International Immunopharmacology 73 (2019) 57–63

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

Teneligliptin inhibits lipopolysaccharide-induced cytotoxicity and inflammation in dental pulp cells

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ARTICLE INFO

Keywords:
Teneligliptin
Lipopolysaccharide (LPS)
Nuclear factor-κB
Human dental pulp cell
Diabetes mellitus

ABSTRACT

Diabetes mellitus is one of the most common health threatening disorders. Patients with chronic diabetes are at high risk of contracting oral diseases, including dental pulp damage. In this study, we reviewed how Teneligliptin, a commonly used anti-diabetic agent, protected dental pulp cells from lipopolysaccharide (LPS)-induced cytotoxicity and improved their viability. The dental pulp cells treated with Teneligliptin were resistant to LPS-induced reactive oxygen species (ROS) and its byproduct 4-hydroxynonenal (4-HNE) generation. The Teneligliptin recovered LPS-induced a reduction of cellular glutathione and produced cytokine including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6). Mechanistically, we found that Teneligliptin suppressed LPS- that caused an expression of the cell surface receptor toll like receptor 4 (TLR-4) and the activation of JNK kinase and activator protein 1 (AP1) as well as the nuclear factor-κB (NF-κB) signal pathways. Collectively, our study demonstrates that the molecular mechanism Teneligliptin is a protective anti-diabetic agent in dental pulp cells and it has the potential to treat diabetes-associated dental pulp diseases.

1. Introduction

Pulpitis (tooth pulp inflammation) is an infective disease caused by accumulation of oral bacteria on the surface of the tooth [1]. A diverse range of bacterial components can invade the dentin and root canal. Lipopolysaccharide (LPS), also termed endotoxin, acts as one of the major components of the outer membrane of gram-negative bacteria [3]. Previous studies have shown that LPS plays a key role in the pathogenesis of pulpitis by activating several intracellular responses to pulpal infection [4]. Human dental pulp cells (HDPCs) have been identified as the major cell type in dental pulp tissue [5]. HDPCs play a key role in regulating host defense and dental pulp regeneration in pulpitis [6]. Upon stimulation with extracellular toxins, HDPCs secrete significant amounts of inflammatory mediators locally to attract and interact with additional immune cells [7]. Exposure to LPS causes extensive inflammatory response in hDPCs by increasing the expression and secretion of various pro-inflammatory cytokines and chemokines, including tumor necrosis factor α (TNF-α), interleukin-1 (IL-1β), and IL-6 [8] via activation of toll-like receptor 4 (TLR-4) and TLR-2 [9]. LPS treatment has been reported to reduce cell viability and increase apoptosis in hDPCs [10]. Importantly, LPS treatment induces the initiation and activation of several intracellular signaling cascades. For example, activation of the c-Jun N-terminal kinase (JNK) is involved in mediating the excessive expression of cytokines in response to LPS stimulation [11]. Additionally, LPS exposure causes the activation of the activator protein 1 (AP-1) and nuclear factor-κB (NF-κB) signaling pathways, both of which have been identified as central regulators of the inflammatory response in various tissues and cells [12]. Amelioration of LPS-induced inflammatory response and cellular damage has become an important strategy for the treatment of pulpitis.

Teneligliptin, an important member of the dipeptidyl peptidase-4 (DPP-4) inhibitor family, has been licensed for the treatment of diabetes mellitus. Administration of teneligliptin can modulate both fasting and post-prandial glucose levels by increasing the plasma concentrations of incretion hormones including glucagon-like peptide 1(GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [13]. Teneligliptin prevents obesity and obesity-related manifestations by increasing energy expenditure, thereby suggesting a potential use case for

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https://doi.org/10.1016/j.intimp.2019.04.059
Received 29 August 2018; Received in revised form 17 April 2019; Accepted 28 April 2019
Available online 09 May 2019
1567-5769/

teneligliptin as a therapeutic treatment option for a broad spectrum of obesity-related metabolic disorders beyond glycemic control [14]. Teneligliptin also displays a cardio-protective effect by improving left ventricular (LV) function and endothelial function in patients with type 2 diabetes mellitus [15]. The pleiotropic effects of teneligliptin have been reported in previous studies [16]. Interestingly, mounting evidence suggests that diabetes is associated with a higher prevalence of periapical infection and oral lesions [17]. Clinical studies have also indicated a higher prevalence of periapical lesions in patients with uncontrolled diabetes. Conversely, an increase in local oral inflammation can intensify the diabetic condition by raising blood glucose levels, thereby placing the patient in an uncontrolled diabetic state [18]. However, the pharmacological function of teneligliptin on LPS-induced inflammatory response and cellular insult in hDPCs has not been reported before. In light of this, in the present study, we investigated the biological effects of teneligliptin in cultured dental pulp cells.

