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Porphyromonas gingivalis Infection Promotes the Expression of Inflammatory Biomarkers in Atherosclerosis and Periodontitis: An Experimental Study Using an Apolipoprotein E-Deficient Mouse Model

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Abstract

Objectives

P. gingivalis, a major periodontal pathogen, accelerates the development of atherosclerosis. We aimed to validate an animal model of atherosclerosis and periodontitis and investigate the inflammatory effects of *P. gingivalis* infection on these diseases.

Methods

We treated 8-week-old apolipoprotein E-deficient (ApoE-/-) mice with PBS supplemented with 2% CMC and *P. gingivalis* every two days for a total of 20 times. Aorta, blood and maxillary bone samples were collected and used for Oil Red O staining, micro-CT, enzyme-linked immunosorbent assays (ELISAs) and real-time PCR.

Results

Micro-CT analysis revealed remarkable resorption of alveolar bone in the maxillary region after *P. gingivalis* infection. Frozen aortic root sections stained with Oil red O revealed lipid deposition and plaque formation. The levels of inflammatory biomarkers such as TNF-a, IFN-γ, IL-6, VEGF and RANTES increased, whereas that of b-FGF decreased. Additionally, the mRNA levels of ACTA2, HO-1, STAT3 and T-bet in the aorta were significantly different between the *P. gingivalis* infection group and the control group.

Conclusion

An animal model of atherosclerosis and periodontitis caused by *P. gingivalis* infection was successfully established, and changes in the levels of inflammatory markers were observed, suggesting that periodontitis may serve as an intervenable risk factor for atherosclerosis. By measuring inflammatory biomarker levels, potential indicators for early clinical risk assessment and prognostic monitoring were identified.

Introduction

Atherosclerosis (AS) is a chronic infectious disease and among the main causes of cardiovascular diseases [1]. Traditional risk factors for AS include an unhealthy lifestyle, smoking, hyperlipidemia, hypertension and alterations in glucose metabolism [2]. In recent years, many studies have shown that chronic infectious diseases are also risk factors for cardiovascular diseases [3]. Studies have also shown that periodontal pathogen infection is an independent risk factor for cardiovascular events [4]. As one of the most important periodontal pathogens, *Porphyromonas gingivalis* (*P. gingivalis*) has been indicated to be closely related to the occurrence and development of AS in many animal experiments [5–7].

Various experimental animal models have been used to evaluate the association between *P. gingivalis* and AS. Rodents are widely used as models because of their genome similarity to humans, low cost, and rapid reproduction [8]. Many studies have investigated the association between *P. gingivalis* and AS in ApoE-/- mice, exhibit spontaneous atherosclerosis that is similar to the process of atherosclerosis in humans [9]. However, different methods have been applied to establish models of experimental periodontitis, such as oral infection, intravenous injection of *P. gingivalis* [10–12], or the use of different strains or different infection durations (from 3 weeks to 12 weeks), which has resulted in atherosclerotic plaque severity and periodontal tissue destruction that significantly differ.

To investigate the occurrence and development of periodontitis and atherosclerosis, identifying the best disease model is crucial. This study aims to establish a modelling method that is simple to implement, has a high success rate, good repeatability and animal tolerance, a low mortality rate, and has already been successful. By inducing chronic periodontitis and atherosclerotic plaques in ApoE-/- mice through oral infection with *P. gingivalis*, the mechanisms of oral infection-mediated inflammation on the destruction of periodontal tissues and the formation of aortic plaques can be explored, which will provide a solid research platform for the future drug screening.

Materials and Methods

Culture of P. gingivalis FDC 381

P. gingivalis FDC 381 was incubated on 5% sheep blood anaerobic agar and cultured under anaerobic conditions (80% N_2, 10% CO_2, and 10% H_2) at 37°C in an anaerobic chamber (Oxoid Ltd., UK) for 3 ~ 5 days. The cultured bacteria were then transferred to brain heart infusion (BHI) broth (Oxoid Ltd., UK) supplemented with 5 mg/L hemin and 0.4 mg/L vitamin K and incubated anaerobically at 37°C for 2 days until an optical density (OD) at 600 nm of 1.0 was reached, as measured with a spectrophotometer (Thermo Scientific, USA); this value corresponds to approximately 10^9 CFU/ml. Cultures were centrifuged at 8,000 × g for 5 min at 4°C, and the supernatant was discarded. The bacterial pellet was then resuspended in phosphate-buffered saline (PBS) to a concentration of 10^9 CFU/ml, followed by suspension in 0.5% carboxymethylcellulose (CMC; Sigma-Aldrich, USA) for subsequent oral infection.

Experimental animals and grouping

This research was approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center (approval number PUIRB-LA2024209). Six-week-old male ApoE knockout (ApoE-/-) mice (C57BL/6) were obtained from Vital River Inc. (Beijing, China) and the company's consent was obtained. After two weeks of adaptive feeding, eight-week-old ApoE-/- mice were randomized into two groups (n = 8 per group): the control (PBS) group, in which ApoE-/- mice were fed a normal diet and orally administered PBS three times per week for 8 weeks; and the *P. gingivalis* oral infection group, in which ApoE-/- mice were fed a normal diet and orally administered *P. gingivalis* three times per week for 8 weeks.

