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Research article

Periodontitis promotes the progression of diabetes mellitus by enhancing autophagy

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ABSTRACT

Objective: This study aims to identify the periodontitis factor that activates excessive autophagy in pancreatic β cells, resulting in organic lesions of pancreatic islet tissues and diminished insulin secretion, thereby accelerating the progression of diabetes mellitus (DM).
Methods: Sprague-Dawley (SD) rats were induced with periodontitis (PD), type 2 diabetes mellitus (T2DM), or the combination of T2DM and PD (DP) through a high-sugar/high-fat diet and ligation of the tooth neck with silk thread. Alveolar bone resorption was assessed using Micro-CT, blood glucose levels were measured with a blood glucose meter, pancreatic tissue pathology was examined through HE staining, and the expression of autophagy-related proteins Beclin1 and LC3II/LC3I was analyzed using Western blotting.
Results: Micro-CT results revealed more pronounced alveolar bone resorption and root bifurcation exposure in the PD and DP groups compared to the control group, with the DP group exhibiting the most severe condition. HE staining demonstrated the formation of periodontal pockets, severe alveolar bone destruction, and abnormal pancreatic islet tissue morphology in the PD and DP groups. The serum levels of IL-6, TNF-α, and IL-1β increased sequentially in the control, DM, PD, and DP groups ($P < 0.05$). Relative expressions of *GCK* and *GLUT-2* mRNA decreased in the PD group compared to the control group ($P > 0.05$), while the mRNA expressions in the DP and DM groups increased ($P < 0.05$), with the DP group exhibiting higher levels than the DM group ($P < 0.05$). Western blot results indicated increased expression levels of autophagy proteins Beclin1 and LC3II/LC3I in the DM and DP groups compared to the control group ($P < 0.05$), with the DP group exhibiting higher levels than the DM group ($P < 0.05$).
Conclusion: The findings demonstrate that periodontal inflammatory factors may promote the enhancement of pancreatic cell autophagy in diabetic rats.

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1. Introduction

Type 2 diabetes mellitus (T2DM) is a metabolic disorder characterized by chronic hyperglycemia with multiple etiologies, yet its full etiology remains incompletely understood. The primary manifestations include poor insulin secretion, low insulin sensitivity, and insulin resistance (IR) [1–3]. Given the high morbidity and mortality associated with diabetes mellitus (DM) and its various complications such as cardiovascular disease (CVD), diabetic nephropathy (DN), and oral diseases, DM has emerged as a global health concern [4,5]. The most significant challenge for medical practitioners currently is the effective control of blood sugar. Prolonged hyperglycemia can lead to periodontal tissue lesions in patients, with scholars identifying periodontitis (PD) as the sixth major complication of DM in 1993 [6].

Our research team has extensively investigated the impact of T2DM on PD and the associated treatment measures [7–9]. In recent years, the reciprocal impact of PD on T2DM has garnered attention. The current understanding suggests that when the periodontal microecosystem becomes imbalanced, bacteria enter the bloodstream through ulceration of local periodontal inflammatory tissues, inducing increases in serum reactive oxygen species (ROS) and proinflammatory cytokine levels, thereby exacerbating glucose metabolism syndrome and IR [10]. T2DM patients with severe PD, compared to those with mild PD, exhibit decreased neutrophil chemotaxis [11] and apoptotic neutrophils [12]. In a study involving 371 T2DM patients receiving basic periodontal treatment, HbA1c levels decreased by an average of 0.40 % at 3–9 months of follow-up [13], suggesting that effective periodontal treatment contributes to blood glucose control to a certain extent [14]. Following the interference of periodontitis factors in the T2DM rat model, glucose tolerance was further reduced compared with diabetic rats alone, supporting the hypothesis that periodontitis worsens glycemic control.

Autophagy, a programmed mechanism wherein cells rely on lysosomes to "self-digest," involves the degradation of longevity proteins, self-senescent cells, and damaged organelles through a highly conserved mechanism [15]. Research by Goginashvili [16] and others indicates that the level of β cell autophagy is directly proportional to the level of insulin, and that autophagy may be implicated in the process of insulin storage and release. Moreover, the addition of an autophagy activator can significantly reduce the number of pancreatic β cells undergoing lipopolysaccharide (LPS)-mediated apoptosis and increase insulin secretion capacity. These findings suggest that proper autophagy plays a crucial role in the physiological functions of pancreatic β cells. LPS may induce excessive autophagy in silicotic macrophages, exacerbating apoptosis in lung tissue through the accumulation of autophagosomes [17]. Marsh [18] demonstrated that up-regulating autophagy levels in pancreatic islet β cells in mice can accelerate intracellular insulin degradation, consistent with Goginashvili's results [16], suggesting that excessive autophagy may lead to relatively inadequate insulin stores. Simultaneously, electron microscope studies by Masini et al. [19] revealed a significantly higher incidence of cells displaying signs of autophagy-induced death in patients with T2DM compared to the control group.

These pieces of evidence suggest that autophagy-induced death may contribute to a decrease in the number of β cells, thereby exacerbating the progression of diabetes. However, whether periodontal inflammatory factors induce excessive autophagy in pancreatic β cells, thereby reducing insulin secretion and promoting the progression of T2DM, remains unclear. To address this gap, our team conducted an experiment in rats to explore these topics.

2. Materials and methods

2.1. Rat model establishment

One hundred healthy male Sprague-Dawley (SD) rats, aged 6 weeks, and weighing between 180 g and 220 g, were individually housed in cages maintained at a temperature of 22 ± 2 °C and relative humidity of 60 ± 5 %, with natural ventilation on a 12-h light/dark cycle. The rats were provided ad libitum access to food and water. Rats in the DM group and the T2DM with PD (DP) group were subjected to a high-fat/high-sugar diet for 6 weeks and intraperitoneally injected with 1 % streptozotocin (STZ) (in citric acid-sodium citrate buffer) at a dose of 35 mg/kg body weight (0.1 mmol/L, pH 4.4, 1 g STZ dissolved in 100 mL). Following a 12-h fast, blood glucose levels were measured from the tail vein using a glucose meter one week and 3 days after STZ injection. Rats with a stable fasting blood glucose (FBG) level ≥11.1 mmol/L for one week were selected as T2DM model rats. A probe was used to separate the buccal-lingual gums from the left and right maxillary first and second molars (Molar1, M1; Molar2, M2) in the PD group and DP group, and the tooth neck was ligated with double-stranded 3-0 silk thread for 8 weeks. The weight of the rats in each group was monitored at the 0th week, 6th week, and 14th week using electronic scales. FBG levels were measured at weeks 0, 6, 7 (1 week after STZ injection), and 14 to observe changes in the FBG levels. At 14 weeks, rats in each group were euthanized, and maxillary alveolar bone and pancreatic tissue were collected for correlation analysis. Alveolar bone resorption was assessed to confirm the success of PD modeling. Animal handling adhered to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised in 1996). This study ([2016]1–013) received approval from the Animal Experiment Ethics Committee of Zunyi Medical University, Guizhou.

2.2. Micro-CT analysis

Three alveolar bone specimens, fixed in 4 % paraformaldehyde, were randomly selected from each experimental group and subsequently sent to the Affiliated Stomatological Hospital of Chongqing Medical University for scanning. The scanning parameters were set as follows: voltage: 70 Kvp, current: 114 μA, power: 8 W, and resolution: 15 μm. The acquired meso-distal tomographic images were analyzed using VGstudio Max3.0 software. The distance between the mesial and distal cemento-enamel junction to the alveolar bone

crest (CEJ-ABC) of the first and second molars was independently measured three times for each specimen. Alveolar bone loss (ABL) was then calculated by averaging these three measurements.

2.3. Pathological analysis

Maxillary alveolar bone specimens were collected and fixed in 4 % paraformaldehyde. Following a 24-h fixation period, the specimens were transferred to 10 % EDTA for decalcification. Successful decalcification was confirmed by the ease of penetration with a needle, indicating the absence of resistance. Concurrently, pancreatic tissue samples, approximately 50 mm × 50 mm in size, were obtained and sent to the Department of Pathology at the Affiliated Hospital of Zunyi Medical University for paraffin embedding, sectioning, staining, and subsequent analysis.

2.4. Enzyme-linked immunosorbent assay (ELISA)

On the 14th weekend, rats from each experimental group were anesthetized with 10 % chloral hydrate. Subsequent to achieving anesthesia, abdominal aortic blood was drawn, allowed to stand for 3 h, and then centrifuged in a high-speed centrifuge at 3000 r/min for 10 min. The resulting serum was collected and stored in a refrigerator at −20 °C. The stored serum was later utilized for analysis in accordance with the manufacturer's protocol.

2.5. Real-time fluorescent quantitative PCR (qPCR)

Cells underwent three washes with ice-cold PBS, and total cellular RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Subsequently, 20 mL of total RNA was reverse transcribed into single-stranded cDNA using reverse transcriptase and oligo-dT primers, following the manufacturer's instructions (Bioengineering Co., Ltd., Shanghai) (Table 1). The cDNA was then PCR amplified, and SYBR Green Mix was employed for quantifying the levels of the amplified products through real-time fluorescent qPCR. Each reaction underwent three repetitions. The GAPDH gene served as an internal control. The reaction conditions for GAPDH were as follows: (1) 95 °C for 30 s; (2) 40 cycles of 95 °C for 5 s, and 60 °C for 30 s; and (3) 95 °C for 10 s, 65 °C for 5 s, and 95 °C for 5 s.

2.6. Western blot analysis

Samples were lysed on ice using RIPA lysis buffer. The resulting supernatant was collected, and the protein concentration was assessed with a BCA kit (Beyotime) following the manufacturer's instructions. Equal amounts of protein were then separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA). Following a 1-h blocking period in 5 % skimmed milk powder in TBST, the membranes were incubated with the primary antibody overnight at 4 °C, followed by treatment with an HRP-conjugated anti-rabbit secondary antibody (Cell Signaling Technology). Subsequently, an enhanced chemiluminescence kit (West Pico; Thermo Fisher Scientific) was employed for signal development. GAPDH served as the internal reference. The Odyssey system was utilized for analyzing the target protein bands and capturing images. The expression level of the target protein was determined, and the experiment was repeated three times.

