

Relationship between apical periodontitis and atherosclerosis in rats: lipid profile and histological study

L. C. Conti¹ , J. J. Segura-Egea² , C. B. M. Cardoso¹ , F. Benetti³ ,
M. M. Azuma⁴ , P. H. C. Oliveira¹ , S. R. M. Bomfim⁵  & L. T. A. Cintra¹ 

¹Endodontic Section, Department of Preventive and Restorative Dentistry, School of Dentistry, São Paulo State University (Unesp), Araçatuba, SP, Brazil; ²Endodontic Section, Department of Stomatology, School of Dentistry, University of Sevilla, Sevilla, Spain; ³Endodontic Section, Department of Restorative Dentistry, School of Dentistry, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil; ⁴Department of Cariology, Restorative Sciences and Endodontics, University of Michigan, Ann Arbor, MI, USA; and ⁵Department Clinic and Surgery and Animal Reproduction, Araçatuba Veterinary Medicine, São Paulo State University (Unesp), Araçatuba, SP, Brazil

Abstract

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Aim To investigate the relationship between apical periodontitis and atherosclerosis in rats by lipid profile and carotid artery intima tunic measurement, and histological and histometric evaluation of periapical lesions.

Methodology Forty male Wistar rats were allocated into four groups: control (C), with apical periodontitis (AP), with atherosclerosis (AT) and with AP and AT (AP + AT). Atherosclerosis was induced using a high-lipid diet associated with a surgical ligature in the carotid artery and a super dosage of vitamin D₃. AP was induced via pulp exposure to the oral environment. At 45 and 75 days, serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured. The maxillary and mandibular jaws and carotid artery were collected and processed for histological analysis. The Kruskal–Wallis or Mann–Whitney test was performed for nonparametric data, and the Tukey's or Student's *t*-test was performed for parametric data ($P < 0.05$).

Results In nonatherosclerotic animals, the induction of apical periodontitis increased TG levels significantly, from $63.1 \pm 11.4 \text{ mg dL}^{-1}$ in group C to $88.2 \pm 7.9 \text{ mg dL}^{-1}$ in the AP group ($P < 0.05$). The induction of AP was associated with a trend for higher TC and LDL-C levels in atherosclerotic animals ($P > 0.05$); however, it only significantly increased TG levels, from $93.2 \pm 18.0 \text{ mg dL}^{-1}$ in AT group to $121.9 \pm 14.5 \text{ mg dL}^{-1}$ in the AP + AT group ($P < 0.05$). Animals in the AP + AT group had a 36.5% increase in the thickness of the carotid intima tunic when compared with the AT group ($P < 0.05$). The intensity of the inflammatory infiltrate was significantly larger in the AP + AT group when compared with AP group ($P < 0.05$). The AP + AT group exhibited significantly greater alveolar bone loss, with a periapical lesion size of $206.4 \pm 56.3 \times 10^4 \mu\text{m}^2$, compared with $151.4 \pm 49.1 \times 10^4 \mu\text{m}^2$ in the AP group ($P < 0.05$).

Conclusion Apical periodontitis influenced triglyceride levels, increasing them even in the absence of atherosclerosis, and influenced the increase in the thickness of the carotid artery intima tunic in the presence of atherosclerosis. Atherosclerosis intensified the inflammatory reaction and increased bone resorption in periapical lesions.

Keywords: apical periodontitis, atherosclerosis, endodontic medicine, systemic disorders.

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Correspondence: Luciano Tavares Angelo Cintra, Endodontic Section, Department of Preventive and Restorative Dentistry, São Paulo State University (Unesp), School of Dentistry, R: José Bonifácio, 1193, Araçatuba, São Paulo, Brazil (e-mail: luciano.cintra@unesp.br).

Introduction

Cardiovascular diseases are the second most common cause of disease-related death worldwide, especially coronary heart disease (CHD) (Tymchuk *et al.* 2006). The underlying cause of CHD is atherosclerosis, a pathological process that results from lipoproteins retention in the subendothelial layer of large and medium arteries. The accumulation of fat plaques triggers innate immune responses. T cells play an important role in defending against lipid proteins, resulting in a slow progression of the disease through a chronic inflammatory process (Gisterå & Hansson 2017). Risk factors such as smoking, hypertension, high low-density lipoprotein (LDL) serum levels, diabetes, obesity, genetic dispositions, gender and socio-economic status are implicated in the aetiology of CHD. Chronic inflammatory processes have also been considered as potential predictors for atherosclerosis in recent years (Willershausen *et al.* 2009). Moreover, chronic oral infections, such as apical periodontitis and periodontal diseases, are potential risk for systemic diseases, including CHD (Segura-Egea *et al.* 2015, Bui *et al.* 2019).

Apical periodontitis (AP) is an inflammatory disease characterized by inflammation and destruction of periradicular tissues due to the interaction between microbial factors and the host immune response (Howait *et al.* 2019). The prevalence of AP is 34–61% (López-López *et al.* 2012), and several studies have investigated a possible correlation between periapical pathosis and various systemic conditions (Segura-Egea *et al.* 2015, Khalighinejad *et al.* 2016, Cintra *et al.* 2018). Considering that AP is associated with increased levels of inflammatory markers such as interleukins, immunoglobulins, asymmetrical dimethyl-L-arginine, and C-reactive protein in humans (Gomes *et al.* 2013) and nitric oxide, leucocyte and lymphocyte levels in experimental animal models (Cintra *et al.* 2014a, Samuel *et al.* 2018), AP could contribute to systemic inflammation, which could be its link to several systemic diseases. In fact, the impact of AP on systemic health seems to be related to the number of foci of infection (Cintra *et al.* 2016, Samuel *et al.* 2018). Accordingly, a previous study reported that the larger number of AP lesions, the greater the probability of detecting quantifiable atherosclerotic lesions and the greater the aortic atherosclerotic burden (Petersen *et al.* 2014). Other studies have demonstrated the influence of these lesions on endothelial flow reserve (Cotti *et al.* 2015)

and impaired flow-mediated dilatation and greater carotid intima-media thickness in subjects with AP (Chauhan *et al.* 2019). In addition, subjects with AP were more likely to have cardiovascular diseases than subjects without AP by 5.3-fold (Segura-Egea *et al.* 2010, An *et al.* 2016). Moreover, several studies have suggested a potential link between AP and atherosclerosis, since they share similar pathogenesis mechanisms and maintain a local and systemic chronic inflammation state if left untreated (Petersen *et al.* 2014, Cotti *et al.* 2015, An *et al.* 2016, Berlin-Broner *et al.* 2017, Chauhan *et al.* 2019).

