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High glucose condition aggravates inflammatory response induced by *Porphyromonas gingivalis* in THP-1 macrophages via autophagy inhibition

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Abstract

Background *Porphyromonase gingivalis* (*P. gingivalis*) is a type of bacteria that causes periodontitis, which is strongly correlated with systemic diseases such as diabetes. However, the effect of hyperglycemia on periodontitis are unclear. The present study examined the effects of high glucose levels on the response to *P. gingivalis* infection.

Results The expression of *P. gingivalis*-induced interleukin-1β (IL-1β) and inflammasomes increased as the glucose concentration increased. High glucose conditions suppressed *P. gingivalis*–induced autophagy in human acute monocytic leukemia cell line (THP-1) macrophages. Zingerone increased autophagy and alleviated *P. gingivalis*-induced inflammatory response in THP-1 macrophages under high glucose conditions. In addition, *P. gingivalis*-induced inflammation in bone marrow-derived macrophages of diabetic mice was higher than in wild-type mice, but a zingerone treatment decreased the levels. Alveolar bone loss due to a *P. gingivalis* infection was significantly higher in diabetic mice than in wild-type mice.

Conclusions High-glucose conditions aggravated the inflammatory response to *P. gingivalis* infection by suppressing of autophagy, suggesting that autophagy induction could potentially to treat periodontitis in diabetes. Zingerone has potential use as a treatment for periodontal inflammation induced by *P. gingivalis* in diabetes patients.

Keywords Autophagy, Hyperglycaemia, Inflammation, Periodontitis, *Porphyromonase gingivalis*

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Background

Periodontitis is an inflammatory disease of the periodontium caused by microbial imbalance between symbiotic commensals and pathogen [1]. *Porphyromonase gingivalis* (*P. gingivalis*) is a major etiological agent in chronic periodontitis [1]. The major virulence factors of *P. gingivalis* include lipopolysaccharides, fimbriae, and gingipains, which are essential for evading host defence systems [2]. *P. gingivalis* is a potent stimulator of immune responses, such as the secretion of pro-inflammatory cytokines and the activation of inflammation-related signaling pathways. Recent studies have shown that periodontitis is strongly associated with systemic diseases, including cardiovascular diseases, diabetes mellitus, respiratory diseases, and Alzheimer's disease [3]. A two-way interrelationship between periodontitis and diabetes has been reported as a high prevalence of severe periodontitis in patients with diabetes may influence glycemic control [4].

Hyperglycemia or high blood glucose levels occur when the body has insufficient insulin or insulin resistance, which impairs the defense mechanisms involving micro- and macro-vasculature; elevated glucose levels in the gingival crevicular fluid with diabetes may alter the growth of some microbial species [5, 6]. The levels of *P. gingivalis* colonization are higher in adults with prediabetes [7]. Moreover, a decrease in neutrophilic phagocytosis has been observed in patients with uncontrolled diabetes, and peripheral blood monocytes from patients with diabetes with periodontal disease, secrete significantly more prostaglandin E2 (PGE2) [6, 8]. Increased inflammatory responses to infection and a reduced healing ability may explain the increased periodontal destruction in diabetes [6].

Activating inflammasome components in response to infection is a potential clinical biomarker of inflammation in periodontal disease [9]. Inflammasomes are cytosolic multi-protein complexes of the innate immune system that regulate the activation of caspase-1 and induce inflammation [10]. The activation and assembly of inflammasomes promote proteolytic cleavage and the maturation and secretion of the pro-inflammatory cytokines, interleukin-1β (IL-1β) and interleukin-18 (IL-18) [10]. Furthermore, the activation of nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3) inflammasome components in patients with periodontitis and uncontrolled type-2 diabetes indicates severe pathological processes [11].

Autophagy is a self-degrading process and an intracellular degradation mechanism that plays a critical role in maintaining cellular integrity and survival by eliminating unnecessary proteins and impaired organelles [12]. Autophagy functions as a modulator of pathogenesis and is a potential therapeutic target in various diseases, such as periodontitis and diabetes by regulating apoptosis, inflammation, pathogen clearance, immune response, and other cellular processes [12]. In addition, high glucose levels suppress autophagy through the AMP-activated protein kinases (AMPK) signalling pathway in chondrocytes, and an autophagy enhancer improves the metabolic profile of ob/ob mice and ameliorates inflammasome activation [13, 14]. However, the inflammatory response and autophagy of oral pathogens in diabetes periodontitis have not been studied. Therefore, the impact of high glucose levels on periodontal inflammation in *P. gingivalis* infections in macrophages must be determined. This study aims to investigate whether high glucose conditions influence the inflammatory response to *P. gingivalis* infection and its mechanism of action.

Materials and methods

Bacterial culture

P. gingivalis (strain 381) was cultured anaerobically in 80% nitrogen, 10% hydrogen, and 10% CO_2 in a Gifu anaerobic medium broth (Nissui Co., Tokyo, Japan) comprising 5 mg/mL hemin and 0.5 mg/mL 3-phytyl-menadione (vitamin K) at 37 °C. The bacteria were washed and resuspended in a cell culture medium to infect the human acute monocytic leukemia cell line (THP-1) and bone marrow-derived macrophages (BMDMs) at a multiplicity of infection of 50.

Cell culture

The human monocyte cell line THP-1 was cultured in RPMI medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum and was differentiated into macrophages using 50 nM phorbol 12-mystristate 13-acetate (Sigma-Aldrich, St. Louis, MO, USA) overnight. The differentiated macrophages were pre-treated with glucose (Sigma-Aldrich) for 24 h and then infected with *P. gingivalis* for 24 h.

Cell death assay

Cell death was measured using a lactate dehydrogenase cytotoxicity assay kit (CytoTox96 nonradioactive cytotoxicity assay; Promega, Madison, WI, USA). The optical densities were measured at 492 nm according to the manufacturer's instructions.

Cytokine assay

The IL-1β levels in the cell supernatant after *P. gingivalis* infection were determined usinga an enzyme-linked immunosorbent assay kit (BioLegend, San Diego, CA, USA). The optical densities were measured at 450 and 570 nm using an enzyme-linked immunosorbent assay reader (Tecan, Männedorf, Switzerland) according to the manufacturer's instructions.

Western blot analysis

The cells were harvested and lysed in RIPA buffer (Cell Signaling Technology, Danvers, MA, USA) containing protease inhibitor cocktail (Sigma-Aldrich). The proteins samples were separated using 10–15% SDS-PAGE and then transferred to membranes (Milli-poreSigma, Burlington, MA, USA). The transferred membranes were cut according to the sizes determined by the protein marker. A cut membranes were probed with primary and secondary antibodies. The membranes were developed using a chemiluminescence solution (GE Healthcare, Chicago, IL, USA) in a LAS-4000 Lumino-imaging unit (Fujifilm, Tokyo, Japan). Immunoblot band intensities were quantified using NIH ImageJ software (Fu-jifilm), and the results are presented as the intensity ratios versus β-actin. The antibodies used were as follows: an apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC; 22 kDa, Cell Signalling, Danvers, MA, USA), absent in melanoma 2 (AIM 2; 40 kDa, Cell Signaling, Danvers, MA, USA), a nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 (NLRP3; 117 kDa, Cell Signalling, Danvers, MA, USA), Beclin-1 (60 kDa, Santa Cruz Biotechnology, Dallas, TX, USA), autophagy related protein 5/12 (ATG5/12; 50 kDa, Santa Cruz Biotechnology, Dallas, TX, USA), light chain 3 (LC3; 18, 16 kDa, Cell Signalling, Danvers, MA, USA), and β-actin (43 kDa, Santa Cruz Biotechnology, Dallas, TX, USA).

Autophagy and lysosome detection via confocal microscopy

Macrophages were stained with fluorescent dyes using the CYTO-ID Autophagy Detection Kit (Enzo Life Sciences, Farmingdale, NY, USA), which comprised a cationic amphiphilic dye that can precisely monitor the autophagic vacuoles (pre-autophagosomes, autophagosomes, and autolysosomes) in green with minimal lysosomal staining and without transfection. The whole macrophages were stained with Hoechst-33,342 (a nuclear stain) to determine the total cell population. The macrophages were incubated at 37˚C for 15 min on a light block and were then observed using a confocal laser scanning microscope (Carl Zeiss, Jena, Germany). The percentage of macrophages containing autophagosomes was calculated by dividing the number of macrophages containing autophagosomes by the total number of macrophages.

Reagents

Rapamycin (CalbioChem, San Diego, CA, USA), an autophagy activator that inhibits the mammalian target of the rapamycin pathway by the direct binding of the mammalian target of rapamycin Complex 1, was used at 1μM per cell to induce autophagy. Zingerone, derived from ginger (ChromaDex, Irvine, CA, USA) was treated with 40 μM for 30 min in THP-1 and BMDMs, respectively.

Animals

Wild-type (WT; C57BL/6J) and diabetic mice (C57BL/6J) were purchased from the Central Lab Animal Inc. (Seoul, Korea). The mice were fed a finely milled autoclaved normal fat diet (under 10 kcal% fat) and were housed in a controlled environment (22 °C and 45–55% relative humidity) under a 12-h light/dark cycle.

Fresh bone marrow macrophages, isolated from femurs and tibiae, were differentiated into BMDMs using an L929 cell-conditioned medium as a source of macrophage colony-stimulating factors. The macrophages were incubated at 37 °C in in an atmosphere containing 5% CO_2. After four days of incubation, the attached macrophages were washed and detached with sterile PBS, then centrifuged at 200 g for 5 min, and used for experiments.

To generate a periodontitis model, mice were randomly divided randomly into a control group and a *P. gingivalis*-infected periodontitis control group (4×10^9 CFU/kg body weight; *P. gingivalis* group). The control group comprised sham-infected mice. *P. gingivalis* (4×10^9 CFU/kg body weight) was suspended in 100 μL of 2% carboxymethyl cellulose and was administered orally using a Zonde needle every two days for two weeks. The mice were euthanized through carbon dioxide (CO_2) inhalation and evaluated on the same day. According to the 2020 guidelines from the American Veterinary Medical Association (AVMA), euthanasia using a CO_2 chamber minimizes discomfort in animals (AVMA 2020, pp. 31). The study protocol was approved by the Institutional Animal Care and Use Committee of the Pusan National University (PNU-2022-0108).

Quantification of alveolar bone loss

The mandibular bone of the mice was prepared to observe alveolar bone resorption. Three-dimensional images were scanned in slices via micro-computed tomography (micro-CT, InspeXio SMX-90CT; Shimadzu Science, Tokyo, Japan) using the following settings: 90 kV, 110 μA, and 0.5 mm aluminum attenuation filter. The scans were reconstructed to generate three-dimensional models, and the region of interest was the cuboidal bone body encompassing the roots; it extended from the most mesial to the most distal aspect of the three lower molar roots. The three-dimensional images were prepared in a standardized identical position according to the defined anatomical reference using TRI/3D Bone (Ratoc, Tokyo, Japan). The amount of lost alveolar bone was summed by the area from the cemento-enamel junction to the alveolar bone crest of each tooth using ImageJ software.