Tissue collection

The mice were fed regular chow and distilled water until 16 weeks of age. All the mice were euthanized by intraperitoneal injection of pentobarbital sodium (100 μg/μl; Merck, Germany). The hearts and aortas were perfused through the left ventricle with heparinized 0.9% cold PBS solution for 10 minutes. Different parts of the heart (including the aortic root) were carefully separated, embedded in OCT compound (Tissue Tek, Torrance, USA), and frozen for sectioning. Maxillary samples were collected, fixed in 4% paraformaldehyde at 4°C overnight, transferred to 70% ethanol and subjected to micro-CT (Inveon MM CT, Siemens, USA) imaging to evaluate alveolar bone loss (Fig. 1).

Microcomputed tomography

Microcomputed tomography was used to visualize the maxillary tissue of the mice. A three-dimensional model was generated using a microcomputed tomography scanner, and bone resorption from the first to third molars of the maxilla of the mice was analysed. The acquisition parameters were as follows: 360° rotation, 360 projections, 1500 ms exposure time, 60 kV tube voltage, 220 μA tube current, and an effective pixel size of 8.82 μm. Three-dimensional images were reconstructed using the filtered back-projection algorithm, generating a 1024×1024×448 matrix, and analysed using Siemens Inveon workstation software. The images were rotated to align the molars (M1 to M3) of the mice, and the cementoenamel junction-alveolar bone crest (CEJ-ABC) distance was measured at six different sites from M1 to M3 to evaluate the degree of alveolar bone resorption using software.

Oil Red O staining

The heart was carefully dissected with the aorta attached and embedded in OCT compound, and frozen sections were prepared for Oil Red O staining. The progression of atherosclerosis was evaluated using the modified Paigen method [13]. Lesions were observed at 100× magnification under an Olympus BX41 microscope. The lesion area and lipid deposition ratio were quantified using Image-Pro Plus software and photographed with the attached camera. An average of 15 frozen sections from each mouse were examined.

Enzyme-linked immunosorbent assays

After ApoE-/- mice were anaesthetized by intraperitoneal injection of pentobarbital sodium (100 microlitres) and euthanized, blood was collected. The serum was separated by centrifugation at 10,000 revolutions per minute for 5 minutes at 4°C. Then, the levels of IL-6, b-FGF, RANTES, TNF-α, VEGF and IFN-γ in the serum of each group were detected using enzyme-linked immunosorbent assays (ELISAs). The protein levels in each group were determined with ELISA kits according to the manufacturer’s instructions.

Real-time quantitative PCR

The mRNA expression levels of the target genes ACTA2, HO-1, STAT3 and T-bet in the aortic tissue were measured real-time quantitative polymerase chain reaction (Table 1).

Table 1: Sequences of primers used for quantitative real-time PCR

Primer name	Forwards	Reverse
ACTA2	GAGCGTGGCTATTCCTTCGT	TTCAAAGTCCAGAGCTACATAACACAGT
STAT3	AGGCTTCTCCTTCCGGGTGT	GTCTGCAGTATTTCCCGAAAGC
T-bet	CAACACAGGAGGCGCACTGG	CCCCCTTGTTGTTTGTGAGCT
HO-1	TGCAGGTGATGCTGACAGAGG	GGGATGAGCTAGTGCTGATCTGG
GAPDH	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG

Statistical analysis

Statistical analyses were performed with SPSS 11.0. The data are presented as the mean ± standard deviation. Two-tailed t- tests, one-way analysis of variance (ANOVA), and the Tukey–Kramer post hoc test were used as appropriate. $P < 0.05$ was considered to indicate statistical significance.

Results

Establishment of an animal model of periodontitis combined with atherosclerosis

To evaluate the progression of atherosclerotic disease, Oil Red O staining was used to assess the atherosclerotic plaque areas in mice. Oil Red O staining revealed that oral infection with *P. gingivalis* resulted in a significant increase in the aortic atherosclerotic plaque area compared with that in the control group (Fig. 2, PBS: 6.09 ± 0.79 vs. *P. gingivalis*: 10.17 ± 0.85; $P < 0.05$). Micro-CT analysis revealed that compared with oral administration of PBS, oral infection with *P. gingivalis* resulted in significantly more maxillary molar alveolar bone resorption (Fig. 3, PBS: 0.17 ± 0.055; *P. gingivalis*: 0.21 ± 0.06; $P < 0.05$). These findings confirmed the successful establishment of an animal model of periodontitis induced by *P. gingivalis* infection combined with atherosclerosis.

Correlation of inflammatory biomarker levels with P. gingivalis infection in the animal model

After the animal model was successfully established, the serum levels of IL-6 (PBS: 12.82 ± 0.44 vs. *P. gingivalis*: 17.94 ± 0.22 pg/mL; P < 0.01), TNF-a (PBS: 11.79 ± 0.51 vs. *P. gingivalis*: 17.68 ± 0.24 pg/mL; P < 0.05), RANTES (PBS: 13.04 ± 0.55 vs. *P. gingivalis*: 19.72 ± 0.61 ng/mL; P < 0.05), VEGF (PBS: 10.08 ± 0.55 vs. *P. gingivalis*: 17.40 ± 0.31 pg/mL; P < 0.05) and IFN-γ (PBS: 13.72 ± 0.55 vs. *P. gingivalis*: 18.51 ± 0.26 pg/mL; P < 0.05) significantly increased compared with those at baseline. In contrast, the level of b-FGF (PBS: 20.34 ± 0.48 vs. *P. gingivalis*: 15.62 ± 0.35 pg/mL; P < 0.05) significantly decreased after *P. gingivalis* infection (Fig. 4).

mRNA expression of ACTA2, STAT3, T-bet and HO-1 aggravates the progression of periodontitis and atherosclerosis