Therefore, the objective of this *in vivo* study was to investigate the relationship between apical periodontitis and atherosclerosis by lipid profile and carotid artery intima tunic measurement and histological and histometric evaluation of periapical lesions. The null hypothesis is that there is no relationship between apical periodontitis and atherosclerosis.

Materials and Methods

Experimental design

The experimental procedures of this study were approved by the Institutional Ethics Committee of the Universidade Estadual Paulista, São Paulo, Brazil, and conducted according to the relevant guidelines of the Ethical Conduct Committee on Animal Experimentation (00358-2016).

Forty male Wistar rats (*Rattus norvegicus albinus*, Wistar), weighing on average 120 g, were housed in mini isolators for rats (Alesco, São Paulo, Brazil). The rats were kept in a temperature-controlled environment and given *ad libitum* access to water. Each study group received a different type of diet.

Sample size was estimated based on data from previous studies (Azuma *et al.* 2017a,b, Dal-Fabbro *et al.* 2019). Considering an alpha error of 0.05% and 95% power to recognize a significant difference of 1 in the median scores, a minimum of seven animals per group was necessary. Considering possible animal deaths, three more animals were added in each group, resulting in ten rats per group (Cosme-Silva *et al.* 2019).

Induction of atherosclerosis

During the experiment, the atherosclerotic group (AT, $n = 20$) received a high-lipid diet composed of 75% of commercial food, 20% lard and 5% sugar (Chen *et al.* 2014). On the 15th day, the rats were anaesthetized

with intramuscular administration of ketamine (87 mg kg⁻¹) (Francotar – Virbac do Brasil Ind e Com Ltda, Roseira, Brazil) and xylazine (13 mg kg⁻¹) (Rompum – Bayer SA, São Paulo, Brazil) for the surgical induction of atherosclerosis. The trichotomy and disinfection with Iodopovidone were performed in the region to be operated. Next, the animals were placed in supine position at surgical tables for rats and an incision was made in the midline of the neck, at the position of the carotid plexus. The right carotid artery was isolated to avoid damage to the blood vessels and nerves. A sterile needle (0.3 mm outer diameter) was placed in parallel and over the artery. A ligature was made around the carotid artery 1.5 cm away from its bifurcation using a suture segment (Nam *et al.* 2009). The needle was then removed, and the ligature remained around the carotid artery. The inner diameter of the stenosis remained approximately 0.3 mm in diameter. One day after the surgical procedure, an oral dose of vitamin D₃ equivalent to 90 000 IU (Apothecário-Boraschi Vieira Ribas e Cia Ltda, Aracatuba, Brazil-Manipulated and equivalent to 45 000 IU per drop or 1 125 000 IU mL⁻¹), was given for two days to the animals with the artificial ligation performed (Wang *et al.* 2015).

The other 20 animals were fed with commercial food (Labina[®]; Purina Agribands do Brasil Ltda, Paulínia, São Paulo, Brazil) and underwent the same surgical procedure. However, the artificial ligature was not performed in these rats, and they were given physiological saline instead of vitamin D₃. This procedure was performed to standardize all procedures and equalize the level of stress and inflammatory mediators.

Induction of apical periodontitis

On the 45th day, apical periodontitis was induced in 20 animals, 10 of them corresponding to the AT group, using surgical round burs (0.1 mm diameter – Broca Ln Long Neck; Maillefer, Dentsply Ind and Com Ltda, Petrópolis, Brazil). The animals were anaesthetized according to the aforementioned protocol. The pulps of the first and second maxillary and mandibular right molars of each animal were exposed to the oral cavity during the entire experimental period (Samuel *et al.* 2018).

After the induction protocols, the rats were divided into 4 groups (10 rats per group):

Group C: control rats

Group AP: rats with apical periodontitis

Group AT: rats with atherosclerosis

Group AP + AT: rats with apical periodontitis and atherosclerosis

Collection of blood samples for determination of serum lipid profile

On the 45th and 75th days, the animals were subjected to fasting for 8–12 h and then anaesthetized according to the aforementioned protocol, and cardiac puncture blood samples were collected from each animal to determine the lipid profile. The blood samples were placed in vacuum tubes for collection, containing clot activator spread on the tube's wall to accelerate the coagulation process and a separator gel to obtain a better quality serum. The samples were then centrifuged immediately after collection at 1800 × *g* for 15 min at 4 °C to obtain the serum. Plasma total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) levels were enzymatically measured using a commercial kit (Cintra *et al.* 2013) (Cholesterol Liquiform Labtest[®], Triglycerides Liquiform Labtest[®], HDL Labtest[®] Cholesterol; Labtest Diagnostica Ind. e Com. Ltda, Lagoa Santa, MG, Brazil). The levels of low-density lipoprotein cholesterol (LDL-C) were obtained by the Friedewald formula: LDL-C = (CT – HDL-C) – (TG/5).