2. Materials and methods

2.1. Human dental pulp cell culture and treatment

Primary human dental pulp cells were purchased from American Type Culture Collection (ATCC). Cells were maintained in 10% serum growth media supplemented with all the growth factors, and used in low passage numbers (< 10 passage). We purchased *Escherichia coli* (*E. coli*) LPS from Sigma-Aldrich (#L4524) and P. gingivalis LPS from InvivoGen (#05H23-SV), USA, both of which have been used for preparing periodontitis disease models in previous studies [19,20]. LPS was dissolved in PBS with 0.1% BSA. Teneligliptin is from Combi-Blocks, Inc. (#COM497515716), USA. The dental pulp cells were treated with LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 2, 24 or 48 h depending on the different assays. All the cell experiments were housed in 5% (v/v) CO_2/95% (v/v) Nitrogen incubator at 37 °C.

2.2. 3-(4,5)-dimethylthiahiazo (−z-y1)-3,5-di-phenytetrazoliumromide (MTT) and lactate dehydrogenase (LDH) assay

The viability of dental pulp cells was measured by MTT assay. Briefly, cells were incubated for 4 h with 0.8 mg/ml of MTT in serum free medium and followed by the addition of dimethyl sulfoxide (DMSO). The stabilized cell-MTT reaction mixture was then transferred in 96-well plates and absorbance was recorded at 560 nm using the microplate spectrophotometer system (Thermo Fisher Scientific, USA). Cytotoxicity was assessed by the LDH leakage into the culture medium. The culture medium was collected in order to obtain the cell free supernatants in different conditions. The type of activity by LDH in the mediums was determined using a commercially available kit from Sigma-Aldrich, USA. The data was presented as the percentage of the control values.

2.3. ROS and 4-HNE assay

Cellular reactive oxygen (ROS) production was measured by a quick staining of cells at different conditions with 2′, 7′–dichlorofluorescin diacetate dye (DCFH-DA). Briefly, cells were loaded with 10 μM DCFH-DA and incubated for 15 min at 37 °C. After 3 washes, a fluorescent microscope captured the Green fluorescent signals. 4-hydroxynonenal (4-HNE) is the major byproduct of lipid peroxidation during oxidative stress. We measured 4-HNE levels using the immunofluorescence method. In summary, the cells on the coated slides grew and were treated at a desired condition. The cells were then fixed by 4% paraformaldehyde and permeabilized by 0.1% triton-x 100, the cells were stained with anti-HNE antibody (Abcam, USA). This was followed by incubation with Alexa 594 that conjugated secondary antibody to visualize the image.

The stained images were quantified by fluorescence density with the software Image J. Briefly, regions of interest (ROI) were defined and the average number of cells present in the previously defined ROI was determined. The integrated density value (IDV) in ROI was assessed. The IDV was divided by the average number of cells and was used to determine the index average level of intracellular ROS and 4-HNE.

2.4. Intracellular glutathione (GSH) level

To assess the reaction result by the cellular oxidative stress, we measured the reduced GSH level in dental pulp cells. We used Ellman's reagent (5, 5′-dithiobis-2-nitrobenzoic acid (DNTB)) that reacted with GSH and measured the reaction substrate at a 412 nm wavelength by a spectrometry reader.

2.5. Quantitative real time PCR analysis

The total RNAs from the dental pulp cells were extracted according to the manufacturer's manual with a micro RNeasy Micro Kit from Qiagen (Hilden, Germany). The RNA's concentrations were quantified by a Nanadrop spectrophotometer from Cole-Parmer (Chicago, IL). A total of 1 μg RNA was used to synthesize the cDNA by iScript™ Reverse Transcription Supermix for RT-qPCR from Invitrogen (Carlsbad, CA). A SYBR real time based PCR experiment was performed to detect the total transcripts of mRNA of TNF-α, IL-1β, IL-6 and TLR-4 by ABI 7500 platform. The following primers were used in this study: TLR4 (forward: 5′-CTTTATCCAACCAGGTGC-3′, reverse: 5′-GGAATGCTGGAAAT CCAG-3′); TNF-α (forward: 5′-GATGGACTCACCAGGTGAG-3′, reverse:′-CTCATGGTGTCCTTTCCAGG-3′); IL-1β (forward: 5′-TACCTGTCCTGCG TGTTGAA-3′; reverse: 5′-TCTTTGGGTAATTTTTGGGATCT-3′); IL-6 (forward: 5′-TTGGGAAGGTTACATCAGATC-3′; reverse: 5′-GGGTTGGT CCATGTCAATTT-3′); GAPDH (forward, 5′-ACT GGC GTC TTC ACC ACC AT-3′; reverse, 5′-AAG GCC ATG CCA GTG AGC TT-3′).

