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Diabetes increases interleukin-17 levels in periapical, hepatic, and renal tissues in rats

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ABSTRACT

Objectives: This study aimed to evaluate the association between endodontic infection and diabetes on interleukin-17 levels in periapical, hepatic, and renal tissues of rats.
Design: Forty male rats were divided into groups: normoglycemic rats (N), normoglycemic rats with apical periodontitis (N-AP), rats with experimental diabetes (ED), and rats with experimental diabetes and apical periodontitis (ED-AP). Diabetes was induced by intravenous streptozotocin injection, and blood sugar levels were monitored to confirm disease development. Apical periodontitis (AP) was induced by pulp exposure to the oral environment during 30 days. After 30 days, hepatic and renal tissues were obtained, and IL-17 levels were quantified by ELISA. The right hemi-jaw was used to quantify IL-17 levels by immunohistochemistry. The values obtained in parametric tests were tabulated and analyzed statistically by analysis of variance (ANOVA) and Tukey tests, and the values obtained for scores were statistically analyzed by using the Kruskal-Wallis and Dun tests. The level of significance was set at 5%.
Results: ED and ED-AP groups expressed significantly higher IL-17 levels in both hepatic and renal tissues ($p < 0.05$), compared to N and N-AP groups. Apical periodontitis (AP) in ED-AP group was significantly more severe than that in N-AP group ($p < 0.05$). Furthermore, there was a significantly larger increase in the IL-17 levels in ED-AP group compared to N group ($p < 0.05$).
Conclusion: Our results indicate that diabetes increases IL-17 levels in hepatic and renal tissues and also enhances IL-17 production in apical periodontitis area of rats.

1. Introduction

Apical periodontitis (AP) is an infectious disease characterized by the destruction of periradicular tissues and is mediated by cytokines secreted from immunocompetent cells that infiltrate the periapical tissues in response to intracanal bacterial infection (Kawashima et al., 1996). Previous studies which investigated the association between oral infections and diabetes have shown that AP and periodontal diseases increased triglyceride levels (Cintra et al., 2013), blood glucose concentrations (Cintra, Samuel, Facundo et al., 2014), serum inflammatory cells (Cintra, da Silva Facundo et al., 2014), as well as altered organs weight of diabetic rats (Cintra et al., 2017). It's well established that diabetes alone exerts deleterious effects in some organs, including kidney and liver, as a consequence of elevated blood glucose levels (Lee et al., 2008). However, to the best of our knowledge, none of them has ever investigated the impact of AP in the inflammatory mediators in those organs of diabetic rats.

Interleukin-17A (IL-17) is the founding member of a novel family of pro-inflammatory cytokines consisting of at least six members, IL-17A-17F (Aggarwal & Gurney, 2002). IL-17 has potent effects on numerous cells of the innate immune system, particularly on the granulocyte lineage, and it is important in bridging the adaptive and innate immune systems (Yu & Gaffen, 2008).

Many reports describe the presence of IL-17 in AP (Marçal et al.,

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2010; Xiong, Wei, & Peng, 2009) and it is known to contribute to the exacerbation of chronic AP (Colić et al., 2014), as it might be linked to the acute process of AP (Marçal et al., 2010). Furthermore, increased IL-17 serum levels have also been previously reported in a study investigating the association between AP and periodontal disease in rats (Cintra, Samuel, Azuma et al., 2014). IL-17 plays an active role in diabetes pathogenesis (Kumar, Natarajan, & Shanmugam, 2013; Wang, Chueh et al., 2011; Wang, Yang et al., 2011), especially in relation to its deleterious role in maintaining the integrity of pancreatic islets and in glycemic control (Kumar et al., 2013; Sumarac-Dumanovic et al., 2013; Wang, Chueh et al., 2011; Wang, Yang et al., 2011). Diabetes is further associated with increasing the severity and progression of AP (Armada-Dias et al., 2016; Fouad & Burleson, 2003; Iwama et al., 2003), resulting in recovery failures following non-surgical endodontic treatments (Brito, Katz, Guelmann, & Heft, 2003; Wang, Chueh et al., 2011; Wang, Yang et al., 2011). Considering that IL-17 has an important role in both diseases, diabetes and AP, it would be interesting to evaluate if diabetes can potentiate the production of IL-17 locally in AP site. It is therefore important to understand the relationship between diabetes and AP with respect to the systemic effects in rats. The aim of this study was to evaluate the influence of diabetes on AP progression and on IL-17 levels in periapical tissues, and also evaluate the association of diabetes and/or AP on IL-17 levels in hepatic and renal tissues.

2. Materials & methods

2.1. Induction of diabetes, apical periodontitis, and sample preparation

Male Wistar rats (*Rattus norvegicus albinus*, n = 40) that weighed 250–300 g and were 6 weeks of age were used in the study. The rats were housed in mini-isolators for rats (Alesco, São Paulo, Brazil), kept in temperature-controlled rooms (25 ± 1 °C) and given *ad libitum* access to water and food. The experimental protocol was approved by and conducted in accordance with guidelines of the institutional ethical committee, and in accordance with the U.K. Animals (Scientific Procedure) Act, 1986.