The mRNA levels of ACTA2, STAT3, T-bet and HO-1 in the aorta were measured by qRT–PCR to clarify the molecular mechanism underlying the inflammatory response in *P. gingivalis*-mediated periodontitis and atherosclerosis. Compared with the control treatment, *P. gingivalis* treatment increased the mRNA levels of ACTA2 (1.04 ± 0.06 vs. *P. gingivalis*, 1.63 ± 0.72 after normalization to the GAPDH mRNA level; P < 0.05), STAT3 (1.25 ± 0.04 vs. *P. gingivalis*, 1.35 ± 0.09 after normalization to the GAPDH mRNA level; P < 0.05) and T-bet (1.33 ± 0.15 vs. *P. gingivalis*, 1.68 ± 0.09 after normalization to the GAPDH mRNA level; P < 0.05), which exacerbated atherosclerosis (AS). Additionally, compared with that in the PBS control group, the level of HO-1, which reduces the superoxide content, in mice infected with *P. gingivalis* was significantly lower (PBS: 1.44 ± 0.08 vs. *P. gingivalis*: 1.31 ± 0.03 after normalization to GAPDH mRNA levels, P < 0.05), suggesting that *P. gingivalis* promotes the stress-induced senescence of vascular endothelial cells by inducing oxidative stress, thereby promoting the progression of periodontitis and atherosclerosis in ApoE-/- mice (Fig. 5).

Discussion

Periodontitis is a chronic infectious disease caused by a combination of environmental factors, host factors, and the presence of pathogenic microorganisms. Periodontitis causes gingival bleeding, loss of periodontal attachment, alveolar bone resorption, tooth mobility, tooth displacement, and even tooth loss. Periodontitis is the leading cause of tooth loss among adults in China and among the major public health problems worldwide [14]. In recent years, correlations between periodontitis and various systemic diseases, such as diabetes, cardiovascular diseases, chronic kidney diseases, and Alzheimer's disease, have been established [15]. Therefore, controlling periodontal inflammation and exploring safe and effective treatment methods for periodontitis have become urgent global health needs.

Studies have shown that the presence of periodontal pathogenic bacteria (such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Actinobacillus actinomycetemcomitans*, and *Prevotella intermedia*) in subgingival plaques is closely related to systemic diseases [16]. Meta-analyses of the relationship between coronary heart disease and periodontitis have indicated that periodontitis is an

independent risk factor for coronary heart disease after accounting for other factors such as smoking, hypertension, and high cholesterol levels, with a relative risk of 1.24 to 1.35[17].

Previous studies have shown that the elevated levels of the inflammatory cytokines IL-6 and TNF-α in the serum of patients with periodontitis are the main reasons for the increased risk of chronic periodontitis and cardiovascular diseases [18]. Our research also revealed that in animal models of atherosclerosis and periodontitis mediated by *P. gingivalis* infection, the levels of the inflammatory factors IL-6 and TNF-α significantly increased, which was consistent with previous research conclusions. Other studies have reported that elevated serum levels of the inflammatory cytokines IL-6 and TNF-α in patients with chronic periodontitis are major contributors to the increased risk of cardiovascular disease.

P. gingivalis is a gram-negative anaerobic bacterium and is the “dominant bacterial group” in periodontitis [19, 20]. *P. gingivalis* is also the most commonly used pathogenic bacterium for establishing periodontitis models owing to its simplicity, as a single strain can induce periodontitis. Several methods are commonly used for modelling, as follows.

1. Subgingival/ligature-associated infection: This method can result in rapid bone resorption, has shown strong reproducibility and is commonly used to assess short-term bone loss and therapeutic efficacy [21]. This method involves both mechanical irritation and infection, so it is not fully comparable to a pure infection model.
2. Local injection (subgingival or intragingival injection): *P. gingivalis* cells or their products (e.g., LPS and proteases) are injected directly into gingival tissue to induce a local inflammatory response [22]. This approach results in a rapid and localized response, making it suitable for mechanistic studies; however, it does not represent true natural colonization, and the pathological process differs.
3. Germ-free or humanized microbiome models: Single *P. gingivalis* strains or mixed human-derived communities are introduced into germ-free animals, which facilitates the analysis of interactions of a single species or community with the host immune response [23]. However, these models have high technical requirements and are costly and less practicable.
4. Oral/gastric gavage (oral gavage or oral inoculation): This method is typically used in mice. High concentrations of *P. gingivalis* suspensions are administered repeatedly over several weeks to several months [24]. This method simulates natural colonization and is suitable for studying immune responses and microbiome dynamics [25]. This was the method we adopted in our research.

The two most commonly used transgenic mouse models of atherosclerosis are the ApoE-/- (apolipoprotein E knockout) model and the LDL receptor knockout (LDLR-/-) model [26], with ApoE-/- mice being more widely used. The advantages of the ApoE-/- model include the development of complex vascular lesions even when feeding a normal chow diet that are comparable to human atherosclerotic lesions (foam cell lesions appear after 8 weeks, and fibrous plaques appear after 15 weeks) [27]. Feeding a high-fat, high-cholesterol diet markedly increases the incidence and severity of atherosclerosis in ApoE-/- mice; lesions most commonly occur at the aortic root, followed by the brachiocephalic artery,

carotid arteries and pulmonary arteries. Because plaques in ApoE-/- mice closely resemble stable human plaques and because the mouse genome has been fully characterized, ApoE-/- mice are frequently used as animal models for studying the pathogenesis and treatment of atherosclerosis.