2.6. Western blot analysis

The dental pulp cells with different conditions were lysed by radioimmunoprecipitation assay (RIPA) buffered with protease inhibitors. A total of 20 μg cell lysates were loaded to 4–20% precasted polyacrylamide gel electrophoresis (PAGE) gel to separate the proteins according to their size. The separated protein mix was transferred to polyvinylidene fluoride (PVDF) members to detect the corresponding protein levels by specific antibodies. The following antibodies were used in this study: TLR-4 (1:2000, #14358, Cell signaling technology, USA), p65 (1:3000, #8242, Cell signaling technology, USA), p-JNK (1:1000, #4668, Cell signaling technology, USA), c-Jun (1:2000, #9165, Cell signaling technology, USA), c-Fos (1:1000, #2250, Cell signaling technology, USA) and β-actin (1:5000, #3700, Cell signaling technology, USA).

2.7. Nuclear extract

The nuclear extracts of the dental pulp cells were extracted using a kit from Thermo Fisher Scientific, USA according to manufactory's instruction. The nuclear protein Lamin B was used for quality control, and the nuclear fraction of p65 protein level was examined to determine NF-κB activation in nuclear.

2.8. ELISA assay

In order to measure the secreted levels of TNF-α, IL-1β, and IL-6 cytokines in the different conditions; the dental pulp culture media was collected for the analyses. Separate ELISA kits (#DTA00C, #DLB50, #D6050) were purchased from R&D systems, USA. The manufacturing instructions were followed to complete the experiments. The data was collected by a 96-plate reader spectrometry. The relative levels of TNF-

α, IL-1β, and IL-6 were normalized to total protein amounts and represented by fold change.

2.9. Promoter assay

The AP1 and NF-κB binding sites containing firefly luciferases vectors were purchased from Thermo Fisher Scientific, USA. Cells were co-transfected with AP1 or NF-κB promoter and a firefly luciferase promoter by Lipofectamine 2000 reagents from Invitrogen (cat # 11668027). 24 h post the transfection, cells were treated with 100 ng/ml LPS in the presence or absence of Teneligliptin at 2.5 and 5 μM concentration for an additional 24 h. The total cell lysates were collected to measure the dual luciferase activity of the renilla and firefly luciferase. The relative luciferase was calculated by normalizing the activity of firefly luciferase to renilla luciferase activity.

2.10. Statistical analysis

Experimental data was presented as mean ± S.E.M. The SPSS statistical software (version 18.0) was used for statistical analyses. The statistical significance between multiple groups was compared using ANOVA followed by Bonferroni post-hoc test. $P < 0.05$ was considered as a significant difference.

3. Results

3.1. Teneligliptin protects against E. coli LPS-induced cytotoxicity in dental pulp cells

To assess the effects of the anti-diabetic agent Teneligliptin on *E. coli* LPS - induced cytotoxicity, we tested the protective effects of two different concentrations of Teneligliptin on dental pulp cells. Compared to non-treated cells, 100 ng/ml of *E. coli* LPS treatment resulted in 60% of cell death. However, the addition of 2.5 and 5 μM Teneligliptin greatly improved the cell viability. Only 30% and 10% of the cell population was lost during these two doses of Teneligliptin treatment respectively (Fig. 1A). Simultaneously, we measured LDH released on different treatments. *E. coli* LPS treated cells released 55% LDH. However, the cells with 2.5 and 5 μM Teneligliptin released about 35% and 15% of LDH (Fig. 1B). These results indicate that Teneligliptin protects dental gulp cells from LPS induced cell toxicity.

3.2. Teneligliptin suppresses E. coli LPS induced oxidative stress in human dental pulp cells

Next, we assessed the influence of Teneligliptin on *E. coli* LPS with an induced generation of reactive oxidative species (ROS). The results showed that there is a very mild ROS production in non-treated cells, and *E. coli* LPS induced about 4 folds high ROS production when compared to non-treated cells. However, the cells treated with 2.5 and 5 μM of Teneligliptin produced only about 2.2 and 1.8 folds high of ROS respectively (Fig. 2A). To help visualize this inhibitory effect of Teneligliptin on ROS, we measured a production of 4-HNE from lipid peroxidation, which is influenced by ROS levels. Compared to non-treated cells, *E. coli* LPS treatment increased about 3 folds higher than 4-HNE production, while the cells treated with 2.5 and 5 μM of Teneligliptin produced about 2 and 1.3 folds high of 4-HNE respectively (Fig. 2B). Glutathione (GSH) acts as an important antioxidant in cytosol. Here, our results indicated that *E. coli* LPS treatment significantly reduced intracellular GSH levels, which were prevented by treatment with Teneligliptin in a dose dependent manner (Fig. 3).