The rats were fasted overnight (14–16 h), and the tail-tip blood used to measure blood glucose levels using a blood glucose monitoring system (Accu-Check® Performa, Roche Diagnostics Corporation, IN, USA). The rats were subsequently intramuscularly anesthetized with 87 mg/kg ketamine (Francotar, Virbac do Brasil Ind. Com. Ltda., São Paulo, Brazil), and 13 mg/kg xylazine (Rompum, Bayer S.A., São Paulo, Brazil). The rats were then randomly assigned into four groups (n = 10 rats/group): normoglycemic rats (N); normoglycemic rats with apical periodontitis (N-AP); rats with experimental diabetes (ED), and rats with experimental diabetes and apical periodontitis (ED-AP). The, either citrate buffer solution or streptozotocin (Sigma- Aldrich Corp., MO, USA) was injected into the penile vein. The citrate buffer solution (0.01 M, pH 4.5) was injected in groups N and N-AP, and streptozotocin, which was dissolved in citrate buffer solution at 35 mg/kg body weight and used for the experimental induction of diabetes, was injected in groups ED and ED-AP (Cintra et al., 2013; Cintra, Samuel, Facundo et al., 2014).

Six days after diabetes was induced, blood samples were collected from each rat and their blood glucose levels determined. Rats with blood glucose levels > 200 mg/dL were used in this study (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Garber, Shabahang, Escher, & Torabinejad, 2009).

Once hyperglycemia was confirmed, the animals were anesthetized as previously described, and endodontic infection was induced in groups N-AP and ED-AP as follows: pulps of the first upper right molars were exposed to the oral eviroment, using surgical round burs (Broca Ln Long Neck-Maillefer, Dentsply Ind. Com. Ltda, Rio de Janeiro, Brazil), for 30 days (Cintra et al., 2013; Cintra, da Silva Facundo et al., 2014; Garber et al., 2009). The rats were killed with an overdose of the anesthetic solution after 30 days. The left kidney and a liver fragment from each rat were immediately collected and preserved in liquid nitrogen to avoid cytokine degradation prior to the determination of IL-17 levels in the hepatic and renal tissues.

2.2. ELISA

Kidney and liver tissue fragments were used to quantify IL-17 levels by the enzyme-linked immune-sorbent assay (ELISA) capture technique. Briefly, 0.2 g of tissues and 800 µl of sterile phosphate-buffered saline (PBS), pH 7.0, were kept on ice and ground in a tissue homogenizer (Ultraturrax T8, IKA, Germany) for approximately 5 min. The resulting homogenate was thereafter centrifuged (10,000*g*, 15 min, 4 °C) and the supernatant immediately stored at −80 °C (Revco, Twinsburg, Ohio, USA). To determine the IL-17 levels, 100 µl of the supernatant was assessed using ELISA commercial kits (Rat IL-17A ELISA MAX™ Deluxe, cat #437904; Biolegend, San Diego, CA, USA) following the manufacturer's instructions.

2.3. Histological analysis

Maxillae from sacrificed rats were removed, post-fixed in neutral buffered formalin for 48 h, decalcified during 4 weeks in buffered (pH 8) 10% EDTA (Sigma Chemical Co, St Louis, MO, USA), rinsed in sterile water, dehydrated in ethanol, cleared in xylene and embedded in paraffin. Serial slices (5 µm) were prepared in a mesiodistal plane and stained with hematoxylin and eosin. The slices were examined sequentially under an optical microscope (DM 4000 B, Leica, Wetzlar, Germany) and the intensity and extension of the inflammatory infiltrate evaluated. The average number of cells per field and the extension beyond the apical foramen (AP groups) were considered. The number of cells in each experimental group (n = 10/group) was calculated (100× magnification). The intensity of inflammation was graded as follows: 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 inflammation was graded as follows: absent (score = 1), mild (inflammatory cells extending up to 300 µm beyond the tooth apical foramen, score = 2), moderate (inflammatory cells extending up to 600 µm beyond the tooth apical foramen, score = 3), and severe (inflammatory cells extending more than 600 µm beyond the tooth apical foramen, score = 4).

For AP and AP-O groups, the area of periapical lesion associated with the distal root of the maxillary first molar was histometrically meausured. The area was calculated by rounding up the lesion boundary, considering the outer external surface of alveolar bone, and it was expressed in square micrometers. For each rat, 5 serial histological sections were histometrically measured by an image processing system that consisted of a light microscope (DM 4000 B, Leica, Wetzlar, Germany), color camera (DFC 500, Leica), color image processor (Leica Qwin V3 software Leica Microsystems, Wetzlar, Germany) and a personal computer (Intel Pentium 4, 2.80 GHZ, Windows XP SP3). The AP areas were determined for each slice, and the average value (mean ± standard deviation) was calculated for each experimental group.

2.4. Immunohistochemistry

For immunohistochemical reactions, antigen retrieval was achieved by immersing the histological slices in buffer solution (Diva Decloaker®; Bio- care Medical, CA, USA) in a pressurized chamber (Decloaking Chamber®, Biocare Medical, CA, USA) at 95 °C for 10 min. The slices were rinsed with 0.1 M PBS (pH 7.4) at the end of each stage of the immunohistochemical reaction. The histological sections were immersed in 3% hydrogen peroxide for 1 h and in 1% bovine serum albumin for 12 h to block endogenous peroxidase activity and nonspecific sites, respectively. The histological slices containing samples from all the experimental groups were divided into three batches, and each batch was incubated with one of the following primary antibodies: