ORIGINAL RESEARCH

The transition from pulpitis to periapical periodontitis in dogs' teeth

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Keywords
dental pulp diseases, dogs, histology, periapical periodontitis.

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doi:10.1111/j.1747-4477.2008.00120.x

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Abstract

The purpose of the study was to histologically analyse transition from pulpitis to periapical periodontitis on dog's teeth. Pulps of mandibular premolars (37 roots) were exposed using a low-speed handpiece. Teeth were left open to the oral environment for 20, 35, 50 and 65 days. After the experimental period animals were sacrificed. Undemineralised teeth with surrounding bone, embedded in methylmetacrylate, were prepared for standard histological analysis. All teeth with pulpitis (five roots), regardless of the experimental period, had acute serose periapical periodontitis. All teeth (15 roots) with partial pulp necrosis had subacute periapical periodontitis. Teeth with complete pulp necrosis (19 roots) had chronic periapical periodontitis and in one case suppurative apical periodontitis. The condition of the pulp correlates with the histopathological findings of periapical tissue in the open types of pulp infection. Acute periapical periodontitis begins during pulpitis and can occur before 20 days of pulp exposure in the dog.

Introduction

It is commonly thought that apical periodontitis follows total pulp necrosis, although a growing number of researches suggest that acute serous apical periodontitis can occur even at the pulpitis stage. Such apical periodontitis is described as a condition limited to the area of periodontal ligament and is reversible in its character; for example, it spontaneously heals after pulpitis therapy (1).

There are a relatively small number of researches focusing on the consecutive events in pulp and periapical tissues, because such pathohistological analyses can be performed only on experimental animals.

When rats are used as experimental animals, the transition from pulpitis to apical periodontitis develops relatively fast. The development of ulcerative pulpitis was observed 2 to 3 days after pulp exposure. At that time, there was an increased cellular infiltration and blood vessel congestion at the periapex. Seven days after pulp exposure necrosis had spread into the root canal, while a more abundant inflammatory cell infiltrate was observed in the periapex coupled with the beginning of alveolar bone resorption (2–4).

Experiments regarding apical periodontitis on larger animals (including dogs) showed a slower rate of progression. These experimenters purposefully planned their experiments either in the way that they caused pulp necrosis and then observed changes in the periapex (5), or pulp extirpation preceded observation of changes in the periapex (6).

Jansson *et al.* described a periapical lesion development in monkeys. After a minimum of 1 month of pulp exposure, they observed only remnants of pulp tissue in the area of apical delta, while there were no inflammatory changes in the periapex. In those teeth where the pulp chamber had been sealed, the root canal was completely devoid of pulp tissue, while in the periapex they observed isolated active osteoclasts at the alveolar bone surface without any other sign of inflammation (7).

Table 1 Pulp diagnoses divided into experimental groups ($N = 37$)

	Diagnose		
Experimental group	Pulpitis (No. of roots)	Partial pulp necrosis (No. of roots)	Pulp necrosis (No. of roots)
1	–	6	2
2	3	6	6
3	2	3	1
4	–	–	8
Sum	5	15	17

Experiments planned in such a way did not give a detailed insight into the histolopathogical events during inflammation spreading from the root canal into the surrounding tissues.

The aim of our research was to establish an experimental model for the analysis of transitional stages from pulpitis to apical periodontitis in dogs' teeth with an infection caused by pulp exposure.

Materials and methods

The experiment protocol was approved by the Croatian Veterinary Chamber (approval No. 111/97).

Nine mongrel dogs aged from 1 to 4.5 years were divided into four experimental groups. Groups 1, 3 and 4 consisted of two animals, whereas group 2 consisted of three animals.

Histological analysis was performed on eight premolar roots in groups 1 and 4, on six roots in group 3, and on 16 roots in group 2. The mesial root of the mandibular first molars served as controls.