Collection and processing of tissues for histologic evaluation

On the 75th day, after the second blood collection, the animals were euthanized with an overdose of the anaesthetic solution. The maxillary and mandibular jaws and carotid artery of each animal were dissected and fixed in 10% formaldehyde at neutral pH for 24 h, and then washed in running water for 12 h to remove the entire fixative solution. The maxillary and mandibular jaws were demineralized with 17% buffered EDTA (Sigma Chemical Co., St. Louis, MO, USA). Subsequently, both the jaws and the carotid arteries were dehydrated with ethanol, diaphonized with xylol and embedded in paraffin. Serial paraffin sections (5 µm) were obtained in the mesial-distal aspects of the right maxillary and mandibular first molars and of the carotid artery in the transverse and longitudinal directions. The tissue sections were stained with haematoxylin–eosin and evaluated under optical microscopy (DM 4000 B; Leica, Wetzlar, Germany).

In the histopathological analysis, the region of interest was a 1200 × 800-µm area located within the periapical tissues of the mandibular first molar mesial root.

Inflammatory infiltrate analysis was conducted using the following guidelines: quality of inflammation and the cellularity pattern of dental and periodontal tissues. Based on these guidelines, we scored the inflammatory infiltrate as follows: score 0 (absent to few inflammatory cells), score 1 (less than 25 cells and mild reaction), score 2 (between 25 and 125 inflammatory cells and moderate reaction) and score 3 (125 or more cells and severe reaction) (Dal-Fabbro *et al.* 2019).

For AP and AP + AT groups, the area of periapical lesion associated was measured histometrically. The area was estimated by rounding up the lesion, considering the outer external surface of alveolar bone, and expressed in square micrometres. For each rat, 7 serial histologic sections were histometrically measured. The AP areas were determined for each side, and the average value (mean \pm standard deviation) was calculated for each group (Azuma *et al.* 2017a).

For histological analysis, the carotid intima tunic thickness (from the innermost of intima to the innermost of media, and the direction of measurement was perpendicular to vessel wall) was measured with a magnification of 100 \times . Five fields were randomly selected, linear values were obtained for each vessel and the average carotid intima tunic thickness values were used for the quantitative analysis, with a magnification of 100 \times .

These measurements used an image processing system, which consisted of a light microscope (DM 4000 B, Leica), a colour image processor (Leica Qwin V3 software, Leica), a colour camera (DFC 500, Leica) (Cintra *et al.* 2014b) and a personal computer (Intel Core I5; Intel Corp, Santa Clara, CA, USA; Windows 10, Microsoft Corp, Redmond, WA, USA). The analyses were performed blindly by a single calibrated operator.

Statistical analysis

Data were tabulated and statistically analysed using Sigmaplot software (San Jose, CA, USA). After the Shapiro–Wilk test of normality, the Kruskal–Wallis or the Mann–Whitney test was performed for nonparametric data and analysis of variance. Tukey's test or Student's *t*-test was performed for parametric data. The level of significance was 5%.

Results

Lipid profile Serum

The results of the dosed fat fractions of lipid profile (TC, TG, HDL-C and LDL-C) are shown in Table 1 and 2. At the

45th day, the animals with induced atherosclerosis had significantly higher TC, TG and LDL-C levels when compared with the normal animals ($P < 0.05$; Table 1). The HDL-C levels were similar amongst all animals ($P > 0.05$). TC (91.58 ± 17.63) and LDL-C levels (33.36 ± 16.63 mg dL⁻¹) in atherosclerotic animals were 1.4 and 4 times and significantly higher, respectively, than those of the normal animals ($P < 0.05$; Table 1).

On the 75th day (30 days after AP induction), the two atherosclerotic groups (AT and AP + AT) still had the highest levels of TC, TG and LDL-C ($P < 0.05$; Table 2). In nonatherosclerotic animals, the induction of apical periodontitis significantly increased TG levels, from 63.05 ± 11.35 mg dL⁻¹ in group C to 88.18 ± 7.89 mg dL⁻¹ in the AP group ($P < 0.05$). The induction of AP increased TC and LDL-C levels in atherosclerotic animals ($P > 0.05$); however, it only increased significantly TG levels, from 93.20 ± 18.00 mg dL⁻¹ in the AT group to 121.85 ± 14.46 mg dL⁻¹ in the AP + AT group ($P < 0.05$; Table 2). The HDL-C levels were similar amongst all groups ($P > 0.05$).

Histological and histometric analysis of the periapical lesions

To evaluate the intensity of the inflammatory infiltrate and bone resorption in apical periodontitis, histologic images of the H&E-stained periapical region were analysed from the various experimental groups (Fig. 1).

In the C and AT groups, no inflammation was observed in the periapical regions. However, in the AP and AP + AT groups, the pulps had signs of total necrosis 30 days after exposure. In these groups, the apical region of the periodontal ligament was disorganized, with the presence of necrotic tissue and underlying inflammation in all teeth. Near the bone tissue, active resorption gaps were found throughout the periphery of the granuloma (Fig. 1).

Histologically, AP + AT animals had significantly more severe periapical inflammatory infiltrates than those in the AP group ($P = 0.006$). In these groups, regions of the cementum surface of most specimens exhibited areas of resorption. All the lesions found had characteristics compatible with periapical granulomas due to pulp necrosis. The histometric analysis revealed that the AP + AT group exhibited the most significant alveolar bone loss, with a periapical lesion size of $206.41 \pm 56.28 \times 10^4$ μm^2 , compared with $151.41 \pm 49.11 \times 10^4$ μm^2 in the AP group ($P = 0.032$; Table 3).

Table 1 Mean and standard deviation (SD) values of the lipid profile at 45 days of the rats in the AT and normal groups.

Animal condition	Lipid profile at 45 days (mg dL ⁻¹)								n
	TC		TG		HDL-C levels		LDL-C levels		
	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	
Normal	66.35 ^a	11.14	60.43 ^a	12.30	43.88 ^a	7.13	8.27 ^a	11.84	20
Atherosclerotic	91.58 ^b	17.63	90.68 ^b	26.17	42.78 ^a	6.04	33.36 ^b	16.63	20

*Same letters indicate the absence of a significant difference amongst the groups ($P > 0.05$); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides levels.

