Effects of Gavage Administration of Porphyromonas gingivalis on Colonic Mechanical and Immune Barriers in Type 2 Diabetic Mice: A Postprint

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Abstract

Background: Porphyromonas gingivalis (Pg.) is the main pathogenic bacterium of periodontitis. Studies have found that Pg. can affect systemic diseases including type 2 diabetes mellitus (T2DM) through the oral-intestinal pathway, but its specific mechanism is not yet fully understood.

Objective: To investigate whether Pg. affects T2DM by altering the intestinal mechanical barrier and immune barrier.

Methods: Forty SPF-grade mice were randomly selected, with 24 used to establish a T2DM model. Among the successfully modeled mice, 16 were selected and divided into a model group (DM group, n=8) and a model plus Porphyromonas gingivalis group (PD group, n=8). The remaining 16 mice were divided into a control group (N group, n=8) and a Pg. group (n=8). After modeling, mouse body weight and fasting plasma glucose (FPG) were observed. At week 5, an oral glucose tolerance test (OGTT) was performed, OGTT curves were plotted, and the area under the curve (AUC) was calculated. From week 7, the Pg. group and PD group were administered Pg. bacterial suspension by gavage for 5 consecutive weeks. Lipopolysaccharide (LPS) was measured by enzyme-linked immunosorbent assay, colonic tight junction proteins and inflammatory factors were detected by real-time fluorescence quantitative PCR, and mouse colonic tissue lesions were observed by hematoxylin-eosin (HE) staining. Pearson or Spearman correlation analysis was used to explore the relationship between mouse FPG and colonic tight junction protein mRNA expression and serum LPS content.

Results: Before gavage, during weeks 2-6, the DM group had higher body weight than the N and Pg. groups, the PD group had higher body weight than the N

group, and during weeks 3-6, the PD group had higher body weight than the Pg. group. During weeks 9-11, the N and Pg. groups had higher body weight than the DM and PD groups, and at week 11, the PD group had lower body weight than the DM group. During weeks 3-6, the PD group had higher FPG than the N and Pg. groups, and during weeks 4-6, the DM group had higher FPG than the N and Pg. groups. During weeks 7-11, the Pg. group had lower FPG than the DM and PD groups, the PD group had higher FPG than the N group, and at weeks 10 and 11, the PD group had higher FPG than the Pg. group. The DM group had higher AUC than the N and Pg. groups, the PD group had higher AUC than the N, DM, and Pg. groups, and the PD group had higher LPS than the N and DM groups. The PD group had lower zonula occludens-1 (ZO-1) than the N group, the DM group had lower Occludin than the N group, the PD group had lower Occludin than the N, DM, and Pg. groups, the PD group had lower interleukin (IL)-17A than the N and Pg. groups, the N group had higher IL-10 than the DM, Pg., and PD groups, the PD group had higher tumor necrosis factor (TNF-α) than the N, DM, and Pg. groups, and the Pg. and PD groups had higher Toll-like receptor 4 (TLR4) than the N group. Correlation analysis results showed that FPG was positively correlated with LPS and negatively correlated with Occludin ($P<0.05$). Pathological results showed that the Pg. and DM groups had connective tissue hyperplasia in the lamina propria with focal lymphocyte infiltration, and the PD group had focal lymphocyte infiltration in the lamina propria.

Conclusion: Pg. may aggravate glucose metabolism disorders in T2DM mice by destroying the intestinal mechanical barrier and immune barrier, leading to LPS entering the bloodstream.

Full Text

Effects of Gavage Administration of *Porphyromonas gingivalis* on Colonic Mechanical and Immune Barriers in Type 2 Diabetic Mice

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Abstract

Background: *Porphyromonas gingivalis* (Pg.) is the primary pathogen associated with periodontitis. Studies have found that Pg. can influence systemic