3.3. Teneligliptin attenuates E. coli LPS - induced reduction of GSH in human dental pulp cells

To test the effect of Teneligliptin on *E. coli* LPS-triggered inflammatory response in pulp cells, we measured the production of three major cytokines in different conditions of *E. coli* LPS and Teneligliptin including TNF-α, IL-1β, and IL-6. As shown in Fig. 4A, compared to the non-treated control. The *E. coli* LPS treatment gave rise to about 4–6 folds high induction of the three cytokines mRNA transcripts, while the addition of 2.5 and 5 μM of Teneligliptin to the culture media resulted in a significant reduction of their mRNAs in a dose dependent manner. The inhibitory role of Teneligliptin was confirmed by the production of TNF-α, IL-1β, and IL-6. As shown in Fig. 4B. *E. coli* LPS treatment induced about 3–4 folds high generation of the three cytokines proteins, while the presence of two doses of 2.5 and 5 μM of Teneligliptin greatly reduced their protein production in a dose dependent manner.

3.4. Teneligliptin suppresses E. coli LPS - induced activation of Toll like receptor 4 (TLR-4)

Toll like receptors act as cytokine-induced inflammatory mediators in dental pulp cells. We tested TLR family receptors and found that Teneligliptin suppressed TLR-4 in response to the *E. coli* LPS treatment. Compared to non-treated pulp cells, *E. coli* LPS treatment induced ~5 folds high of the TLR-4 at mRNA level, however, the presence of 2.5 and 5 μM of Teneligliptin allowed for only ~3 and ~2 folds of TLR-4 induction respectively (Fig. 5A). This inhibition was confirmed at protein level. At protein level and compared to non-treated control, *E. coli* LPS gave rise by ~3.5 folds higher than TLR-4, but 2.5 and 5 μM of Teneligliptin allowed only ~2 and ~1.5 folds high of TLR-4 protein to be generated respectively (Fig. 5B). Thus, we concluded that Teneligliptin suppressed LPS-induced and TLR-4 induction in dental pulp cells.

3.5. Teneligliptin inhibits E. coli LPS - induced activation of JNK and AP1 pathway

The JNK/AP1 pathway has been reported to be involved in *E. coli* LPS-mediated inflammatory reactions. Compared to non-treated

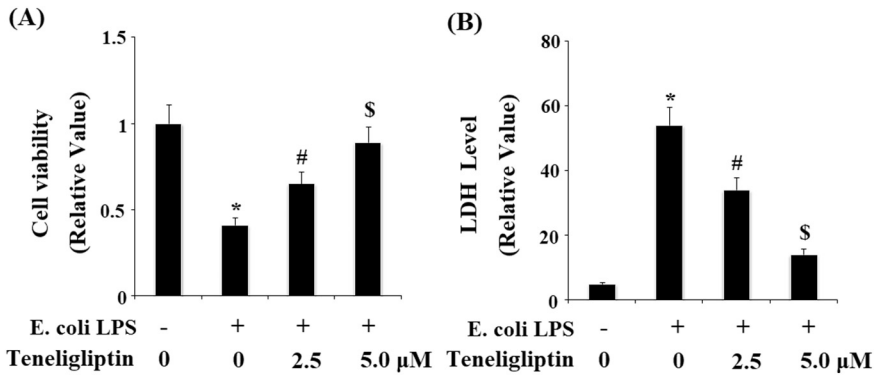

Fig. 1. Teneligliptin protects *E. coli* LPS - induced reduction of cell viability and cellular LDH release in human dental pulp cells. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 48 h. (A). Cell viability was determined by MTT assay; (B). The LDH released was determined using a commercial kit (*, #, $, $P < 0.01$ vs. previous column group, $n = 4–5$).