Animals were, 1 day before operation, premedicated with 0.2–0.5 mL of 0.5% atropine (Belupo, Koprivnica, Croatia). Animals were anaesthetized with Ketanest® (ketamine hydrochloride, 35 mg kg^{-1}; Goedecke/Parke-Davis, Berlin, Germany) and Rompun® (xylazine, 10 mg kg^{-1}; Bayer AG, Leverkusen, Germany). The pulps of the mandibular premolars were exposed using a low-speed handpiece. The teeth were left open and pulps exposed to the oral environment for 20 days (group 1), 35 days (group 2), 50 days (group 3) and 65 days (group 4) to induce pulpitis and apical periodontitis. The pulps of the mesial root of mandibular first molar (control) were not exposed to the oral environment. Animals were sacrificed at the end of respective experimental periods by intracardial injection of T61® (0.2 g embutramid, 0.05 g mebezonijjodid, 0.005 g tetracainclorid in aqueous solution; concentration 0.3 mg kg^{-1} of body mass) (Hoechst Veterinär GmbH, München, Germany).

Histological preparation and evaluation

The mandibles were removed and resected mechanically into blocks, each block containing premolars and first molar with the surrounding bone. Undemineralised blocks were embedded in methyl methacrylate and prepared for histological analysis (8). Since enamel complicates the preparation of undemineralised teeth for sectioning, it was removed from the experimental teeth *in situ* by diamond-coated burs mounted on a high-speed handpiece with water spray cooling.

Tissue blocks were sectioned longitudinally in a buccolingual direction through the central parts of the teeth. Sections 5–7 (m thick were stained with Toluidine blue (TB) and examined using a light microscope. Five sections of each tooth that included the region of the apical delta were used to evaluate histologically the pulp and periapex.

Results

Histological analysis of the pulp

Histological assessment showed various pictures from pulpitis to pulp necrosis in the experimental period from 20 to 50 days (Table 1). After 20 days of pulp exposure (group 1), six roots had partial pulp necrosis with abundant bleeding and neutrophil diapedesis into the connective tissue, while two roots had a total pulp necrosis. In group 2 (35 days of pulp exposure) three roots had pulpitis; one of those roots had distinctive changes in the apical third that included disruption of odontoblast layer, blood vessel dilatation and early neutrophil diapedesis (Fig. 1). Six roots had total pulp necrosis and six had partial pulp necrosis. In group 3 (50 days of pulp exposure) two roots had pulpitis with abundant serous exudate, three roots had partial pulp necrosis and one tooth had total pulp necrosis. In group 4 (65 days of exposure) all of the pulps (eight) had total pulp necrosis.

Histological analysis of the periapex

The correlation between the histological picture of pulp and periapical tissues was determined, regardless of the experimental period (Table 2).

All teeth diagnosed with pulpitis had acute serous apical periodontitis. Although the connective tissue at the

