Assessment of Gingival Mucosa of Infant Rats during Teething

Evamiris Vasques de França Landim, Maria Goretti Freire de Carvalho, Rejane Andrade de Carvalho Ana Flávia Granville-Garcia, Rennaly de Freitas Lima, Edja Maria Melo de Brito Costa

ABSTRACT

Aim: The aim of the present study was to perform a histological analysis of the gingival mucosa in infant rats undergoing the teething process.

Materials and methods: Eighteen Wistar rats between 8 and 15 days of life were distributed among three groups: group A—without teething; group B—eruption of incisors; and group C—eruption of incisors and molars. The samples included teeth and periodontal tissue from the region of the incisors and molars of each animal. Fragments were processed for histological analysis and submitted to immunohistochemical analysis.

Results: In the 8-day-old rats, mild inflammatory infiltrate predominated with mononuclear cells in the pericoronal follicles of the incisors and molars. At 12 days of age, all animals exhibited moderate inflammation in the pericoronal follicles and epithelium of the incisors and mild inflammatory infiltrate with predominantly mononuclear cells in the molars. At 15 days of age, moderate neutrophilic exudate was found in the pericoronal follicles and epithelium of the incisors and molars. Immunohistochemical analysis revealed positivity for interleukin-1b in the pericoronal follicles in the pre-eruption phase.

Conclusion: An inflammatory reaction with progressive intensity occurs during the teething process, the response of which is preceded by the release of interleukin-1b.

Clinical significance: Morphological proof of events that occur during teething that can affect the dynamics of the physiologic process manifesting as clinical symptoms.

Keywords: Pediatric dentistry, Cell physiology, Pain, Animal research.

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INTRODUCTION

The eruption of the first primary teeth is an important moment in the life of infants. Teething requires care from those who deal directly with the health of these children, such as parents, dentists and pediatricians1,2 due to the possible effects on the health of the child. However, this association has been the object of debate in recent years3,4 and studies addressing this issue have involved different methodologies and populations, with insufficient samples for scientific validation and generalization to the larger population.1 A number of studies consider systemic signs merely coincident with period of teething.5,9

The different clinical phenomena that accompany this process include irritability, hypersalivation, diarrhea, gingivitis, reduced appetite, restless sleep, rashes, coughing, vomiting and fever.1,4,10-12 The fever response is considered an integral part of the acute inflammatory reaction and is produced by interleukin-1 (IL-1), which is an endogenous pyrogen that activates specialized receptors located in the hypothalamus.13 IL-1 is produced by different cells, including macrophages, monocytes, osteoclasts, A cells, neutrophils, fibroblasts and epithelial cells14,15 and is secreted in the extracellular fluid in two molecular forms, alpha and beta, both of which have proinflammatory effects. The main effects of IL-1 are to activate macrophages, stimulate the biosynthesis of prostaglandins by macrophages and fibroblasts, stimulate the synthesis of antibodies, be chemotactic for polymorphonuclear neutrophils, stimulate the synthesis of lymphokines by T lymphocytes, cause fever through the stimulus of hypothalamic cells and stimulate bone resorption.18

For the identification of biological data that may explain local and systemic repercussions during tooth eruption, the
aim of the present study was to assess the occurrence of inflammation and monoclonal anti-IL-1β labeling in the alveolar and gingival mucosa in infant rats undergoing the teething process.

MATERIALS AND METHODS

An experimental study was carried out with histological and immunohistochemical analyses of tooth and periodontal tissues in infant rats. This study received approval from the Ethics Committee of the Universidade Potiguar (Brazil) under process number 142/2006.

The sample was made up of 18 male and female Wistar rats (Rattus norvegicus albinus) between 8 and 15 days of life obtained from the animal lodging facilities of the university. For the histological and immunohistochemical analyses, the left upper incisors and molars were considered separately, totaling 36 specimens.

A pilot study was first conducted to determine the tooth eruption phases in newborn rats. The results determined that the upper incisors erupt on the 12th day of life and the upper molars erupt between the 15th and 16th days of life. Based on these findings, the animals in the main study were divided into three groups according to age and tooth eruption: group A (8 days)—without teething (n = 6); group B (12 days)—eruption of the upper incisors (n = 6); and group C (15 days)—eruption of upper incisors and molars (n = 6).

At the end of each period, the rats were sacrificed in a carbon gas chamber and decapitated. The maxillae were dissected and fixed in 10% formalin for at least 24 hours. The de-mineralization process was then performed in 5.5% nitric acid for 24 to 36 hours. The de-mineralized material was sliced, with sagittal cuts in the region of the upper left incisors and coronal cuts in the region of the upper left molars.

For the histological analysis, the specimens were processed, cut to a thickness of 4 μm and stained with hematoxylin and eosin. For the immunohistochemical analysis, histological cuts measuring 3 μm in thickness were performed. The slices were placed on slides with organosilane and labeled with monoclonal anti-IL-1β antibody. Tissue phenomena were analyzed under a light microscope coupled to a digital camera (Olympus CX31, Olympus, Tokyo, Japan). The microscopic analysis involved (1) the presence/absence of inflammatory cells; (2) type of inflammatory cells (neutrophils or mononuclear cells); and (3) intensity of the inflammatory infiltrate. The latter variable was analyzed using the following scores: absent (–); mild (+, presence of 6 to 8 inflammatory cells); moderate (++, presence of 9 to 20 inflammatory cells); and intense (+++, presence of more than 20 inflammatory cells).

The Kruskal-Wallis test was used to determine the significance of the variables. Dunn’s test was used to detect variables significant differences. The level of significance was set to 5% (p < 0.05).

