Influence of Apical Periodontitis on Stress Oxidative Parameters in Diabetic Rats

Annelise Katrine Carrara Prieto, MSc, João Eduardo Gomes-Filho, PhD,* Mariane Maffei Azuma, MSc,* Gustavo Sivieri-Araújo, PhD,* Luis Gustavo Narciso, MSc,† Jucilene Conceição Souza, MSc,† Paulo César Ciarlini, PhD,† and Luciano Tavares Angelo Cintra, PhD**

Abstract

Introduction: In this study, we investigated if apical periodontitis (AP) associated with diabetes influenced the levels of endogenous antioxidants, the total antioxidant capacity (TAC), and the oxidant parameter in the serum of Wistar rats. **Methods:** Forty male rats were divided into 4 equal groups: normal rats (N), rats with AP (AP), diabetic rats (D), and diabetic rats with AP (D + AP). Diabetes was induced by alloxan (150 mg/kg). AP was induced by exposing the pulpal tissue to the oral environment. After 36 days, blood and maxillae were collected. Albumin, bilirubin, uric acid, TAC, and malondialdehyde (MDA) levels were measured, and histologic analysis of the maxillae was performed. $P < .05$ was set as the threshold for statistical significance. **Results:** Uric acid levels were higher in the D + AP group when compared with that of the N, D, and AP groups ($P < .05$). The MDA concentration was higher in the D and D + AP groups when compared with the N and AP groups ($P < .05$). The level of albumin was lower in the D + AP group when compared with the N, AP, and D groups. Inflammatory infiltration was more intense in the periapical region in the D + AP group compared with that in the AP group ($P < .05$). **Conclusions:** Our findings indicate that diabetes may change the antioxidant status, increase the concentration of MDA and uric acid, and decrease albumin levels in the serum. In addition, AP can potentiate the effects of diabetes by reducing the levels of albumin and increasing the levels of uric acid. *(J Endod 2017;■:1–6)*

Key Words

Albumin, apical periodontitis, bilirubin, diabetes mellitus, oxidative stress, uric acid

Significance

Our findings indicate that diabetes may change the antioxidant status, increase the concentration of MDA and uric acid, and decrease albumin levels in the serum. Apical periodontitis can potentiate the effects of diabetes by reducing the levels of albumin and increasing the levels of uric acid.

The bidirectional relationship between diabetes and apical periodontitis (AP) has been widely studied. Diabetes might influence the pathogenesis of AP by increasing the number of inflammatory cells (1) and contributing to the expansion of lesions (1, 2). In addition, AP can potentiate the systemic effects of diabetes by increasing the serum levels of triglycerides (3), creatinine (4), inflammatory cells (1), and proinflammatory cytokines such as interleukin 17 (5) and decreasing the glycemic control (2, 6). Considering that AP is an inflammatory process that causes tissue destruction (7), it is important to find newer strategies to decrease the inflammation. In this context, understanding the oxidative metabolism of the body is essential to unravel the mechanisms involved in tissue damage caused by diabetes as well as AP (8).

Oxidative stress occurs because of the deficiency of antioxidants or an increase in the production of free radicals (9). It is well-known that diabetes increases the oxidative stress, especially because hyperglycemia is directly related to the production of advanced glycation end products as well as reactive oxygen species (10). In overwhelming concentrations, reactive oxygen species can damage the cellular macromolecules, causing destruction of membranes, loss of cellular homeostasis, and, consequently, cell death (11). It may explain in part why AP is higher and more aggressive in diabetics compared with normoglycemic rats (5). In addition, our hypothesis is that the presence of AP may exacerbate oxidative stress systemically by increasing oxidative stress parameters, as observed in periodontal disease (12). Although AP and periodontal disease share important similarities with the microbial spectrum and the inflammatory response (13), until now, there was no study available in the literature relating the impact of AP on oxidative stress systemically. Thus, it would be interesting to investigate if AP can potentiate the production of antioxidants and free radicals in diabetic rats.

This understanding will also aid in the development of new therapies with an aim to modulate oxidative stress (14, 15). These therapies might help reduce the symptoms related to periapical lesions (16), periodontal disease (17), and diabetes (18, 19). Therefore, the aim of this study was to evaluate if AP alters the oxidative stress

From the *Department of Endodontics, School of Dentistry, São Paulo State University (Unesp) and †Department of Clinic and Surgery and Animal Reproduction, Veterinary Medicine, São Paulo State University (Unesp), Araçatuba, São Paulo, Brazil.

Address requests for reprints to Prof Luciano Tavares Angelo Cintra, Department of Endodontics, School of Dentistry, São Paulo State University (Unesp), Araçatuba, São Paulo, Brazil. E-mail address: lucianocintra@foa.unesp.br

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parameters such as albumin, bilirubin, uric acid, the total antioxidant capacity (TAC), and malondialdehyde (MDA) in normoglycemic and diabetic rats.

Materials and Methods

Experimental Design

The study was approved by the Institutional Ethics Committee of Universidade Estadual Paulista, São Paulo, Brazil. Experiments were conducted in accordance with the relevant guidelines. Forty male Wistar rats (*Rattus norvegicus albinus*), 6 weeks old and weighing 200–250 g, were used in this study. The animals were housed in a mini-isolator for rats (Alesco, São Paulo, Brazil) in temperature-controlled rooms and were given *ad libitum* access to water and food.