2.7. Statistical analysis

The data are presented as the mean ± standard deviation. Statistical analyses were conducted using t-tests for two groups or one-way ANOVA (Tukey's test) for multiple groups with Prism 7 software (GraphPad Software, San Diego, CA, USA). Each experiment was repeated in triplicate, and a significance level of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Establishment of rat T2DM and PD models

No significant differences were observed in body weight or fasting blood glucose (FBG) levels among rats from each group at the

Table 1
Primer sequences for the target genes.

Gene	Primer sequence
GAPDH	Upstream: AATGGGCAGCCGTTAGGAAA Downstream: GCGCCCAATACGACCAAATC
GCK	Upstream: ACTCACAACGGGCACATGAA3 Downstream: AGCACAAGTCGTACCAGCTC
UCP-2	Upstream: TGTGGTAAAGGTCCGCTTCC Downstream: TGAGCTTGCTTTATGGGCGA
GLUT-2	Upstream: AATTCACCTCCGCTTGCTCC Downstream: ACCATTCCGCCTACTGCAAA

start of the experiment (0 weeks) ($P > 0.05$). By the 6th week, the weight of the PD group was comparable to that of the control group ($P > 0.05$), while the DM group and the DP group exhibited increased weights ($P < 0.05$) compared to the control. There were no significant differences in weight between the DM group and the DP group ($P > 0.05$) (Fig. 1-A). By week 7, FBG levels showed no significant difference between the PD group and the control group ($P > 0.05$). However, FBG levels in both the DM group and the DP group were significantly elevated ($P < 0.05$). There were no significant differences in FBG levels between the DM group and the DP group ($P > 0.05$) (Fig. 1-B). At the 14th week, compared to the control group, the weights of the PD group, the DM group, and the DP group displayed a declining trend ($P > 0.05$), with the weight of the DP group being lower than that of the DM group ($P < 0.05$). Additionally, in comparison to the control group, both the DM group and the DP group exhibited a significant increase in FBG levels ($P < 0.05$), and the FBG level of the DP group was higher than that of the DM group ($P < 0.05$) (Fig. 1-A, B).

3.2. *Pathological changes in periodontal and pancreatic tissues of rats from each group*

In the control group (Fig. 1-Ca) and DM group (Fig. 1-Cb), the gingival epithelium of rats remained intact, the combined epithelium was situated at the enamel-cementum boundary, collagen fibers were neatly arranged, and the alveolar crest height was normal. Conversely, in the PD group (Fig. 1-*Cc*), the combined epithelium extended to the root, forming a deep periodontal pocket; the arrangement of collagen fibers was disordered, and the height of the alveolar bone was reduced. These findings indicated the successful construction of the PD rat model, with more severe alveolar bone resorption observed in both the DP and PD groups (Fig. 1-Cd).

In both the control group and the PD group, the structure of the islets was normal, with evenly distributed and densely arranged cells. The peritoneum was abundant, and a clear separation existed between the islets and exocrine glands, with the surrounding glands tightly arranged (Fig. 1-Da, Dc). In the DM group, the boundary between pancreatic islet cells and exocrine glands became unclear, islet cells were loosely arranged, exhibiting vacuolar degeneration, and the surrounding gland cells were more loosely arranged (Fig. 1-Db). In the DP group, the islet volume was significantly smaller, with noticeably fewer islet cells, and the surrounding glandular cells were more loosely arranged compared to the DM group (Fig. 1-Dd).

