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Diabetes triggers the loss of tooth structure associated to radiographical and histological dental changes and its evolution to progressive pulp and periapical lesions in rats

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ABSTRACT

Objectives: The aim of this study was to evaluate the putative influence of diabetes without metabolic control in the loss of tooth structure as well as histological changes in dentin and pulp tissue in rats.
Design: Diabetes was induced in Wistar rats ($n = 25$) by intravenous administration of alloxan (42 mg/kg). Diabetic and non-diabetic control rats were evaluated at 1, 3, 6, 9 and 12 months of follow-up. In order to evaluate the presence and progression of dental caries and periapical lesions, hemimandibles were removed and submitted to radiographical, histological, and morphometrical procedures.
Results: Dental caries were detected after radiographical and histological evaluations in diabetic group from the third month of diabetes onset, increasing gradually in frequency and severity in periods. Diabetic rats dental pulps also presented significant reduction in volume density of collagen fibers and fibroblasts at third month, parallel with a trend towards the increase in inflammatory cells volume density. Diabetic rats presented a generalized pulp tissue necrosis after 6 months of diabetes induction. Moreover, periapical lesions were not detected in control group, while these lesions were observed in all rats after 3, 6, 9, and 12 months of diabetes induction.
Conclusions: Uncontrolled diabetes seems to trigger the loss of tooth structure, associated to histological dental changes and mediates its evolution to progressive severe pulp and periapical lesions in rats. Therefore, diabetes may be considered a very important risk factor regarding alterations in dental pulp, development of dental caries, and periapical lesions.

1. Introduction

Diabetes mellitus (DM), one of the most common chronic diseases, is characterized by abnormal glucose and lipids metabolism resulting in hyperglycemia and hyperlipidemia. The abnormal metabolism is associated to diabetic complications, which include microanginopathy, macroanginopathy with accelerated arteriosclerosis, neuropathy involving neuromuscular dysfunction and decreased resistance to infection (American Diabetes Association, 2013). Diabetes is also associated to oral complications such as xerostomia, tooth loss, gingivitis, periodontitis, and oral mucosa lesions (Lamster, Lalla, Borgnakke, & Taylor, 2008).

The association between diabetes and periodontal disease is well described, showing that microbial and immunological changes support the increased severity of periodontal disease in diabetic patients (Mealey & Oates, 2006). However, the association between dental caries, pulp tissue changes and DM remains unknown. Some authors have reported higher prevalence of dental caries in diabetic patients in the absence of glycemic control (Siudikiene, Machiulskiene, Nyvad, Tenovuo, & Nedzelskiene, 2006). When compared to non-diabetic subjects, increased prevalence of dental caries in uncontrolled diabetic patients is observed under similar conditions of oral hygiene and salivary flow (Miralles et al., 2006). Conversely, other studies failed to demonstrate increased caries levels in the presence of diabetes (Amaral, Ramos, & Ferreira, 2006; Miralles et al., 2006; Tagelsir, Cauwels, van Aken, Vanobbergen, & Martens, 2011), and no associations were found between glycemic control and dental

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caries (Miralles et al., 2006; Siudikiene et al., 2006). Experimental models in rats have been used to evaluate the effects of diabetes in the prevalence and severity of dental caries (Kodama et al., 2011; Reuterving, Hagg, & Gustafson, 1986; Sano, Matsuura, Ozaki, & Narama, 2011), but these studies are usually limited to specific experimental periods and did not demonstrate the kinetics of caries development in different periods after diabetes onset.

Despite the possible effect on caries development, diabetes could directly affect the pulp tissue (Bender & Bender, 2003). In fact, metabolic alterations in pulp tissue such as altered levels of nitrite and kallikrein have been shown in diabetic rats (Catanzaro, Dziubecki, Lauria, Ceron, & Rodriguez, 2006). Decreased collagen concentration has also been shown (Catanzaro et al., 2006), however other components of pulp tissue were not evaluated in this study (Catanzaro et al., 2006). In fact, long-term diabetes mediated alterations of other components of dental pulp tissue, such as vascular changes (thickened basement membrane). Also, calcifications in pulp tissue were more frequent in presence of diabetes (Russell, 1966). Conversely, other authors did not find vascular changes in dental pulp of diabetics and non-diabetics patients (Bissada, El-Kafrawy, & El-Mostehy, 1969).

Despite the wide discussion concerning the association between diabetes and oral health, the influence of diabetes on the dental caries and pulp tissue is not totally described. In addition, development and progression analyses of caries lesions in different periods of experimental diabetes may be necessary to evaluate the kinetics of diabetic influence. Therefore, the aim of this study was to evaluate the presence and severity of dental caries and periapical lesions in rats after 1, 3, 6, 9, and 12 months of diabetes induction without glycemic control.