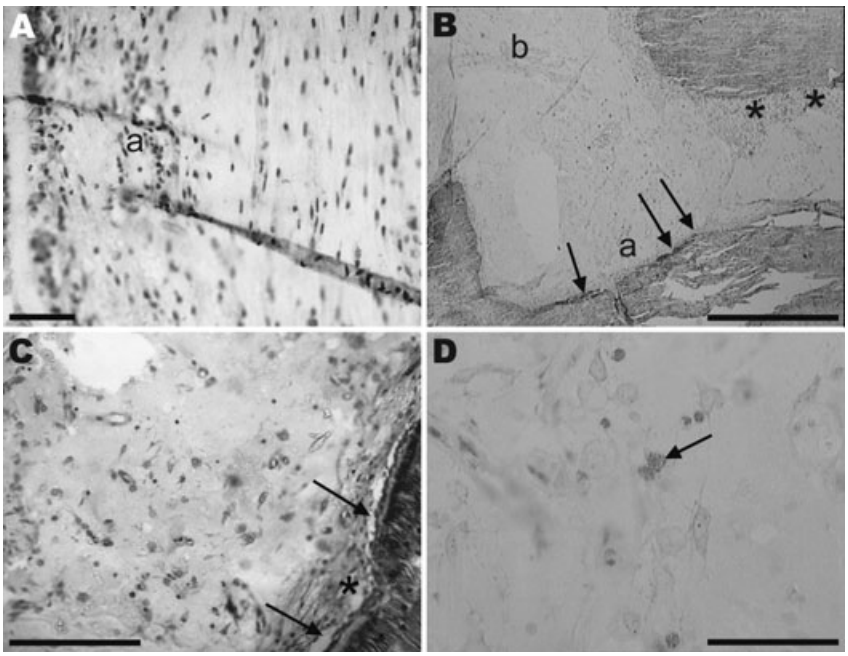

Figure 1 Initial stage of pulpitis. (A) Apical part of the pulp. Disrupted odontoblast layer and typical arrangement of pulp cells. Blood vessels are dilatated and surrounded by neutrophils that pass into the surrounding tissue by diapedesis (a). Toluidine blue, ×20. (B) Periapical region of the same tooth. Periodontal ligament at the root surface is preserved (a). Pronounced bone resorption and exudate with low cell count dominate the periapical lesion (b). Toluidine blue, ×4. (C) Region near the arrows in Figure 1B. Osteoclasts at the bone surface (a) are interposed between palisade-forming osteoblasts (b). Toluidine blue, ×40. (D) The exudate from Figure 1B. Mast cells prevail, whose membrane is stained methachromatically purple (arrow). Beside mast cells, neutrophils (white block arrow) and macrophages (black block arrow) are dominant. Toluidine blue, ×40 (scale bar in all pictures = 100 μm).

Table 2 Periapical diagnoses in relation to pulp diagnosis ($N = 37$)

Pulp diagnosis	No. of roots	Periapical diagnosis	No. of roots
Pulpitis	5	Acute serous apical periodontitis	5
Partial diagnosis	15	Subacute apical periodontitis	15
Necrosis	17	Chronic apical periodontitis	16

root surface was still preserved, the alveolar bone surface was resorbed while abundant serous exudate dominated the periapical lesion with few cells including mast cells, neutrophils and macrophages present (Fig. 1). All teeth with partial pulp necrosis had subacute apical periodontitis (Fig. 2). Those teeth had a relatively small quantity of exudate in the periapical lesion centre and we observed young granulomatous tissue proliferation typical of chronic inflammation.

Teeth with complete pulp necrosis had chronic apical periodontitis and developed periapical granulomas (Fig. 3. In one case (in group 3) a periapical abscess formed. Neutrophils, that dominated the inflammatory cell infiltrate, gathered mostly around apical delta openings, marking the main pathway where factors causing apical periodontitis exit the root into periapical tissue (Fig. 4).

Discussion

Our experiment confirmed that pulpal changes, caused by pulp exposure, correlate with histopathological changes in the periapex. Acute apical periodontitis with alveolar bone resorption begins as early as during pulpitis and probably develops before the 20th day of pulp exposure in dog's teeth.

The 65-day period of pulp exposure shows sequential stages from pulpitis to completely developed chronic apical periodontitis in dog's teeth. There are four distinct histological pictures in such pulp infection caused by exposure to the oral environment. Pulpitis is coupled with acute serous apical periodontitis. Partial pulp necrosis is accompanied with subacute apical periodontitis, while total necrosis has a chronic and in some cases acute