diseases including type 2 diabetes mellitus (T2DM) through the oral-intestinal pathway, though the specific mechanisms remain incompletely understood. **Objective:** To investigate whether Pg. affects T2DM by altering intestinal mechanical and immune barriers. **Methods:** Among 40 SPF-grade mice, 24 were randomly selected to establish a T2DM model. Sixteen successfully modeled mice were divided into a model group (DM group, n=8) and a model plus *Porphyromonas gingivalis* group (PD group, n=8). The remaining 16 mice were divided into a control group (N group, n=8) and a Pg. group (n=8). Following modeling, body weight and fasting plasma glucose (FPG) were monitored weekly. At week 5, oral glucose tolerance tests (OGTT) were performed, curves were plotted, and area under the curve (AUC) was calculated. From week 7, the Pg. and PD groups received gavage administration of Pg. bacterial suspension for 5 consecutive weeks. Lipopolysaccharide (LPS) levels were measured by enzyme-linked immunosorbent assay. Colonic tight junction proteins and inflammatory factors were detected by real-time quantitative PCR. Colonic tissue lesions were observed via hematoxylin-eosin (HE) staining. Pearson or Spearman correlation analysis was used to explore relationships between FPG and colonic tight junction protein mRNA expression and serum LPS content. **Results:** Before gavage (weeks 2-6), DM group body weight was higher than N and Pg. groups, while PD group weight exceeded N group and was higher than Pg. group during weeks 3-6. During weeks 9-11 post-gavage, N and Pg. groups had higher body weight than DM and PD groups, with PD group lower than DM group at week 11. FPG in PD group was higher than N and Pg. groups during weeks 3-6 pre-gavage, and DM group FPG exceeded N and Pg. groups during weeks 4-6. During weeks 7-11 post-gavage, Pg. group FPG was lower than DM and PD groups, while PD group was higher than N group and exceeded Pg. group at weeks 10 and 11. DM group AUC was higher than N and Pg. groups, while PD group AUC exceeded all other groups. PD group LPS levels were higher than N and DM groups. PD group tight junction protein 1 (ZO-1) was lower than N group. DM group occludin was lower than N group, while PD group occludin was lower than N, DM, and Pg. groups. PD group interleukin (IL)-17A was lower than N and Pg. groups. N group IL-10 was higher than DM, Pg., and PD groups. PD group tumor necrosis factor (TNF-α) was higher than N, DM, and Pg. groups. Pg. and PD groups Toll-like receptor 4 (TLR4) was higher than N group. Correlation analysis showed FPG was positively correlated with LPS and negatively correlated with occludin ($P<0.05$). Pathological results showed connective tissue hyperplasia with focal lymphocyte infiltration in the lamina propria of Pg. and DM groups, with more pronounced focal lymphocyte infiltration in PD group. **Conclusion:** Pg. may aggravate glucose metabolism disorders in T2DM mice by disrupting intestinal mechanical and immune barriers, leading to LPS translocation into the bloodstream.

Keywords: Diabetes mellitus, type 2; Glucose metabolism disorders; *Porphyromonas gingivalis*; Intestinal tight junction proteins; Intestinal immunity

Introduction

Periodontitis (PD) is a chronic inflammatory disease of periodontal tissues caused by plaque microbial infection. It represents a major cause of tooth loss in adults and is closely associated with various metabolic, inflammatory, and autoimmune diseases including type 2 diabetes mellitus (T2DM), hyperlipidemia, atherosclerotic vascular disease, and rheumatoid arthritis [1]. T2DM is the fastest-growing metabolic disease globally [2], characterized by relative insulin deficiency and insulin resistance, with mortality rates second only to cardiovascular disease and cancer. Research has demonstrated a bidirectional relationship between periodontitis and T2DM, wherein T2DM constitutes a significant risk factor for periodontitis, while periodontitis may also influence T2DM development and progression [3,4].

Recent studies have confirmed that periodontitis-specific microbiota can affect systemic multi-organ diseases through the intestinal microbiota and intestinal barrier, following an "oral-gut-multi-organ" pattern, including conditions such as colitis, arthritis, encephalopathy, diabetes, and hyperlipidemia [5-7]. *Porphyromonas gingivalis* (Pg.), the primary pathogen in periodontitis, can enter the intestine through swallowing, altering intestinal microbial composition and triggering inflammatory responses in organs including the liver, spleen, and gut [8]. The anaerobic and high-pH environment of the colonic mucosa facilitates Pg. adhesion [9]. Once dysbiosis occurs and pathogenic bacteria dominate, host intestinal immune status and barrier function become compromised, allowing bacterial toxins and metabolites to enter systemic circulation and damage distant tissues and organs [10], thereby increasing the risk of other systemic diseases characterized by low-grade inflammation. However, the specific mechanisms by which Pg. disrupts intestinal barrier integrity and immune balance remain incompletely understood.

