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Research Article

A quantitative radiological and histopathological study of periapical inflammatory lesions associated with experimentally induced diabetes and osteoporosis

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Abstract. The increased prevalence of periapical inflammatory lesions (PL) is a common problem especially in patients with metabolic disorders such as diabetes or osteoporosis. Objectives: To investigate in a quantitative manner periapical tissue changes through digital radiographs and histopathology at different times in an animal model. Material and methods: Periapical lesions were experimentally created in the first mandibular molars of 63 Wistar rats, which were divided into 3 experimental groups: group I: healthy animals with experimentally induced PL, group II: animals with experimentally induced type 2 diabetes mellitus and PL, group III: female animals with osteoporosis induced following bilateral ovariectomy and experimental PL, receiving zoledronic acid (ZOA). The periapical status of the first mandibular molars was quantitatively assessed radiologically and histopathologically. The progress of PL was examined by X-ray and light microscopy, after an adequate preparation of the tissues at three different time points: T1 - 14 days, T2 - 30 days and T3 - 60 days in each experimental group. At each time point analyzed, 7 animals of each experimental group were sacrificed, and radiological parameters of the PL were evaluated on digital radiographs and histopathological parameters were also assessed in histological sections. Regarding radiological parameters, scores were assigned to the visibility degree of periapical structures – periapical ligament (POL), lamina dura (LD), alveolar bone (AB), the severity degree of lesions (PAI), and the radiological area A(Rx) of each lesion was measured in digital radiographs. Concerning histopathological parameters, scores were attributed to periodontal ligament space (PLS), external root resorption (ERR), and the histological area A(H) of the lesions was measured in mm^2 on histological sections. Data were analyzed by statistical tests. Results: There were significant statistical differences between experimental group regarding radiological and histological parameters evaluated during the entire experimental period. Conclusion: The radiological and histopathological parameters allow a better assessment of the progression of periapical lesions in the teeth of healthy animals, animals with type 2 diabetes mellitus and those with osteoporosis under ZOA therapy.

Key Words: periapical pathology, radiology, histopathological parameters

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Introduction

Periapical lesions (PL) are inflammatory conditions caused by various injuries of the dental pulp, such as bacterial infection, physical and iatrogenic trauma, or by incorrect endodontic therapy. Pathogenic microorganisms reaching the root canal system progress continuously to the periapical region, causing tissue destruction and enlargement of PL (Torabinejad&Kiger 1980). The infections caused by gram-negative bacteria species have an important role in the severity degree of periapical inflammation and in the degree of destruction of alveolar bone (Martinho 2014).

The inflammatory response consists of reactive periapical granulomas and cysts, with simultaneous resorption of alveolar bone surrounding the teeth with necrotic pulp. The regulation of periapical inflammation is very complex, involving immune components such as antibodies and complement, cytokines, arachidonic acid metabolites, and neuropeptides (Stashenko et al 1998). Also, systemic factors factors such as metabolic disorders including osteoporosis and diabetes can affect the extent and severity of PL (Lerner 2006, Lopez-Lopez et al 2013, Lopez-Lopez et al 2011, Kohsaka et al 1996).

Osteoporosis is a systemic skeletal disorder characterized by micro-architectural deterioration and low bone mass with consequences in decreasing bone strength, with risks of bone fractures. Total skeletal mass reduction is frequently encountered in postmenopausal women and may include jaw bones, particularly the mandible (Pizzo et al 2010). This is why osteoporosis has recently received increasing attention, being associated with

periodontal disease in postmenopausal women (Lerner 2006, Geurs 2007). A number of studies have shown that osteoporosis represents a risk indicator that may influence progression of periodontal disease (Tezal et al 2000). There are also studies showing that zoledronic acid (ZOA) can diminish PL development (Wayama et al 2015).

Diabetes mellitus is a metabolic disorder affecting millions of patients of all ages (Rowers 2012). Diabetes consists of hyperglycemia with wound healing difficulties and other systemic and oral manifestations. It is a chronic progressive disease, characterized by partial or total deficiency in insulin production, with consequences in metabolism of carbohydrates, fats and proteins. Signs and symptoms of diabetes are polydipsia, polyuria, polyphagia and glycosuria (Bender&Bender 2003). Patients with diabetes are susceptible to bone metabolism alterations, peripheral neuropathy, vascular insufficiency and autonomic dysfunction (Lima et al 2013). The complex metabolic changes present in diabetes cause an increased susceptibility to infections. It is important to mention that these patients are prone to develop oral complications such as caries, pulp and periapical pathosis, and especially periodontal disease (Lima et al 2013). The direct effect of diabetes mellitus on dental pulp consists of various alterations in pulp structure, mainly caused by impaired collateral circulation with risk of necrosis, but also a reduction of collagen concentration, increased thickness of the basement membrane of blood vessels, angiopathy, macrophage function inhibition, impairment of cellular growth and vitamin D metabolism, and many others (Lima et al 2013).