Table 2 Mean and standard deviation (SD) of the lipid profile at 75 days of the all groups.

Groups	Lipid profile at 75 days (mg dL ⁻¹)								n
	TC		TG		HDL-C levels		LDL-C levels		
	Mean*	SD	Mean*	SD	Mean*	SD	Mean*	SD	
C	69.40 ^a	9.47	63.05 ^a	11.35	31.13 ^a	5.08	25.72 ^a	8.13	10
AP	68.88 ^a	9.06	88.18 ^b	7.89	28.61 ^a	5.50	22.69 ^a	6.14	10
AT	94.05 ^b	21.64	93.20 ^b	18.00	28.86 ^a	4.13	46.55 ^b	18.23	10
AP + AT	101.40 ^b	23.40	121.85 ^c	14.46	32.29 ^a	3.86	54.60 ^b	17.54	10

*Same letters indicate the absence of a significant difference amongst the groups ($P > 0.05$); AP, group with apical periodontitis; AP + AT, group with AP and AT; AT, group with atherosclerosis; C, control group; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglycerides levels.

Histological and histometric analysis of the carotid artery

To evaluate the morphology of the carotid artery, H&E-stained histological images from the various experimental groups were analysed (Fig. 2). The histological analysis of C and AP groups sections revealed a normal and intact carotid intima tunic. Endothelial cells were orderly and continuing organized, without morphological alterations. In atherosclerotic animals (groups AT and AP + AT), disorganized and foam cell layers were detected. Moreover, calcified and necrotic areas were found in the carotid intima tunic of some specimens. Vacuoles were observed in regions where adipocyte cells were probably present. In AT and AP + AT groups, the carotid intima tunic was significantly thicker than in groups C and AP ($P < 0.05$) with exacerbated proliferation of the endothelial cells (Fig. 2, Table 3). In addition, the AP + AT group had a 36.5% increase in the thickness of the carotid intima tunic when compared with AT group ($P < 0.05$). There was no significant difference between group C and group AP ($P > 0.05$; Table 3).

Discussion

This appears to be the first experimental study analysing the possible relationship between apical periodontitis and

atherosclerosis in animals. The atherosclerotic Wistar rats with induced periapical lesions had exacerbated periapical bone resorption, increased inflammatory infiltrate intensity and increased triglyceride levels compared with nonatherosclerotic animals, suggesting a bidirectional relationship between endodontic infection and atherosclerosis. Moreover, apical periodontitis increased triglyceride levels even in the absence of atherosclerosis and increased the thickness of the carotid artery intima tunic in the presence of atherosclerosis. Thus, the null hypothesis was rejected.

The methodology used to develop atherosclerosis in rats has been validated previously. The animals that underwent atherosclerosis induction with a high-lipid diet (Chen *et al.* 2014) associated with the surgical procedure performed on the right carotid artery (Nam *et al.* 2009) and with a highly concentrated dose of vitamin D₃ (Wang *et al.* 2015) had significant alterations in the morphology of the carotid, resulting in modification of the carotid intima tunic, increasing its thickness, and in the formation of the foam cell layer. These induction methods have been used in several animal studies with success (Chen *et al.* 2014), since they can reproduce several aspects of atherosclerosis that occur in humans. In the present study, both local changes in the carotid arteries and systemic changes in lipid profile proved the success of the animal model used.