Induction of Diabetes and AP

The Wistar rats were divided into 4 groups of 10 rats each: normal (N), AP, diabetic (D), and diabetic with AP (D + AP). The rats were then administered anesthesia via intramuscular injections of ketamine (87 mg/kg, Francotar; Virbac do Brazil Ind e Com Ltda, Roseira, São Paulo, Brazil) and xylazine (13 mg/kg, Rompum, Bayer AS, São Paulo, Brazil). The rats were randomly assigned to receive intramuscular injections containing either citrate buffer solution (N and AP groups, $n = 20$) or alloxan (D and D + AP groups, $n = 20$) dissolved in a citrate buffer solution at 150 mg/kg body weight for the experimental induction of diabetes (day 0). After 6 days, all rats showed blood glucose levels more than 250 mg/dL, which was the standard levels stablished for diabetic rats (1, 5, 6).

To induce AP (day 6), surgical round burs (Drill LN Long Neck; Dentsply Ind e Com Ltda, Petrópolis, Brazil) were used to expose the pulp on the mesial surfaces of the right upper first molars to the oral environment (AP and D + AP groups) (1, 3–5).

Blood Sample Collection

Thirty days after the induction of AP (day 36), the rats were anesthetized using a protocol described previously, and a cardiac puncture was performed to collect 5 mL blood from each rat. The blood samples were centrifuged to obtain plasma, which was stored at −20°C and protected from light. Quantification of albumin, bilirubin, uric acid, TAC, and MDA was subsequently performed.

Histologic Analyses

After blood collection, the animals were killed with an overdose of the anesthetic solution. Maxillae were removed, postfixed in neutral buffered formalin for 48 hours, decalcified in buffered (pH = 8) 17% EDTA (Sigma-Aldrich, St Louis, MO), rinsed again in sterile water, dehydrated in ethanol, cleared in xylene, and embedded in paraffin. Serial slices (5-μm thickness) were prepared in the mesiodistal plane and stained with hematoxylin-eosin. The slices were examined sequentially under an optical microscope (DM 4000 B; Leica Microsystems, Wetzlar, Germany).

Analyses were performed by a single calibrated operator who was blind to the specimen's group affiliation. The inflammatory infiltrate was evaluated for intensity and extension. The average number of cells per field and the extension that went beyond either of the apical foramen (AP groups) were considered. For each experimental group, the number of cells was calculated as the average of 10 separate areas (×400 magnification). The intensity of the inflammatory infiltrate was graded as absent (0 to few inflammatory cells: score 1), mild (<25 cells: score 2), moderate (25–125 cells: score 3), and severe (>125 cells: score 4). The extension of the inflammatory infiltrate was graded as absent (0 to few inflammatory cells: score 1), mild (cells occupying up to a 300-μm length: score 2), moderate (cells occupying up to a 600-μm length: score 3), and severe (cells occupying >a 600-μm length: score 4).

For the AP and D + AP groups, the area of the periapical lesion was measured histometrically. For each rat, 5 serial histologic sections were analyzed by an image processing system that consisted of a light microscope (DM 4000 B, Leica), a color camera (DFC 500, Leica Microsystems), a color image processor (Leica Qwin V3 software, Leica Microsystems), and a personal computer (Intel Corel I5 [Intel, Santa Clara, CA] with Windows 10 [Microsoft, Redmond, WA]).

Measurement of Oxidative Stress

All the biochemical tests were performed with the use of commercial reagents (BioSystems, Barcelona, Spain) at 37°C in an automated analyzer (BTS-370 Plus, BioSystems), which had been previously calibrated. The blood concentration of albumin was determined by the bromocresol green assay, bilirubin by using diazotized sulfanilic acid, and uric acid by the uricase/peroxidase enzymatic assay.

The serum level of TAC was quantified in an automated analyzer by inhibiting 2.2′-azino diethylbenzothiazoline sulfonic acid cation formation. TAC analyses were monitored with a standard antioxidant specific for automation.

Blood lipid peroxidation was determined by quantifying the plasma thiobarbituric acid reactive substances. Absorbance at 530 nm was measured in a plate reader (Spectra Count Reader; Packard BioScience, Meriden, CT) in accordance with the manufacturer's recommendations, starting with a standard commercial MDA solution (500 mmol/L) and using a computer program (GraphPad Prism, version 4; GraphPad Software, San Diego, CA). A curve for final concentrations of 0, 0.625, 1.25, 2.5, 5, 10, 25, and 50 nmol/mL MDA was developed. Each point on the curve was obtained from the mean value of 10 repetitions.

Statistical Analyses

The values obtained in parametric tests were tabulated and presented as the means observed in each group. Statistical differences between groups were determined by analysis of variance followed by the

TABLE 1. Weight (g) and Blood Glucose (mg/dL) in Rats from All Groups (Mean and Standard Deviation [SD*])

	Weight (g) ± SD*		Blood glucose (mg/dL) ± SD*		
Groups	Day 0	Day 36	Day 0	Day 6	Day 36
N	269.8 ± 20.7[a]	410.0 ± 31.1[a]	73.7 ± 9.8[a]	79.8 ± 11.1[a]	95.0 ± 7.3[a]
AP	268.1 ± 23.6[a]	381.1 ± 31.5[a]	79.8 ± 9.1[a]	82.8 ± 9.0[a]	89.0 ± 8.8[a]
D	273.5 ± 11.7[a]	210.0 ± 38.3[b]	73.5 ± 12.3[a]	597.3 ± 6.3[b]	586.0 ± 21.0[b]
D + AP	260.0 ± 24.2[a]	211.4 ± 30.5[b]	74.4 ± 7.9[a]	555.4 ± 51.9[c]	600.0 ± 0.0[b]

AP, apical periodontitis; D, diabetic; D + AP, diabetic with apical periodontitis; N, normal.

*Different letters in the columns indicate statistical differences, $P < .05$.