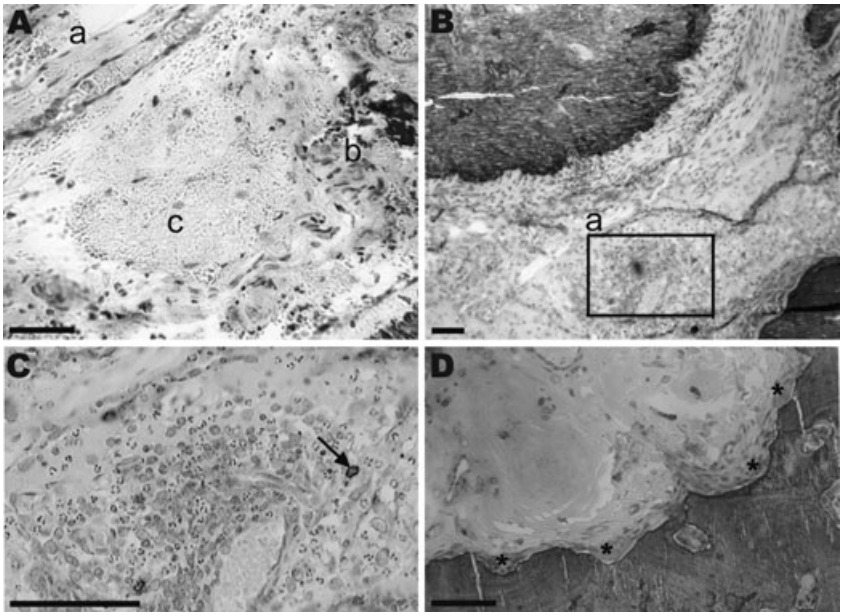

Figure 2 Partial pulp necrosis. (A) Pulp tissue. A complete disruption of typical arrangement of pulp cells. Blood vessels are dilatated and filled, beside erythrocytes, with leukocytes. Mostly neutrophils passed into the surrounding tissue by diapedesis and are found inside the pulp stroma (a). There is an abundant bleeding into the tissue (b). Toluidine blue, ×20. (B) Periapical region of the same tooth. The inflammation is of mixed exudative and proliferative type. Toulidine blue, ×10. (C) The lesion periphery and surrounding bone lacunae. Besides neutrophils and macrophages, mast cells are visible in even greater numbers than in group 1, as well as increased number of lymphocytes and plasma cells (a). Toluidine blue, ×40. (D) Bone surface at the lesion periphery. Numerous multinucleated osteoclasts are visible in the resorptive lacunae (a). Toluidine blue, ×20 (scale bar in all pictures = 100 μm).

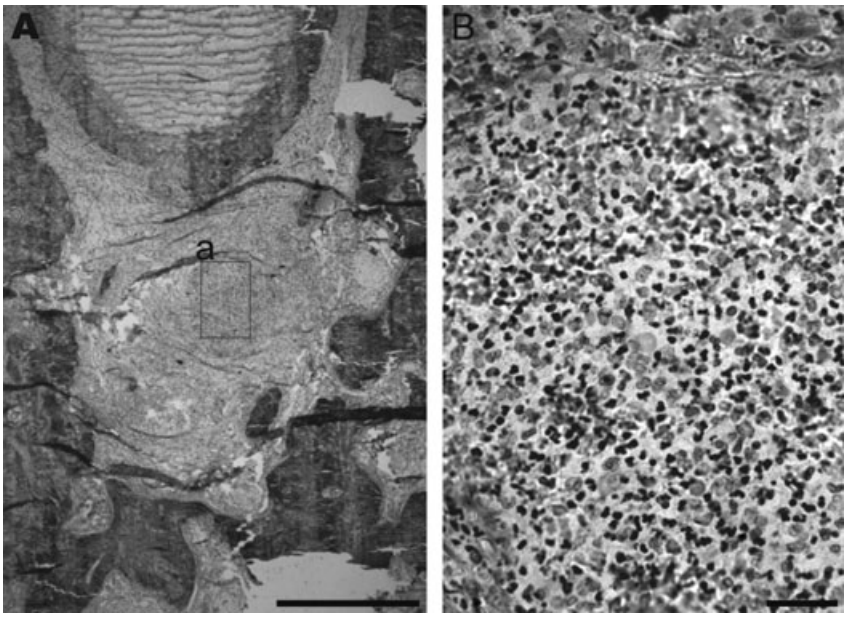

Figure 3 Chronic apical periodontitis. (A) Periapical region. Toluidine blue, ×4. (B) The periapical region as in (a), on higher magnification. Chronic inflammatory cells prevail: lymphocytes, macrophages and plasma cells. Toluidine blue, ×20 (scale bar in all pictures = 100 μm).