The *P. gingivalis* FDC 381 strain used in this study is among the most virulent of the numerous *P. gingivalis* strains discovered thus far [28]. The periodontitis model induced by oral swabbing with this strain has the advantages of stability and high repeatability. Micro-CT was used to analyse alveolar bone resorption in M1 to M3, and the distance from the cementoenamel junction to the alveolar bone crest (ABC) was measured to assess the degree of alveolar bone resorption [29]. Compared with the healthy control group, the infection group showed significant alveolar bone resorption, confirming successful establishment of the periodontitis model. Oil Red O staining was performed on frozen sections of the aortic root of the heart to examine macrophage infiltration, lipid deposition, and plaque formation in the aorta. The lesion area and percent lipid deposition were measured using analysis software, and significant differences between the experimental and control groups were detected, verifying the successful establishment of a combined ApoE -/- mouse atherosclerosis and experimental periodontitis model.

b-FGF (basic fibroblast growth factor) is an important growth factor that activates downstream pathways (MAPK/ERK, PI3K/Akt, PLCγ, etc.) via FGF receptors [30, 31] to regulate cell proliferation, migration, differentiation, and angiogenesis. During atherosclerosis development, b-FGF plays important and complex roles in vascular smooth muscle cells (VSMCs), endothelial cells (ECs) and macrophages. In this study, compared with that in the PBS group, the serum expression of b-FGF in the *P. gingivalis*-infected group increased. This finding verified that b-FGF interacted with inflammatory factors, thereby altering the polarization of macrophages and the production of cytokines and indirectly affecting plaque formation and bone resorption.

VEGF participates in angiogenesis and repair by stimulating endothelial cell proliferation, migration, and lumen formation and increasing vascular permeability [32]. In the pathogenesis of atherosclerosis, VEGF has dual spatiotemporal effects on plaque formation and stability [33]. In most studies, VEGF, primarily VEGF-A, is upregulated in chronic periodontitis tissues, gingival crevicular fluid (GCF), and saliva [34]. VEGF-A expression is positively correlated with clinical indicators such as the degree of inflammation, bleeding, and probe depth. In our study, the increased expression of VEGF was accompanied by the upregulation of the expression of adhesion molecules and chemokines, which participate in the recruitment and activation of monocytes/macrophages.

IFN-γ is an important proinflammatory factor in the progression of atherosclerosis and periodontitis. IFN-γ promotes atherosclerosis by inducing M1 macrophage polarization, inhibiting the synthesis of collagen by smooth muscle cells, increasing the activity of matrix-degrading enzymes, and inducing apoptosis [35]. Our study also revealed that the expression of the proinflammatory factors IL-6, TNF-α, and IFN-γ increased and that these factors interacted with each other to synergistically regulate and amplify the inflammatory response.

ACTA2 encodes α-smooth muscle actin, which is specific to vascular smooth muscle cells (VSMCs) and a key cytoskeletal protein that maintains the contractile phenotype of VSMCs and the tension of the vascular wall. Studies have shown that in established animal models, the downregulation of ACTA2 expression is accompanied by aggravated inflammation, which may be due to a phenotypic transformation of VSMCs from contractile to synthetic or towards macrophage-like/foam cells, which play important roles in plaque formation and instability [36].

Previous studies have shown that in ApoE-/- or LDLR-/- mice, increased expression of HO-1 reduces plaque formation, decreases inflammation and stabilizes plaques [37]. HO-1 and its products can inhibit the production of proinflammatory cytokines (such as IL-1β, TNF-α, and IL-6) and suppress NF-κB signalling, thereby reducing local inflammation [38]. In the ApoE-/- mouse model of periodontitis and atherosclerosis, we found that after *P. gingivalis* infection, HO-1 expression significantly decreased, further demonstrating that HO-1 has multiple functions, such as antioxidative, anti-inflammatory, antiapoptotic, antiproliferative, and vascular protective effects.

Signal transducer and activator of transcription 3 (STAT3) is an important transcription factor in the JAK/STAT pathway that is involved in inflammatory responses and cell survival, differentiation, and metabolism [39]. In our animal model, the expression of the STAT3 gene decreased, which may be related to STAT3-mediated M1 macrophage polarization, which causes proinflammatory factors to promote disease progression.

T bet (encoded by the TBX21 gene) is a key transcription factor that induces Th1 differentiation and IFN-γ expression, is expressed mainly in T cells and certain innate immune cells (such as ILC1s and NK cells), and is associated with proinflammatory Th1 responses [40]. T bet (Tbx21) is the main regulatory factor that drives CD4 + T cells to differentiate into Th1 cells. The Th1 immune response is generally considered to promote the progression of atherosclerosis and plaque instability. Most studies support that in atherosclerosis mouse models (such as ApoE-/- or LDLR-/- mice), inhibiting or deleting T bet is often accompanied by a weakened Th1 response, decreased IFN-γ levels, and reduced lesion areas. Our study also revealed that the expression of the T bet gene increased in the model mice, which was accompanied by increased expression of the associated proinflammatory factors.

Our research group previously explored the mechanism by which live *P. gingivalis* injected via the tail vein promoted atherosclerosis in ApoE knockout (ApoE-/-) mice and established a periodontal disease model through oral infection with *P. gingivalis* [41, 42]. These findings were published in international journals, and we have accumulated considerable experience in establishing animal models. In this study, 8-week-old ApoE-/- mice were orally administered 100 μL of PBS containing 2% CMC and 10^9 CFU of *P. gingivalis* every two days for a total of 20 treatments. When the mice were 15 weeks old, they were sacrificed, and blood, gingival tissue, and maxillary bone samples were collected for micro-CT analysis to confirm the successful establishment of the periodontitis disease model. Frozen sections of the aortic root of the heart were stained with Oil Red O. The lesion area and percent lipid deposition were measured using

analysis software and differed significantly from those in the control group, verifying the successful establishment of the combined atherosclerosis and experimental periodontitis animal model.