The periapical inflammatory process is characterized radiographically by a periapical radiolucent area that results from bone loss caused by the interaction between a microbial challenge and immune response, involving recruitment of inflammatory cells, generation of cytokines, elaboration of lytic enzymes and activation of osteoclasts, which lead to alveolar bone resorption (Bender & Seltzer 2013, Liu et al 2010). In the evaluation of the apical periodontium, bone density changes present in radiographs are the most consistent feature of the presence, progression or resolution of periapical inflammation (Ridao-Sacie et al 2007). Radiographic examinations are very important in diagnosis, treatment, planning and monitoring of periapical inflammatory lesions, but the complex anatomy of the teeth and their related structures may render these tasks difficult (Grondahl & Huumonen 2004, Huumonen&Ostavik 2002). Also, the various evolution stages of lesions cannot be accurately differentiated based on radiological examination alone. It is histopathological differentiation in serial sections that provides detailed information about the microscopic structure and the real size of the lesions (Saraf et al 2014).

The purpose of this study was to investigate periapical tissue changes by radiology and histology at different time intervals after experimental induction of PL in the molar teeth of Wistar rats.

Materials and methods

The experimental study was approved by the Ethics Committee of the "Iuliu Haţieganu" University of Medicine and Pharmacy Cluj-Napoca and complied with international standards on animal research for scientific purposes. The study was performed on 63 Wistar rats with an average weight of 180 grams, which were examined radiologically and histopathologically over a 60-day period. 7 animals of each group were evaluated at 3 different time points: T1 - 14 days, T2 - 30 days and T3 - 60 days. Three animal groups were investigated:

• Group I: rats with experimentally induced PL in the first right mandibular molars (n=21)

• Group II: rats with experimentally induced type 2 diabetes mellitus and PL in the first right mandibular molars (n=21)

•Group III: female rats with osteoporosis following bilateral ovariectomy and PL in the first right mandibular molars, treated with ZOA (n=21).

Radiological analysis

In the end of each time point the animals were euthanized and their mandibles were collected and examined radiologically. Digital periapical radiographs were taken immediately with a digital dental X-ray device Endos AC/ACP (Lia Medical Systems, Buccinasco, Italy), having the following acquisition parameters: 70 Kv, 8 mA, 0.10 seconds, with the radiographic tube placed 10 cm away from the right hemimandible. The radiographic images obtained were analyzed using the Easy Dent Viewer visualization software.

The radiological parameters evaluated at three different times (T1 - 14 days, T2 - 30 days, T3 - 60 days) were the following:

• Degree of visibility of periapical structures (periodontal ligament (POL), lamina dura (LD), alveolar bone (AB)). Each structure was assigned a score on a scale from 1 to 5. Thus, score 1 - very good visibility; 2 - good visibility; 3 - moderate visibility; 4 - weak visibility and 5 - invisible structures.

• Degree of severity of periapical tissue damage using the modified periapical index (PAI), described by Orstavik in humans: PAI score 1 - normal periapical structures; 2 - minimal changes in AB; 3 - changes in bone structure with bone mineral loss and 4 - well defined radiolucent PL.

• Radiological area A(Rx) of PL measured in mm2 using the image analysis software Digimizer 4.3.1.

Histopathological analysis

Histopathological examination was performed in serial sections of right hemimandibles including the molar teeth with their surrounding tissues. The hemimandible samples were kept in 4% buffered formaldehyde solution for 48 hours. Then, they were decalcified by treatment with formic acid/hydrochloric acid (1/1), diluted to 8%, for 3 weeks, followed by their embedding in paraffin wax. Serial sections 3 mm thick were cut with the microtome and were stained with hematoxylin-eosin (HE). Histopathological parameters were evaluated by assigning different scores at 3 different times (T1 - 14 days, T2 - 30 days, T3 - 60 days), using the Olympus Stream 1.9 image analysis software. The parameters were the following:

• Thickness of the periodontal ligament space (PLS) according to the distance between root surfaces in the third apical part and surrounding bone tissue: normal aspect (0); slightly increased (1); moderately increased (2); heavily increased (3).