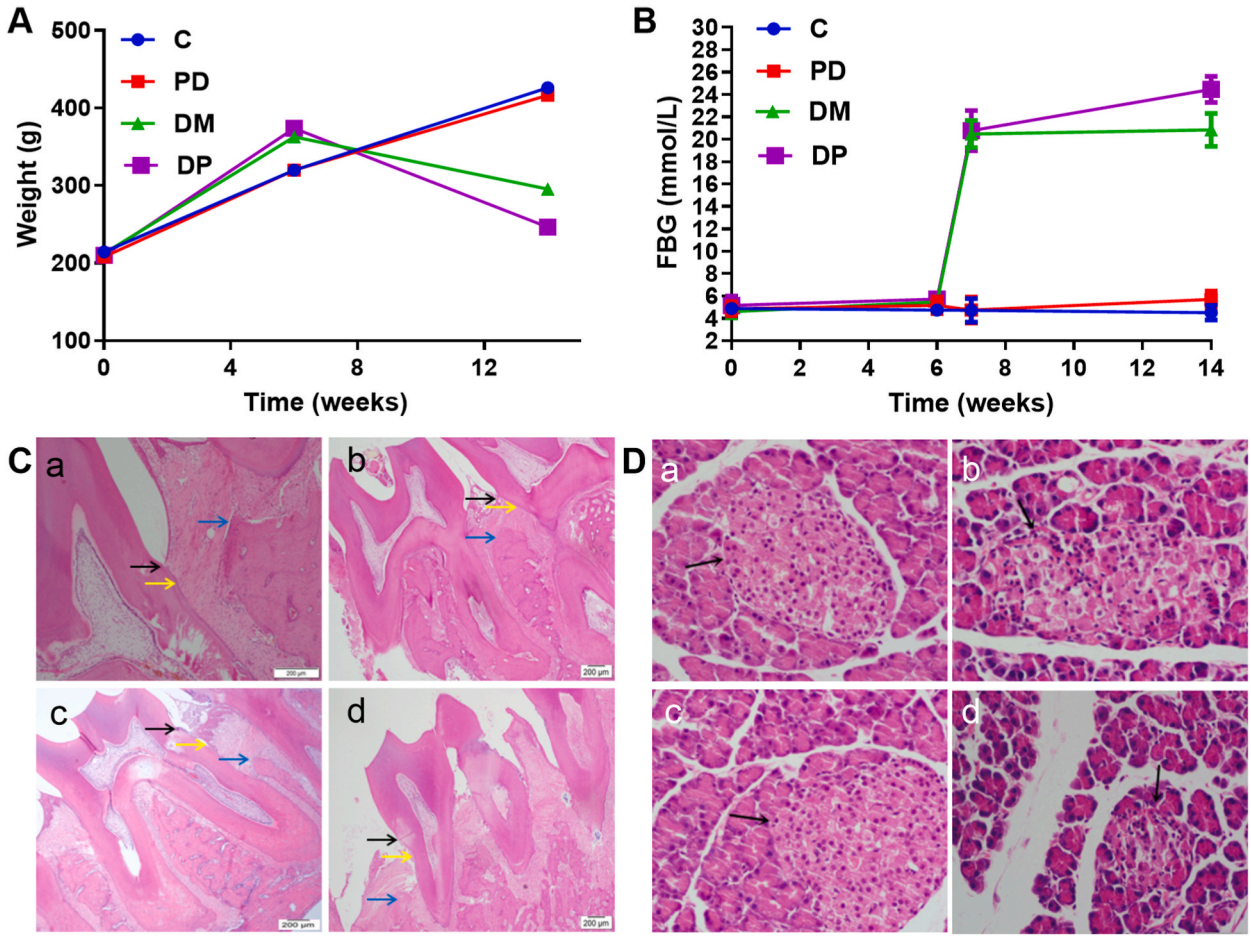

Fig. 1. (A) Changes in the body weight of rats in each group. (B) Changes in the FBG levels of rats in each group. (C) Pathological changes in rat periodontal tissue, (400x). (D) Histopathological changes in the rat pancreas. (a: control group; b: DM group; c: PD group; d: DP group).

3.3. Micro-CT analysis of alveolar bone resorption in each group of rats

The 3D scan results revealed no evident resorption in the alveolar bone of the first and second molars in both the control group and the DM group (Fig. 2-Aa, Ab). In contrast, the PD group and the DP group exhibited exposed alveolar bone roots, with the most severe root tissue damage observed in the DP group (Fig. 2-Ac, Ad). The distance between the cemento-enamel junction and the alveolar bone crest (CEJ-ABC) was measured (Fig. 2-Ba), and it was determined that the alveolar bone resorption value of the DM group did not significantly differ from that of the control group ($P > 0.05$). However, the alveolar bone resorption value increased in both the PD group and the DP group ($P < 0.05$). Furthermore, compared to the DM group, the alveolar bone resorption value was higher in both the PD group and the DP group ($P < 0.05$), with the DP group exhibiting a higher value than the PD group ($P < 0.05$) (Fig. 2-Bb).

3.4. Analysis of serum inflammatory factor levels in rats from each group

The expression levels of serum inflammatory factors, including IL-6, IL-1β, and TNF-α, in the DM group, PD group, and DP group were significantly higher than those in the control group ($P < 0.05$). Moreover, the expression levels of these serum inflammatory cytokines (IL-6, IL-1β, and TNF-α) in both the PD group and the DP group were elevated compared to the DM group ($P < 0.05$). Notably, the expression levels of these factors in the DP group were higher than those in the PD group ($P < 0.05$) (Fig. 3-A, B, C).

3.5. RT-qPCR analysis of the expression of the insulin secretion-related genes glucokinase (GCK), glucose transporter 2 (GLUT-2), and uncoupling protein 2 (UCP-2) in the pancreatic tissues of rats from each group

The relative expression levels of the *GCK* and *GLUT-2* genes in the PD group, DM group, and DP group were found to be lower than those in the control group ($P < 0.05$). Specifically, the expression of the *GCK* and *GLUT-2* genes in the PD group was higher than that in the DM group ($P < 0.05$), while the relative expression of *GCK* and *GLUT-2* in the DP group was lower than that in the DM group ($P < 0.05$). Furthermore, the expression of *UCP-2* in both the DM group and DP group, as well as the PD group, was higher than that in the