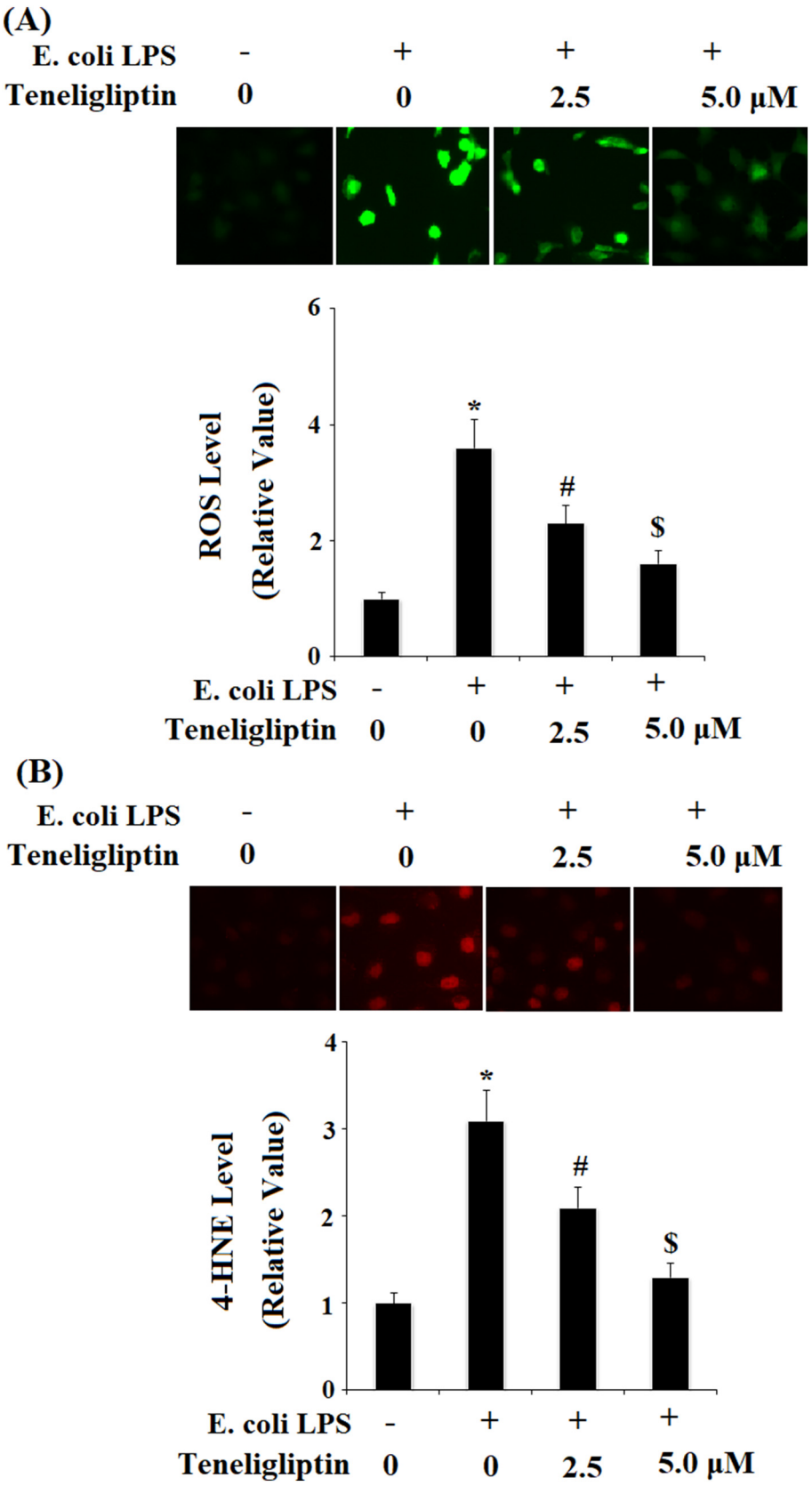

Fig. 2. Teneligliptin ameliorates *E. coli* LPS-induced oxidative stress in human dental pulp cells. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. (A). Intracellular ROS was determined by DCFH-DA staining; (B). 4-HNE was determined by immunoassaying. Scale bar, 100 μM (*, #, $, $P < 0.01$ vs. previous column group, $n = 4–5$).

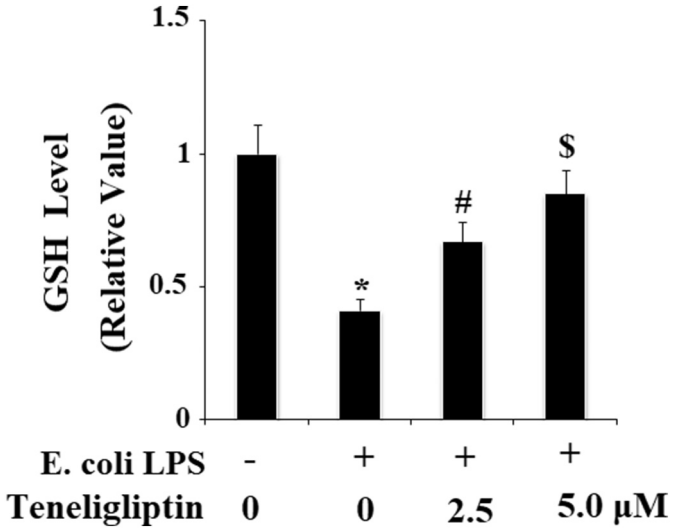

Fig. 3. Teneligliptin recovers *E. coli* LPS-induced reduction of GSH levels in human dental pulp cells. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. GSH was determined and quantified (*, #, $, P < 0.01 vs. previous column group, n = 4–5).