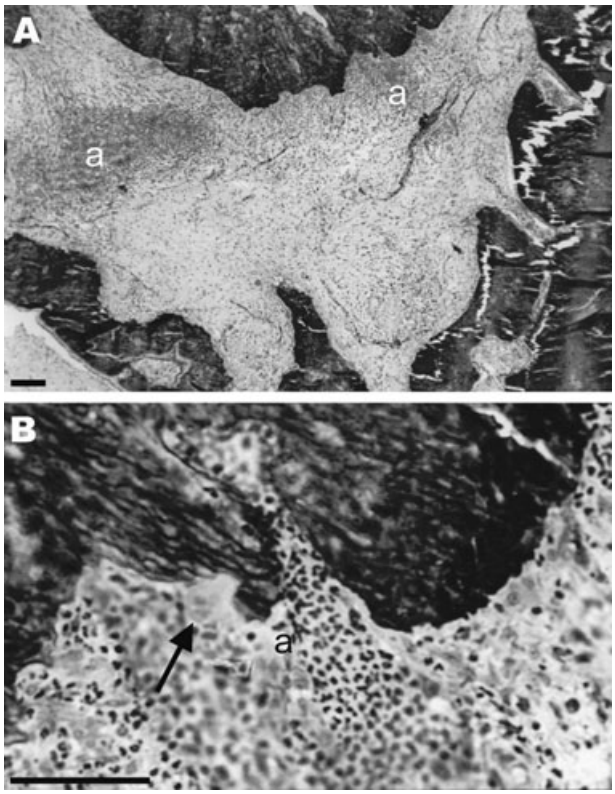

Figure 4 Suppurative apical periodontitis. (A) Periapical region. Numerous microabscesses (a) communicate with apical delta openings, but can also be seen at the lesion periphery close to the bone surface. Connective tissue rich with capillaries is found between those abscesses. Toluidine blue, ×20. (B) Root apex. Osteoclasts (cementoclasts) at the root cementum surface (black arrow). Toluidine blue, ×20 (scale bar in all pictures = 100 μm).

suppurative apical periodontitis as a consequence. In the first three experimental groups (20, 35 and 50 days of exposure), all of the histological pictures described were observed, while in the group where the experimental period was 65 days, all teeth were diagnosed with total pulp necrosis with chronic apical periodontitis.

In the first phase of apical periodontitis that is coupled with developed pulpitis, all of the morphological characteristics typical for an inflammation caused by mast cell degranulation were present: blood vessel dilatation, increased vascular permeability, neutrophil accumulation and also the gathering of plasma cell-derived defence mechanisms around pathogens. The same mechanism, Walsh described in chronic inflammatory disorders in the oral cavity (9). Until now, mast cells have been described in the literature as cells typical for chronic phases of apical periodontitis, most frequently found at the granuloma's periphery, close to its capsule (10). Beside exudation, alveolar bone resorption was present in that phase. We presume that it was as a result of macrophages, neutrophils, lymphocytes and mast cells mediated activity (11–13).

In those teeth where the root canals contained partially necrotic pulp tissue, subacute apical periodontitis was observed. Together with fibroblasts, fibrous elements and capillary proliferation, various inflammatory cells appeared in significant numbers, including lymphocytes and plasma cells. Neutrophils persisted in affected periapical tissues, particularly in central parts surrounded by granulomatous tissue. The appearance of granulomatous tissue is the main characteristic of this stage, although other cells like macrophages, polymorphonuclearleukocytes and mast-cells of acute inflammation are also still present. Bone resorption in this group was even more pronounced, although there were some osteoblast palisades interposed among active osteoclasts.