• External root resorption (ERR) according to the degree of destruction of radicular dentin and cementum; absent (0), present (1).

• Histological area A(H) of PL (mm2) measured on the histological sections.

Statistical analysis

Data were statistically analyzed using Stats Direct v.2.7.2. software and Excel application (Microsoft Office 2010).Shapiro-Wilk test was used was used to assess normal distribution. For data following a normal distribution, Student's t test was used, while for non-normally distributed data, non-parametric Mann-Whitney (U) test was used. For the analysis of more than two samples, ANOVA or Kruskal-Wallis tests were used. The significance level was $\alpha = 0.05$ (5%). To measure the statistical dependence between two variables the Pearson (r) correlation coefficient or the Spearman's rank correlation coefficient (rho) were used.

Results

The results of the radiological parameters are presented in Tables I, II, III, IV. The visibility of POL was good to moderate in group I at T1, and poor at T2, T3 (mean scores 2.7-4), with statistically significant differences ($p<0.001$) (Table I). In group II, at T1, POL visibility was poor, and at T2, T3, POL was invisible (mean visibility score 4-5), with statistically significant differences ($p<0.001$). In group III, the visibility of POL was moderate at all time points (mean scores 2.7-3.3), with statistically significant differences ($p=0.02$) (Table I). The visibility of the LD in group I was moderate at T1, T2 and poor at T3 (mean scores 3.3-4), with statistically significant differences ($p=0.002$). In group II, at T1, T2 and T3, LD was invisible at all 3 time points (mean score 5). Group III presented good to moderate visibility at T1, T2 and T3 (mean scores 2.7-3.3), with statistically significant differences ($p=0.024$).

AB had a good to moderate visibility in group I at T1, T2 and T3 (mean scores 2.5-3.3). The same was observed in group II (mean scores 2.7-3.3). AB in group III had a good visibility at T1, T2 and a poor visibility at T3 (mean scores 2.3-4), with statistically significant differences ($p<0.001$) (Table I).

The results of statistical analysis indicate the differences in the degrees of visibility of periapical structures on digital radiographs in the experimental groups between the three times points (Table I) and also, the statistical differences between all groups of animals at the three time points (Table II).

In group I, the severity of PL evaluated by PAI increased progressively from time T1 to time T3 (mean ± SD; 1.5 ± 0.52 vs 3.5 ± 0.52), with statistically significant differences ($p <0.001$). In group II, the severity degree of PL was increased at all 3 time points (T1 3.6 ± 0.51, T2 3.2 ± 0.42, T3 3.7 ± 0.48), with no statistically significant differences. In group III, mean PAI scores were 2.4 ± 0.51 at T1 compared with 2.7 ± 0.48 at T3 (Table III). In group I, A (Rx) increased progressively from time T1 (1.33 ± 0.46) to time T3 (2.40 ± 0.20), with significant statistical differences between the analyzed time points ($p = 0.001$) (Table III) (Figure 1). In group II, A (Rx) increased at all time points, with no statistically significant differences. In group III, A (Rx) decreased progressively from T1 (1.04 ± 0.53) to T3 (0.54 ± 0.23), with statistically significant differences between the time points ($p = 0.003$) (Table III) (Figure 1).

A comparative statistical analysis between experimental groups in terms of PAI and A (Rx) at different time points is presented in Table IV.

The correlation analysis between radiological parameters showed the following correlations in each group of animals. In group I, POL visibility scores were positively correlated with LD visibility scores at T2 ($r = 0.682$) and T3 ($r=1.000$). There was a good correlation between POL and AB at T2 ($r = 0.682$) and T3 ($r = 0.621$). The severity of periapical tissue damage was positively correlated with the visibility of POL at T2 ($r = 0.637$) and T3 ($r = 0.621$). LD visibility was positively correlated with PAI at T1 ($r=0.655$) and T3 ($r=0.621$). AB visibility correlated well with A (Rx) at T1 ($r=0.749$).

