

Short Communication

Spontaneous Gingivitis Related to Hair Penetration in Rats

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Abstract: Maxillary gingivae from male and female Crl:CD(SD) rats at 12, 16, 21, and 34 weeks of age were examined histologically. The incidence of gingivitis was approximately 40%, with no age or sex predilection, and was most frequent between the first and second molar. Lesions were characterized by acute focal neutrophilic infiltration into the gingival mucosa, occasionally with inflammatory exudate. In severe cases, inflammation extended to the periodontal ligament with abscess formation, and adjacent alveolar bone destruction/resorption. The most characteristic finding was the presence of hair shafts associated with the lesion, which was observed in approximately 80% of the rats with gingivitis. These findings suggest that molar gingivitis occurs in rats from an early age and persists thereafter, and that the main cause of gingivitis in rats is hair penetration into the gingiva. It would be prudent to keep these background lesions in mind as potential modifiers in toxicity studies. (DOI: 10.1293/tox.25.229; J Toxicol Pathol 2012; 25: 229–232)

Key words: molar, gingivitis, hair shaft

The esophagus and stomach are routinely examined histologically, but the oral mucosa is most commonly not. Thus there is limited background information available about spontaneous lesions in the oral mucosa. Hair impaction in the gingiva and sulci of teeth with localized granulomatous inflammation and fibrosis around the affected teeth has been described in rats¹. However, epidemiologic information such as incidence and age distribution, to our knowledge, is not available. We examined maxillary the molar gingiva from rats of various age groups, and found a high incidence of acute gingival inflammation related to hair penetration, irrespective of age.

Maxillary gingivae from male and female Crl:CD(SD) rats at 12 (n=59), 16 (n=20), 21 (n=79) and 34 (n=97) weeks of age supplied by Charles River Laboratories Japan, Inc. (Shiga, Japan) for 3 different studies were examined in this study. The animals were housed individually in steel cages with an artificial lighting cycle of 12 hr (07:00 to 19:00), a temperature range of 20–26°C and a humidity range of 35 to 75%. Animals were fed approximately 21 g/day (in males) or 14 g/day (in females) of solid chow CRF-1 (Oriental Yeast

Co., Ltd., Tokyo, Japan) or Powdered CRF-1 chow (Oriental Yeast Co., Ltd., Tokyo, Japan) once a day. Sterilized well water was provided from an automatic water dispenser or bottle *ad libitum*. The study protocol was approved by the Laboratory Animal Care and Use Committee and was performed in compliance with Laboratory Animal Policy of Eisai Co., Ltd.

All animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia and then subjected to complete necropsy. The maxilla, consisting of alveolar bone, molars and gingival tissue, was collected from all animals and fixed in 10% neutral buffered formalin, decalcified with 5% formic acid in 5% formalin, trimmed longitudinally to include three molars and adjacent gingival tissues, embedded in paraffin, sectioned and stained with hematoxylin and eosin.

Severity, location and incidence of gingival inflammation were evaluated for each age group. Severity of gingival inflammation was graded as slight, moderate or severe, taking into account the size of the affected area and degree of extension into adjacent tissues. In “slight” gingival inflammation, inflammatory cell infiltration, primarily neutrophils and lymphocytes, was limited to the gingival epithelium or submucosal connective tissue. In “moderate” gingival inflammation, inflammatory cell infiltration involved deeper submucosal areas or multiple inflammatory lesions in one interdental area. In “severe” gingival inflammation, multiple foci/areas of inflammatory cell infiltration were observed with destruction of adjacent connective tissues. The

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location of gingival inflammation was divided into four interdental areas as (a) rostral to M1, (b) M1–M2, (c) M2–M3, and (d) caudal to M3 as shown in Fig. 1.

Inflammation in the molar gingiva was observed with various degrees of severity in all age groups. In general, inflammation was associated with hair shafts in the mucosal layer or periodontal area of molar teeth (Fig. 2A). In severe cases, gingival inflammation was often accompanied by caries with marked necrotic inflammatory cells and multiple hair shafts (Fig. 3). Abscess formation in a deeper part of connective tissue containing hair shafts and bacterial colonies were occasionally seen (Fig. 2B), and was associated with destruction/resorption of adjacent alveolar bone (Fig. 4). There were some cases in which hair penetrated verti-



Fig. 1. Longitudinal sections from the upper jaw including three molars. The interdental gingiva was divided into four areas (a–d), and each area was individually evaluated for inflammation. M1: first molar; M2: second molar; M3: third molar. HE stain. Bar=1 mm.

