Periodontitis and diabetes interrelationships in rats: biochemical and histopathological variables

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Abstract

Background A two-way relationship between diabetes and periodontitis is now clinically established. Both conditions share common mechanisms of pathogenesis that are related to altered immune-inflammatory responses at local and/or systemic levels. The aim of this study is to investigate whether periodontitis is associated with the development and progression of diabetes and to evaluate the health impact of coexistence of both diseases.

Material and methods Male Sprague Dawley rats (10 weeks old) were randomized into seven groups ($n = 12$): Group 1) Control; Groups 2 and 6) Periodontitis induced at two or six weeks; Groups 3 and 5) Diabetes induced at two or six weeks; Groups 4 and 7) Periodontitis followed by diabetes, and diabetes followed by periodontitis at two or six weeks. For diabetes induction, animals received a one-time intravenous injection of streptozotocin (50 mg/kg). Periodontitis was induced by Lipopolysaccharide injection (20 µg/rat) through the palatal gingival and by placing a ligature of 0/0 braided silk around the cervix of the upper right second molar. Body weight, glycaemia as well as urine were collected weekly. Rats were sacrificed at the end of week 10, gingival tissue was removed, fixed in formaldehyde and processed for histopathological study.

Results Body weight was significantly decreased (25%) following diabetes induction ($p < 0.01$). 40% weight loss was observed when diabetes was induced first compared to 30% when periodontitis was first induced in the animals ($p < 0.01$). Rats treated with streptozotocin showed a three-fold increase in diabetes compared to the control group ($p < 0.01$). In rats where periodontitis was induced after diabetes, glucose levels increased significantly (450 mg/dL) compared to glucose levels (410 mg/dL) where periodontitis was induced first ($p < 0.01$). Histopathological studies showed greater alveolar bone loss when both diabetes and periodontitis were present.

Conclusion When periodontitis occurs after diabetes it aggravates the symptoms of the two pathologies. When diabetes is induced after periodontitis, no symptoms aggravation is observed for diabetes, although periodontitis gets worse.

Keywords Periodontitis · Diabetes · Rats · Blood glucose · Weight · Histopathology

Introduction

Diabetes (DB) is a global epidemic that affects approximately 171 million people worldwide and is projected to increase to 366 million in 2030 [1]. Diabetes represents a public health problem due to its high prevalence, severe morbidity and mortality. It is considered a metabolic disorder characterized by chronic hyperglycemia due to insulin deficiency or due to insulin inability to promote its effects. Diabetes includes a clinically and genetically heterogeneous group of disorders that affect the metabolism of carbohydrates, lipids and proteins [2, 3]. According to the American Diabetes Association [4] the current classification of diabetes is based on the pathophysiology of each form of the disease. The most common forms of diabetes include type 1 and type 2, respectively.

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Diabetes is associated with a number of metabolic risk factors that contribute to a high rate of micro- and macro-vascular events [5–10]. The risk factors and mechanisms that contribute to the onset and development of these complications are poorly defined. On another hand, the use of reliable biomarkers is becoming increasingly important for the improved management of patients with diabetes and diabetic complications especially cardiovascular injuries. Diabetes is the leading cause of heart disease, stroke, hypertension, blindness and other eye problems, kidney disease, amputation of lower extremities and oral diseases. The heavy burden of diabetes among the population and the trend of increasing prevalence of this condition, present challenges for health care management. The basic mechanisms of diabetes and its complications are not understood and the treatments remain sub-optimal [6–15].

Periodontitis (PD) has been considered as the sixth complication of diabetes after retinopathy, nephropathy, neuropathy, cardiovascular disease and peripheral vascular disease [16]. It had been reported in several studies that patients with diabetes have an increased risk of PD [17–19]. Periodontitis is an inflammatory disease of the periodontium, primarily caused by bacterial infection [20]. In advanced cases of PD, degradation of the connective tissue and destruction of the bone occur, ultimately leading to tooth loss in adults [21]. Current evidence shows that diabetes and persisting hyperglycemia will lead to altered immune-inflammatory response to the periodontal bacterial challenges, which will result in more rapid and severe periodontal tissue destruction [20].