Animal models serve as bridges between translational medicine and regenerative medicine [43]. Therefore, establishing the correct animal model is of paramount importance. Only once an accurate animal model is established can we clearly reveal the pathogenesis of human diseases and provide a solid research platform drug screens. Model establishment by intravenous injection is relatively expensive, is characterized by high technical sensitivity, exhibits a high animal mortality rate, and significantly differs from the clinical observations. Constructing an experimental animal model of periodontal disease by oral infection with *P. gingivalis* is more economical and simpler. Therefore, developing a simple, very successful, and highly reproducible modelling method that is well tolerated by animals with a low mortality rate is urgent.

Conclusion

In this study, an animal model of coexisting periodontitis and atherosclerosis was established by local administration of *P. gingivalis* FDC 381 to the oral cavity of ApoE-/- mice. This approach led to the establishment of a model that is more similar to patients with both periodontitis and atherosclerosis in clinical practice. Moreover, oral infection is more consistent with the pathogen colonization pattern of clinical periodontitis patients. Bacteria infecting the oral cavity affect distant organs of the body through the periodontal local area; thus, this model represents a stable animal model for exploring the correlation between periodontitis and atherosclerosis and offering a basic guarantee for future research on the potential mechanisms and treatments for these two diseases. Compared with the commonly used method of tail vein injection of *P. gingivalis* in recent studies, this model uses the oral administration of a high-concentration live bacterial suspension, which has the advantages of simple operation, low technical sensitivity, and relatively low modelling cost. In terms of the success rate and effect of the modelling method, this this model exhibits the stable induction of severe periodontal destruction and significant atherosclerotic plaques, with the advantages of easy repeatability, controllable quality, a high success rate, high replicability, and increased clinical significance. In addition, the *P. gingivalis* FDC 381 strain used in this study is among the most virulent strains of the numerous *P. gingivalis* strains discovered thus far. The periodontitis model induced by oral application of this strain has the advantages of stability and high repeatability.

Our results revealed increased serum levels of IL-6, RANTES, TNF-a, VEGF, and IFN-γ after oral infection with *P. gingivalis* as well as decreased mRNA levels of ACTA2, STAT3 and T-bet in the aorta and decreased expression of b-FGF and HO-1. Further experimental studies are warranted to confirm our findings and to investigate the possible mechanisms through which the inflammatory state plays a role in these two diseases. Human studies should be carried out to assess whether IL-6, RANTES, TNF-α, VEGF, and IFN-γ levels are related to atherosclerosis in periodontal patients. If so, interventional trials would be the next step to determine whether periodontal treatment could lower the circulating levels of

these biomarkers and, therefore, benefit the vascular health of periodontal patients with other comorbidities.

Declarations

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Author Contributions

Conceptualization, Yan Xuan; methodology, Yan Xuan, Liu Yuanqing and Haijiao Liu; data curation, Yan Xuan and Congcong Lou; formal analysis, Huang Hao and Sun Tong; writing original draft, Yan Xuan and Huang Hao; review, editing and supervision, Qingxian Luan. All the authors have read and agreed to the published version of the manuscript.

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Data availability

The data are contained within the article. Additional data may be available upon reasonable request from the corresponding author.

Clinical trial number

Not applicable.

Ethics approval and consent to participate

ApoE knockout (ApoE-/-) mice (C57BL/6) were obtained from Vital River Inc. (Beijing, China) and the company's consent was obtained. The Institutional Animal Care and Use Committee of Peking University Health Science Center approved all the animal protocols (approval number PUIRB-LA2024209).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