control, *E. coli* LPS significantly increased the levels of phosphorylated JNK (p-JNK), however, the presence of 2.5 and 5 μM Teneligliptin only allowed ~3 and ~1.8 folds high of p-JNK expression respectively (Fig. 6). Similarly, we found that by measuring the expression levels of two subunits of AP1 and AP-1 luciferase promoter activity, Teneligliptin also suppressed *E. coli* LPS - induced AP-1 activation. At protein level and compared to non-treated cells, *E. coli* LPS induced ~3–4 folds high of c-Jun and c-Fos expression, however, the addition of 2.5 and 5 μM of Teneligliptin greatly suppressed their expression (Fig. 7A). By transfecting the AP-1 promoter into the cells, we were able to confirm the inhibitory effects of Teneligliptin on AP-1 promoter activity. Compared to the basal non-treated condition, *E. coli* LPS induced > 30 folds of AP-1 luciferase promoter activity, but the addition of 2.5 and 5 μM Teneligliptin only allowed for ~20 and ~5 folds high of AP-1 promoter activity (Fig. 7B). These results indicate that Teneligliptin suppresses the activation of JNK and AP-1 transcriptional activity in pulp cells.

3.6. Teneligliptin inhibits E. coli LPS - induced activation of NF-κB

NF-κB is the major regulator of inflammatory responses in various types of cells. We tested whether or not Teneligliptin has an influence on the NF-κB activation. Indeed, our results proved that Teneligliptin potentially suppresses NF-κB signals in pulp cells. We examined the influence of Teneligliptin on nuclear p65 protein accumulation and the NF-κB promoter activation. The nuclear p65 expression, compared to non-treated cells of *E. coli* LPS treatment induced ~2.7 folds high of p65. However, the addition of 2.5 and 5 μM Teneligliptin only resulted in ~1.8 and ~1.4 fold high of p65 in nuclear (Fig. 8A). When transfecting NF-κB luciferase promoter into the cells, the *E. coli* LPS treatment promoter activity, rised to ~26 folds higher but 2.5 and 5 μM Teneligliptin only allowed for ~13 and ~5 folds high of NF-κB promoter activity (Fig. 8A). This data demonstrated the inhibitory effect of Teneligliptin on NF-κB activation.

3.7. The protective effects of Teneligliptin against P. gingivalis LPS in dental pulp cells

P. gingivalis has been positively linked with destructive periodontal disease [21]. Therefore, P. gingivalis LPS was also used in this study. The results in Supplementary Fig. 1A demonstrate that P. gingivalis LPS significantly increased the release of LDH from dental pulp cells, which was prevented by treatment with Teneligliptin. Interestingly, the presence of Teneligliptin inhibited P. gingivalis LPS-induced expression of TNF-α, IL-1β, and IL-6 (Supplementary Fig. 1B). Additionally, we found that Teneligliptin prevented P. gingivalis LPS-induced increased TLR2 expression. Importantly, treatment with Teneligliptin also reduced P. gingivalis LPS-induced increased NF-κB luciferase activity, which suggests that Teneligliptin prevented the activation of NF-κB.

4. Discussion

Patients that are chronically diabetic have a high risk of dental diseases, including dental pulp diseases. The effective treatment of dental infections caused by diabetes can improve the quality of life for diabetic patients. Gliptins are a class of glucose-lowering agents to treat Type 2 diabetes [22]. Recently, several types of Gliptins have shown to be effective in improving diabetes induced vascular complications, to reduce oxidative and pro-inflammatory state, and to exert additional beneficial effects besides its glucose reducing capability [23,24]. The newly developed Teneligliptin has a unique structure, with five consecutive rings and pharmacokinetic characteristics compared to other Gliptins [25]. Teneligliptin appears to have highly potent pleiotropic, long-lasting effects on glycemic control, and reduces the complications of hypoglycemia in preclinical studies [26].