Recent clinical studies have indicated higher prevalence, severity, and progression of PD in patients with diabetes when compared to patients without diabetes, as well as a positive association between blood sugar and the severity of periodontitis. In this context meta-analyses of adjusted estimates showed that diabetes increased the risk of incidence or progression of periodontitis by 86% (RR 1.86 [95% CI 1.3–2.8]). However, there is scarce information on the association between diabetes and periodontal destruction [22–25]. Both PD and DB share common mechanisms of pathogenesis that are related to altered immune-inflammatory responses at local and/or systemic levels [26]. However, despite the remarkable clinical evidence of the negative influence of diabetes on periodontal breakdown, to date there are scarce data concerning the cascade of immune-inflammatory mediators at sites of chronic periodontitis in patients with diabetes [27–33]. Therefore, the precise cellular and molecular mechanisms that could explain the high levels of periodontitis in patients with DB when compared to patients without diabetes remain unclear. In addition, the mechanisms that could lead to a more severe PD in patients with poorly controlled DB when compared to patients with better-controlled DB still remain unknown [22–25]. New evidence regarding the immune-inflammatory profile of the PD associated with

DB may provide a wider therapeutic window for periodontitis in patients with diabetes.

As such, it is important to explore the ‘two-way’ relationship between diabetes and periodontitis, and to compare the effect of periodontitis on diabetes and that of diabetes on periodontitis. Diabetes can be a risk factor for periodontitis, and periodontitis could have a negative effect on glycaemic control. For this purpose, the present study explored the effect of STZ-induced diabetes on ligature-induced periodontitis and vice-versa in rats in terms of body weight, glycemia, glucosuria, ketone bodies and histopathological examination.

The objective of this study was to evaluate the role of periodontitis in the initiation and progression of diabetes and its complications as well as the role of diabetes in the initiation and progression of periodontitis in a rat model using streptozotocin (STZ) to induce diabetes and teeth ligature to mimic gum diseases.

Material and methods

Animals

Male Sprague Dawley rats, weighing 300 ± 20 g were used. Animals were housed in standard polypropylene cages and maintained under standard conditions: a constant temperature of 22 ± 2 °C and humidity ($55 \pm 5\%$) with fixed 12:12-h light-dark cycle. They were fed regular rat chow and tap water ad libitum. Body weight was measured weekly using an electronic balance. The animals used in the present study were maintained in accordance with the principles and guidelines of the Canadian Council on Animal Care as outlined in the Guide for the Care and Use of Laboratory Animals. All the experiments were approved by the Ethics committee of the Lebanese university under the code CUMEB/D 126/ 132,018.

Experimental design

The animals were distributed randomly into seven equal groups ($n = 12$ for each group) and treated for 10 weeks as follows (Fig. 1):

- Group 1: Control rats without any intervention
- Group 2: PD induction in rats at week two
- Group 3: DB induction at week six
- Group 4: PD induction at week two, followed by DB induction at week six
- Group 5: DB induction at week two
- Group 6: PD induction at week six
- Group 7: DB induction at week two, followed by PD induction at week six

Fig. 1 Experimental protocol.

Group 1: Control group of rats without any intervention.
Protocol I: Periodontitis before Diabetes, Group 2: PD induced at week 2 with LPS; Group 3: DB induced with STZ at week 6; Group 4: PD induced at week 2 followed by DB induced at week 6; **Protocol II: Diabetes before Periodontitis**, Group 5: DB induced at week 2 with STZ; Group 6: PD induced at week 6 with LPS; Group 7: DB induced week 2 followed by PD induced at week 6. *PD: periodontitis, DB: diabetes, LPS: Lipopolysaccharide, STZ: streptozotocin*

Induction of diabetes

Diabetes was induced by intraperitoneal (i.p.) injection of streptozotocin (STZ) at a dose of 50 mg/kg (Sigma-Aldrich Inc., USA), dissolved in 0.1 M of a citrate buffer (pH 4.5) freshly prepared. Nicotinamide (100 mg/kg) was injected 15 min before STZ. STZ is a vitamin B3 (niacin) derivate with antioxidant capacity reducing the cytotoxic actions of STZ which does not require exogenous insulin to survive [26]. Citrate buffer was injected alone in control group of rats.