Table I: Radiological visibility degree of periapical structures on digital radiographs in the experimental groups at T1 - 14 days, T2 - 30 days, T3 - 60 days (statistical significance p – one way Anova / Kruskal-Wallis and Student t tests)

Groups		POL			LD			AB	
I	T1-T2-T3 < 0.001	T1-T2	0.008	T1-T2-T3 0.002	T1-T2	NS	T1-T2-T3 0.009	T1-T2	NS
		T1-T3	0.008		T1-T3	0.016		T1-T3	NS
		T2-T3	-		T2-T3	0.016		T2-T3	NS
II	T1-T2-T3 < 0.001	T1-T2	0.002	T1-T2-T3 NS	T1-T2	-	T1-T2-T3 0.013	T1-T2	NS
		T1-T3	0.002		T1-T3	-		T1-T3	NS
		T2-T3	-		T2-T3	-		T2-T3	NS
III	T1-T2-T3 0.021	T1-T2	0.031	T1-T2-T3 0.024	T1-T2	NS	T1-T2-T3 < 0.001	T1-T2	NS
		T1-T3	NS		T1-T3	NS		T1-T3	0.004
		T2-T3	NS		T2-T3	NS		T2-T3	0.002

Table II: Comparative statistical analysis of the visibility degrees of periapical structures between the experimental groups at different time points (T1 - 14 days, T2 - 30 days, T3 - 60 days) (statistical significance p – t Student or Mann Whitney tests)

Time	POL (Rx)				LD				AB			
	I-II-III	I-II	I-III	II-III	I-II-III	I-II	I-III	II-III	I-II-III	I-II	I-III	II-III
T1	0.001	0.001	NS	< 0.001	< 0.001	< 0.001	0.037	< 0.001	0.007	0.007	NS	0.0025
T2	< 0.001	< 0.001	0.003	< 0.001	< 0.001	< 0.001	NS	< 0.001	0.002	0.037	0.0013	NS
T3	< 0.001	< 0.001	0.0031	< 0.001	< 0.001	< 0.001	0.003	< 0.001	< 0.001	0.032	< 0.001	< 0.001

Table III: PAI and A (Rx) of the lesions in the experimental groups at three time points (T1 - 14 days, T2 - 30 days, T3 - 60 days) (statistical significance p – one way Anova / Kruskal-Wallis and t Student tests)

Groups		PAI			A(Rx)	
I	T1-T2-T3	T1-T2	0.002	T1-T2-T3	T1-T2	0.002
	< 0.001	T1-T3	0.002	0.001	T1-T3	0.002
		T2-T3	NS		T2-T3	0.048
II	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	NS
	NS	T1-T3	NS	NS	T1-T3	NS
		T2-T3	NS		T2-T3	NS
III	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	NS
	NS	T1-T3	NS	0.003	T1-T3	0.020
		T2-T3	NS		T2-T3	0.006

In group II, the visibility of POL was positively correlated with the visibility of LD at T1, T2 and T3 (r=1.000) and with the visibility of AB at T1, T2 (r = 0.682) and T3 (r=1.000). The visibility of POL was also positively correlated with the severity of lesions at T1 (r=0.636), T2 (r = 0.758) and T3 (r=0.682). LD visibility was positively correlated with AB visibility at T1 (r=0.682), T2 (r = 0.682) and T3 (r=1.000) and also, with PAI at T1 (r=0.636), T2 (0.758) and T3 (r=0.682). AB visibility was also positively correlated with PAI at T3 (r=0.682).

In group III, the visibility of POL positively correlated with the visibility of LD at T2 (r = 0.764). Also, POL visibility scores were positively correlated with AB visibility at T3 (r=0.682) and with PAI at T2 (r=0.655).

In group III, radiological LD visibility scores were negatively correlated with AB visibility scores at T1 (r= -0.986) and positively correlated at T3 (r = 0.682). LD visibility was also negatively correlated with PAI at T3 (r= -1.000). Radiological AB visibility positively correlated with PAI at T3 (r = 0.682).

Table IV: Comparative analysis of PAI and A (Rx) of the lesions between groups of animals at three time points (statistical significance p – t Student or Mann Whitney tests)

Time	PAI				A(RX)			
	I-II-III	I-II	I-III	II-III	I-II-III	I-II	I-III	II-III
T1	< 0.001	< 0.001	0.005	0.001	< 0.001	< 0.001	NS	< 0.001
T2	0.003	NS	0.005	0.014	< 0.001	NS	< 0.001	< 0.001
T3	0.001	NS	0.009	0.0013	< 0.001	NS	< 0.001	< 0.001

The results obtained from the analysis of histopathological parameters are presented in Tables V and VI. In group I, the mean value of POL scores showed that POL thickness was moderately increased at T1 and T3 (1.9 ± 0.56 vs 2.2 ± 0.78), with no statistically significant differences (p = 0.59). In group II, the mean value showed a moderate to intense increase of POL thickness at T1 and T3 (2.7 ± 0.48 vs 2.5 ± 0.52). The mean value for group III showed a slight increase of POL thickness at T1 and T3 (1.4 ± 0.51 vs 0.7 ± 0.48), with statistically significant differences (p = 0.004) (Table V).