A single-blinded examiner performed all the measurements. A larger area implies more severe bone loss.

Statistical analysis

The data were analyzed using the SPSS (version 21.0; IBM Co., Armonk, NY, USA). The groups were compared using the Student's t-test or analysis of variance, where relevant. The results are expressed as means±standard deviations, and statistical significance was set for unpaired *P*-values at <0.050.

Results

High glucose conditions regulated *P. gingivalis*–induced inflammasome activation and autophagy in THP-1 macrophages

This study examined the expression of inflammasome-related molecular proteins, such as NLRP3, absent in melanoma 2, pro-IL-1β, and ASC, in THP-1 macrophages to determine the impact of glucose on *P. gingivalis*–induced inflammation. The cytotoxicity of *P. gingivalis* at a high glucose concentration (50 mM) was higher than that at a low glucose concentration (5 mM) (Fig. 1A). At 5mM glucose, the inflammasome-related protein levels increased gradually the glucose concentration was increased by *P. ginigivalis* infection compared to the levels in the control group (Fig. 1B). The IL-1β level produced by *P. gingivalis* under high-glucose conditions was significantly higher than that under low-glucose conditions (Fig. 1C).

The effects of high glucose levels on *P. gingivalis*–induced autophagy in THP-1 macrophages were examined by measuring, the levels of autophagy-related proteins. The levels of ATG5/12, Beclin1, and LC3-II expression were increased by *P. gingivalis* but decreased according to the glucose concentrations (Fig. 1D). For the *P. gingivalis* infection, the number of Cyto-ID puncta