Humans secrete 1.0-1.5 L of saliva daily. Under physiological conditions, salivary microbiota rarely reaches the intestine due to gastric acid and alkaline bile protection. However, the salivary microbiota of periodontitis patients differs significantly from that of orally healthy individuals [11,12], with substantially higher abundance of pathogenic bacteria such as Pg. in periodontal disease patients [13]. Young adults with severe periodontitis (ages 21-23) swallow approximately 10^{12}-10^{13} Pg. organisms daily [14]. Due to its acid resistance, Pg. may survive gastric acid to reach the intestine and disrupt intestinal homeostasis [10].

The intestinal barrier comprises microbial, mucus, physical, and immune components that constitute the first line of defense against external pathogens. Disruption of any barrier layer may lead to "leaky gut," allowing intestinal bacteria and metabolites to enter the bloodstream and exacerbate various systemic diseases including arthritis, liver disease, encephalopathy, T2DM, inflammatory bowel disease (IBD), and obesity [7,15-17]. This study established a T2DM mouse model and simulated Pg. entry into the intestine through swallowing in

periodontitis patients via gavage administration, observing changes in intestinal inflammation and tight junction proteins and their relationship with glycemic changes to explore whether Pg. affects glucose metabolism by altering intestinal immune and mechanical barrier function.

Materials and Methods

1.1 Experimental Animals and Materials 1.1.1 Animals and Reagents

From May 2022 to February 2023, forty 6-8-week-old male C57BL/6J mice (SPF grade, 20-22 g) were purchased from Beijing Huafukang Bioscience Co. (Animal License No. 110322220102485742). Mice were acclimated for one week under a 12-hour light/dark cycle at (24$±1)°*Cand*(60±$10)% humidity with free access to food and water. All animal experiments were conducted at the Laboratory Animal Center of Guizhou Medical University and approved by the Institutional Animal Care and Use Committee (Approval No. 2201457).

Key reagents included: streptozotocin (STZ) (Solarbio, Cat. No. S8050); *Porphyromonas gingivalis* strain (ATCC33277) (Beina Biology, Cat. No. BNCC236547); mouse lipopolysaccharide (LPS) ELISA kit (Huamei Biology, Cat. No. CSB-E09308H); brain heart infusion (BHI) solid medium (Solarbio, Cat. No. LA0360); Trizol (Takara, Cat. No. 9108); cDNA reverse transcription kit (Prime Script™ RT Master Mix, Takara, Cat. No. RR036A); TB Green Premix Ex Taq (Takara, Cat. No. RR820A); and HE staining kit (Wuhan Servicebio, Cat. No. G1003). Major instruments included: Roche Accu-Chek glucose meter; real-time quantitative PCR system (Bio-RAD); Infinite F50 microplate reader (TECAN); anaerobic chamber (AW500TG, ELECTROTEK); low-speed centrifuge (Thermo Fisher); and high-speed cryogenic tissue grinder (Wuhan Servicebio).

1.2 Experimental Procedures 1.2.1 Animal Grouping and Modeling

After one week of acclimation, 24 mice were randomly selected for T2DM modeling using a random number table method. Mice received a high-fat diet (standard 60% fat-derived energy purified diet) for 4 weeks with free feeding. The remaining 16 mice were divided into control (N group, n=8) and Pg. groups (n=8) and fed control diet (35% fat-derived energy diet) during the same period. T2DM model mice received intraperitoneal injections of 30 mg/kg 2% STZ three times within one week. The N group received equivalent volume of 0.1 mmol/L citrate buffer (pH=4.5). Successful T2DM modeling was defined as two fasting blood glucose measurements $ $11.1 mmol/L or random glucose $ $16.7 mmol/L. Twenty-one successful models were obtained, from which 16 were randomly divided into model (DM group, n=8) and model plus *Porphyromonas gingivalis* (PD group, n=8). After successful modeling, all groups were switched to 35% fat-derived energy diet.

1.2.2 Pg. Preparation

Porphyromonas gingivalis was cultured on BHI solid plates at 37°C in anaerobic conditions (80% N_2, 10% H_2, 10% CO_2) for 7 days, then transferred to BHI liquid medium for 16-18 hours of enrichment. During logarithmic growth phase, bacteria were centrifuged at 3,500 rpm for 5 minutes (radius 8.6 cm), supernatant was discarded, and bacterial pellets were resuspended in sterile phosphate-buffered saline (PBS). Suspension absorbance was measured and concentration adjusted to 1$×10^{9}$ CFU/mL.

