Effects of aging on the evolution of Apical Periodontitis lesions in rats

Efeitos do envelhecimento na evolução das lesões de Periodontite Apical em ratos

Efectos del envejecimiento sobre la evolución de las lesiones de Periodontitis Apical en ratas

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Abstract
This study aimed to evaluate the effects of aging on the evolution of Apical Periodontitis (AP) lesions in rats. Elderly males (study group, n = 12) and young males (control group, n = 12) Wistar rats were used. When the study group were 24 months old and the control group were 3 months old, AP lesions were induced by creating pulp exposure of the mandibular left first molars. After 21 and 40 days, the animals were euthanized and the mandibles were removed. The left hemi-mandibles were radiographed and the AP lesions of the mesial roots of the first molars were measured using ImageJ software (National Institute of Mental Health, Bethesda, USA). The AP lesions were significantly larger in the Elderly (E) group (p = 0.0006) compared to the Control (C) group (E 21: 218.8 ± 72.96, C 21: 94.77 ± 9.44, E 40: 237.8 ± 57.20, C 40: 85.23 ± 6.63). The lesions of the animals in the elderly groups were significantly larger than the lesions in the younger animals. These findings suggest that the bone and immunological changes caused by aging can influence the progression of AP lesions.

Keywords: Apical periodontitis; Elderly; Mandible.

Resumo
Este estudo teve como objetivo avaliar os efeitos do envelhecimento na evolução das lesões de Periodontite Apical (PA) em ratos. Foram utilizados ratos Wistar machos idosos (grupo estudo, n = 12) e jovens (grupo controle, n = 12). Quando o grupo de estudo tinha 24 meses e o grupo controle tinha 3 meses, as lesões PA foram induzidas através da exposição pulpar dos primeiros molares inferiores esquerdos. Após 21 e 40 dias, os animais foram eutanasiados e as
mandíbulas removidas. As hemimandíbulas esquerdas foram radiografadas e as lesões PA das raízes mesiais dos primeiros molares foram medidas com o software ImageJ (National Institute of Mental Health, Bethesda, EUA). As lesões PA foram significativamente maiores no grupo Idoso (E) (p = 0,0006) em comparação ao grupo Controle (C) (I 21: 218,8 ± 72,96, C 21: 94,77 ± 9,44, I 40: 237,8 ± 57,20, C 40: 85,23 ± 6,63). As lesões dos animais dos grupos idosos foram significativamente maiores do que as lesões dos animais mais jovens. Esses achados sugerem que as alterações ósseas e imunológicas causadas pelo envelhecimento podem influenciar na progressão das lesões de PA.

Palavras-chave: Periodontite apical; Idoso; Mandíbula.

1. Introduction

Apical Periodontitis (AP) is an inflammatory disease that affects tissues that surround the dental root (Tibúrcio-Machado et al., 2021). Microorganisms that infect the root channel, especially bacteria, are the main cause involved in this pathogenesis (Karamifar et al., 2020). The host mechanism is involved in the development of AP through its immune system, which in an attempt to contain the infection at the root canal level, releases chemical mediators that stimulate bone resorption and help form the AP lesion (Neto et al., 2018).

Different hosts present dissimilar responses to periradicular infections and to the same type of endodontic treatment; furthermore, the diverse conditions of individuals may influence the susceptibility of the disease (Segura-Egea et al. 2005). These individualistic variations may be referred to as disease modifiers, which are not the cause of AP but can influence the development, diagnosis, and severity of the disease as well as affect the response to treatment (Segura-Egea et al., 2015).

Physiological aging is a natural, progressive, and irreversible phenomenon and its effects on the body are a series of changes in organic functions. At more advanced ages, the body loses its ability to maintain a homeostatic equilibrium and all physiological functions gradually start to decline (Allman & Miller, 2005; Shaw et al., 2013). Furthermore, the elderly undergo changes in their bone metabolism and immune system, which lead to increased bone resorption, reduced repair capabilities, and at the same time an inflammatory state is installed that is characterized by an increase in plasma levels of proinflammatory cytokines (Duque & Troen, 2008).