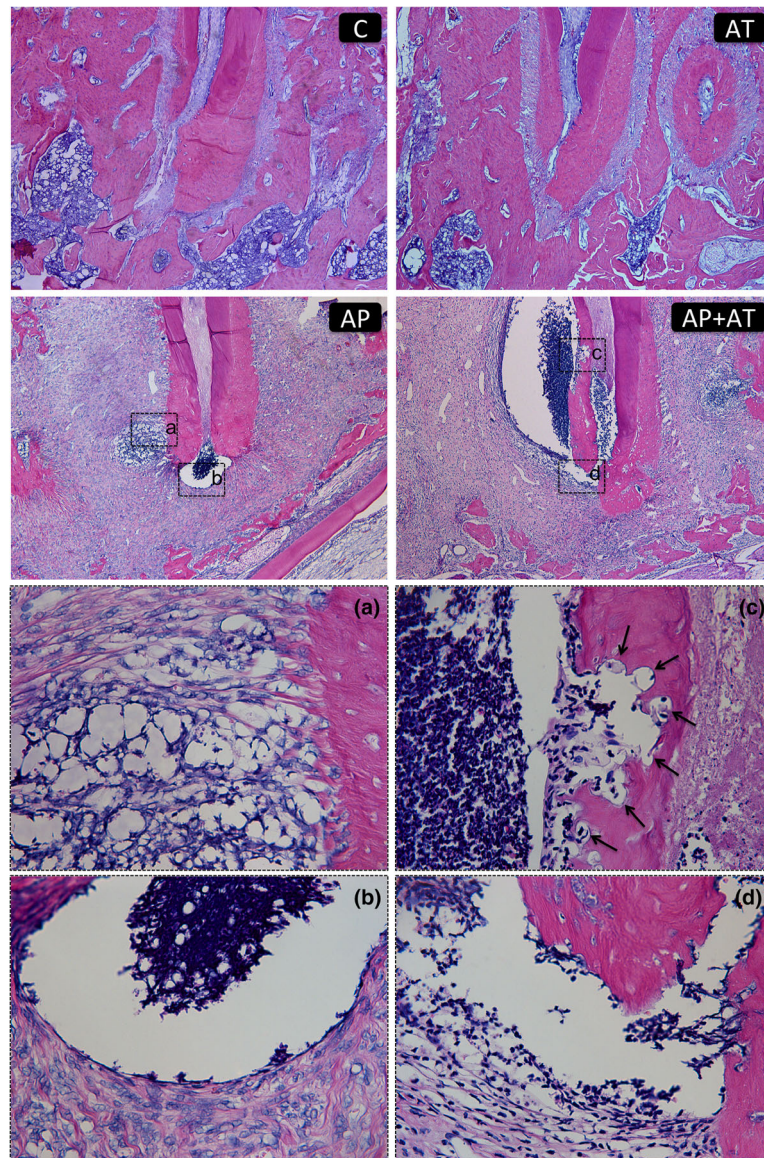


Figure 1 Histologic images of periapical lesions at 30 days after pulp exposure to the oral environment (H&E staining). (C) Group C. The apical and periapical regions are free of inflammatory infiltrates (H&E staining, $\times 100$). (AT) Group AT. Similar to C, the pulp and periodontal tissues are shown with normal aspect (H&E staining, $\times 100$). (AP, a and b) Group AP. (AP) A inflammatory cell infiltration area surrounding tooth apex, severe disorganization of the periodontal ligament and large area of bone resorption were visible (H&E staining, $\times 100$). (a) A moderate inflammatory cell infiltration near the dentine region can be observed (H&E staining, $\times 1000$). (b) Presence of acute inflammatory cell concentrations near the tooth apex region (H&E staining, $\times 1000$). (AP + AT, c and d) Group AP + AT. (AP + AT) A inflammatory cell concentration near the tooth apex region can be observed, and large bone resorption area is visible (H&E staining; $\times 100$). (c) Severe inflammatory cell concentrations and presence of intense dentine resorption (black arrows) (H&E staining, $\times 1000$). (d) Moderate acute inflammatory cell concentrations near the tooth apex region, and large bone resorption (H&E staining, $\times 1000$).

Oral infection was induced using a consolidated method described in previous studies (Samuel *et al.* 2019). Molar pulps of rats were exposed in the

oral cavity for 30 days, a sufficient period for the formation of apical periodontitis, as well as in previous studies (Astolphi *et al.* 2013). The induction model

Table 3 Scores, median, mean and standard deviation (SD) in the periapical region, periapical lesion size and carotid intima tunic thickness of all groups.

Analysis parameters	Groups				P value	
	C	AP	AT	AP + AT		
Inflammatory Infiltrate	1 – Absent	10/10	0/10	10/10	0/10	<i>P</i> = 0.006
	2 – Mild	0/10	1/10	0/10	0/10	
	3 – Moderate	0/10	7/10	0/10	4/10	
	4 – Severe	0/10	2/10	0/10	6/10	
	Median*	1 ^a	3 ^b	1 ^a	4 ^c	
Periapical Lesion size (x10 ⁴ μm ² ± SD)*	–	151.41 ± 49.11 ^a	–	206.41 ± 56.28 ^b	<i>P</i> = 0.032	
Carotid intima tunic thickness (μm ± SD)*	33.58 ± 6.78 ^a	35.22 ± 4.19 ^a	90.52 ± 29.54 ^b	123.50 ± 50.51 ^c	<i>P</i> = 0.032	

*Different letters indicate significant differences in rows (*P* < 0.05).

AP, group with apical periodontitis; AP + AT, group with AP and AT; AT, group with atherosclerosis; C, control group; SD, standard deviation.

was confirmed by histological images of the pulp that revealed necrosis and of the apical third of the root that contained periapical lesions, with inflammatory infiltrate.

One of the objectives of the present study was to analyse possible changes in lipid profile after the induction of apical periodontitis. Hyperlipidaemia is considered a risk factor for atherosclerosis (Beverly & Budoff 2019). The results of CT, TG and LDL-C measurements performed at both periods (45 and 75 days) revealed that the AT-induced animals had higher values than the others. These results, along with the morphological changes observed in the carotid arteries of these animals, are consistent with evidence that hypercholesterolaemia can cause pathosis such as endothelial damage and changes in the myocardium (Stapleton *et al.* 2010). In addition, on the 75th day, the AP + AT group had the highest values of TC, TG and LDL-C; however, only the TG value was significantly different when compared with the other groups.

Previous studies have reported that apical periodontitis associated with periodontal disease increases triglycerides levels in normal rats (Cintra *et al.* 2013), as observed in the presence of multiple foci of apical periodontitis (Azuma *et al.* 2018). Thus, the increase in lipid profile levels, observed in the AP + AT group, mainly TG, could be explained by the association of apical periodontitis with the atherosclerotic process.

The reduction in HDL-C levels and the increase in LDL-C levels observed between 45 and 75 days agrees with Gobalakrishnan *et al.* (2016), who induced atherosclerosis in rats by the administration of high-lipid diet. High HDL-C levels prevent atherosclerotic

plaque formation, being associated with lower occurrence of cardiovascular diseases. On the other hand, high LDL values are associated with main complications of atherosclerosis, such as cerebrovascular disease, coronary heart disease and peripheral vascular disease (Gobalakrishnan *et al.* 2016).

The present findings are consistent with those in previous experimental animal studies that presented atherosclerosis as a systemic alteration (He *et al.* 2014, Wang *et al.* 2015). The marked (36.5%) increase in the carotid intima tunica thickness observed in atherosclerotic animals after induction of apical periodontitis (group AT + AP) indicate that AP did affect significantly the thickness of the carotid intima tunic. This significant increase is compatible with the results of a previous human study investigating the association between apical periodontitis and cardiovascular disease using noninvasive methods (Chauhan *et al.* 2019). This study revealed impaired flow-mediated dilation and greater intimal-to-average carotid thickness in individuals with apical periodontitis. These findings support evidence that apical periodontitis may aggravate the progression of atherosclerosis.