1.2.3 Gavage Administration and Monitoring

From week 7, Pg. and PD groups received 200 L of Pg. suspension (1$×10^{9}$ CFU/mL) by gavage twice weekly for 5 consecutive weeks. DM and N groups received equivalent volume of sterile PBS. General condition (mental status, activity, fur appearance, bedding moisture) was monitored daily or every other day post-modeling and post-gavage. Body weight and FPG were measured weekly. At week 5, OGTT was performed after 12-hour overnight fasting: mice received 25% glucose solution at 2 g/kg, and blood glucose was measured via tail vein at 0, 30, 60, 90, and 120 minutes using a glucose meter. OGTT curves were plotted and AUC calculated.

1.2.4 Sample Collection

After 5 weeks of gavage, mice were anesthetized with 1.25% tribromoethanol (0.2 mL/10 g) via intraperitoneal injection. Cardiac blood was collected, followed by euthanasia with 5% chloral hydrate (0.2 mL/10 g). Colonic tissue (proximal to cecum) was harvested: portions were fixed in 4% paraformaldehyde, and portions were placed in RNA stabilization solution at -80°C for subsequent analysis.

1.2.5 Serum LPS Measurement

Cardiac blood was collected in 1.5 mL tubes, stored overnight at 4°C, then centrifuged at 1,000×g for 15 minutes at 2-8°C. Supernatant was collected, and LPS levels were determined following the mouse LPS ELISA kit instructions. Absorbance at 450 nm was measured using an automated microplate reader, and standard curves were used to calculate concentrations.

1.2.6 Quantitative PCR (qPCR) Analysis

Colonic tight junction proteins and inflammatory factors were measured by qPCR. Approximately 0.1 g colonic tissue was ground using a cryogenic tissue grinder. Total RNA was extracted using Trizol method and quantified using a micro-volume nucleic acid analyzer. RNA was reverse-transcribed to cDNA using the reverse transcription kit. qPCR was performed using TB Green™ Premix Ex Taq™ II with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as internal reference. Each sample was run in triplicate, and relative mRNA expression was calculated. Primers were synthesized by Sangon Biotech; sequences are shown in Table 1 .

1.2.7 Histopathological Examination

Fixed colonic samples underwent gradient ethanol dehydration, routine paraffin

embedding, and sectioning. HE staining was performed, and colonic tissue lesions were observed under light microscopy.

1.3 Statistical Analysis Data were analyzed using SPSS 27.0 software and graphed using GraphPad Prism 9.0. Normally distributed continuous data are expressed as mean±standard deviation ($\bar{x}\pm s$). Comparisons among multiple groups were performed using repeated measures ANOVA or one-way ANOVA, with pairwise comparisons using LSD test or Bonferroni correction. Non-normally distributed data are expressed as median (P25, P75), with pairwise comparisons using Kruskal-Wallis H test. Pearson or Spearman correlation analysis was used to examine relationships between FPG and colonic tight junction protein mRNA expression and serum LPS levels. $P<0.05$ was considered statistically significant.

Results

2.1 General Condition and Body Weight N and Pg. groups exhibited vigorous activity, soft and glossy fur, and dry bedding. DM and PD groups showed depressed mental status, reduced activity, moist bedding, dull fur, pungent odor, and increased food and water intake.

Pre-gavage body weight comparisons during weeks 2-6 showed significant differences ($P<0.05$). DM group weight was higher than N and Pg. groups during weeks 2-6, while PD group weight exceeded N group and was higher than Pg. group during weeks 3-6. No significant differences were observed among groups at week 1 ($P>0.05$) (Table 2).

Post-gavage repeated measures ANOVA revealed significant interaction between group and time ($F=32.544$, $P<0.01$), with significant main effects of group ($F=23.272$, $P<0.01$) and time ($F=42.092$, $P<0.01$). During weeks 9-11, N and Pg. groups had higher body weight than DM and PD groups, with PD group lower than DM group at week 11 (Table 3).

