to be involved in the pathogenesis and the role of local inflammation has been associated with bone resorption¹⁻⁴⁾. An inflammatory cytokine called Interleukin-1 is involved in tissue breakdown and expansion of inflammatory response. It enhances the production of collagenase and prostaglandins indirectly via cytokine production and it is also involved in inflammatory reaction and bone destruction. It has also been suggested that cytokines produced by the inflammatory tissue at the apex of the tooth induce osteoclasts to act on osteoblast lineage to inhibit bone formation^{5,6)}. We thought to verify this by establishing a simple animal experiment clarifying the pathogenesis of apical periodontitis.

Although other authors reported the induction of periapical lesion by allowing pulp exposure of rat molars^{7,8)}, studies on

laboratory animals using m-CT over time are very few⁵⁾. In conventional R_mCT, the x-ray tube and sensor are fixed therefore the image is difficult to aim since the tail and head would contact the tube while rotating in vivo, which is essential in sample preparation. However, Arai et al.⁹⁻¹²⁾ developed an R_mCT with a stage holding small animals such as laboratory mice stable. In this way the sensor and tube can be rotated thus, it is possible to obtain a three-dimensional image of 100 million pixels in a short recording time of 17 seconds. With a minimum resolution of 10x10x10 micron, it is possible to observe a sharp image of the entire head of an anesthetized rat from 100×100×100 microns and above. Hence, it is the best way to confirm the presence of inflammatory lesion at the apex of the tooth established in the present experiment. Again using R_mCT for follow-up treatment, pre- and immediate post-operative observation showed normal physiological conditions of the root apex. However, two weeks later, R_mCT revealed a slight radiolucent image at the root apex. The radiolucency continued to grow after 4 weeks and the root apex protruded into the radiolucent area. The radiolucent image is relatively uniform in shape. Since it is possible to observe the animals in vivo as well as the formation of inflammatory apical lesion, the combined use of R_mCT and this experiment system is very effective.

Kimberly et al.⁷⁾ showed in their study that exposure of the pulp in rat molars would lead to necrosis of the entire pulp and granulation tissue formation six days post-operatively and necrotic pulp tissue remains until 5 weeks which then proceeds to the formation of granulation tissue at the apex of the tooth.

In this study, it is clear that the cavity opened towards the pulp cavity of the crown and radiolucency emerged at the mesial root apex 4 weeks later using R_mCT. Furthermore, when the maxillary first molar was removed for histopathological examination, an abscess bounded mostly by immature and fibrotic granulation tissue was observed. The periapical lesion fabricated experimentally could be considered equivalent to the clinical chronic apical periodontitis.

Moreover, Tsujigawa et al.¹¹⁾ established an experiment on GFP bone marrow transplanted mice locating the cells that migrated to various tissues using mesenchymal cells in the bone marrow. The study also revealed that the mesenchymal stem cells differentiated into a variety of cell types in their local destination¹²⁻¹⁵⁾. Therefore, the present experiment system together with GFP bone marrow transplant mice established by Tsujigawa et al.¹⁰⁾ would contribute greatly to the understanding of the mechanism in the formation of chronic apical periodontitis.

The present method of establishing chronic apical inflammatory lesions is relatively simple using mouse. Further studies with chronic apical periodontitis using this experiment system should be considered in the future.

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