In this study, we tested the beneficial effects of the anti-diabetic agent Teneligliptin in cultured dental pulp cells. Our data indicated that the presence of Teneligliptin in dental pulp cells; prompts the cells to be resistant to LPS-induced toxic stress and inflammatory response. The protective effects of Teneligliptin can be noted in its capability to reduce cellular LDH release, and promote overall survival of dental pulp cells. In cellular level, Teneligliptin reduces LPS-induced ROS production and its byproduct Glutathione, as well as major inflammatory cytokine production. Our study reveals the multiple molecular pathways of this suppression. From its upstream signal, Teneligliptin appears to directly suppress the cell membrane and the bound pattern recognition receptor –TLR-4. TLR-4 has been documented as one of the major mediators of dental infections for LPS-induced innate immune and acts as the first line of defense for dental pulp infection [27]. Additionally,

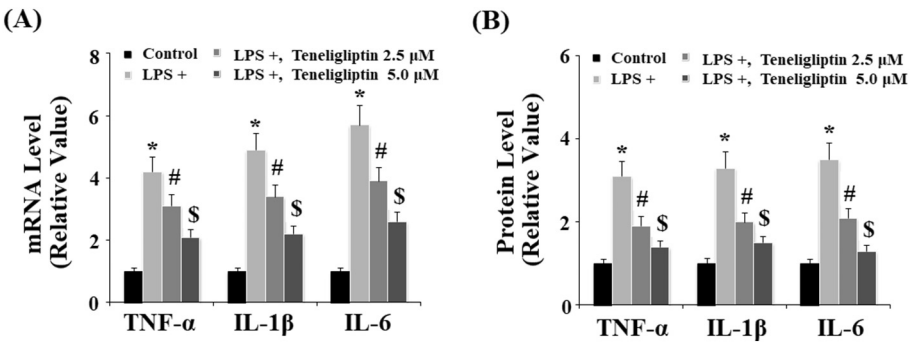

Fig. 4. Teneligliptin reduces LPS-induced generation of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in human dental pulp cells. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. (A). mRNA levels of TNF-α, IL-1β, and IL-6; (B). Protein levels of TNF-α, IL-1β, and IL-6 determined by the Elisa assay (*, #, $, P < 0.01 vs. previous column group, n = 4–5).

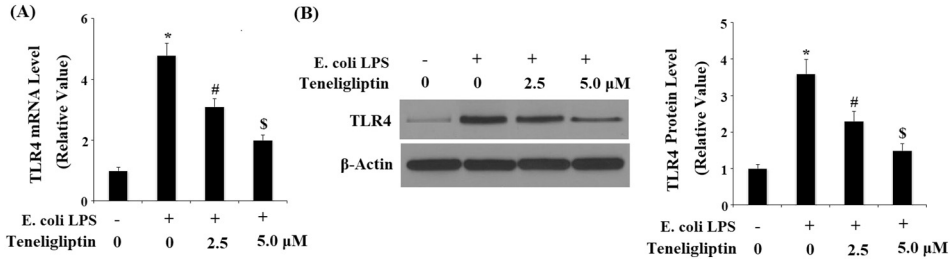

Fig. 5. Teneligliptin inhibits *E. coli* LPS-induced activation of TLR4. Cells were treated with LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. (A). mRNA levels of TLR4; (B). The protein level of TLR4 was determined by the western blot analysis (*, #, $, P < 0.01 vs. previous column group, n = 4–5).

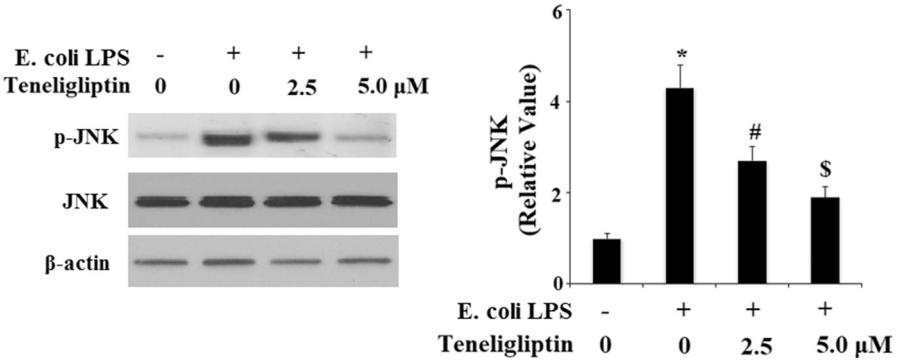

Fig. 6. Teneligliptin suppresses *E. coli* LPS - induced expression of JNK. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 2 h. Phosphorylated JNK was measured by the western blot analysis (*, #, $, P < 0.01 vs. previous column group, n = 4).