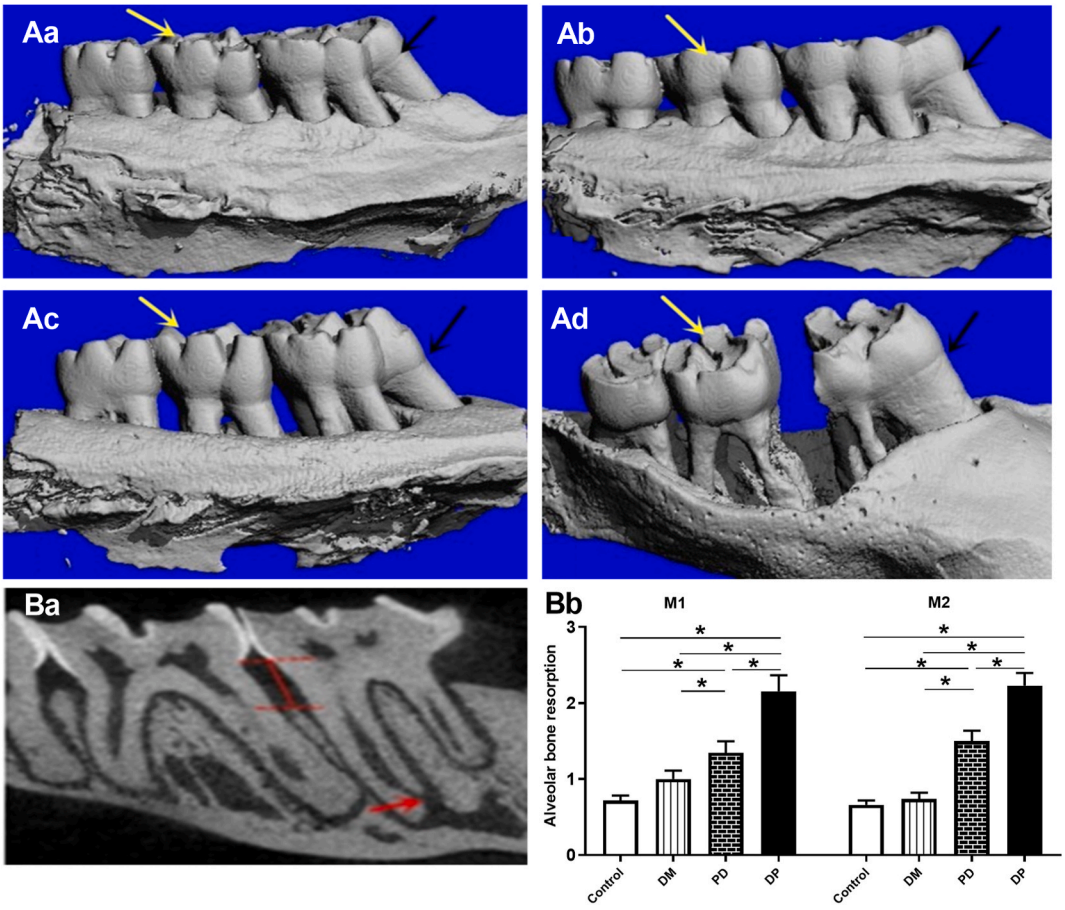

Fig. 2. (A) 3D diagram of alveolar bone resorption in rats from each group. (B) A diagram showing the CEJ-ABC distance (200x). (a: control group; b: DM group; c: PD group; d: DP group).

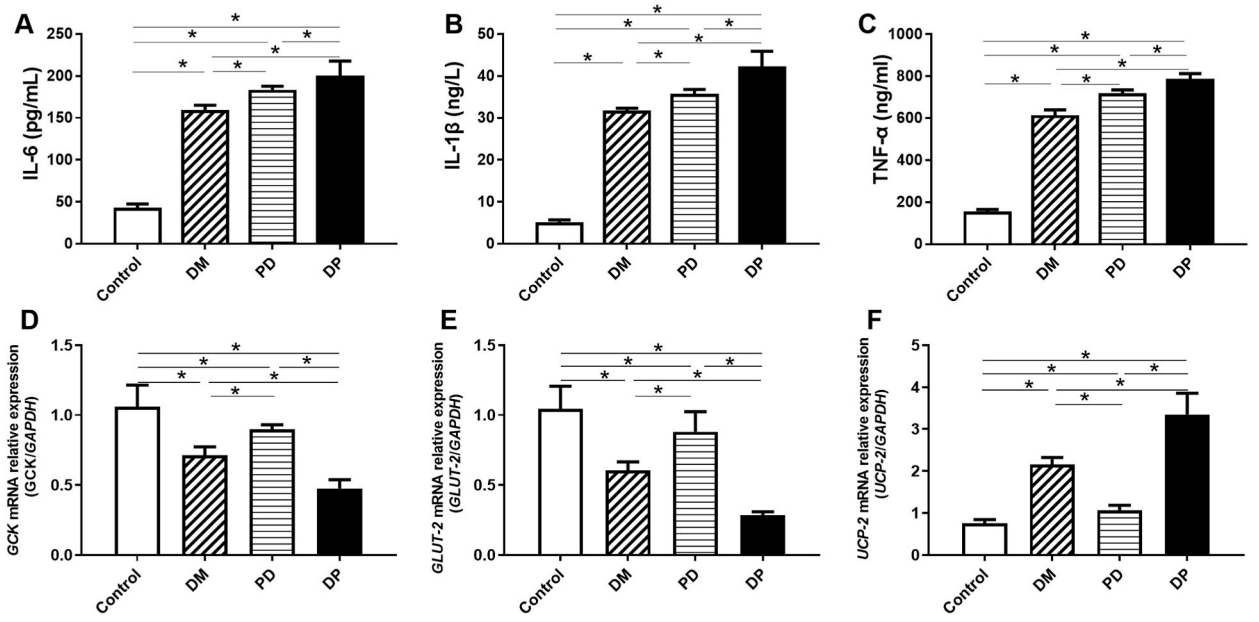

Fig. 3. The levels of IL-6 (A), IL-1β (B), TNF-α (C), The mRNA levels of *GCK* (D), *GLUT-2* (E) and *UCP-2* (F) in the serum of rats in each group. The data are expressed as the mean ± SD (n = 6) (*$P < 0.05$).