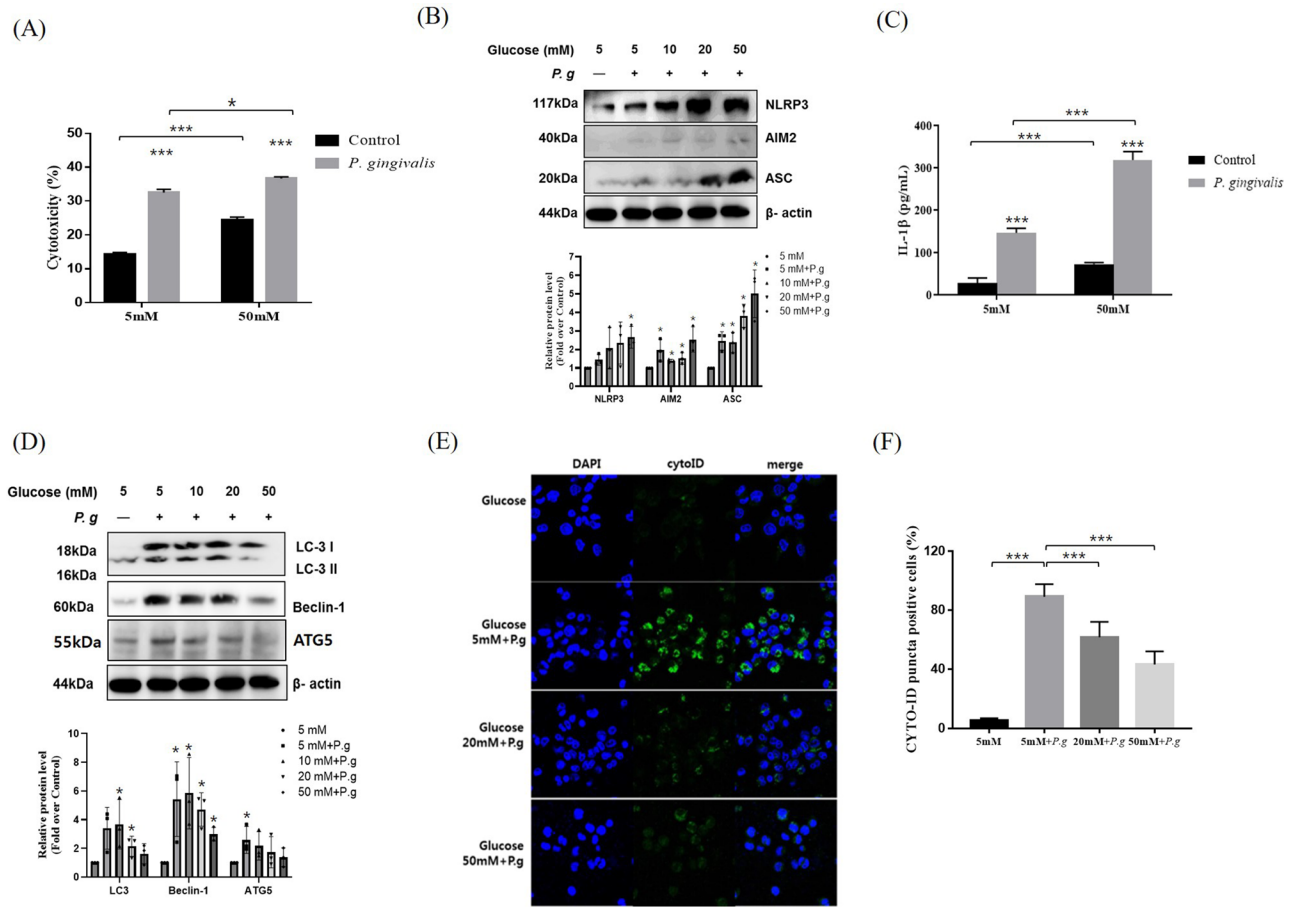

Fig. 1 Impact of high glucose levels on inflammasome activation and autophagy in *P. gingivalis* -infected THP-1 macrophages. (**A**-**F**) THP-1 macrophages were pre-treated with the indicated glucose concentrations and were then infected with *P. gingivalis* for 24 h. (**A**) Cytotoxicity was determined using a lactate dehydrogenase (LDH) assay. (**B**) Western blot analysis was performed to determine the expression of inflammasome-related proteins. Densitometry of the band intensity was performed. (**C**) IL-1β levels in cell supernatants were determined using enzyme-linked immunosorbent assay (ELISA). (**D**) A western blot analysis was performed to determine the expression of autophagy-related proteins. Densitometry of the band intensity was performed. (**E** and **F**) The macrophages were stained with Cyto-ID agents and were analyzed using confocal microscopy (× 200 magnification), and the total number of Cyto-ID puncta per cell was counted. **P* < 0.050, ***P* < 0.010, and ****P* < 0.001

increased at low glucose levels, whereas that of Cyto-1D puncta decreased markedly at high glucose levels (Fig. 1E and F).

These results suggest that high glucose conditions promote *P. gingivalis*-induced inflammation by activating the inflammasome signalling pathway and inhibiting *P. gingivalis*–induced autophagy in the THP-1 macrophages.

Autophagy alleviated inflammasome activation in *P. gingivalis*-infected THP-1 macrophages under high glucose conditions

P. gingivalis-infected THP-1 macrophages were treated with rapamycin, an autophagy activator, to determine if autophagy influences inflammasome activation under high glucose conditions. Rapamycin had no cytotoxic effects (Fig. 2A). Under high glucose conditions, the expression of inflammasome-related molecules was increased by a *P. gingivalis* infection and decreased by rapamycin (Fig. 2B). Furthermore, the increased IL-1β secretion after the *P. gingivalis* infection was inhibited by rapamycin under high glucose conditions (Fig. 2C).

Zingerone, which showed no observable cytotoxicity against THP-1 macrophages, was used to develop a natural candidate for autophagy activation with anti-inhibitory effects (Fig. 2D). The expression of the inflammasome complex produced by *P. gingivalis* infection was suppressed by zingerone (Fig. 2E). Moreover, zingerone inhibited IL-1β secretion significantly (Fig. 2F). Autophagy suppresses inflammasome activation in *P. gingivalis*-infected THP-1 macrophages under high glucose conditions.

P. gingivalis infection aggravated inflammatory response and bone resorption in diabetic mice

Inflammasome expression in WT and diabetes mice was examined to determine if *P. gingivalis* induces inflammation in diabetes cases. After *P. gingivalis* infection, the levels of inflammasome-related proteins in the BMDMs of the diabetic mice were higher than in those of the WT mice, and the levels of autophagy-related proteins were lower in the BMDMs of the diabetic mice than in those of the WT mice (Fig. 3A). IL-1β levels in the BMDMs of the diabetic mice were higher than in those of the WT mice, regardless of the presence of *P. gingivalis* infection (Fig. 3B). Furthermore, the area of the root surface exposed to *P. gingivalis* infection was greater in the diabetic mice than in the WT mice (Fig. 3C). These results suggest that the diabetic conditions increase *P. gingivalis*-induced inflammation and suppress *P. gingivalis*- induced autophagy. Moreover, diabetic conditions increased the susceptibility to *P. gingivalis* infection in an in vivo animal model.