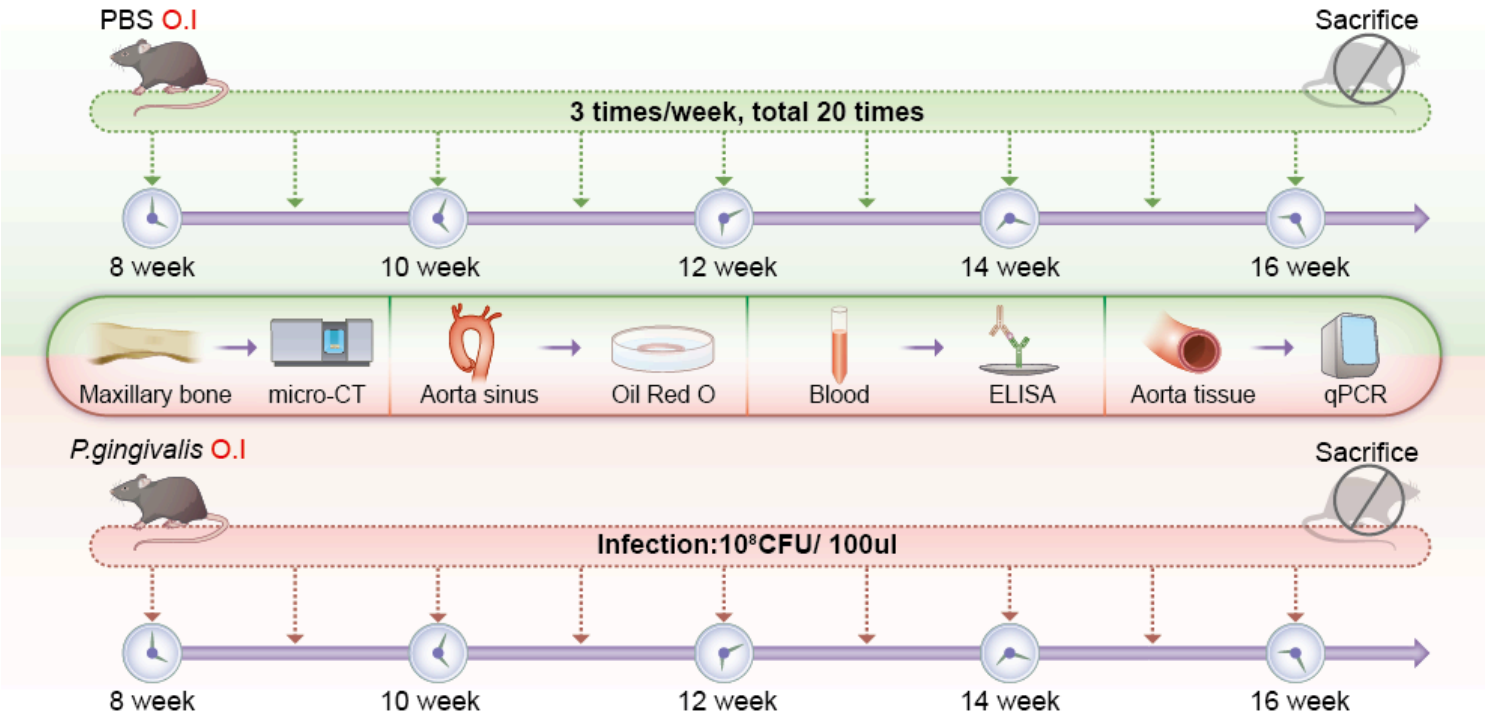


Figure 1

Experimental groups and flow chart of the experiment.

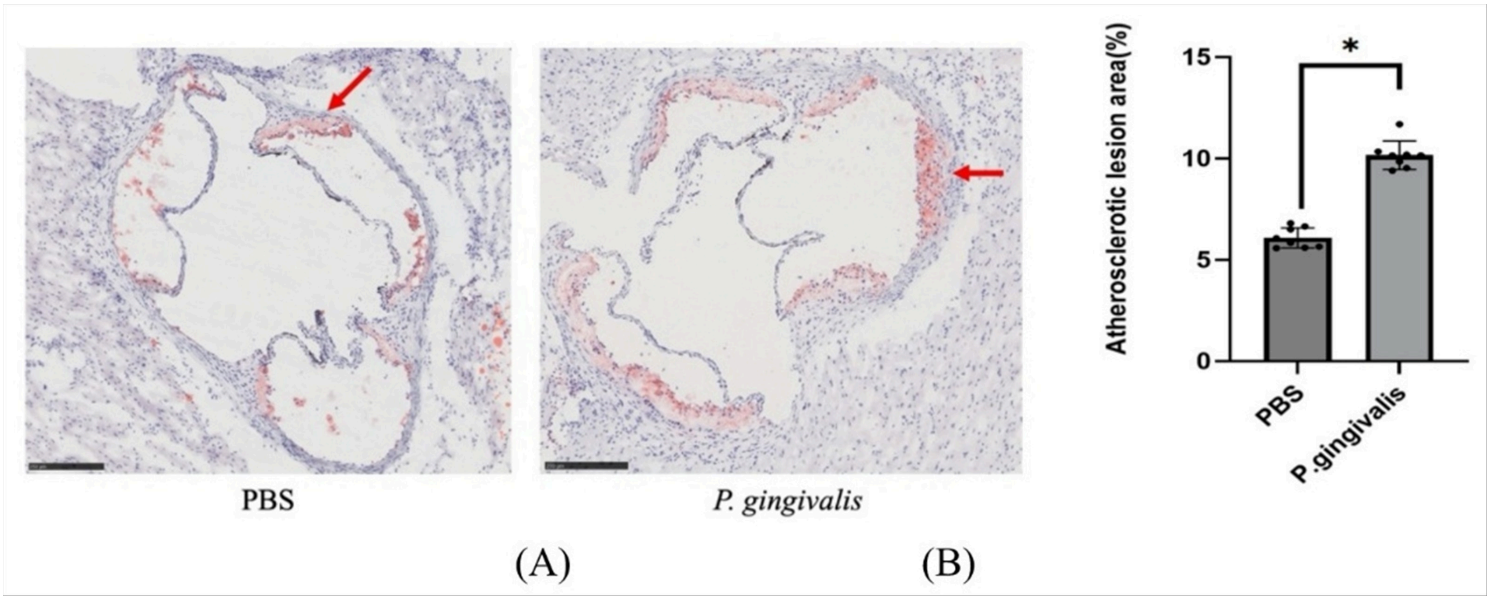


Figure 2

Oil red O staining images showing lipid deposition. The arrows indicate typical lipid deposition in the atherosclerotic areas of the aortic sinus in different groups of ApoE−/− mice (original magnification 100×) (A). The percentage of lesion area in the aortic sinus at 16 weeks is shown (B). The data are presented as the mean ± standard deviation (n = 8). * P < 0.05 compared with the *P. gingivalis* infection group.