ERR was observed in group I at T3. In group II, ERR was present at all time points analyzed, with no statistically significant differences. In group III, ERR was absent at all time points, with statistically significant differences (p = 0.001).

In group I, A(H) progressively increased from T1 (1.24 ± 0.49) to T3 (1.80 ± 0.57), with statistically significant differences between the time points (p = 0.034) (Figure 2). In group II, A (H) was increased at all time points, with no statistically significant differences (Figure 2). In group III, A (H) decreased from T1 (0.71 ± 0.50) to T3 (0.36 ± 0.15), with statistically significant differences (p = 0.018) (Table V).

The PL area was evaluated both histologically and radiologically by measurements using image analysis software. In group I, the PL area was larger on digital X-rays compared to microscopic sections at 60 days (Figs. 1, 2). In the group with diabetes mellitus and PL, the lesion area was larger on radiological images compared to histological images at 14 and 30 days.

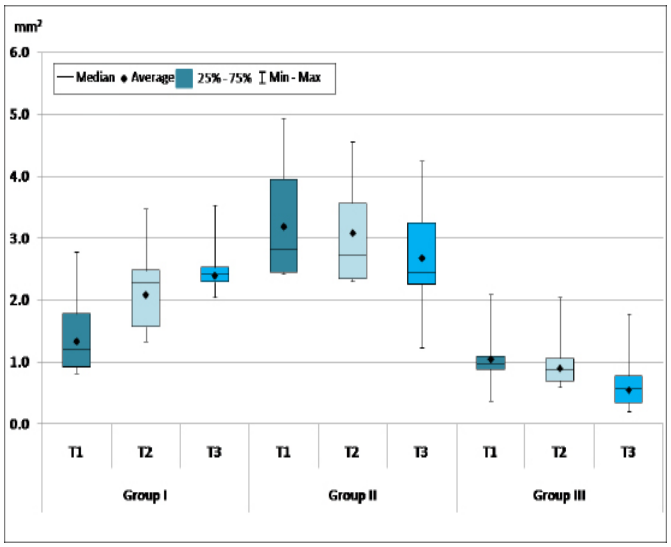

Figure 1: Radiological area A(Rx) of PL (mm^2) in the experimental groups at different time points

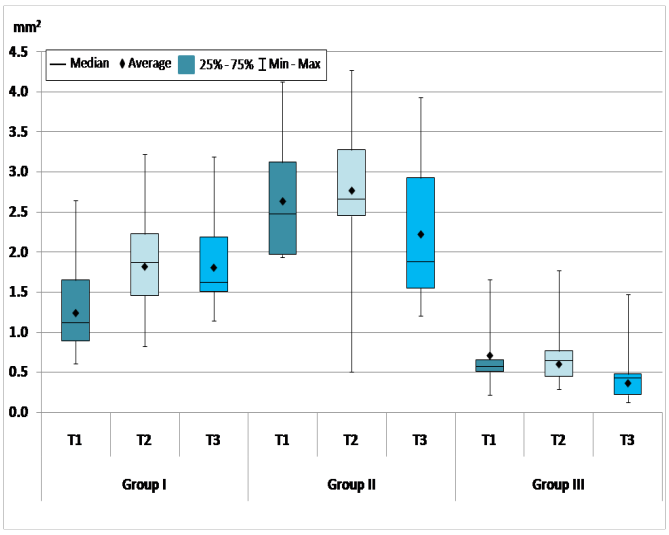

Figure 2: Histological area of A(H) (mm^2) in the experimental groups at three time points

Table V: Histological parameters of the lesions in the experimental groups at the three time points (statistical significance p – one way Anova / Kruskal-Wallis and t Student)