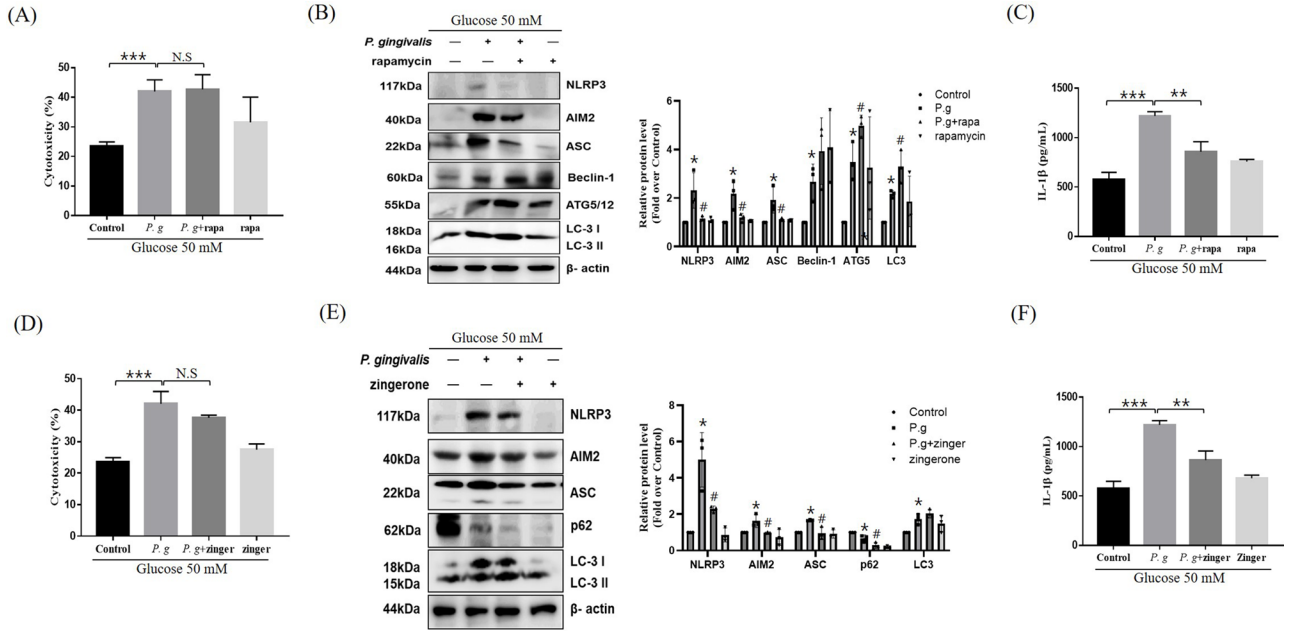

Fig. 2 Inhibitory effects of autophagy on inflammasome activation in *P. gingivalis* -infected THP-1 macrophages under high glucose conditions. (**A**-**C**) THP-1 macrophages were pre-treated with rapamycin (1 μM) and were then infected with *P. gingivalis* for 24 h at a 50 mM glucose concentrations. (**A**) Cytotoxicity was assessed using an LDH assay. (**B**) A western blot analysis was performed to determine the expression of proteins. Densitometry of the band intensity was performed. (**C**) IL-1β levels in cell supernatants were determined using ELISA. (**D**-**F**) THP-1 macrophages were pre-treated with zingerone (40 μM) and were then infected with *P. gingivalis* for 24 h at 50 mM glucose concentrations. (**D**) Cytotoxicity was determined using an LDH assay. (**E**) Western blot analysis was performed to determine the expression of proteins. Densitometry of the band intensity was performed. (**F**) IL-1β levels in cell supernatants were determined using ELISA. N.S: Not Significant. $^{**}P < 0.010$ and $^{***}P < 0.001$. $\#p < 0.05$ *P. gingivalis* infection versus *P. gingivalis* with rapamycin or zingerone

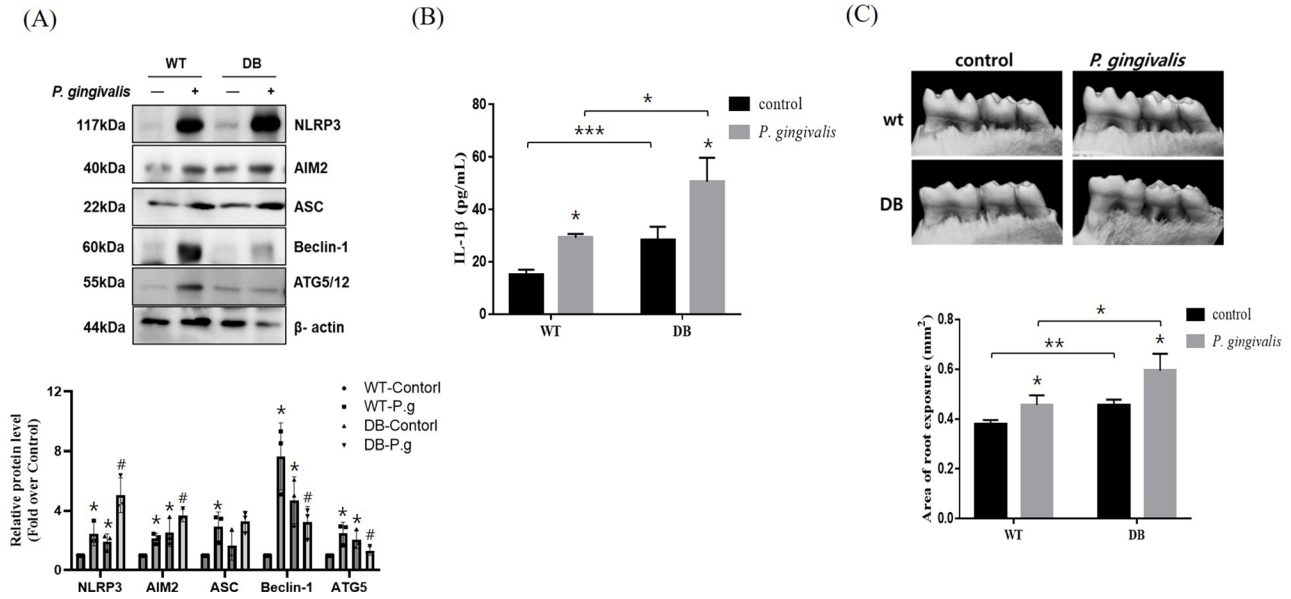

Fig. 3 Inflammatory response induced by *P. gingivalis* in diabetic mice. (**A** and **B**) The BMDMs of WT and diabetic mice were infected with *P. gingivalis* for 24 h. (**A**) Western blot analysis was performed to determine the expression of proteins. Densitometry of the band intensity was performed. (**B**) IL-1β levels in the cell supernatant were determined using ELISA. (**C**) Alveolar bone loss was measured using micro-CT in WT and diabetic mice. Representative images (left) and the areas between the cemento-enamel junction and alveolar bone crest of the three molars were calculated using ImageJ and were presented as the area of root exposure in the bar graph (right). *$P < 0.050$, **$P < 0.010$, and ***$P < 0.001$. #$p < 0.05$ WT-*P. gingivalis* infection versus DB-*P. gingivalis* infection

Zingerone alleviated inflammatory response in *P. gingivalis*-infected BMDMs in high glucose conditions

BMDMs in the WT mice were prepared to confirm the anti-inflammatory effects of zingerone on *P. gingivalis*-inflammation under high glucose concentrations, in which, the expression of inflammasome-related molecules was increased by *P. gingivalis* infection and was decreased by zingerone (Fig. 4A). Furthermore, zingerone increased the levels of autophagy-related proteins (Beclin-1, ATG5/12, and LC3) and suppressed *P. gingivalis*-induced IL-1β production under high glucose conditions (Fig. 4B).