control group ($P < 0.05$). Interestingly, the expression of *UCP-2* in the PD group was lower than that in the DM group ($P < 0.05$), and the expression of *UCP-2* in the DP group was higher than that in the DM group ($P < 0.05$) (Fig. 3-D, E, F).

3.6. Protein expression of Beclin1 and LC3II/LC3I in the rat pancreas

In comparison to the control group, the expression of the autophagy-related protein Beclin1 and the LC3II/LC3I ratio in the PD group exhibited an upward trend, although the difference was not statistically significant ($P > 0.05$). Conversely, the expression of Beclin1 and the LCII/LC3I ratio increased significantly in both the DM group and DP group ($P < 0.05$), with the DP group demonstrating a higher expression than the DM group ($P < 0.05$) (Fig. 4-A, B, C).

4. Discussion

PD exerts an adverse impact on metabolic control in individuals with T2DM, thereby compromising blood sugar regulation in these patients [20]. Moreover, individuals afflicted with PD face an increased susceptibility to various complications associated with T2DM [21]. The influence of PD on blood glucose management in T2DM may be attributed to its impact on the metabolic status of affected individuals. Elevated levels of inflammatory mediators in the plasma of PD patients, which possess an antagonistic effect on insulin, are counterproductive to effective blood glucose control in T2DM [22]. Furthermore, in a T2DM rat model, interference with periodontal virulence factors resulted in a more pronounced reduction in glucose tolerance compared to the normal T2DM rat model. This suggests that PD exacerbates the impairment of blood glucose control in the T2DM rat model [23]. Research has substantiated the crucial role of autophagy in preserving the structure and function of pancreatic β cells and mitigating IR [24]. Nevertheless, the precise mechanisms

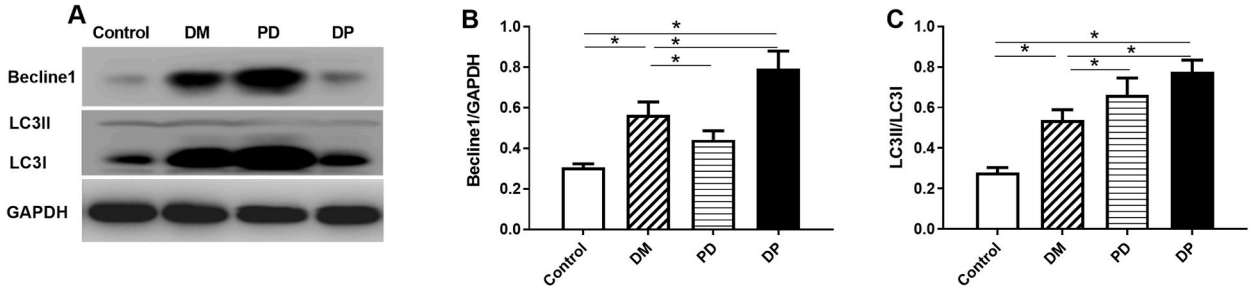

Fig. 4. The protein levels of Beclin1 (B) and the LC3II/LC3I ratio (C). The expression level of Beclin1 and the LC3II/LC3I ratio in the pancreatic β cells of rats from each group. The data are expressed as the mean ± SD (n = 6) (*$P < 0.05$).

governing the interactions between autophagy and pancreatic β cell function, as well as between autophagy and IR, remain unclear. In patients with T2DM, inflammatory mediators and ROS can disrupt pancreatic β cell autophagy, resulting in functional defects and even apoptosis of β cells [25]. Consequently, our study explores and observes the promotional effect of PD on the development of T2DM, shedding light on the intricate connections between periodontal health, metabolic regulation, and pancreatic β cell function.

PD, T2DM, and a combined DP rat model have been well-established [26,27]. After a comprehensive review of the literature, it became evident that SD rats were a reliable and judicious choice for our research. Specifically, the Zucker Diabetic SD rat serves as a well-established animal model designed for preclinical investigations in the realm of T2DM. This unique strain originated through crossbreeding Charles River Crl; SD rats with Zucker Diabetic Fatty (ZDF) lean homozygous wild-type ($^{+/+}$) rats, devoid of the leptin receptor mutation (fa) [28,29]. Rigorous selective inbreeding spanning over 30 generations solidified the establishment of the SD rat [28]. Notably, the phenotypic and genotypic attributes of the SD rat render it potentially more advantageous for the translation of preclinical findings into clinical applications compared to the ZDF strain. For instance, the absence of the leptin receptor mutation (fa/fa) in SD rats aligns more closely with the physiological characteristics of humans, as leptin receptor mutations are infrequent in the human population [30]. Consequently, the mechanisms underpinning T2D in SD rats may exhibit greater similarity to those observed in humans. Moreover, Zucker Diabetic-Sprague Dawley rats manifest age-dependent and reproducible progression, mirroring qualitative parallels to prediabetic alterations in blood glucose concentration and insulin sensitivity observed in humans [28,31,32]. This alignment in progression enhances the translational relevance of findings derived from this rat model to human T2D scenarios.