Model for experimental periodontitis

We employed an established rat model of periodontal disease induced by *A. actinomycetemcomitans* LPS. Experimental periodontitis was induced under ketamine (70 mg/kg, i.p.) and xylazine anesthesia (10 mg/Kg, i.p.). LPS from *Aggregatibacter actinomycetemcomitans* (strain Y4, serotype B) was diluted in phosphate-buffered saline (PBS) [27, 28]. Each rat was injected with 20 µg/rat LPS through the palatal gingival between the maxillary 1st and 2nd molars 3 times (at day 8, 10 and 12). Other groups of rats were injected with PBS. A ligature of 0/0 braided silk was placed around the cervix of the upper right second molar.

Glycemia, glucosuria and ketone bodies monitoring

Blood glucose levels were measured in a drop of blood from the tail vein by glucose oxidase method using a glucometer (OneTouch, Ultra, LifeScan, USA) once a week during all of the experimental period. Diabetes was confirmed when the random glucose levels are >200 mg/dl [29].

Fresh urine samples were collected in metabolism cages during week 1, 6 and 10. The presence of glucose in the samples was detected with urine test paper (Biolabo, France). The urinary ketone body was tested using a test stick used clinically for humans Ketostix®.

Histopathological examination

At the end of the experiment, animals were euthanized using high-dose of ketamine. Histopathological examination was performed on the mandibles of all rats. The samples were fixed in 10% neutral-buffered formalin, and subsequently decalcified in a 5% solution of ethylenediaminetetraacetic acid tetrasodium salt (EDTA-4Na) for 2 weeks at 4 °C. After decalcification, the specimens were trimmed, dehydrated in a sequential ethanol series using an automated processor and embedded in paraffin wax. Serial 7-µm-thick sections through the centers of all molars on the mesiodistal plane were obtained and stained with hematoxylin and eosin and examined using light microscopy. The periodontal lesions (gingivitis, marginal periodontitis and apical periodontitis) were evaluated histopathologically by a specialized histopathologist. Samples were classified according to the following parameters: Inflammatory cells, cellular infiltration, alveolar resorption, hyperkeratosis, fibrosis and destruction of cementum. We adopted the following score: 0 = no pathological changes observed; 1 = mild lesions changes covering 10 to 25% of tissue section; 2 = moderate lesions changes covering 25 to 45% of tissue section; 3 = Severe lesions changes covering 45% to 75% of tissue section. To avoid observer bias, the histopathologist was blinded to the study groups, and the data were recorded with respect to the sample codes [30–32].

Statistical analysis

Data were analyzed using the SPSS version 20.0 for windows computer software package (SPSS Inc., Chicago, IL, USA). One way Anova test was used to compare differences in mean change of glycemia and weight across the 7 experimental groups as well as the difference in glycemia and weight at each week across the 7 experimental groups. They were followed by univariate analyses and Bonferonni post hoc test. The degree of organ pathology was then compared between genders using Fisher's exact test; *p*-values smaller than 0.05 ($p < 0.05$) were considered to be statistically significant.

Results

The effect of periodontitis and periodontitis in combination with diabetes on body weight of treated rats during 10 weeks is observed in Fig. 2 (upper panel). Experimental periodontitis (group 2) decreased body weight significantly (359 g) as compared to the control group (409 g) at week 10 ($p < 0.01$). After induction of diabetes, group 3 injected with STZ alone and group 4 injected with STZ after PD showed significantly lower body weight (307 g and 287 g respectively) as compared to groups 2 and 3 ($p < 0.01$). The effect on body weight of STZ alone (week two), PD alone (week 6), and STZ in combination with PD is observed in Fig. 2 (middle panel). During 10-week experiment, PD alone (group 5) induced at week 6 decreased body weight to 354 g and STZ alone induced at week 2 (group 6) decreased body weight to 305 significantly ($p < 0.01$) as compared to the control group (409 g). STZ induction before PD (group 7) displayed a significantly higher weight loss (247 g) when compared with normal and STZ groups ($p < 0.01$). Percent changes of body weight between all groups of rats at week 10 are showed in Fig. 2 (lower panel). Thus a 40% decrease in body weight was observed in group 7 (STZ before PD) compared to a 30% decrease in group 4 (PD before STZ). However this difference is not significant.