Finally, this study examined the impact of zingerone on the BMDMs of diabetic mice infected with *P. gingivalis*. Moreover, the zingerone treatment enhanced the expression of autophagy-related proteins produced by *P. gingivalis* (Fig. 4C). The IL-1β level, which was increased by *P. gingivalis*, was inhibited by zingerone (Fig. 4D).

These results suggest that zingerone, an autophagy inducer, suppresses IL-1β production and inflammasome activation in WT and diabetic BMDMs infected with *P. gingivalis.*

Discussion

Several research and clinical studies have reported an association between periodontitis and diabetes [4]. Hyperglycemia is a well-known risk factor and the most widely used clinical diagnosis of diabetes [5]. We have previously reported that glucose levels are significantly elevated in the saliva of patients with periodontitis [15]. Patients with poorly controlled diabetes are at a high risk of periodontitis and alveolar bone loss [16]. This study examined the impact of high glucose levels on the host cell response to *P. gingivalis* infection.

Initially, this study examined whether high glucose levels affect inflammation in *P. gingivalis*-infected THP-1 macrophages. Inflammasome activation is a response that leads to inflammation by forming a multi-complex in innate immunity [17]. In THP-1 macrophages, inflammasome-related proteins and the IL-1β levels were elevated in response to glucose (5–50 mM) (Suppl. A-C). After the *P. gingivalis* infection, these responses were markedly higher at high glucose levels than at low levels (Fig. 1A-C). A previous study reported that NLRP3 inflammasome components were overexpressed in the gingival tissues of patients with periodontitis and uncontrolled diabetes [18]. The present study showed that inflammasome-related proteins and IL-1β levels were higher in the BMDMs of diabetic mice than in WT mice (Fig. 3A and B). These results indicated that high glucose levels accelerate *P. gingivalis*-induced inflammation via the inflammasome signaling pathway.

Autophagy is a highly conserved cellular pathway for the lysosomal degradation and recycling of long-lived proteins and organelles, playing an essential in the maintaining the cytoplasmic quality and homeostasis under normal and pathological conditions [19]. Another study reported that high glucose concentrations suppressed