In this investigation, a successful construction of a T2DM model was achieved by subjecting rats to a high-fat/high-sugar diet and intraperitoneally injecting them with STZ. Additionally, a chronic PD model was effectively established by ligating the tooth neck with a silk thread. Furthermore, a combination of both conditions, a Diabetic-Periodontal (DP) model, was developed by implementing a high-fat/high-sugar diet, intraperitoneal STZ injection, and tooth neck ligation with a silk thread. PD, characterized by alveolar bone loss, is known to influence bone mass and trabecular bone microstructure. In a relevant overseas study, researchers utilized a silk thread to ligate the M2 molar neck for two months, observing a significantly greater amount of alveolar bone absorption in the experimental group compared to the control group. This method was deemed effective in establishing a PD model [27]. In our experiment, mechanical damage was applied to the gingival junction epithelium of bilateral M1 and M2 molars. Subsequently, tooth neck ligation and burial in the gingival sulcus were executed to expedite the accumulation of food residue, formation of dental plaque, and attachment to the tooth neck. Rats subjected to silk thread ligation exhibited evident PD lesions in maxillary alveolar bone pathological sections, including epithelium migration to the root in periodontal tissue, loss of attachment, and alveolar bone resorption. Micro-CT scans demonstrated a significant increase in alveolar bone resorption in rats with PD, with the root bifurcation exposed. Notably, the most severe changes were observed in the DP group ($P < 0.05$), affirming the successful establishment of a chronic PD model.

The approach by David et al. involved feeding 8-week-old male rats a high-sugar/high-fat diet for 56 weeks, coupled with periodic low-dose STZ injections. Results indicated a significant increase in blood sugar levels, a notable reduction in pancreatic islet cell count, and a propensity for bleeding or bleeding failure [33]. These findings closely paralleled our experiment, wherein both the DM and PD groups exhibited a sharp increase in blood sugar levels at 6 weeks under high-sugar diet feeding, surpassing 11.1 mmol/L at 14 weeks. Remarkably, the DP group demonstrated a significantly higher blood sugar level than the DM group. At eight weeks, the control group and PD group displayed higher weights than the DM group and DP group, with a subsequent reversal of this trend. HE staining of the pancreas revealed unclear separation of pancreatic islet cells and exocrine glands in the DM group, accompanied by loosely arranged cells with vacuolar degeneration. These changes were more pronounced in the DP group.

In accordance with the inflammation theory, T2DM is posited to alter the inflammatory response of the body's cells to oral pathogens. Stimulation of monocytes from individuals with both PD and T2DM with LPS derived from *P. gingivalis* revealed elevated levels of inflammatory factors such as TNF-α and IL-1β in peripheral blood. Concurrently, serum cholesterol and triglyceride levels increased, accelerating the progression of T2DM [34]. Scientific investigations have established that IL-1β can promote an upsurge in Fas/Fas ligand (FasL) expression in β cells, inducing β cell apoptosis through T cell participation. TNF-α, on the other hand, activates nuclear factor kappa-B (NF-κB), causing abnormal serine phosphorylation of insulin receptor substrate-1 (IRS-1). This, in turn, inhibits tyrosine phosphorylation, disrupts signal transmission following insulin-receptor binding, hampers glycogen synthesis, and reduces insulin sensitivity, resulting in IR [35]. In our study, both the T2DM and PD models exhibited increased serum levels of the inflammatory cytokines IL-1β, IL-6, and TNF-α. Notably, these levels were higher in the PD model, suggesting that, when periodontal tissue is compromised, stimulating factors may enter the bloodstream through the disrupted periodontal pocket. This entry leads to an augmentation in serum inflammatory factor contents. The combined presence of PD and T2DM resulted in the highest serum inflammatory factor levels. It is postulated that in T2DM, inflammatory factors entering the systemic circulation from periodontal tissue affected by PD may synergistically exacerbate the existing low-grade inflammatory response, culminating in an increased expression of inflammatory cytokines in the serum. This proposition aligns with the research findings of scholar Frühbeck [36].