2.2 Blood Glucose Parameters Pre-gavage FPG comparisons during weeks 3-6 showed significant differences ($P<0.05$). PD group FPG was higher than N and Pg. groups during weeks 3-6, while DM group exceeded N and Pg. groups during weeks 4-6. No significant differences were found during weeks 1-2 ($P>0.05$) (Table 4).

Post-gavage repeated measures ANOVA indicated significant group-time interaction ($F=2.187$, $P=0.021$), with significant main effects of group ($F=358.936$, $P<0.01$) and time ($F=6.371$, $P<0.01$). During weeks 7-11, Pg. group FPG was lower than DM and PD groups, while PD group was higher than N group and exceeded Pg. group at weeks 10 and 11 (Table 5).

OGTT analysis showed significant group-time interaction (F=3.902, P=0.013), with significant main effects of group (F=52.580, P<0.01) and time (F=63.235, P<0.01). At all time points (0-120 min), DM group glucose was higher than N and Pg. groups, while PD group exceeded N, DM, and Pg. groups. At 120 minutes, Pg. group was higher than N group (Table 6).

OGTT AUC and serum LPS comparisons revealed significant differences among groups (P<0.01). DM group AUC was higher than N and Pg. groups, while PD group AUC exceeded all other groups. PD group LPS levels were higher than N and DM groups (Table 7).

2.3 Colonic Tight Junction Protein and Inflammatory Factor mRNA Expression Significant differences were observed among groups for ZO-1, occludin, IL-17A, IL-10, TNF-α, and TLR4 (P<0.05). PD group ZO-1 was lower than N group. DM group occludin was lower than N group, while PD group occludin was lower than N, DM, and Pg. groups. PD group IL-17A was lower than N and Pg. groups. N group IL-10 was higher than DM, Pg., and PD groups. PD group TNF-α was higher than N, DM, and Pg. groups. Pg. and PD groups TLR4 were higher than N group (Table 8).

2.4 Correlation Analysis Correlation analysis revealed that FPG was positively correlated with LPS (r=0.635, P<0.01) and negatively correlated with occludin (r=-0.377, P<0.01) (Table 9).

2.5 Histopathological Findings HE staining of colonic sections showed normal crypt depth and morphology in N group, with abundant intestinal glands and no obvious inflammatory cells. Pg. and DM groups exhibited connective tissue hyperplasia in the lamina propria with focal lymphocyte infiltration and occasional interstitial lymphocytes. PD group showed more pronounced focal lymphocyte infiltration in the lamina propria, mild submucosal edema, sparse connective tissue arrangement, shallow colonic crypts, and atypical crypt morphology (Figure 1 [Figure 1: see original paper]).

Discussion

The intestinal barrier comprises microbial, mucus, physical, and immune components that form the first line of defense against external pathogens. Disruption of any barrier layer may lead to "leaky gut," allowing intestinal bacteria and metabolites to enter the bloodstream and exacerbate systemic diseases including arthritis, liver disease, encephalopathy, T2DM, inflammatory bowel disease, and obesity [7,15-17]. This study demonstrates that Pg. gavage impairs intestinal immune and mechanical barrier function, reducing glycemic control and insulin sensitivity in T2DM mice and further aggravating glucose metabolism disorders on top of hyperglycemia.

Intestinal immune regulatory cells serve as a "bridge"between gut microbiota and diabetes. Destruction of immune balance is accompanied by disruption of T lymphocyte and cytokine homeostasis, adversely affecting immune status, glucose-lipid metabolism, and insulin sensitivity by influencing pancreatic, hepatic, and other endocrine organs [18]. CD4+ T helper (Th) cells play a crucial role in maintaining intestinal immunity, with balance between CD4+IL-17+ Th17 cells and CD4+CD25+Foxp3+ regulatory T cells (Treg) forming the foundation of immune homeostasis. Intestinal immune imbalance typically manifests as altered Th17/Treg ratios and related cytokines, with increased Th17-associated pro-inflammatory factors (IL-6, TNF-α, IL-17A, IL-17F) and decreased Treg-associated anti-inflammatory factors (TGF-β, IL-10). This study found that Pg. gavage in T2DM mice increased colonic pro-inflammatory factor TNF-α expression while further reducing anti-inflammatory IL-10 gene expression. HE staining revealed more pronounced inflammatory pathological changes in the colonic lamina propria and interstitium of PD group compared to DM and Pg. groups, suggesting more significant Th17/Treg imbalance and intestinal immune homeostasis disruption.