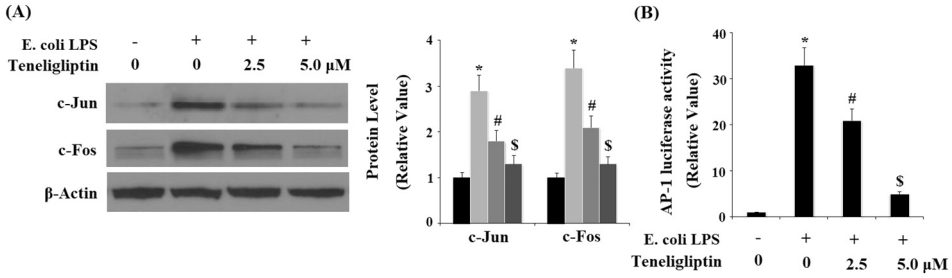

Fig. 7. Teneligliptin inhibits *E. coli* LPS - induced activation of transcriptional factor AP-1. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. (A). Expressions of c-Jun and c-Fos were determined by the western blot analysis; (B). AP-1 luciferase activity (*, #, $, P < 0.01 vs. previous column group, n = 4).

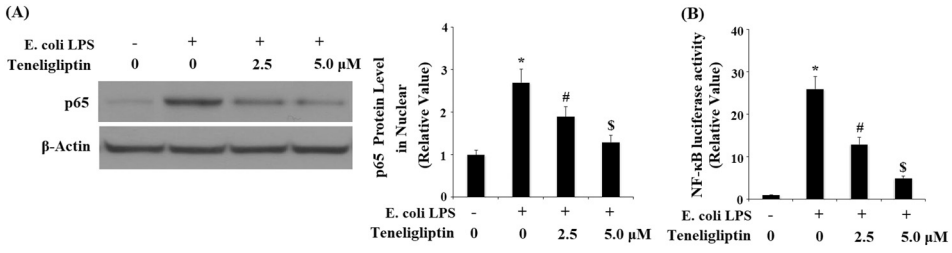

Fig. 8. Teneligliptin suppresses *E. coli* LPS-induced activation of NF-κB. Cells were treated with *E. coli* LPS (100 ng/ml) in the presence or absence of Teneligliptin (2.5 and 5.0 μM) for 24 h. (A). Nuclear level of p65 was determined by the western blot analysis; (B). NF-κB luciferase activity (*, #, $, P < 0.01 vs. previous column group, n = 4–5).

Teneligliptin shows significant suppression of JNK/AP1/NF-κB signal pathways in dental pulp cells. The TLR-4 mediated activation of JNK, AP1, and NF-κB to control inflammatory responses has been well studied in different cell types [28]. The inhibitory role of these inflammatory signals on Teneligliptin further confirmed its anti-oxidative and anti-inflammatory role in dental pulp cells. Furthermore, the suppression of Teneligliptin on LPS-caused major cell cytokine production including TNF-α, IL-1β, and IL-6. The inhibition of these pro-inflammatory molecules on Teneligliptin contributes to its cellular protection on the survival of dental pulp. The lack of awareness of the oral complications of the diabetic condition has led to the lag of research in this field. It has called for the attention of doctors and dentists to be aware of the various oral manifestations of diabetes in order to make an early diagnosis.

A study shows that another member of Gliptins and Sitagliptin, reduces pro-inflammatory genes such as MMP-9, NOS2, and IL-1β in gingiva tissue of the experimental periodontal rat model [29]. Several studies have indicated that Teneligliptin has an independent protective function other than reducing blood glucose. Teneligliptin can improve cardiovascular functions in pre-diabetic and atherosclerotic animal models [30,31]. Another group showed that Teneligliptin reduces the expression of NADPH oxidase subunit Nox-4 in perivascular adipocytes [32]. In human subjects, administration of Teneligliptin in diabetes patients increased eNOS expression levels and improved patients' vascular function and heart function [33,34].

In addition to dental pulp cells, dental pulp consists of several other types of cells, including odontoblasts, fibroblasts, undifferentiated mesenchymal stem cells (MSCs), and immune cells. Activation of immune cells such as macrophages plays a key role in the pathogenesis of pulpitis by mediating both innate and acquired immune responses [35]. Along with the progression of caries in human pulp tissues, there is an increase in the number of macrophages [36]. Activated macrophages

produce more TNF-α and IL-1β, so inflamed pulp has higher levels of cytokines as compared with healthy pulp [37]. Importantly, it has been reported that administration of the DPP-4 inhibitor teneligliptin remarkably suppressed foam cell formation and inflammatory response in macrophages [38]. Another study reported that teneligliptin treatment suppressed intrarenal TNF-α expression and induced macrophage polarization towards the anti-inflammatory M2 phenotype in an in vivo acute kidney injury (AKI) model [39]. These findings suggest that the protective effects of teneligliptin in the context of pulpitis may be mediated by other cell types.

In conclusion, our study revealed that Teneligliptin is a promising agent among the Gliptins family to have beneficial effects in dental pulp cells. A more detailed study of preclinical and clinical trials that include oral disease as a variable, could shed more light on the pleiotropic role of Gliptins including Teneligliptin.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.intimp.2019.04.059.

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