2. Material and methods

2.1. Induction of diabetes and collection of samples

Adult male Wistar rats, 10–12 weeks old, weighing approximately 250 g were obtained from Central Biotery of Sao Paulo State University, Botucatu, Brazil. Animals were submitted to diabetes induction by intravenous administration of alloxan (Sigma Chemical Co., St Louis, USA) in a single dose of 42 mg/kg of body weight into the caudal vein. Only rats showing two successive determinations of blood glucose levels (7 and 14 days after alloxan injection) greater than 250 mg/dL were considered diabetic and included in the experiment. Glucose levels during the experiment were typically 400–450 mg/dL in diabetic rats whereas serum glucose levels of the control rats ranged from 90 to 130 mg/dL during all the experiment. Glucose measurements were performed with 12 h of fasting using enzymatic method of glucose oxidase (CELM, Barueri, São Paulo).

The animals were housed in metabolic cages in groups of four per cage, and fed a standard rat chow and water *ad libitum*. Study protocol was approved by the Experimental Committee of Bauru School of Dentistry, University of São Paulo. Each diabetic and control group was composed of 25 animals, subdivided into five subgroups with 5 rats each, and analyzed at 1, 3, 6, 9, and 12 months after diabetes onset. On their respective sacrifice dates, the animals were anaesthetized with 30 mg/kg of sodium pentobarbital (Cristália Chemical Products—Itapira/SP) and the hemimandibles were removed and fixed in 10% formalin solution for 7 days.

2.2. Histological analyses

The hemimandibles were decalcified for 8 weeks in 4.13% EDTA, washed, dehydrated and embedded in paraffin. Serial sections (5 semi-serial sections of each hemimandible) with 5 mm thickness were cut and stained with hematoxilin and eosin. All histological sections were identified with a random numerical sequence in order to codify experimental periods and groups during the analysis procedures, and the 1st, 3rd and 5th were analyzed by a single calibrated investigator with a binocular microscope (Olympus Optical Co., Tokyo, Japan). The examiner was previously trained and calibrated by an experienced professional in the histology area. Twenty histological sections were evaluated and the results were compared between these examiners. These results showed significant interexaminer concordance (Student t test, $p > 0.05$). After calibration, the blind evaluation initiated, which was performed by replacing the histological section identification by a random number sequence.

Histological analysis was based on qualitative and quantitative evaluations. Initially, the quantification of tooth structure using linear and area measurements was performed to evaluate the loss of tooth structure. Dentin and pulp tissue area and linear measurements were obtained using KS 300, 3.0 software (Kontron Electronic GmbH, Carl Zeiss, Germany). Linear measurements were also performed using the same software as described: M1: measurement from the mesial apex of the mesial cuspid to the mesial alveolar bone crest and M2: measurement from the mesial alveolar bone crest to the apex of mesial root.

A qualitative analysis was also performed to assess the presence of dental caries, which were characterized by the presence of tubules invaded by bacteria, as well as liquefaction foci (Kidd & Fejerskov, 2004). Such histological characteristics are relevant to differentiate lesions caused by occlusal wear and dental caries. The incidence and severity of dental caries was graded as follows: absent; slight, dentin caries localized in occlusal surface of dentin; mild, dentin caries extended into dental pulp with pulpitis and/or pulp necrosis; moderate, dental crowns were partially decayed; and severe, dental crowns were completely decayed (only molar roots remained) (Nakahara, Sano, Kodama, Ozaki, & Matsuura, 2013a). The incidence and severity of apical lesions was also graded as follows: absent, absence of inflammatory cells and bone resorption; mild, localized inflammatory cell infiltration and bone resorption in the apical area, and moderate, extensive inflammatory cell infiltration and bone resorption in the apical area (Nakahara, Sano, Kodama, Ozaki, & Matsuura, 2012; Nakahara, Ozaki, Sano, Kodama, & Matsuura, 2014).

Incidence and severity of periapical lesions were graded as follows: −, absence of inflammatory cells; +, localized inflammatory cell infiltration in the apical area; ++, extensive inflammatory cell infiltration in the apical area (Nakahara et al., 2012, 2014).

Morphometrical pulp tissue analysis was carried out using a 100× immersion objective and a Zeiss kpl 8 X eyepiece containing a Zeiss II integration grid (Carl Zeiss Jena GmbH, Jena, Germany) with 10 parallel lines and 100 points in a quadrangular area. The grid image was successively superimposed on approximately 8 histological fields per histological section, comprising all the pulp area. For each animal, 3 sections were counted and averaged for each component. The points were counted (Pi) to match the images of the following components of pulp tissue (i): collagen fibers, fibroblasts, blood vessels, inflammatory cells, odontoblasts, and necrosis area. Dentin and cement scores were not considered since the region of interest was limited to the pulp tissue. The total number of points (Pt) was then obtained. Volume density was calculated by the equation: Vvi = Pi/Pt. Results were presented as the mean volume density for each structure considered in each examined group.

2.3. Radiographical evaluation

After fixation, the hemimandibles were radiographed with X-707 (Yoshida Dental MFC Co., Ltd., Tokyo, Japan) at 70 kvp, 7 mA with exposure time of 0.17 s and 40 cm source-to-film distance