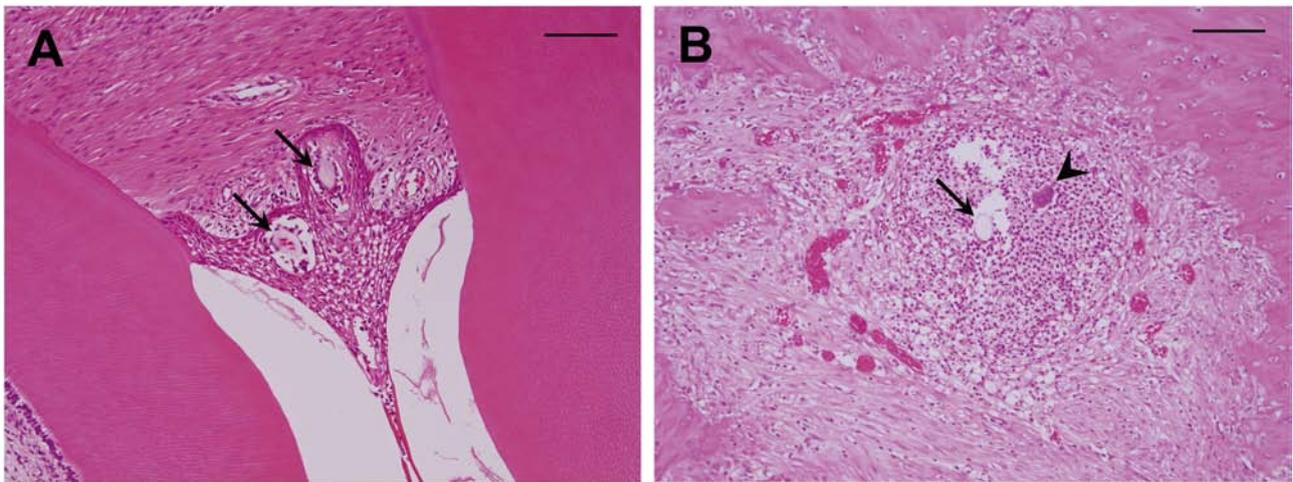


Fig. 2. A: Slight gingival inflammation between M1 and M2. Inflammatory cells consisted of neutrophils and lymphocytes focally infiltrating the gingival epithelium. Arrows indicate hair shafts penetrating the gingival mucosa. B: Slight inflammation between M1 and M2. Abscess formation, associated with a hair shaft (arrow) and bacterial colonies (arrowhead) in the deeper part of connective tissue surrounded by neovascularization. HE stain. Bar=100 μ m.

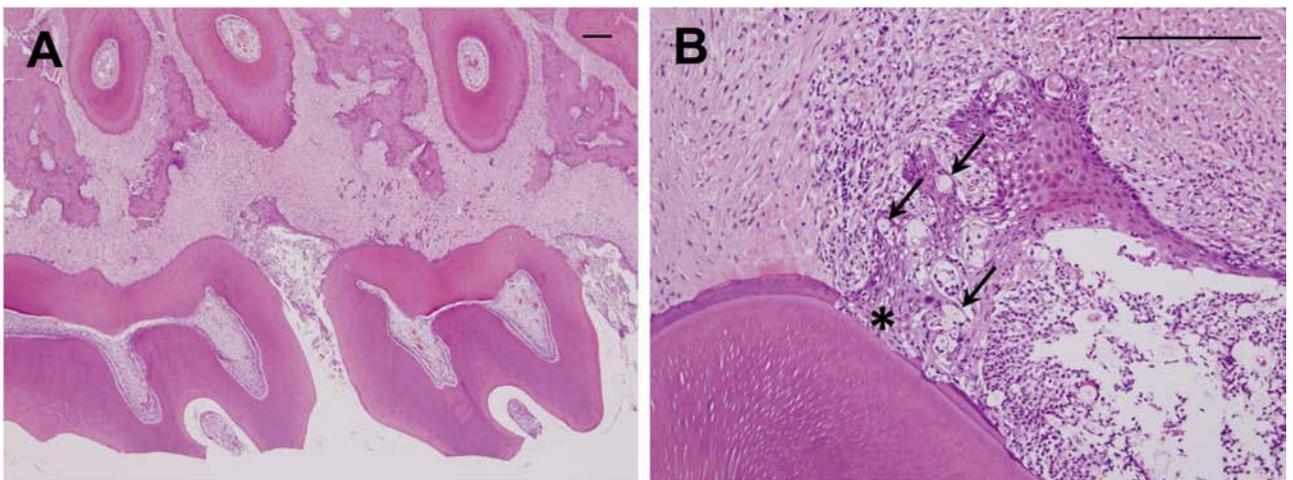


Fig. 3. A: Severe gingival inflammation between M1 and M2. The interdental gingiva is depressed with marked necrotic inflammatory cells and multiple hair shafts. A few hair shafts were surrounded by a reactive proliferated squamous epithelium. B: High magnification of A. The inflammatory changes are primarily seen in the upper mucosal connective tissue accompanied by a fibrous reaction. The molar cementum is partially destroyed by the inflammatory reaction (*). Arrows indicate penetrated hair shafts. HE stain. Bar=200 μ m.

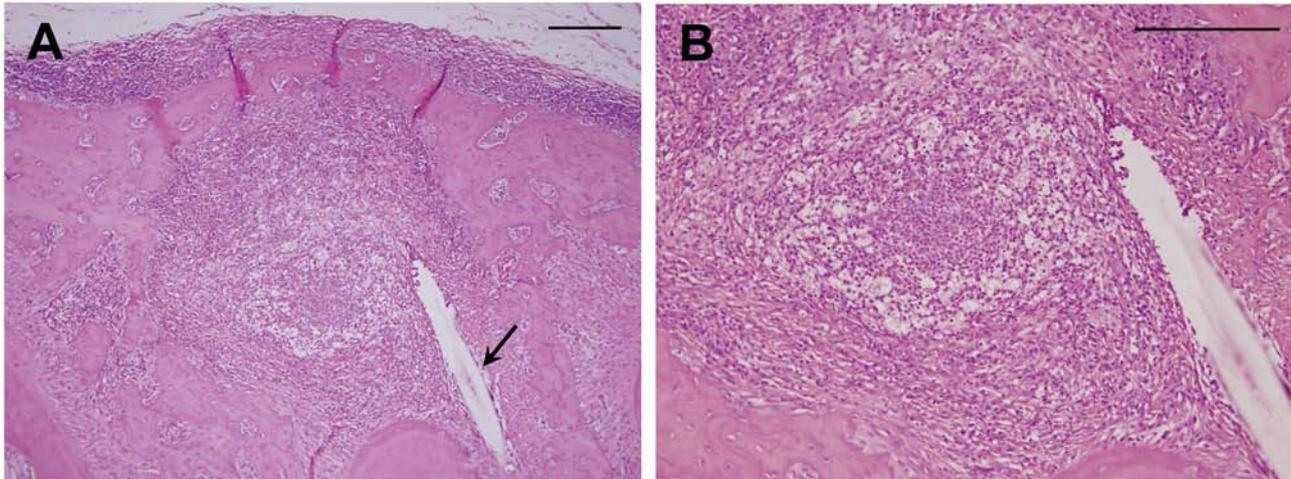


Fig. 4. A: Severe gingival inflammation between M2 and M3. The inflammatory reaction is located deep in the connective tissue of the upper jaw and affects adjacent alveolar bone. The arrow indicates a vertically penetrated hair shaft in the deep connective tissue. B: Higher magnification of A. Abscess adjacent to the penetrated hair shaft. HE stain. Bar=200 μ m.

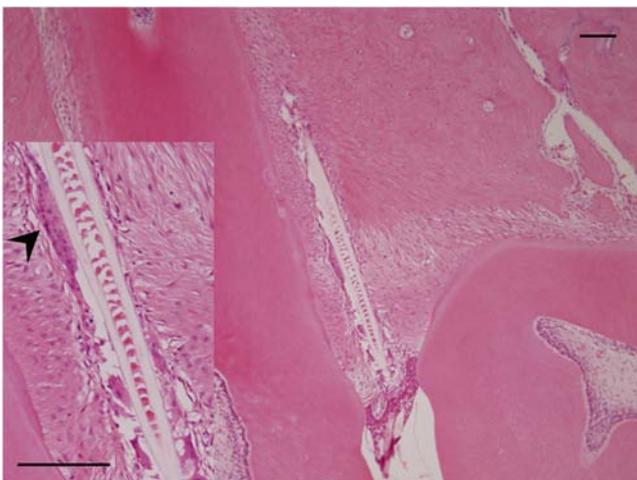


Fig. 5. Hair shaft vertically penetrated into the gingiva between M1 and M2 with little inflammatory reaction, but surrounded by foreign-body giant cells (arrowhead). HE stain. Bar=100 μ m.

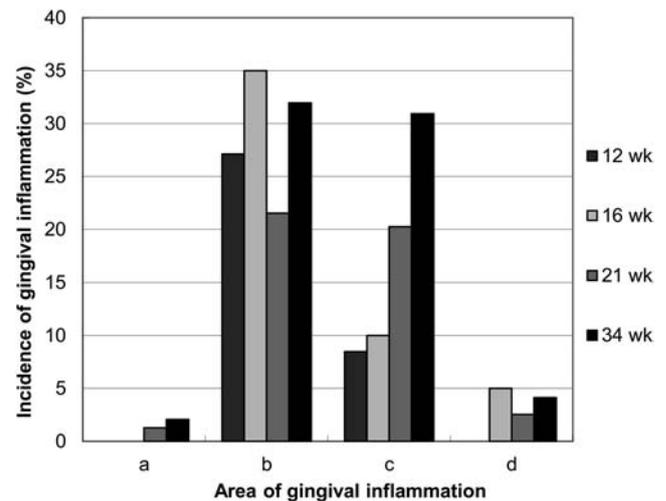


Fig. 6. Distribution of gingival inflammation in the upper jaw. Gingival area **b** (between M1 and M2) shows the highest incidence irrespective of age, followed by area **c** (between M2 and M3).