Animals without AP (groups C and AT) had no inflammation and no bone resorption in the periapical area. Periapical lesions induced by pulp exposure to the oral environment were significantly greater in the AP + AT group than in the AP group. Despite the inexistence of previous experimental studies on apical periodontitis and atherosclerosis, several studies have investigated the association between apical periodontitis and diabetes, which is characterized by an angiopathy with endothelial dysfunction. The results

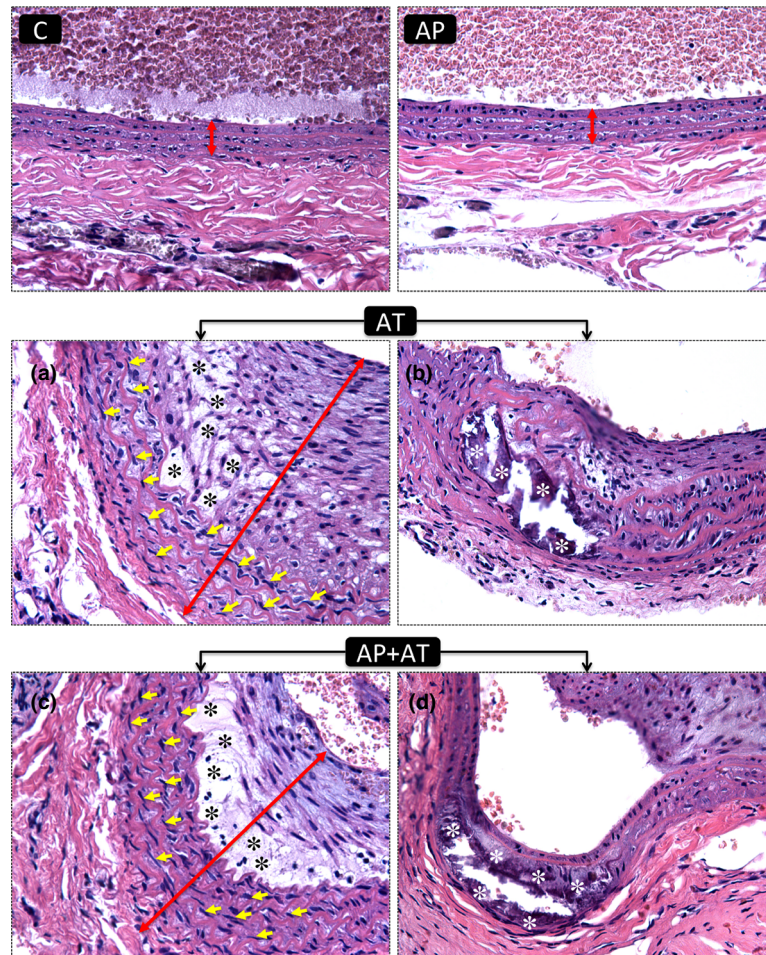


Figure 2 Histological images corresponding to 75 days after the surgical procedure performed on the right carotid artery of the rats of different experimental groups (H&E staining). (C) Group C and (AP) Group AP. Normal and intact appearances of carotid artery. Carotid intima tunic was smooth and thin (red arrow). Endothelial cells were arranged orderly and continuous organized, without morphological alterations (original magnification, x100 and x400, respectively). (a and b) Group AT and (c and d) Group AP + AT. In these two groups, there was a notable thickening of the carotid intima tunic (red arrow), with exacerbated proliferation of the endothelial cells. Endothelial cells were arranged and lined disorderly (yellow arrows). Disorganization of the cell layers and presence of the foam cell layer was also detected. Calcification areas and necrosis were found in the carotid intima tunic of some specimens (white asterisks). Vacuoles were observed in regions where adipocyte cells were probably present (black asterisks).

of these studies also demonstrated larger periapical lesions in diabetic rats when compared to control rats with apical periodontitis (Cintra *et al.* 2014c, Prieto *et al.* 2017, Azuma *et al.* 2017b). The alteration of the vascular endothelium could partially explain the larger size of the periapical lesions in both diabetic and atherosclerotic animals. In fact, apical periodontitis has been associated with early endothelial dysfunction in humans, documented by the reduced endothelial flow reserve (Cotti *et al.* 2011, 2015,

Bergandi *et al.* 2019). Another two human studies have found that bone loss over 40% due to periodontal disease was associated with a threefold increase in mortality from cardiovascular diseases (Beck *et al.* 1996), as well as a positive correlation between alveolar bone loss, assessed by panoramic radiographs, with increased calcification in the carotid artery internal wall (Persson *et al.* 2002).

Regarding inflammatory infiltrate, as expected, microscopic examination revealed no inflammation in

C and AT groups. Histologically, the periapical inflammatory infiltrates were more intense in the AP + AT group, with more inflammatory cells when compared with the AP group. These results are consistent with those of previous studies investigating the association between apical periodontitis and other systemic diseases (Cintra *et al.* 2014b, Samuel *et al.* 2018). Studies in humans indicate that periodontal disease may influence atherogenesis (Beck *et al.* 2001) and cardiovascular events (Beck *et al.* 2001, Dietrich *et al.* 2018). Studies have suggested that several mechanisms can trigger or aggravate atherosclerotic processes in periodontal patients, such as innate immune system activation, bacteraemia – due to dental intervention – and inflammatory mediator involvement – activated by antigens of dental infections in atheromas, as well as common predisposing factors that influence both pathologies (Bartova *et al.* 2014). Despite the different aetiology and pathogenesis, periodontal disease and apical periodontitis are chronic oral infections with a common microbiota (Siqueira & Rôças 2014). Moreover, the mechanisms that relate endodontic disease as a risk for coronary heart disease may be similar to the hypothetical association between periodontal disease and coronary heart disease. In both cases, the presence of the bacterial infection generates a localized inflammatory response that release systemic cytokines, which may lead to subsequent deleterious vascular effects (Caplan *et al.* 2006, Segura-Egea *et al.* 2015). Therefore, apical periodontitis can be associated with the same systemic disorders as periodontal disease (Segura-Egea *et al.* 2015).