RESULTS

Thirty-six specimens of gingival and periodontal tissue from the region of the upper left incisor and molar of infant rats were examined. Tables 1 and 2 display the results of the type of inflammatory infiltrate and its distribution in the pericoronal follicles and epithelium in the incisor and molar regions.

In group A (8 days of life), mild to moderate neutrophil infiltration was found in the pericoronal follicles and epithelium of the incisor region and mild mononuclear infiltrate was found in the pericoronal follicles and molar regions. In group B (12 days of life), moderate infiltration of neutrophils and mononuclear cells was found in the pericoronal follicles and epithelium of the incisor region. In group C (15 days of life), neutrophil infiltration ranged from moderate to intense in the pericoronal follicles and epithelium of the incisor and molar regions (Fig. 1).

Statistically significant differences were found between the three groups (Kruskal-Wallis test, p < 0.05). Statistically significant differences were found between groups A and C regarding neutrophil infiltrate in the epithelium of the molar region as well as in the pericoronal follicles

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<th>Table 1: Distribution of scores related to neutrophil infiltration according to group, region (incisor or molar) and tissue layer (pericoronal follicles and epithelium)</th>
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Incisors (I), Molars (M), Absent (–), Mild (+), Moderate (++), Intense (+++)
well as between groups A and C. Statistically significant differences were found regarding mononuclear infiltrate in the pericoronal follicles of the molar region between groups A and C as well as between groups B and C (Dunn’s test, p < 0.05). No significant differences between groups were found regarding mononuclear infiltrate in the epithelium of the molar region (Dunn’s test, p = 0.11).

In the immunohistochemical analysis, positive labeling for the IL-1ß antibody was found in the interstitial matrix of the pericoronal follicles, with subepithelial and periodontal localization in the region of nonerupted incisors and molars. Brown coloration denoted positive labeling (Fig. 2). Immunolabeling was negative in erupted teeth after breaking through the mucosa.

**DISCUSSION**

The tooth eruption traditionally has been the explanation for a variety of symptoms and signs in the early childhood. The information about that topic usually is subjective, since the result of parental reports and opinions. Today it is still not clear if tooth eruption leads to local and systemic disturbances or simply or would be haphazard events, once coincidentally; primary tooth eruption begins when infants lose maternal antibody protection against bacteria and viruses, making the baby more vulnerable to infectious processes.

The tooth germs are localizes in the dental sac or follicle, connectives tissues that limit the bone crypt. Its movement is tightly regulated by eruption molecules secreted by the dental follicle and many histological changes are observed along the eruption pathway. The present study analyzed the tissue reactions that occur during the eruption of primary teeth, including the type and intensity of the inflammatory response and the presence of immunolabeling for IL-1ß in the gingival mucosa of infant rats.

Gingival inflammation has been considered one of the clinical manifestations most commonly associated with teething. When the crown emerges into the oral cavity
the vessels exhibit signals of increase vascular permeability, suggesting the presence of inflammation in the gingival. The histological findings of the specimens analyzed demonstrated inflammatory reaction of increasing intensity in the periodontal tissue until tooth eruption. Inflammatory cells have been found in the conjunctive tissue adjacent to the teeth, with neutrophils disseminated among the epithelial cells. Similar findings are reported in the present study, in which an inflammatory response was found in the periodontal tissues of recently erupted teeth, with neutrophil infiltrate in the epithelium.

The histological exam of the periodontal tissues of infant rats demonstrated a mild mononuclear inflammatory reaction in all animals in the pre-eruption phase, coinciding with positive immunolabeling for IL-1β. This finding suggests the possibility that these mononuclear cells in the pericoronal follicles of the mucosa and gingival tissue release IL-1β, which, in turn, activates other cells that release proinflammatory substances, thereby facilitating the migration of the tooth until its final eruption into the oral cavity. Another histological finding that supports this affirmation is the disappearance of this interleukin from the tissues after the tooth breaks through the mucosa. Other authors also believe that this cytokine is related to the pre-eruption phase. IL-1β is considered more pyrogenic cytokines and febrile response related to the newborn, regardless of bacterial infection. Some authors reported correlations between IL-1β levels and some of the clinical symptoms of teething, like fever, sleep disturbances, gastrointestinal disturbances and appetite disturbances. This interleukin is associated with the synthesis of prostaglandins, which are potent triggering factors of pain and systemic inflammatory phenomena. In the present study, IL-1β immunolabeling was detected in the pericoronal follicles of the incisor and molar regions in the pre-eruption phase, demonstrating the presence of an acute-phase proinflammatory mediator. The presence of this interleukin in the pre-eruption phase underscores the fact that clinical signs and symptoms are more intense immediately prior to tooth eruption.

In the period corresponding to the tooth breaking through the mucosa, a mononuclear and neutrophil inflammatory reaction was observed, with a predominance of neutrophils in the mucosal epithelium, especially in the junctional epithelium. This neutrophil exocytosis may stem from the release of pro-inflammatory substances and/or the attractive-chemotactic action of possible local microbiota.

The contribution of this research relates to the possibility to elucidate biochemical events occurring in the physiological process of tooth eruption, which are likely to be externalized through clinical symptoms, stressing the mothers and interfering in behavior of infants.

**CONCLUSION**

Inflammation occurs during teething, the intensity of which progressively increases until the tooth breaks through the mucosa.

Primary tooth eruption is preceded by the release of IL-1β in the chorion.

After the eruption of the tooth, the inflammatory response is predominantly neutrophilic.

**CLINICAL SIGNIFICANCES**

The contribution of the present study resides in the morphological proof of events that occur during teething that can affect the dynamics of this physiologic process. Such events may be manifested as clinical symptoms, which are a source of stress for mothers and affect the behavior of infants. Further studies should be carried out with the aim of identifying other chemical substances involved in this process and clear up the controversy regarding this issue.

**REFERENCES**

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