IL-10 not only exerts anti-inflammatory effects in immune responses but also plays a protective role in diabetes development. Yuan et al. [19] found significantly reduced Th17/Treg cell percentages and related cytokines (primarily IL-10 and TGF-β) in T2DM patients. STZ-induced non-obese diabetic mice treated with bacterial strains carrying IL-4 and IL-10 coding plasmids showed effective reduction of hyperglycemia and pancreatic islet destruction [20]. We hypothesize that reduced IL-10 after Pg. gavage may affect blood glucose not only through intestinal immunity but also via direct effects of IL-10 on glucose metabolism.

Intestinal Th17/Treg immune imbalance is typically accompanied by increased IL-17A. In collagen-induced arthritis models, oral Pg. administration shifts intestinal immune patterns toward Th17 dominance with altered gut microbiota, aggravating arthritis symptoms [21]. However, IL-17A can exert both pro- and anti-inflammatory effects in various tissues [22]. In the intestine, IL-17A promotes epithelial cell proliferation, upregulates antimicrobial peptides and tight junction protein expression, thereby protecting intestinal mucosa from pathogen infection [23]. IL-17A or IL-17 receptor inhibitors can impair intestinal barrier function and exacerbate colitis [24]. Additionally, reduced intestinal Th17/IL-17 in high-fat diet mice correlates with increased body weight, glucose intolerance, and insulin resistance [25]. Studies have demonstrated that reduced gut microbiota diversity and decreased IL-17/IL-22 are associated with metabolic diseases including insulin resistance [26].

Besides Th17 cells, natural killer cells, $\gamma\delta$ T cells, CD8+ T cells, and other cell types also secrete IL-17. In this study, colonic IL-17A showed a decreasing trend in diabetic mice after Pg. gavage, consistent with changes in tight junction proteins, suggesting Pg. may affect IL-17 secretion by other immune cells to influence colonic tight junction proteins, though specific mechanisms require

further investigation.

Toll-like receptors (TLRs) are pathogen-associated molecular pattern recognition receptors expressed not only on innate immune cells and specialized antigen-presenting cells but also on CD4+ T cells. TLR4 activation can trigger NF- B signaling, causing intestinal Th17/Treg imbalance and insulin resistance [27,28], thereby affecting diabetes progression.

Occludin and ZO-1 are major protein components of intestinal tight junctions [29] and serve as key indicators of intestinal mechanical barrier function. This study found reduced colonic occludin mRNA expression in both DM and PD groups compared to N group, with more pronounced reductions in both tight junction proteins in PD group. These findings indicate that both diabetes alone and Pg. gavage alone can affect intestinal mechanical and immune barriers, while combined Pg. gavage in T2DM mice causes more severe barrier disruption and immune imbalance, with significantly increased serum LPS levels. The negative correlation between blood glucose changes and occludin/ZO-1 mRNA expression, and positive correlation with LPS levels, further support that LPS translocation due to intestinal barrier disruption may be a key factor mediating glycemic changes.

In summary, Pg. gavage disrupts colonic mechanical and immune barriers, with more severe effects in hyperglycemic conditions, potentially leading to LPS translocation into the bloodstream and further impairing glycemic regulation and insulin sensitivity in diabetic mice. In vitro studies have shown that LPS can regulate insulin signaling in adipocytes through X-box binding protein 1 modulation of insulin receptor substrate 1, phosphoinositide-dependent protein kinase 1, and protein kinase B pathways [30], suggesting LPS may be an important target through which Pg. affects distal organ glucose-lipid metabolism via the gut. The mechanisms of Pg.-induced intestinal barrier disruption may involve both direct effects of altered intestinal microbiota and metabolites [31] and indirect effects through broken immune balance (reduced IL-17A and IL-10, increased TLR4 and TNF-α). The specific mechanisms by which Pg. affects diabetes development through the oral-intestinal axis, and how LPS influences systemic glucose-lipid metabolism after entering circulation, require further exploration.

References

[1] YAN Fuhua. Research progress on the impact of periodontitis on systemic diseases and health [J]. Stomatology, 2018, 38(7): 577-581. DOI:10.13591/j.cnki.kqyx.2018.07.001.