Furthermore, genes associated with insulin secretion play a crucial role in modulating blood sugar levels. GCK, a hexokinase predominantly present in pancreatic β cells, exhibits heightened activity in response to elevated blood sugar levels, thereby accelerating glucose metabolism [37]. GLUT-2, a membrane protein responsible for glucose transport, serves as the primary glucose transporter for pancreatic β cells. Under normal conditions, GLUT-2 facilitates glucose entry into cells and, in conjunction with GCK, forms glucose receptors that regulate insulin synthesis and secretion [38]. UCP-2, a transport protein located on the inner mitochondrial membrane, induces uncoupling of oxidative phosphorylation, leading to reduced intracellular adenosine triphosphate (ATP) levels [39]. Given that insulin secretion by pancreatic β cells is contingent on ATP, high expression of UCP-2 diminishes intracellular ATP production, resulting in decreased insulin secretion and ultimately promoting the onset and progression of T2DM [40]. In our study, the expression of *GCK*, *GLUT-2*, and *UCP-2* genes in pancreatic tissues from PD model rats did not significantly differ from that in

pancreatic tissues from the control group. We posit that PD alone may not suffice to induce abnormal insulin secretion by pancreatic β cells. In rats with both T2DM and PD, the mRNA expression of *GCK*, *GLUT-2*, and *UCP-2* in pancreatic tissues was elevated, disrupting the insulin secretion function of pancreatic β cells. We postulate that inflammatory mediators associated with PD may exacerbate this disruption and contribute to the development of T2DM. Previous studies have indicated a strong correlation between impaired insulin secretion in experimental animal models of T2DM and reduced expression of GLUT-2 in pancreatic β cells [41]. Additionally, in rats subjected to an 8-week high-fat diet, the protein expression of GLUT-2 and GCK in pancreatic β cells significantly decreased, indicating impaired glucose-stimulated insulin secretion (GSIS) and IR [42]. These findings align with our speculative observations.

The mechanism underlying the impact of bacteria is believed to be correlated with autophagy and its association with T2DM, representing an early response of β cells [43]. In animal models induced by a high-fat diet [44] and in the pancreatic β cells of T2DM patients [45], upregulated expression of endoplasmic reticulum stress markers and increased glucose levels have been observed. A high-fat diet tends to enhance β cell autophagy [44]. Excessive autophagy or prolonged organelle degradation may cause cells to lose specificity, eventually leading to cell death [46]. While there is limited evidence categorizing autophagy, characterized by cytoplasmic vacuolation, as a form of programmed cell death, it is identified by the appearance of numerous vacuolar structures enclosing cytoplasm and organelles, with subsequent degradation by lysosomal enzymes [47]. Studies have indicated that downregulation of the transcription factor pancreatic-duodenal homeobox-1 (PDX-1) in pancreatic islet cell lines exacerbates the degree of autophagy, and the administration of various autophagy inhibitors significantly enhances cell survival [48]. Another study found that endoplasmic reticulum stress downregulates insulin receptor expression and induces IR in 3T3-L1 mouse adipocytes by increasing autophagy [49]. This evidence suggests that autophagy is crucial for maintaining the structural and functional integrity of pancreatic β cells [50,51]. However, excessive autophagy leads to the over-degradation of intracellular proteins and organelles, disrupting pancreatic β cell function and structure, potentially resulting in autophagic cell death [52,53]. Our experimental findings revealed that protein levels of Beclin1 and LC3II in the PD group were not significantly different from those in the control group. However, Beclin1 and LC3II protein expression increased over time in the PD group, DM group, and DP group, especially in the DP group. These results suggest that chronic PD alone is insufficient to increase autophagic activity in normal rat pancreatic islet cells. Interestingly, autophagy indicators LC3II and Beclin1 were elevated in pancreatic tissues from the DP group. We hypothesize that PD-related inflammatory factors regulate changes in endogenous molecular mechanisms, leading to excessive autophagy in pancreatic tissue in T2DM. This excessive autophagy may result in the over-degradation of intracellular organelles, impair insulin secretion by pancreatic β cells, and exacerbate T2DM.

Certainly, autophagy is a complex and multifaceted process with various mechanisms. The focus of our study was on the most classic pathway; however, it's important to acknowledge that there could be interaction effects between different pathways. The intricate nature of cellular processes suggests that there might be additional pathways involved, and further in-depth and thorough research is warranted to explore and understand these potential interactions. Comprehensive investigations into the cross-talk and interplay between different autophagic pathways will contribute to a more nuanced and comprehensive understanding of the role of autophagy in the context of our study and its implications for conditions such as T2DM and PD.

5. Conclusion

In conclusion, the factors associated with PD, when overlaid on a foundation of diabetes, may contribute to the heightened autophagy of pancreatic β cells, thereby accelerating the advancement of diabetes. Understanding the intricacies of how PD influences the trajectory of T2DM holds promise for novel perspectives in the prevention and treatment of T2DM. However, the enduring consequences of prolonged periodontitis and the feasibility of timely interventions to potentially reverse the progression of diabetes remain uncertain, necessitating further investigation. Ongoing research endeavors will be crucial in shedding light on the long-term implications of persistent periodontitis and exploring the potential for interventions to mitigate or reverse the course of diabetes.

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Ethical approval

This study ([2016]1–013) was approved by the Animal Experiment Ethics Committee of Zunyi Medical University, Guizhou.

Data availability statement

The raw measurements are available in the Supplemental Files.

CRediT authorship contribution statement

Zhiguo Cai: Writing – review & editing, Writing – original draft, Data curation. **Shasha Du:** Writing – review & editing, Data curation. **Na Zhao:** Writing – review & editing, Data curation. **Nanqu Huang:** Writing – original draft. **Kun Yang:** Writing – review & editing, Funding acquisition. **Liu Qi:** Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24366.

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