Although generally accepted classifications of apical periodontitis distinguish acute and chronic phase 1, our aim was to put an emphasis onto this transient phase, because the histological picture suggests the possibility that when there is partial necrosis of the pulp, in the periapex there evolves a sequence as a consequence of immunological reactions to the altered pulp tissue, similarly as in the first phase of pulpitis, but possibly also because of the influence of microorganisms. Because the presence of micro-organisms within periapical lesions is usually connected with total pulp necrosis (14), the probable explanation for this phenomenon is that this situation is mediated by their toxins or some of the cell wall components. This hypothesis should be tested using microbiological analyses of pulp and periapical tissues, as well as immunohistochemical analyses.

At the time of total pulp necrosis, the pathway for infection spread is wide open and typical forms of chronic apical periodontitis develop, accompanied with periapical granuloma formation and micro-abscesses in the granuloma's centre. Humoral and cellular immunity are activated, their main role being to constrain further inflammation, lesion circumscription and tissue repair (11,12). At the lesion's periphery plasma cells and lymphocytes were observed. Such a histological picture was typical for group 4, where pulp exposure lasted for 65 days, but it was also observed in those roots in groups 1 and 2 that had total pulp necrosis.

One of the roots in group 3 with total pulp necrosis had suppurative apical periodontitis. We assumed that in this case the pulp chamber was closed at a certain time point, probably blocked by food. This led to an anaerobic microflora prevalence (15). Another possibility is that such different pathophysiological processes occurred because of biodiversity.

The majority of studies involved with transition of pulpitis into apical periodontitis were performed on rats. Although in rat studies inflammatory changes develop rapidly so that ulcerative pulpitis is present after 2–3 days and total pulp necrosis after 7 days, studies by Tagger and Massler (2) and Yamasaki *et al.* (3) confirmed that apical periodontitis develops before the pulp is completely necrotic. However, in their studies, alveolar bone resorption began after total pulp necrosis (2,3). Histological diagnoses of the pulp and periapex are highly correlated, while the duration of the experiment does not correlate to the expression of pathological changes (2), both of which were confirmed in our experiment.

In this experiment, 16 out of 17 teeth diagnosed with total pulp necrosis were correlated with the diagnosis of chronic apical periodontitis in the periapical tissues. In Tagger and Massler's experiment (2), acute suppurative and chronic granulomatous apical periodontitis was present in similar numbers. Beside an explanation that micro-organism virulence and food impaction affect the type of periapical inflammation, it is also obvious that body's immune defensive forces, biodiversity and preservation of a drainage path play a key role in apical periodontitis development.

We assume that the reactivity of dog's periapical tissues, which is a bigger mammal, is more similar to that of humans, especially because unlike in humans (16), periapical lesions in rats decrease in size under occlusal load (17). Additionally, as a bigger mammal, dogs are more suitable experimental animal because the inflammation develops more slowly, unlike rats, where total necrosis is already present after 7 days in all teeth. Similar stages of inflammation can be found in dogs after 65 days. This enables a more detailed analysis of transitional phases from pulpitis to apical periodontitis. However, dogs are rarely used for such studies. In one of the few, Torneck and Tulananda (6) analysed changes in the periapex after pulp extirpation. Consequently, an analysis of transitional stages from pulpitis to apical periodontitis was impossible to analyse.

Besides dogs, there are descriptions of the histological picture of apical periodontitis development in monkeys. There was a great difference in both inflammation type and rate of inflammation progression between the teeth with closed pulp chambers and those with open chambers (7). In experiments on monkeys performed by Jansson *et al.*, results led to the conclusion that there are no distinctive transitional phases between pulpitis and apical periodontitis (7). While pulp tissue is still present inside the root canal, there were no changes in the periapex, both in teeth with open and closed pulp chambers. In the teeth with closed chambers during the first month of induction, there were just few active osteoclasts on the alveolar bone surface. After 2 months granulomas had developed, together with a very pronounced bone resorption (7). This result was interpreted that they either did not 'catch' the transitional phases, or the pathophysiology of apical periodontitis development differs in monkeys compared with rats and dogs.

Conclusion

Apical periodontitis with bone resorption in dogs starts during the phase of pulpitis and would have the influence on the plan of therapeutic procedures. This should be further analysed.

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