Groups	PLS			ERR			A(H)		
I	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	0.004
	NS	T1-T3	NS	NS	T1-T3	NS	0.034	T1-T3	0.016
		T2-T3	NS		T2-T3	NS		T2-T3	NS
II	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	NS
	NS	T1-T3	NS	NS	T1-T3	-	NS	T1-T3	NS
		T2-T3	NS		T2-T3	NS		T2-T3	NS
III	T1-T2-T3	T1-T2	NS	T1-T2-T3	T1-T2	0.0313	T1-T2-T3	T1-T2	NS
	0.004	T1-T3	0.016	0.001	T1-T3	NS	0.018	T1-T3	0.020
		T2-T3	0.016		T2-T3	0.004		T2-T3	0.001

The analysis of correlation between the histopathological parameters showed that in group I, PLS scores were positively correlated with ERR at T3 (r = 0.564). In group II, PLS scores were positively correlated with ERR at T1, T2 and T3 (r~0.6), and ERR and the histological area of PL were positively correlated at T1, T2, T3 (r ~ 0.5). In group III, PLS thickness was correlated with ERR at T1 (r = 0.802) and T3 (r = 0.682).

Discussion

In this study, a radiological and histometric analysis of PL induced in the first mandibular molars of experimental rats at three different time points was performed, which was aimed at accurately assessing the destruction degree of periapical tissues based on radiological and histopathological parameters in early and advanced stages of the lesions. So far, there are no clear radiological and histological criteria for establishing an accurate diagnosis of PL. The procedure of experimental induction of PL was identical to that of other studies, which confirms the fact that this animal model is useful for the study of PL.

The radiological parameters analyzed in this experimental study presented different aspects depending on the stages of evolution and associated systemic pathologies. In group I, with no associated systemic pathology, the highest POL radiotransparency was observed at 30 and 60 days ($p<0.001$), and LD radiopacity was more diminished at 60 days ($p<0.002$), suggesting a normal radiological progression of PL.

In group II with diabetes and PL, AB radiopacity was constant at all three time points evaluated, similarly to group I (no significant statistical differences), but POL radiotransparency was observed sooner, more exactly at 14 days ($p<0.001$). Likewise, LD radiopacity was diminished even at 14 days, unlike in group I, in which radiopacity was diminished only at 60 days ($p=0.002$).

In group III with osteoporosis under ZOA therapy, POL radiotransparency was slightly reduced at all three time points analyzed, in a constant manner, and LD radiopacity was maintained constant at all three time points analyzed ($p=0.02$).

The statistical analysis between groups at different times (Table II) shows that POL and LD visibility progressed in a similar manner, compared to AB visibility, which presented significant statistical differences only at time T3, suggesting that AB changes were highest only at time T3.

Table VI: Comparative analysis of histological parameters in all groups of animals following the same time point evaluation

Time	PLS				ERR				A(H) of lesions			
	I-II-III	I-II	I-III	II-III	I-II-III	I-II	I-III	II-III	I-II-III	I-II	I-III	II-III
T1	0.001	0.011	NS	0.001	0.001	NS	0.020	0.003	0.001	NS	0.020	0.003
T2	0.022	NS	NS	0.006	NS	NS	NS	NS	NS	NS	NS	NS
T3	< 0.001	NS	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

Regarding the severity degree of the lesions evaluated using PAI, differences were observed between experimental groups and time points. These differences were also confirmed by radiological evaluation of the visibility grade of POL presented above. It was confirmed one more time that lesion severity was higher in the groups of animals with PL and diabetes, at all three time points analyzed, compared with the PL severity grade in the other studied groups. Severity gradually increased from T1 to T3 in group I ($p<0.001$) compared with group II, in which severity was high at T1 and T3, with no significant statistical differences. In group III, the severity of lesions was moderate and slightly increased from T1 to T3, with no significant statistical differences, proving the protective effect of ZOA.

Regarding A (Rx) of PL, in group II, the area was about two times larger than the area measured in group I and group III ($p<0.001$), with no significant statistical differences from T1 to T3, proving that diabetes mellitus affects in a negative way the development of PL. Furthermore, in group I, the area increased from 14 days to 60 days ($p=0.001$), which was not observed in group III, in which a slight decrease of the area from 14 days to 60 days ($p=0.003$) was found. The decrease of the area suggests a reduction and a chronicity of inflammatory infiltrate until the end of the experimental period in the case of the group receiving ZOA. The reduced values of the measured areas in group III compared with groups I and II are probably caused by the protective effect of ZOA administered during the entire experimental period; ZOA has an inhibitory effect against osteoclasts from AB, which are activated during the development of PL (Wayama et al 2015).