Figure 3 (upper panel) shows that there is no significant difference between blood glucose levels of the 4 groups before the administration of STZ. In the control group, blood glucose level stayed around 100 mg/dL. Periodontitis alone induced at week 2 (group 2) had no significant effect on blood glucose levels as compared to the control group (100 mg/dL). At week 6, after injection of STZ, blood glucose levels of diabetic animals with PD (group 3) increased significantly (380 mg/dL) as compared to the control groups ($p < 0.01$). Severe hyperglycemia (410 mg/dL) continued through the last monitoring day in all rats in group 4 (PD + STZ) and displayed significant difference compared to groups 1, 2 and 3 ($p < 0.01$). Figure 3 (middle panel) shows that PD alone (group 5) induced at week 6 had no significant effect on blood glucose levels as compared to the control group. In group 6, STZ alone

induced at week 6 exhibits a significant increase in blood glucose levels (320 mg/dL) when compared to control group (100 mg/dL) ($p < 0.01$). This change was more marked in group 7 at the 10th week following STZ + PD induction (450 mg/dL) when compared with normal and STZ groups ($p < 0.05$). In addition, a mortality of 3 rats was observed at week 10 in the STZ + PD group.

Percent changes of blood glucose between all groups of rats at week 10 are showed in Fig. 3 (lower panel). Results shows statistically significant differences in blood glucose among (PD + STZ) and (STZ + PD) groups. (STZ + PD) induced significantly higher glycemia (450%) in comparison with (PD + STZ) (410%) ($p < 0.01$) Table 1.

The effect of STZ and/or PD induction in rats on urinary volume, glycosuria and ketone bodies is showed in Table 2. Non-diabetic rats with PD displayed values within normal ranges concerning urinary volume, absence of glycosuria and ketone bodies. The group injected with STZ at week 2 showed significant increase in urinary volume (11.56 ± 0.21) at week 10, in addition to a mild presence of glycosuria and ketone bodies. Urinary volume increased significantly ($p < 0.01$) when PD is followed by STZ (PD + STZ) (9.66 ± 0.39) as compared to the control group (5.28 ± 0.30) at week 10. This increase was significantly higher when STZ is followed by PD (STZ + PD) (14.26 ± 0.21) in comparison with Control, STZ and (PD + STZ) groups, in addition to a high presence of glycosuria and ketone bodies at week 10 in (STZ + PD) treated group.

Histological analysis of control group shows that the structure of the periodontium is normal and that the gingiva, periodontal ligament, alveolar bone and cementum can be observed; animals of this group presented a score of 0 (Fig. 4 and Table 2). Rats treated with PD or PD + STZ showed accentuated cellular infiltration, accentuated degradation of the alveolar process and partial destruction of the cementum; animals of this group presented a score of 2 (Fig. 4 and Table 2). The periodontal histopathology of rats subjected to STZ + PD revealed inflammatory cell infiltration coupled with severe destruction of the cementum and alveolar process; animals of this group presented a score of 3 (Fig. 4 and Table 2).

Discussion

Periodontitis is one of the major reasons for adult tooth loss [33] while diabetes is associated with a reduced life expectancy, significant morbidity due to specific diabetes-related microvascular complications, increased risk of macrovascular complications and a diminished quality of life [34, 35].

An epidemiological link between diabetes and periodontitis was established in 1960, and the interaction is classified by age and type of diabetes in most studies [36]. Diabetes has been confirmed as a major risk factor for periodontitis [37,

Fig. 2 Weight. Protocol I: Effect of DB in rats with PD on body weight (upper panel). Group 1: Control (C); Group 2: PD induced by LPS at week 2 (LPS); Group 3: DB induced by STZ at week 6 (STZ) Group 4: PD induced at week 2 followed by DB induced at week 6 (PD + STZ). **Protocol II:** Effect of PD in rats with DB on body weight (middle panel). Group 1: Control (C); Group 5: DB induced at week 2 (STZ); Group 6: PD induced by LPS at week 6 (PD); Group 7: DB induced at week 2 followed by PD induced at week 6 (STZ + PD). **Comparison between all groups at week 10: Percentage of body weight changes (lower panel).** * $p < 0.01$ vs Control. # $p < 0.01$ vs PD. † $p < 0.01$ vs STZ. PD: periodontitis, DB: diabetes, STZ: streptozotocin, LPS: lipopolysaccharide

38]. Findings from the Third National Health and Nutrition Examination Survey (NHANES III) in the US indicated that the prevalence of diabetes among people with periodontal disease was about two-fold higher than that of periodontally healthy diabetic subjects [39]. The level of glycemic control is of key importance in determining increased risk. Studies also showed an association between the severity of periodontitis and glucose intolerance, signs of metabolic syndrome, and additional diabetes-related complications, such as cardiovascular problems [40, 41].