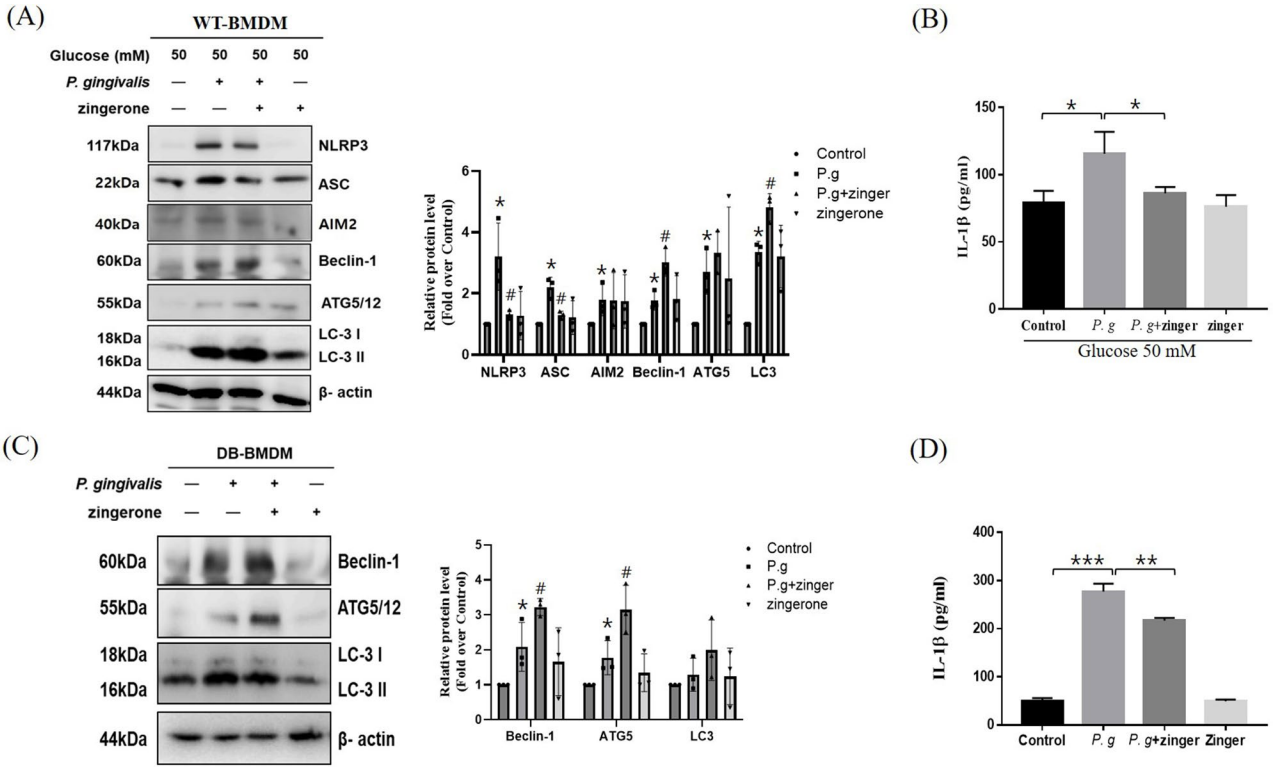

Fig. 4 Effect of zingerone on *P. gingivalis* -induced inflammatory response in high glucose-treated WT BMDMs and diabetic BMDMs. (**A** and **B**) The BMDMs of WT mice were pre-treated with zingerone (40 μM) and were then infected with *P. gingivalis* for 24 h at 50 mM glucose concentrations. (**A**) A western blot analysis was performed to determine the expression of proteins. Densitometry of the band intensity was performed. (**B**) IL-1β levels in cell supernatants were determined using ELISA. (**C** and **D**) The BMDMs of diabetic mice were pre-treated with zingerone (40 μM) and were then infected with *P. gingivalis* for 24 h. (**C**) A western blot analysis was used to determine the expression of proteins. Densitometry of the band intensity was performed. (**D**) IL-1β levels in cell supernatants were determined using ELISA. #$p < 0.05$ *P. gingivalis* infection versus *P. gingivalis* with zingerone

autophagy in chondrocytes [13]. In the present study, the number of autophagy-related proteins and Cyto-ID punctate-positive cells was reduced dramatically at high glucose concentrations (Suppl. D-F). Previously, we reported that *P. gingivalis*-infected macrophages induced autophagy by increasing the expression of autophagy-related proteins [20]. However, we found that *P. gingivalis*–induced autophagy was suppressed in a glucose concentration-dependent manner (Fig. 1D-F). Furthermore, zingerone restored the autophagic response that was suppressed under diabetic conditions (Figs. 2 and 4).

Moreover, many studies reported that autophagy modulates inflammatory response [21, 22]. We previously reported that *P. gingivalis*-induced autophagy in macrophages restrict the excessive inflammatory responses by downregulating IL-1β production [20]. In this study, *P. gingivalis*-induced inflammasome proteins and IL-1β secretion levels were reduced by rapamycin and zingerone, which are autophagy activators in THP-1 macrophages (Fig. 2) and in the BMDMs of WT mice (Fig. 4A and B). Furthermore, in the BMDMs of diabetes mice, zingerone augmented *P. gingivalis*-induced autophagy-related proteins expression and IL-1β secretion.

In addition, we found that basal bone loss of control in DB mice was higher than in WT mice (Fig. 3c). In a clinical report, alveolar bone loss in diabetic patients (who were not diagnosed with periodontitis) was significantly higher compared to healthy individuals [23]. These results provide evidence that diabetes is a risk factor for periodontitis. Furthermore, alveolar bone destruction was higher in diabetes mice than in WT mice infected with *P. gingivalis*. Consistent with these results, another study showed that hyperglycemia in diabetes mellitus suppresses osteoblast-mediated bone formation; however, it promotes osteoclast-mediated bone resorption [24]. Therefore, glucose control may prevent alveolar bone destruction in diabetic mice.

We acknowledge that the long-term therapeutic effects and side effects of zingerone treatment are uncertain and require more work to be conducted to verify autophagy activity compared to chemical autophagy activator by exploring the formation of autophagolysosome and bacterial survival rate in diabetic macrophages infected with *P. gingivalis*.

In this study, we demonstrated that the inflammatory response caused by live *P. gingivalis* in macrophages was

suppressed by activating autophagy under high glucose for the first time. This study provides a new perspective for better understanding the mechanism aggravating periodontal inflammation in diabetes patients.

Conclusions

High glucose exacerbated *P. gingivalis*-induced inflammation by suppressing autophagy and the inflammation was reduced by zingerone which activates autophagy. Thus, zingerone may be potential treatment for periodontal inflammation caused by *P. gingivalis* in patients with diabetes.

Abbreviations

AIM 2	Absent in melanoma 2
AMPK	AMP-activated protein kinases
ASC	Apoptosis-associated speck-like protein containing a caspase recruitment domain
ATG5/12	Autophagy-related protein 5/12
BMDMs	Bone marrow-derived macrophages
DB	Diabetes
IL-1β	Interleukin-1β
IL-18	Interleukin-18
LC3	Light chain 3
NLRP3	Nucleotide-binding oligomerization domain-like receptor containing pyrin domain 3 inflammasome
P. gingivalis	*Porphyromonase gingivalis*
Rapa	Rapamycin
THP-1	Human acute monocytic leukaemia cell line macrophages
WT	Wild type
Zinger	Zingerone

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-024-00655-7.

Supplementary Material 1

Supplementary Material 2: (A-F) THP-1 macrophages were treated with the indicated concentrations of glucose. (A) Cytotoxicity was determined using an LDH assay. (B) A western blot analysis was performed to determine the expression of inflammasome-related proteins. (C) IL-1β levels in cell supernatants were determined using ELISA. (D) A western blot analysis was performed to determine the expression of autophagy-related proteins. (E and F) Macrophages were stained with Cyto-ID agents and analyzed using confocal microscopy (× 200 magnification), and the total number of Cyto-ID puncta per cell was counted.

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None.

Author contributions

YR.S. and JJ.K. contributed equally to the work and should be regarded as co-first authors. YR.S. and JJ.K. performed experiments, analyzed the data and wrote the manuscript. H.S.N. and S.Y.K. performed the acquisition of data, data analysis and interpretation. J.C. and SH.S. were responsible for conception and design of study, revising the manuscript. All authors have read and approved the manuscript.

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Data availability

All data generated or analysed during this study are included in this published article (and its supplementary information files). The original datasets analysed are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Institutional Animal Care and Use Committee of the Pusan National University (PNU-2022-0108).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Hajishengallisa G, Lambris JD. Complement and dysbiosis in periodontal disease. Immunobiol. 2012;217:111–1116.
2. Jia L, Han N, Du J, Guo L, Luo Z, Liu Y. Pathogenesis of important virulence factors of Porphyromonas gingivalis via Toll-Like receptors. Front Cell Infect Microbiol. 2019;9:262.
3. Olsen I, Singhrao SK. Porphyromonas gingivalis infection may contribute to systemic and intracerebral amyloid-beta: implications for Alzheimer's disease onset. Expert Rev Anti Infect Ther. 2020;18:1063–6.
4. Nascimento GG, Leite FRM, Vestergaard P, Scheutz F, López R. Does diabetes increase the risk of periodontitis? A systematic review and meta-regression analysis of longitudinal prospective studies. Acta Diabetol. 2018;55:653–67.
5. Kocher T, König J, Borgnakke WS, Pink C, Meisel P. Periodontal complications of hyperglycemia/diabetes mellitus: epidemiologic complexity and clinical challenge. Periodontol 2000. 2018;78:59–97.
6. Daniel R, Gokulanathan S, Shanmugasundaram N, Lakshmigandhan M, Kavin T. Diabetes and periodontal disease. J Pharm Bioallied Sci. 2012;4(Suppl 2):S280–2.
7. Demmer RT, Jacobs DR Jr, Singh R, et al. Periodontal Bacteria and Prediabetes Prevalence in ORIGINS: the oral infections, glucose intolerance, and insulin resistance study. J Dent Res. 2015;94(Suppl 9):S201–11.
8. Shetty N, Thomas B, Ramesh A. Comparison of neutrophil functions in diabetic and healthy subjects with chronic generalized periodontitis. J Indian Soc Periodontol. 2008;12:41–4.
9. Yilmaz Ö, Lee KL. The Inflammasome and Danger Molecule Signaling: At the Crossroads of Inflammation and Pathogen Persistence in the Oral Cavity. Periodontol. 2000. 2015;69:83–95.
10. de Zoete MR, Palm NW, Zhu S, Flavell RA, Inflammasomes. Cold Spring Harb Perspect Biol. 2014;6:016287.
11. García-Hernández AL, Muñoz-Saavedra ÁE, González-Alva P, et al. Upregulation of proteins of the NLRP3 inflammasome in patients with periodontitis and uncontrolled type 2 diabetes. Oral Dis. 2019;25:596–608.
12. Tan YQ, Zhang J, Zhou G. Autophagy and its implication in human oral diseases. Autophagy. 2017;13:225–36.
13. Wang B, Shi Y, Chen J, et al. High glucose suppresses autophagy through the AMPK pathway while it induces autophagy via oxidative stress in chondrocytes. Cell Death Dis. 2021;12:506.
14. Lim H, Lim YM, Kim KH, et al. A novel autophagy enhancer as a therapeutic agent against metabolic syndrome and diabetes. Nat Commun. 2018;9:1438.
15. Kim S, Kim HJ, Song Y, Lee HA, Kim S, Chung J. Metabolic phenotyping of saliva to identify possible biomarkers of periodontitis using proton nuclear magnetic resonance. J Clin Periodontol. 2021;48:1240–9.
16. Soskolne WA, Klinger A. The relationship between periodontal diseases and diabetes: an overview. Ann Periodontol. 2001;6:91–8.
17. Mariathasan S, Newton K, Monack DM, et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. Nat Nat. 2004;430:213–8.
18. Koonen DP, Jacobs RL, Febbraio M, et al. Increased hepatic CD36 expression contributes to dyslipidemia associated with diet-induced obesity. Diabetes. 2007;56:2863–71.

19. Kobayashi S, Xu X, Chen K, Liang Q. Suppression of autophagy is protective in high glucose-induced cardiomyocyte injury. Autophagy. 2012;8:577–92.
20. Park MH, Jeong SY, Na HS, Chung J. Porphyromonas gingivalis induces autophagy in THP-1-derived macrophages. Mol Oral Microbiol. 2017;32:48–59.
21. Houtman J, Freitag K, Gimber N, Schmoranzer J, Heppner FL, Jendrach M. Beclin1-driven autophagy modulates the inflammatory response of microglia via NLRP3. EMBO J. 2019;38:99430.
22. Ma K, Li Y, Dong X, Guo J. AMPK-mediated autophagy modulates the inflammatory cytokine expression in intestinal epithelial macrophages induced by high glucose. Eur J Inflamm. 2022;20.
23. Afsheen Tabassum. Alveolar bone loss in Diabetic patients: a case-control study. Eur J Dent. 2024;18:168–73.
24. Wongdee K, Charoenphandhu N. Osteoporosis in diabetes mellitus: possible cellular and molecular mechanisms. World J Diabetes. 2011;2:41–8.

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