Considering the correlation results between periapical structures affected during the inflammatory process at different time points in group I, the inflammatory process affects in a similar manner POL/LD and POL/AB at 30 and 60 days. Also, at T2 and T3, there is a similarity concerning the severity and visibility of POL. In the case of group II, a good similarity between POL and LD visibility at all three time points analyzed can be observed. It can also be observed between POL and AB visibility and between POL visibility and PAI at all three time points analyzed. Therefore, in group II, with PL and associated diabetes, lesions seem to evolve similarly for all three anatomical structures analyzed, from time T1 to time T3, compared to group I, in which this similarity is seen only at times T2 and T3. In the case of group III, a good similarity can be observed regarding evolution at all three time points analyzed or only at two time points like in group I. This can be explained by the fact that ZOA offers protection particularly for the bone parts

of periapical structures, soft parts continuing to be affected by bacterial infection. The negative correlation between LD visibility and AB at time T1 (r= -0.986) and the good positive correlation at time T3 (r=0.682) can suggest the fact that under ZOA therapy, LD is affected faster than AB; consequently, at time T3 they will be similarly affected.

So far, only one experimental study in mice proposed 4 histological criteria for grading PL in terms of extension of AB and POL destruction after experimental exposure of the dental pulp of mandibular M1. Experimental mice had in a proportion of 66% moderate and intense alveolar bone resorption corresponding to histological grades 2 and 3 of periapical destruction (Velickovic et al 2013).

Optical microscopy analysis improves visualization of apical and periapical structures, particularly the disarray of collagen fibres in the POL and the extent of root and bone resorbtion in both initial and late extensive PL (De Rossi et al 2007). In our study, the assessment of histological parameters evidenced differences in the evolution of PL depending on the associated systemic pathology. Histopathological analysis revealed that in group I with PL, without associated pathology, PLS was moderately increased at all three time points evaluated, with no significant statistical differences. In group II, the same difference between the evaluated times were found, but PLS was highly increased, confirming the fact that diabetes negatively affects the evolution of PL. In group III, a decrease in PLS thickness from time T1 to time T3 was seen, and the PLS aspect at all three time points was slightly decreased compared to the group I and II, proving one more time the protective effect of ZOA, and probably a chronicity of inflammatory infiltrate in this group.

ERR of endodontic origin occurring as a consequence of PL has an important clinical significance in endodontic practice (Laux et al 2000), potentially influencing the success of the treatment of these lesions. In group I, root resorption was observed only at time T3, compared to group II, in which ERR was seen at all three time points analyzed, and compared to group III, in which no ERR was observed at any of the three time points analyzed. These results evidence once more the negative effect of type 2 diabetes and the positive effect of ZOA in the evolution of PL.

From all parameters assessed in this study, in terms of the method used, only the radiological area can be well compared with the histopathogical area. As can be observed in Fig. 1 and Fig. 2, radiological analysis of PL does not represent a very sensitive method in showing the real dimensions of the lesions (Paula-Silva et al 2009), which is proved by histopathological analysis (Fig. 2). Overall, areas measured using radiological images are slightly larger than areas measured using histological analyses, but they increase (group I) or decrease (group III) or remain constant (group II) in a similar manner regardless of the evaluation method used.

In the case of group III, even a decrease of the PL area can be observed based on both radiological and histopathological analysis, which can demonstrate that ZOA may slow AB destruction.

The analyses of correlation between histological parameters show that PLS and ERR evolve differently according to the associated pathology. In group II, with diabetes, good similarities exist regarding evolution of PL at all three time points analyzed between the size of PLS, ERR and A(H). In groups I and III, these similarities are not seen; only some similarities can be observed at T3 in group I between PLS and ERR, and at T1 and T2 in group III between PLS and ERR.

The assignment of scores to the radiological and histological parameters of the lesions and the numerical presentation of data were aimed at eliminating subjectivity and the possibility of making errors in data processing.

Conclusion

The structures involved in experimental periapical inflammatory lesions are affected in different proportions depending on the various degrees of evolution and severity of the lesions, as well as on the associated general pathology. The area of PL increases with their severity. The severity of PL is higher and evolution is more rapid in animals with PL and type 2 diabetes mellitus compared to healthy animals with PL. The size of POL and AB resorption are more reduced in animals with PL and osteoporosis receiving ZOA compared to healthy animals.

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