Our data showed that diabetes reduced body weight, which is in accordance with findings by Mishima et al. [42]. Periodontitis affected body weight but to a lesser degree than diabetes, which is consistent with the findings of Bezerra et al. [43], but not those of Cetinkaya et al. [44]. The induction of periodontitis following diabetes aggravates the reduction in body weight in comparison with the induction of diabetes after periodontitis. This effect appeared immediately after STZ injection; worsen at six weeks and even further at eight weeks. In this context, a validation of the diabetes model was

Fig. 3 Glycemia. Protocol I: Effect of DB in rats with PD on blood glucose level (upper panel). Group 1: Control (C); Group 2: PD induced by LPS at week 2 (LPS); Group 3: DB induced by STZ at week 6 (STZ); Group 4: PD induced at week 2 followed by DB induced at week 6 (PD + STZ). **Protocol II:** Effect of PD in rats with DB on blood glucose level (middle panel). Group 1: Control (C); Group 5: DB induced at week 2 (STZ); Group 6: PD induced by LPS at week 6 (PD); Group 7: DB induced at week 2 followed by PD induced at week 6 (STZ + PD). **Comparison between all groups at week 10: Percentage of blood glucose changes (lower panel).** * $p < 0.01$ vs Control. # $p < 0.01$ vs STZ. † $p < 0.01$ vs (PD + STZ). PD: periodontitis, DB: diabetes, STZ: streptozotocin, LPS: lipopolysaccharide

evidenced by sustained hyperglycemia after the 50 mg/kg STZ injection. These observations are in agreement with previous results in rats [45, 46].

Likewise, periodontitis superimposed on diabetes affected glucose levels more than diabetes after periodontitis in our model. Our findings are in accordance with the results of Holzhausen et al. [45] that reported significantly higher glucose levels in molar ligated diabetic rats than in diabetic rats.

Periodontitis alone did not alter glucose levels in this study, as demonstrated by the absence of differences between ligated and control rats, which is in line with observations by Gomes et al. [46]. In the present study however, the increase in urinary volume, glycosuria and ketone bodies were more pronounced 10 weeks following the induction of DB and PD.

Histological examination showed increased alveolar bone inflammation and alveolar bone loss in the periodontium of

Table 1 Effect of diabetes (DB) and/or periodontitis (PD) induction in rats on urinary volume, glycosuria and ketone bodies

Groups	Week	Parameters		
		Urinary Volume (mL)	Glycosuria	Ketone bodies
Control	1	5.44 ± 0.26	–	–
	6	5.57 ± 0.27	–	–
	10	5.28 ± 0.30	–	–
PD induction week 2	1	5.32 ± 0.22	–	–
	6	5.17 ± 0.26	–	–
	10	5.47 ± 0.28	–	–
DB induction week 6	1	5.41 ± 0.27	–	–
	6	7.83* ± 0.23	+	–
	10	8.42* ± 0.32	+	+
PD + STZ	1	5.37 ± 0.27	–	–
	6	8.92* ± 0.33	+	–
	10	9.66* ± 0.39	+	+
STZ induction week 2	1	5.34 ± 0.29	–	–
	6	8.63* ± 0.30	+	+
	10	11.56* ± 0.21	+	+
PD induction week 6	1	5.30 ± 0.26	–	–
	6	5.22 ± 0.22	–	–
	10	5.32 ± 0.31	–	–
STZ + PD	1	5.39 ± 0.24	–	–
	6	11.33* ^{#†} ± 0.25	+	+
	10	14.26* ^{#†} ± 0.21	++	++

Group 1: control; **group 2:** PD induced at week 2; **group 3:** DB induced at week 6; **group 4:** PD induced at week 2 followed by DB induced at week 6; **group 5:** dB induced at week 2; **group 6:** PD induced at week 6 and **group 7:** DB induced at week 2 followed by PD at week 6

* $p < 0.01$ vs Control. # $p < 0.01$ vs STZ. † $p < 0.01$ vs (PD + STZ)

PD periodontitis, DB diabetes, LPS Lipopolysaccharide, STZ streptozotocin

rats treated with STZ and presenting periodontitis. The combination of diabetes and periodontitis led to greater bone loss than periodontitis alone. The present finding is in accordance with the radiographic findings by Gomes et al. [46]. However, it contradicts results by Holzhausen et al. [45] that showed

similar bone loss severity between diabetic ligated and non ligated molar in rats.