[2] LING C, BACOS K, RÖNN T. Epigenetics of type 2 diabetes mellitus and weight change - a tool for precision medicine? [J]. Nat Rev Endocrinol, 2022, 18(7): 433-448. DOI:10.1038/s41574-022-00671-w.

[3] STÖHR J, BARBARESKO J, NEUENSCHWANDER M, et al. Bidirectional association between periodontal disease and diabetes mellitus: a systematic review and meta-analysis of cohort studies [J]. Sci Rep, 2021, 11(1): 13686. DOI:10.1038/s41598-021-93062-6.

[4] MIRNIC J, DJURIC M, GUSIC I, et al. Effects of nonsurgical periodontal therapy on salivary 8-hydroxy-deoxyguanosine levels and glycemic control in diabetes mellitus type 2 patients [J]. Biomedicines, 2022, 10(9): 2269. DOI:10.3390/biomedicines10092269.

[5] KITAMOTO S, NAGAO-KITAMOTO H, JIAO Y Z, et al. The intermucosal connection between the mouth and gut in commensal pathobiont-driven colitis [J]. Cell, 2020, 182(2): 447-462.e14. DOI:10.1016/j.cell.2020.05.048.

[6] FENG Y K, WU Q L, PENG Y W, et al. Oral P. gingivalis impairs gut permeability and mediates immune responses associated with neurodegeneration in LRRK2 R1441G mice [J]. J Neuroinflammation, 2020, 17(1): 347. DOI:10.1186/s12974-020-02016-0.

[7] ZHOU N, ZOU F G, CHENG X, et al. Porphyromonas gingivalis induces periodontitis, causes immune imbalance, and promotes rheumatoid arthritis [J]. J Leukoc Biol, 2021, 110(3): 461-473. DOI:10.1002/JLB.3MA0121-045R.

[8] LIU Y M, HUANG W K, DAI K, et al. Inflammatory response of gut, spleen, and liver in mice induced by orally administered Porphyromonas gingivalis [J]. J Oral Microbiol, 2022, 14(1): 2088936. DOI:10.1080/20002297.2022.2088936.

[9] DONALDSON G P, LEE S M, MAZMANIAN S K. Gut biogeography of the bacterial microbiota [J]. Nat Rev Microbiol, 2016, 14(1): 20-32. DOI:10.1038/nrmicro3552.

[10] MULHALL H, HUCK O, AMAR S. Porphyromonas gingivalis, a long-range pathogen: systemic impact and therapeutic implications [J]. Microorganisms, 2020, 8(6): 869. DOI:10.3390/microorganisms8060869.

[11] BAO J, LI L L, ZHANG Y H, et al. Periodontitis may induce gut microbiota dysbiosis via salivary microbiota [J]. Int J Oral Sci, 2022, 14(1): 32. DOI:10.1038/s41368-022-00183-3.

[12] SHI C, CAI L T, XUN Z, et al. Metagenomic analysis of the salivary microbiota in patients with caries, periodontitis and comorbid diseases [J]. J Dent Sci, 2021, 16(4): 1264-1273. DOI:10.1016/j.jds.2020.12.002.

[13] MA J L, KAGEYAMA S, TAKESHITA T, et al. Clinical utility of subgingival plaque-specific bacteria in salivary microbiota for detecting periodontitis [J]. PLoS One, 2021, 16(6): e0253502. DOI:10.1371/journal.pone.0253502.

[14] HE J Y, HUANG W J, PAN Z W, et al. Quantitative analysis of microbiota in saliva, supragingival, and subgingival plaque of Chinese adults with chronic periodontitis [J]. Clin Oral Investig, 2012, 16(6): 1579-1588. DOI:10.1007/s00784-011-0654-2.

[15] NASCIMENTO J C, MATHEUS V A, OLIVEIRA R B, et al. High-fat diet induces disruption of the tight junction-mediated paracellular barrier in the proximal small intestine before the onset of type 2 diabetes and endotoxemia [J]. Dig Dis Sci, 2021, 66(10): 3359-3374. DOI:10.1007/s10620-020-06664-x.

[16] JIN R, NING X Q, LIU X, et al. Porphyromonas gingivalis-induced periodontitis could contribute to cognitive impairment in Sprague-Dawley rats via the P38 MAPK signaling pathway [J]. Front Cell Neurosci, 2023, 17: 1141339. DOI:10.3389/fncel.2023.1141339.