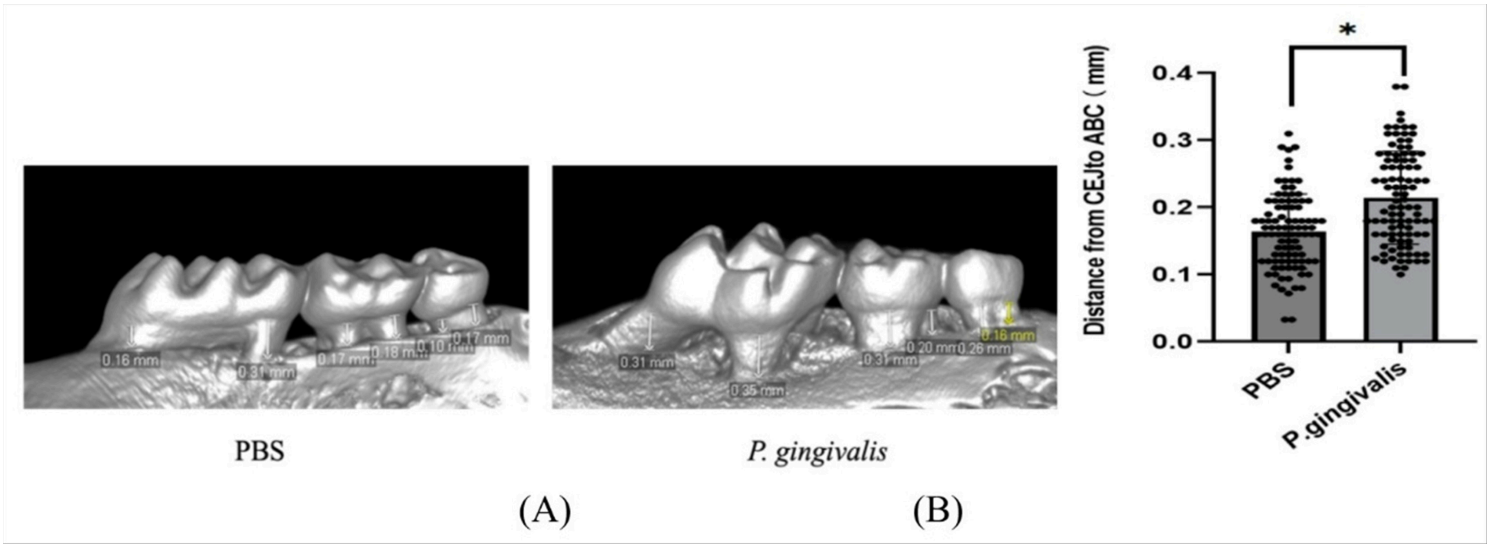


Figure 3

The degree of alveolar bone resorption in mice was examined by micro-CT. (A). Distances from the CEJ to the ABC. (B). The data are presented as the mean ± SD (n=8). * P < 0.05 compared with the *P. gingivalis* infection group.