Endodontic medicine is investigating the interrelationship between endodontic disease and systemic diseases (Segura-Egea *et al.* 2015, 2019), with an increasing number of articles linking apical periodontitis with various body disorders (Segura-Egea *et al.* 2015, Cintra *et al.* 2018). However, these results should be carefully interpreted, since many risk factors shared by both systemic diseases and oral infections could act as confounding variables (Khalighinejad *et al.* 2016), also considering that association does not imply causation (Segura-Egea *et al.* 2019). A recent symposium on endodontics and systemic diseases, held during the ESE Congress in Vienna, evidenced the need to conduct experimental animal studies on the association of apical periodontitis and systemic diseases, such as diabetes and coronary heart disease. The associations between endodontic disease and several systemic conditions

found in epidemiological studies in humans should be further investigated in animal models. The present study is the first experimental contribution in the investigation of a possible association between endodontic infection and atherosclerosis.

Conclusion

Given the conditions of the current experiment, apical periodontitis and atherosclerosis were interconnected in a rat model. The results suggest that apical periodontitis influenced triglyceride levels, increasing them even in the absence of atherosclerosis, and influenced the increase in the thickness of the carotid artery intima tunic in the presence of atherosclerosis. Atherosclerosis intensified the inflammatory reaction and increased bone resorption in periapical lesion.

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Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

References

- An GK, Morse DE, Kunin M *et al.* (2016) Association of radiographically diagnosed apical periodontitis and cardiovascular disease: a hospital records-based Study. *Journal of Endodontics* **42**, 916–20.
- Astolpho RD, Curbete MM, Colombo NH *et al.* (2013) Periapical lesions decrease insulin signal and cause insulin resistance. *Journal of Endodontics* **39**, 648–52.
- Azuma MM, Gomes-Filho JE, Ervolino E *et al.* (2017a) Omega 3 fatty acids reduce bone resorption while promoting bone generation in rat apical periodontitis. *Journal of Endodontics* **43**, 970–6.
- Azuma MM, Gomes-Filho JE, Prieto AKC *et al.* (2017b) Diabetes increases interleukin-17 levels in periapical, hepatic, and renal tissues in rats. *Archives of Oral Biology* **83**, 230–5.
- Azuma MM, Gomes-Filho JE, Cardoso CBM *et al.* (2018) Omega 3 fatty acids reduce the triglyceride levels in rats with apical periodontitis. *Brazilian Dental Journal* **29**, 173–8.
- Bartova J, Sommerova P, Lyuya-Mi Y *et al.* (2014) Periodontitis as a risk factor of atherosclerosis. *Journal of Immunology Research* **2014**, 1–9.