Diabetes was thought to mediate the periodontal status through the direct impact of hyperglycemia and by modulating advanced glycation end-products (AGEs), adducts from

Table 2 Histological analysis

Groups Parameters	Control	PD week 2	STZ week 6	PD + STZ	STZ week 2	PD week 6	STZ + PD
Inflammatory cells	0	2	1	3	1	2	3
Cellular infiltration	0	2	1	3	1	2	3
Alveolar resorption	0	1	0	2	0	1	3*
Hyperkeratosis	0	2	0	2	0	2	3*
Fibrosis	0	1	0	2	0	1	3*
Destruction of cementum	0	1	0	2	0	1	3*

Group 1: control; **group 2:** PD induced at week 2; **group 3:** DB induced at week 6; **group 4:** PD induced at week 2 followed by DB induced at week 6; **group 5:** dB induced at week 2; **group 6:** PD induced at week 6 and **group 7:** DB induced at week 2 followed by PD at week 6. The parameters were scored as follows: (0) absent, (1) slight, (2) moderate, (3) marked and represented as median values

* $p < 0.05$ versus PD + STZ

PD periodontitis, DB diabetes, LPS Lipopolysaccharide, STZ streptozotocin

Fig. 4 Histopathological analysis. Sections were stained with H&E. **a** normal periodontium; **b** periodontium from a rat treated with streptozotocin (STZ) showing moderate cellular infiltration and minor alveolar resorption and intact cementum; **c** accentuated cellular infiltration and severe destruction of the cementum of rat presenting Diabetes before Periodontitis (STZ + PD). (KL) keratinized layer, (CT) connective tissue, (SL) spiny layer, (F) fibroblast, (IC) inflammatory cell, (Hk) hyperkeratosis

the glycation and oxidation of proteins and lipids, [47] leading to an overall impairment of wound healing and changes in periodontal tissues. Sustained hyperglycemia impacts the periodontium by altering microvasculature, increasing collagenase activity, prolonging the inflammatory response and impairing host defense against microbial challenges, wound healing and new bone formation. It has been suggested that periodontitis severity might be greater in diabetics with more severe systemic complications [48].

While the effects of diabetes on periodontitis are more clearly elucidated, there is still limited information regarding how periodontal diseases influence diabetic states. Periodontitis is primarily an oral infection caused by gram-negative anaerobes. The main virulence factors of these microorganisms are endotoxins in the form of LPSs [49]. The pathogenesis is triggered by recognition of pathogen-associated molecular patterns from Toll-like receptors (TLRs), which release ROS from defending cells and subsequently induce oxidative stress, proinflammatory cytokines, and immunoregulatory complexes through the NF- κ B pathway [50, 51]. Periodontitis may induce systemic conditions through translocation of periodontal microorganisms and their products from periodontal biofilms or direct cytokinemia into the circulation [52].

Severe periodontitis at baseline was associated with an increased risk of poor glycemic control. In addition, various studies have reported that the prevalence and severity of non-oral diabetes-related complications, including retinopathy, diabetic neuropathy, proteinuria and cardiovascular complications are correlated with the severity of periodontitis [53]. However, our data showed that PD following DB aggravates symptoms of the two pathologies, in accordance with previous studies [22–25].

Our study demonstrated that coexistence of STZ-induced diabetes followed by ligature induced periodontitis significantly decreased body weight, significantly increased

glycemia, urinary volume, glycosuria, ketone bodies and the severity of alveolar bone loss observed in rats.

Based on the link between diabetes and periodontitis, clinical guidelines were developed to predict undiagnosed diabetes cases. The potential mechanistic interrelationships between diabetes and periodontitis may also direct future therapeutic interventions. In summary, an in-depth understanding of the possible mechanisms linking periodontal disease and diabetes, in terms of periodontal destruction and periodontal healing, is essential to the future development of treatment strategies for patients with diabetes and periodontal disease.

Author's contribution ZS conceived the study and manage funds, RC designed, performed the experiments and analyze the data, CC participate to experiments and wrote the paper; PZ review the paper. All authors read and approved the final version.

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Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Abbreviations DB, Diabetes; PD, Periodontitis; STZ, Streptozotocin; LPS, Lipopolysaccharide; SD, Standard deviation

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