[17] CHOPYK D M, GRAKOUI A. Contribution of the intestinal microbiota to human health and disease [J]. Gastroenterology, 2020, 159(3): 849-863. DOI:10.1053/j.gastro.2020.04.077.

[18] LI Q W, GAO Z Z, WANG H, et al. Intestinal immunomodulatory cells (T lymphocytes): a bridge between gut microbiota and diabetes [J]. Mediators Inflamm, 2018, 2018: 9830939. DOI:10.1155/2018/9830939.

[19] YUAN N, ZHANG H F, WEI Q, et al. Expression of CD4+CD25+Foxp3+ regulatory T cells, interleukin 10 and transforming growth factor β in newly diagnosed type 2 diabetic patients [J]. J Endocrinol Invest, 2018, 126(2): 96-101. DOI:10.1055/s-0043-113454.

[20] PREISSER T M, DA CUNHA V P, SANTANA M P, et al. Recombinant Lactococcus lactis carrying IL-4 and IL-10 coding vectors protects against type 1 diabetes in NOD mice and attenuates insulitis in the STZ-induced model [J]. J Diabetes Res, 2021, 2021: 6697319. DOI:10.1155/2021/6697319.

[21] SATO K, TAKAHASHI N, KATO T, et al. Aggravation of collagen-induced arthritis by orally administered Porphyromonas gingivalis through modulation of the gut microbiota and gut immune system [J]. Sci Rep, 2017, 7(1): 6955. DOI:10.1038/s41598-017-07196-7.

[22] FENG Y, CHEN Z, TU S Q, et al. Role of interleukin-17A in the pathomechanisms of periodontitis and related systemic chronic inflammatory diseases [J]. Front Immunol, 2022, 13: 862415. DOI:10.3389/fimmu.2022.862415.

[23] CHEN L, RUAN G C, CHENG Y, et al. The role of Th17 cells in inflammatory bowel disease and the research progress [J]. Front Immunol, 2022, 13: 1055914. DOI:10.3389/fimmu.2022.1055914.

[24] DENG Z Z, WANG S F, WU C F, et al. IL-17 inhibitor-associated inflammatory bowel disease: a study based on literature and database analysis [J]. Front Pharmacol, 2023, 14: 1124628. DOI:10.3389/fphar.2023.1124628.

[25] HONG C P, PARK A, YANG B G, et al. Gut-specific delivery of T-helper 17 cells reduces obesity and insulin resistance in mice [J]. Gastroenterology, 2017, 152(8): 1998-2010. DOI:10.1053/j.gastro.2017.02.016.

[26] ZHOU X, JOHNSON J S, SPAKOWICZ D, et al. Longitudinal analysis of serum cytokine levels and gut microbial abundance links IL-17/IL-22 with

Clostridia and insulin sensitivity in humans [J]. Diabetes, 2020, 69(8): 1833-1842. DOI:10.2337/db19-0592.

[27] GEORGE L, RAMASAMY T, SIRAJUDEEN K, et al. LPS-induced apoptosis is partially mediated by hydrogen sulphide in RAW 264.7 murine macrophages [J]. Immunol Invest, 2019, 48(5): 451-465. DOI:10.1080/08820139.2019.1566355.

[28] JIA L, WU R Q, HAN N N, et al. Porphyromonas gingivalis and Lactobacillus rhamnosus GG regulate the Th17/Treg balance in colitis via TLR4 and TLR2 [J]. Clin Transl Immunology, 2020, 9(11): e1213. DOI:10.1002/cti2.1213.

[29] KUO W T, ODENWALD M A, TURNER J R, et al. Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival [J]. Ann N Y Acad Sci, 2022, 1514(1): 21-33. DOI:10.1111/nyas.14798.

[30] LU Jiayi, WU Qianqi, CHEN Yiyan, et al. Mechanism of lipopolysaccharide from Porphyromonas gingivalis regulating adipocyte insulin signaling pathway through X-box binding protein 1 [J]. West China Journal of Stomatology, 2022, 40(2): 148-154. DOI:10.7518/hxkq.2022.02.004.

[31] READ E, CURTIS M A, NEVES J F. The role of oral bacteria in inflammatory bowel disease [J]. Nat Rev Gastroenterol Hepatol, 2021, 18(10): 731-742. DOI:10.1038/s41575-021-00488-2.

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