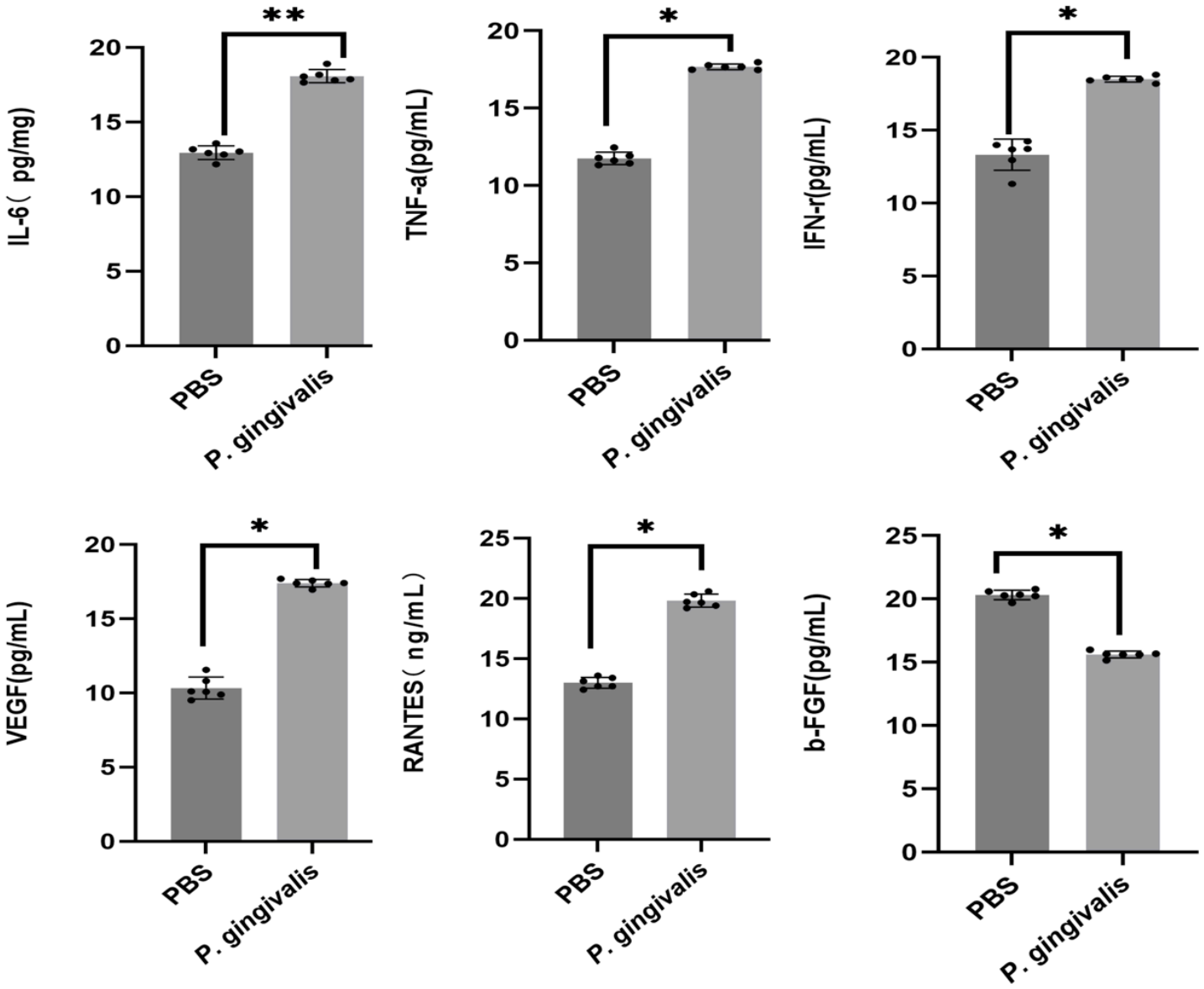


Figure 4

Serum cytokine levels were detected by enzyme-linked immunosorbent assays. The protein levels of IL-6, TNF-α, VEGF, IFN-γ, RANTES, and b-FGF in the serum were measured in blood samples collected at the 16th week. The data are presented as the mean ± standard deviation (n = 8). *P < 0.05, **P < 0.01 compared with the group infected with *P. gingivalis*.

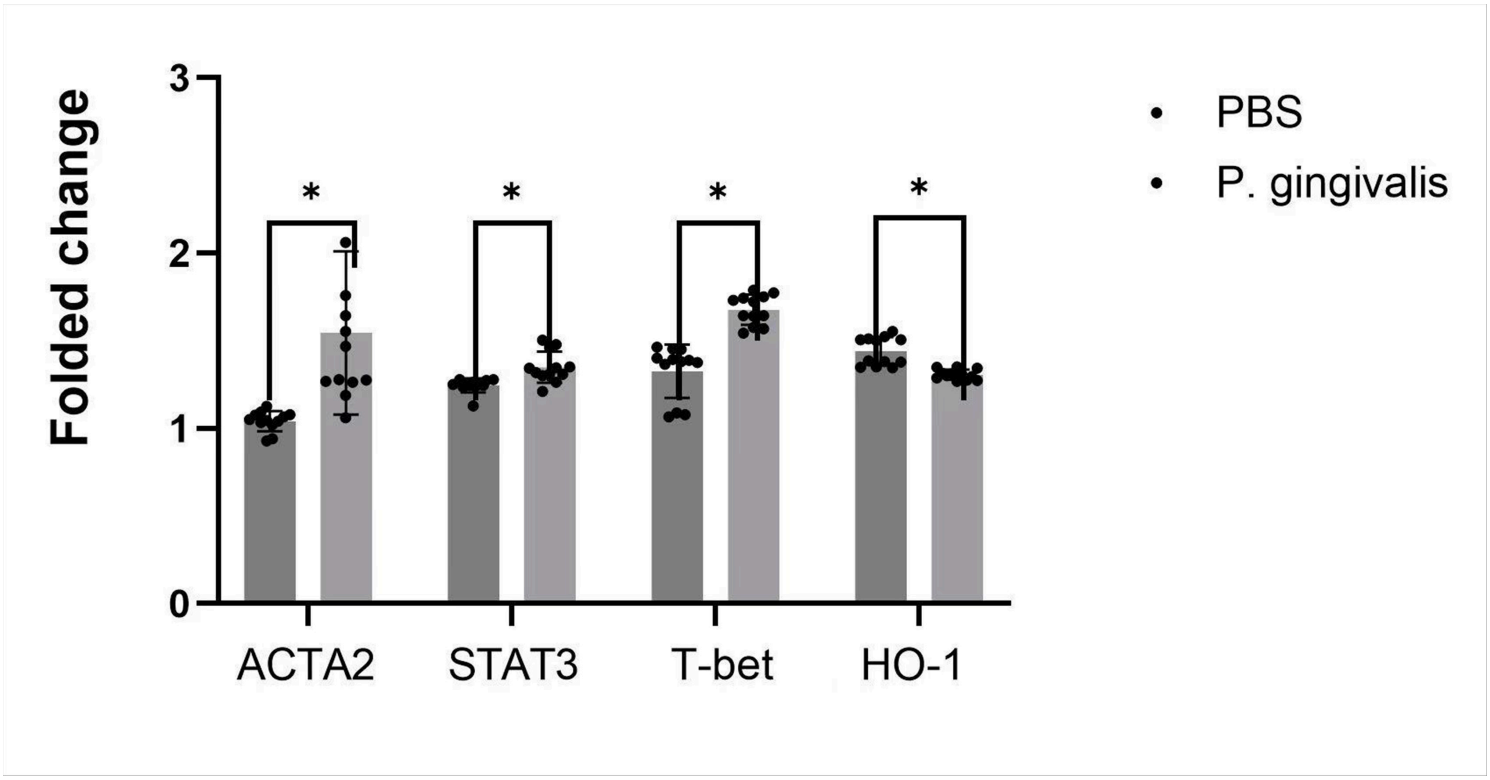


Figure 5

mRNA expression levels of certain mediators in aortic tissue determined through quantitative real-time PCR. The relative mRNA expression levels of the inflammatory mediators in the aortic tissue were normalized to those of GAPDH using real-time PCR. The data are expressed as the fold increase in mRNA level compared with that in the PBS control group or the *P. gingivalis*infection group. The values represent the mean ± standard deviation (n = 8). *P < 0.05 compared with the *P. gingivalis* infection group.

Supplementary Files

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