Table 1. Incidence of Gingival Inflammation in Each Age Group

Weeks of age	12 wk	16 wk	21 wk	34 wk
Number of animals	59	20	79	97
Incidence of gingival inflammation (%)	18 (30.5)	8 (40)	24 (30.4)	50 (51.6)
Slight (%)	13 (22.0)	7 (35)	20 (25.3)	43 (44.3)
Moderate (%)	2 (3.4)	1 (5)	3 (3.8)	5 (5.2)
Severe (%)	3 (5.1)	0 (0)	1 (1.3)	2 (2.1)
Hair penetration ratio in inflammation (%)	14 (77.8)	7 (87.5)	21 (87.5)	46 (92.0)

cally into the deep connective tissue of the gingiva without neutrophilic reaction but was surrounded by foreign-body giant cells (Fig. 5).

The incidence of gingival inflammation is shown in Table 1. The incidence of gingival inflammation was approximately 40% in all age groups, and approximately 80% of these lesions were associated with hair shafts. In each age group, most gingival inflammation was slight, and the incidence of moderate and severe inflammation was low. From these results, we conclude that the incidence and severity of gingival inflammation is similar between all age groups and types of chow (data not shown) and is not aggravated by aging.

The distribution of inflammation by area of the gingiva is shown in Fig. 6. The highest incidence of gingival inflammation occurred between the first and second molars in all age groups followed by the area between the second

and third molars. Inflammation rostral to the first molar and caudal to the third molar was uncommon, but was observed in the animals over 21 weeks of age.

The present investigation revealed that gingival inflammation is a common spontaneous background lesion in rats between 12 and 34 weeks of age. Gingival inflammation was frequently accompanied by hair penetration and was occasionally accompanied by abscess formation with bacterial colonies. It is thus reasonable to consider that oral bacterial flora with the penetrated hair aggravate the gingival inflammation to varying degrees. Lawson² reported the etiopathogenesis of mandibulofacial and maxillofacial abscess in mice and pointed out that hair fragment penetration into the oral submucosa through the gingival sulcus resulted in bacterial colonization of *staphylococcus aureus* and abscess development. Our present data further suggest this hypothesis is also true in the rat. Hair penetration into the molar gingiva during daily grooming and mastication is considered as an initial event for the induction of rodent gingival inflammation.

The incidence and severity of gingival inflammation were similar irrespective of age, and there were some cases of residual hair shafts with little inflammation; however, the hair shafts were surrounded by foreign-body giant cells, which is considered to be a repair process after an inflammatory reaction. These facts indicated that gingival inflammation caused by penetration of hair occurs repeatedly throughout life as the rat continues hair grooming and mastication, the triggering event for inflammation.

Ca-channel antagonists and Cyclosporine A are known to induce gingival overgrowth in laboratory animals^{3,4}. Lenz *et al.*⁵ reported a dose-related increase in oral squamous carcinoma in a 2-year rat carcinogenicity study with a novel calcium channel antagonist that was not a direct tumorigenic effect of the drug but was attributed to severe periodontal disease due to diet and the class-related gingival overgrowth. It is believed that drug-induced gingival overgrowth increased the opportunity for entrapment and penetration of foreign bodies (e.g., food particles, hairs), because the swollen gingiva was more susceptible to mechanical injury⁵. Based on our data, gingival inflammation, which is constantly and repeatedly occurring in rats, likely contributed to induction and exacerbation of the periodontal lesions.

It is well known that plasma cell hyperplasia is a common spontaneous finding in the rodent submandibular lymph nodes⁶, which drain the oral and nasolabial tissues⁷. Plasma cell hyperplasia is often seen in lymph node draining areas in chronic infection (e.g., abscess or necrotic tumors)⁸. Our

present data indicate that antigenic stimuli associated with gingival inflammation as well as inflammation in the nasal cavity⁹ likely contribute to the common spontaneous plasma cell hyperplasia in submandibular lymph nodes.

The present study showed that in rats gingival inflammation due to penetrated hair shafts occurs throughout life, occasionally resulting in secondary bacterial infection. In routine toxicity studies of myelosuppressive or immunosuppressive agents, systemic bacterial infection in the absence of a primary infectious lesion is sometimes observed (unpublished data or reference). It would be wise to keep in mind that the molar gingival tissues may be one of the main routes for secondary bacterial infection in rodents.

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