Changes in bone activity of older individuals probability alter the quantity and density of the mandibular bone, and therefore can even interfere in the pathophysiology of Apical Periodontitis (AP) lesions. This study aimed to evaluate the effects of aging on the evolution of AP in rats.

2. Methodology

An experimental study was carried out to evaluate the relationship between aging and AP.
Ethical Aspects

The project is in accordance with The Ethical Principles for Animal Experimentation according to the College of Animal Experimentation (COBEA), and the study obtained approval of the Ethics and Animal Research Committee (CEPA) of the Fluminense Federal University under protocol No. 594.

Sample Selection

Elderly males (study group, n = 12) and young males (control group, n = 12) Wistar rats were kept in cages under a controlled ambient temperature (25 to 27°C), constant humidity and a 12 hour light/dark cycle (6:00 a.m. to 18:00p.m.). Water and feed (Nuvarab, Sogorb Industry and Comércio Ltda, SP, Brazil) were provided ad libitum.

Apical periodontitis induction

When the study group were 24 months and the control group 3 months, all were anesthetized with thiopental (0.1 ml / 100g of body weight). A ½ spherical carbide bur (KG Sorensen, SP, Brazil) activated by a low-speed motor (Dentec, CS 421, RJ, Brazil) was used to expose the pulp of the left mandibular first molars. A single operator performed these procedures in a standardized manner following the previously published protocol (Brasil et al., 2017; Brasil et al., 2021).

Experimental groups

There were 2 experimental periods, which were: 21 and 40 days after pulp exposure. Thus, 4 experimental groups were formed: C21 - Control with 21-day lesions, n = 6; E21 - Elderly with 21-day lesions, n = 6; C40 - Control with 40-day lesions, n = 6, and E40 - Elderly with 40-day lesions, n = 6. At the end of each experimental period, the animals were euthanized by exsanguinated under anesthesia with thiopental (0.2 mL/100g body weight), and their mandibles were removed.

Radiographic procedures

The left hemi-mandibles were washed and immersed in saline. After this process, radiographs were taken with a digital radiograph (Model Radioefera, Siemens, SP, Brazil) in a standardized position on a radiographic film, to avoid distortions. The areas of AP lesion at the mesial root apexes of the first molars were measured and quantified in pixels by means of the ImageJ software (National Institute of Mental Health Bethesda, USA). Image analysis was performed separately by two calibrated evaluators who were blinded to the groups analysed.

Statistical analysis

The comparative analysis of the data was performed using the Graphpad Prism 6 for Windows (Graphpad Software, San Diego, CA, USA) (www.graphpad.com). The Kruskal-Wallis test was applied and the statistical significance taken into consideration was p <0.05.

3. Results

The size of the AP lesions (pixel) on the radiographs (C21: 94.77 ± 9.44 and E21: 218.8 ± 72.96; C40: 85.23 ± 6.63, and E40: 237.8 ± 57.20) clearly showed that the E group had significantly larger lesions (p = 0.0006) for both experimental periods as can be seen in Figure 1.
Figure 1. Comparative analysis of the apical periodontitis lesion size (pixel). C: Control group, E: Elderly. Values are expressed as mean ± standard deviation. p value refers to the Kruskal-Wallis test.

![Graph showing the comparison of lesion sizes between groups](image)

Source: Authors.

Figure 2 shows examples of the images obtained using the digital radiograph. Arrows indicate the AP lesions in the mesial roots of the left mandibular first molars. Its possible to observe larger lesions in E group for both experimental periods.

Figure 2. Radiographs of rat mandible. A: C21 days; B: E21 days; C: C40 days; D: E40 days. C: Control group, E: Elderly group.