- Beck J, Garcia R, Heiss G *et al.* (1996) Periodontal disease and cardiovascular disease. *Journal of Periodontology* **67**, 1123–37.
- Beck JD, Elter JR, Heiss G *et al.* (2001) Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study. *Arteriosclerosis Thrombosis and Vascular Biology* **21**, 1816–22.
- Bergandi L, Giuggia B, Alovise M *et al.* (2019) Endothelial dysfunction marker variation in young adults with chronic apical periodontitis before and after endodontic treatment. *Journal of Endodontics* **45**, 500–6.
- Berlin-Broner Y, Febbraio M, Levin L (2017) Apical periodontitis and atherosclerosis: Is there a link? Review of the literature and potential mechanism of linkage. *Quintessence International* **48**, 527–34.
- Beverly JK, Budoff MJ. (2020) Atherosclerosis: Pathophysiology of insulin resistance, hyperglycemia, hyperlipidemia, and inflammation. *Journal of Diabetes* **12**, 102–4.
- Bui FQ, Almeida-da-Silva CLC, Huynh B *et al.* (2019) Association between periodontal pathogens and systemic disease. *Biomedical Journal* **42**, 27–35.
- Caplan DJ, Chasen JB, Krall EA *et al.* (2006) Lesions of endodontic origin and risk of coronary heart disease. *Journal of Dental Research* **85**, 996–1000.
- Chauhan N, Mittal S, Tewari S, Sen J, Laller K (2019) Association of apical periodontitis with cardiovascular disease via noninvasive assessment of endothelial function and subclinical atherosclerosis. *Journal of Endodontics* **45**, 681–90.
- Chen Z, Li S, Zhao W *et al.* (2014) Protective effect of co-administration of rosuvastatin and probucol on atherosclerosis in rats. *Canadian Journal of Physiology and Pharmacology* **92**, 797–803.
- Cintra LT, da Silva Facundo AC, Azuma MM *et al.* (2013) Pulpal and periodontal diseases increase triglyceride levels in diabetic rats. *Clinical Oral Investigations* **17**, 1595–9.
- Cintra LT, Samuel RO, Azuma MM *et al.* (2014a) Apical periodontitis and periodontal disease increase serum IL-17 levels in normoglycemic and diabetic rats. *Clinical Oral Investigations* **18**, 2123–8.
- Cintra LT, da Silva Facundo AC, Prieto AK *et al.* (2014b) Blood profile and histology in oral infections associated with diabetes. *Journal of Endodontics* **40**, 1139–44.
- Cintra LT, Samuel RO, Facundo AC *et al.* (2014c) Relationships between oral infections and blood glucose concentrations or HbA1c levels in normal and diabetic rats. *International Endodontic Journal* **47**, 228–37.
- Cintra LT, Samuel RO, Azuma MM *et al.* (2016) Multiple apical periodontitis influences serum levels of cytokines and nitric oxide. *Journal of Endodontics* **42**, 747–51.
- Cintra LTA, Estrela C, Azuma MM, Queiroz ÍOA, Kawai T, Gomes-Filho JE (2018) Endodontic medicine: interrelationships amongst apical periodontitis, systemic disorders, and tissue responses of dental materials. *Brazilian Oral Research* **18**(Suppl. 1), 68e.
- Cosme-Silva L, Dal-Fabbro R, Cintra LTA *et al.* (2019) Systemic administration of probiotics reduces the severity of apical periodontitis. *International Endodontic Journal* **52**, 1738–49.
- Cotti E, Dess C, Piras A *et al.* (2011) Association of endodontic infection with detection of an initial lesion to the cardiovascular system. *Journal of Endodontics* **37**, 1624–9.
- Cotti E, Zedda A, Deidda M *et al.* (2015) Endodontic infection and endothelial dysfunction are associated with different mechanisms in men and women. *Journal of Endodontics* **41**, 594–600.
- Dal-Fabbro R, Marques-de-Almeida M, Cosme-Silva L, Ervolino E, Cintra LTA, Gomes-Filho JE (2019) Chronic alcohol consumption increases inflammation and osteoclastogenesis in apical periodontitis. *International Endodontic Journal* **52**, 329–36.
- Dietrich T, Jimenez M, Krall Kaye EA *et al.* (2018) Age-dependent associations between chronic periodontitis/edentulism and risk of coronary heart disease. *Circulation* **117**, 1668–74.
- Gisterå A, Hansson GK (2017) The immunology of atherosclerosis. *Nature Reviews Nephrology* **13**, 368–80.
- Gobalakrishnan S, Asirvatham SS, Janarthanam V (2016) Effect of silybin on lipid profile in hypercholesterolaemic rats. *Journal of Clinical and Diagnostic Research* **10**, 1–5.
- Gomes MS, Blattner TC, Filho MS *et al.* (2013) Can apical periodontitis modify systemic levels of inflammatory markers? a systematic review and meta-analysis. *Journal of Endodontics* **39**, 1205–17.
- He B, Hao J, Sheng W *et al.* (2014) Fistular onion stalk extract exhibits anti-atherosclerotic effects in rats. *Experimental and Therapeutic Medicine* **8**, 785–92.
- Howait M, Albassam A, Yamada C *et al.* (2019) Elevated expression of macrophage migration inhibitory factor promotes inflammatory bone resorption induced in a mouse model of periradicular periodontitis. *Journal of Immunology* **202**, 2035–43.
- Khalighinejad N, Aminoshariae MR, Aminoshariae A, Kulild JC, Mickel A, Fouad AF (2016) Association between systemic diseases and apical periodontitis. *Journal of Endodontics* **42**, 1427–34.
- López-López J, Jané-Salas E, Estrugo-Devesa A *et al.* (2012) Frequency and distribution of root-filled teeth and apical periodontitis in an adult population of Barcelona, Spain. *International Dental Journal* **62**, 40–6.
- Nam D, Ni CW, Rezvan A *et al.* (2009) Partial carotid ligation is a model of acutely induced disturbed flow, leading to rapid endothelial dysfunction and atherosclerosis. *American Journal of Physiology Heart and Circulatory Physiology* **297**, 1535–43.
- Persson RE, Hollender LG, Powell VL *et al.* (2002) Assessment of periodontal conditions and systemic disease in

- older subjects. II. Focus on cardiovascular diseases. *Journal of Clinical Periodontology* **29**, 803–10.
- Petersen J, Glaßl EM, Nasser P *et al.* (2014) The association of chronic apical periodontitis and endodontic therapy with atherosclerosis. *Clinical Oral Investigations* **18**, 1813–23.
- Prieto AKC, Gomes-Filho JE, Azuma MM *et al.* (2017) Influence of apical periodontitis on stress oxidative parameters in diabetic rats. *Journal of Endodontics* **43**, 1651–6.
- Samuel RO, Gomes-Filho JE, Azuma MM *et al.* (2018) Endodontic infections increase leukocyte and lymphocyte levels in the blood. *Clinical Oral Investigations* **22**, 1395–401.
- Samuel RO, Ervolino E, de Azevedo Queiroz ÍO, Azuma MM, Ferreira GT, Cintra LTA (2019) Th1/Th2/Th17/Treg balance in apical periodontitis of normoglycemic and diabetic Rats. *Journal of Endodontics* **45**, 1009–15.
- Segura-Egea JJ, Jimenez-Moreno E, Calvo-Monroy C *et al.* (2010) Hypertension and dental periapical condition. *Journal of Endodontics* **36**, 1800–4.
- Segura-Egea JJ, Martín-González J, Castellanos-Cosano L (2015) Endodontic medicine: connections between apical periodontitis and systemic diseases. *International Endodontic Journal* **48**, 933–51.
- Segura-Egea JJ, Cabanillas-Balsera D, Jiménez-Sánchez MC, Martín-González J (2019) Endodontics and diabetes: association versus causation. *International Endodontic Journal* **52**, 790–802.
- Siqueira JRJF, Rôças IN (2014) Present status and future directions in endodontic microbiology. *Endodontic Topics* **30**, 3–22.
- Stapleton PA, Goodwill AG, James ME *et al.* (2010) Hypercholesterolemia and microvascular dysfunction: interventional strategies. *Journal of Inflammation* **7**, 54.
- Tymchuk CN, Hartiala J, Patel PI *et al.* (2006) Nonconventional genetic risk factors for cardiovascular disease. *Current Atherosclerosis Report* **8**, 184–92.
- Wang J, Wang L, Yang H *et al.* (2015) Prevention of atherosclerosis by Yindan Xinnaotong capsule combined with swimming in rats. *BMC Complementary and Alternative Medicine* **15**, 109.
- Willershausen B, Kasaj A, Willershausen I *et al.* (2009) Association between chronic dental infection and acute myocardial infarction. *Journal of Endodontics* **35**, 626–30.