![Radiographs of rat mandible](image)

Source: Authors.
4. Discussion

Aging is characterized by changes in various physiological processes of the body (Allman & Miller, 2005), such as changes in bone metabolism, including loss of bone mass and microstructural deterioration of bone tissue, which are both common characteristics (Zeng et al., 2014). In addition, aging also has a significant effect on the immune system (Cunha et al., 2020), such as the impairment of neutrophil and macrophage functions (Kovacs et al., 2014). These changes possibly have an influence on the development of AP lesions.

Several studies have used animal models to evaluate changes in age-related bone metabolism (Raisz & Rodan, 2003; Lin & Lane, 2006; Pietschmann et al., 2007; Duque & Troen, 2008; Zeng et al., 2014). In the present study, the radiographic parameters of the bone pattern were investigated in elderly male Wistar rats (24 months old) that were the study group. The control group consisted of 3-month-old male Wistar rats.

The development of the AP lesions and the choice of 21 and 40 day periods to evaluate the progression of the lesions followed the protocol used by Brasil et al. (2017) and Brasil et al. (2021). Periradicular bone resorption, induced by pulp exposure, was observed radiographically in the experimental groups.

Other studies that have used elderly animal models selected rats that were 22 to 24 months old (Chelvarajan et al., 2005; Pietschmann et al., 2007; Singh et al., 2008). In the present study, only 24-month-old animals were selected, which is based on the study by Andreollo et al. (2012), who affirms that 24-month-old Wistar rats are equivalent to 60-year-old humans. According to the World Health Organization (2015), a 60-year-old individual is considered elderly.

The AP lesions were significantly larger in the elderly rats compared to the younger animals. This result probably occurred due to deregulated inflammation, which is characteristic of elderly individuals (Franceschi et al., 2000; Shaw et al., 2013). The immune system undergoes changes with age and is characterized by persistent inflammatory responses involving various types of immune and nonimmune cells (Cunha et al., 2020). In general, studies in elderly rats (more than 24 months old) (Chelvarajan et al., 2005; Singh et al., 2008) and in humans over 65 years old (Franceschi et al., 2000; Cohen et al., 2003) show that activation of the aged innate immune system results in deregulated inflammation. Evidence indicates that older individuals have high levels of proinflammatory cytokines, and the term inflammaging is used to describe this phenomenon. Teixeira et al. (2022) observed higher expression of IL-1β, IL-6 and TNF-α in the AP lesions of the elderly than in the adult control.

The increased production of cytokines, such as TNF-α, which inhibits the differentiation of osteoblasts and stimulates the production of RANKL, is believed to contribute to reduced bone formation and senile osteoporosis in elderly animals (Pietschmann et al., 2007).

According to Kovacs et al. (2009), the impairment of multiple neutrophil functions, as well as macrophage functions, such as decreases in their phagocytic and chemotactic capacities are observed in the elderly. Our findings may also be related to decreases in the capacity of the Elderly Immune System to combat aggressor agents. Almeida et al. (2017) observed lower expression of macrophages in the AP lesions of the elderly, suggesting that infections in these individuals are more serious and that they may be more susceptible to failure in endodontic treatment.

In the present study, larger lesions were observed in the elderly rats. This finding corroborates with the results found by Cao et al. (2003), that showed the increased RANKL expression and a reduced expression of osteoprotegerin in the elderly. In addition, they also observed greater concentrations of osteoclastic precursors in the bone marrow of elderly animals. These changes can contribute to the gradual loss of bone that occurs in elderly. In addition, elderly patients are more susceptible to systemic diseases and these diseases may be related to increased bone resorption, as already identified by Segura-Egea et al. (2016).
5. Conclusion

As the lesions of the animals in the elderly groups were significantly larger than the lesions in the younger animals, the conclusion was that most probably the bone and immunological changes caused by aging played a role in the development of these lesions. These results suggest that aging may have acted as a disease modifier. However, despite the fact that aging is not the main cause for the development of AP lesions, it probably influences their development. More studies, especially clinical trials, should be carried out to elucidate such issues.

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References


