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Effects of high-glucose environment on periodontal ligament cell Proliferation and apoptosis via NF-κB signaling pathway

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ABSTRACT

The purpose of this study was to discuss the function of the high-glucose environment on the periodontal ligament cell (PDLC) proliferation and apoptosis and the action mechanism of the NF-κB signaling pathway in this process. For this purpose, the human PDLCs were cultured in vitro using 5.5 mM (control group)/24.0 mM glucose (HG group) of glucose and 10 μM of QNZ+24.0 mM of glucose (HG+QNZ), respectively, and the cell proliferation level was checked through CCK-8 assay. TUNEL assay was used to perform cell apoptosis. ELISA was utilized to explore the secretion levels of the proinflammatory factors interleukin (IL)-1β and IL-6 proteins. The p65 and p50 proteins level were tested via the Western blotting (WB) assay. Results showed that in comparison with the control group, 24.0 mM of glucose could significantly decrease the proliferation ability of the PDLCs ($p<0.01$), cause cell apoptosis ($p<0.05$) and promote the secretion of IL-6 and IL-1β ($p<0.05$). The expressions of p65 and p50 proteins were up-regulated obviously in the high-glucose environment ($p<0.05$). QNZ could exert a specific inhibitory effect on the NF-κB activity to significantly down-regulate the expressions of p65 and p50 proteins ($p<0.05$) and reverse the effects of the high-glucose environment on the cell apoptosis and proliferation ($p<0.05$). In conclusion, hyper-glucose may affect PDLC proliferation and apoptosis by suppressing the NF-κB signaling pathway activity.

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Introduction

Periodontal disease is the sixth major complication caused by diabetes (1-3). In recent years, more and more researchers have started paying attention to the action mechanism of diabetes in the progression of inflammation in periodontal diseases. Hyperglycemia, which is triggered by diabetes, can modulate multiple cell functions so as to influence cell proliferation and apoptosis (4-6). In the pathogenesis of periodontal diseases, the diabetes-induced high-glucose environment can change the cell functions in the periodontal tissues, e.g., forming adverse impacts on the physiological activities of gingival fibroblasts and periodontal ligament cells (PDLCs) (7,8)

PDLCs refer to the cells existing in the periodontium and participating in periodontal regeneration. Under the actions of mechanical stimulus, environmental factors and various cell growth factors, PDLCs can further regulate the absorption and regeneration of the periodontal tissues by secreting a variety of inflammatory factors (9). PDLCs can express diversified IL-1, IL-6, and TNF-α under environmental changes and take a part in the bone remodeling process of the periodontal tissues (10).

The NF-κB signaling pathway induces the generation of multiple cytokines and growth factors (11). As an important pathway regulating cell expressions and secreting numerous inflammation-related genes, NF-κB is important in the genesis and development mechanisms of various kinds of diseases (12). It is one of the key regulatory factors for cellular physiological activities, including cell apoptosis, cell growth and differentiation (13). However, few studies have been conducted to explore its action mechanism on PDLC proliferation and apoptosis under a high-glucose environment. This study aims to probe into the function of the high-glucose environment on PDLC proliferation and apoptosis and the role played by the NF-κB signaling pathway in this process, thus providing a theoretical basis for studies regarding periodontal diseases and proposing a potential clinical treatment method.

Materials and Methods

Main materials

PDLCs (Shanghai, China), DMEM, FBS and phosphate buffer solution (PBS) (Gibco), glucose powder (Sigma), p65, p50 and β-Actin antibodies and horseradish peroxidase (HRP)-labeled second antibodies (Abcam), QNZ (Selleck), TUNEL assay kit (green fluorescence, Beyotime), ELISA assay kits for IL-1β and IL-6 (Raybio), and 0.22 μm pinhole filter (Millipore).

Experimental methods

PDLC culture

The PDLCs were cultured with the DMEM (containing

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10% FBS) in an incubator at 37°C with 5% CO_2. Rinsed using PBS twice and digested using 0.25% trypsin, followed by the cell passage at the density of 1:3. The cells of the passages P5-P7 were utilized for the follow-up experiment.

PDLC culture at different glucose concentrations

Glucose powders were added into the DMEM containing 10% FBS to prepare the culture media of different final glucose concentrations: 5.5 mM glucose (control group), 24 mM glucose (HG group) and 24 mM of glucose+10 μM of QNZ (HG+QNZ). The PDLCs grew in the culture plate. The above-prepared culture media of different final glucose concentrations were added into the culture dish to continue to culture cells.

CCK-8 assay

The PDLCs were cultured in a 96-well plate using 5.5 mM of glucose and 24 mM of glucose separately for 1, 3, 5 and 7 d, and then the cells were cultured using 24 mM of glucose+10 μM of QNZ for 1 day. Added 10 μL CCK-8 solution into each well, incubating in the incubator for 2 h, and determined 450 nm absorbance via a microplate reader.

Cell apoptosis detection via one-step TUNEL assay

The PDLCs were cultured in a 6-well plate using 5.5 mM of glucose, 24 mM of glucose and 24 mM of glucose+10 μM of QNZ, respectively, for 24 h. The experiment was conducted according to the instructions of the one-step TUNEL apoptosis assay kit: 50 μL TUNEL solution was added to the sample, which was incubated at 37°C for 60 min, and rinsed with PBS 3 times. Photographed under a fluorescence microscope following the mounting using the anti-fluorescence quenching mounting medium.

Detection of proinflammatory factors via ELISA

The PDLCs were cultured in the 6-well plate with 5.5 mM of glucose, 24 mM of glucose and 24 mM of glucose+10 μM of QNZ, respectively, for 24 h. The cultured supernatant resulting from different treatments was collected. The post-centrifugation supernatant was collected, and the contents of IL-1β and IL-6 in the supernatant were detected through the ELISA assay kits.

Western blotting (WB)

The 5.5 mM of glucose, 24 mM of glucose and 24 mM of glucose+10 μM of QNZ were utilized to culture the PDLCs in a 60 mm culture dish for 24 h. Followed by the cell lysis using the cell lysis solution, extraction of total proteins and determination of the protein concentrations via the BCA method. Separated protein with 8% SDS-PAGE. Transferred to the PVDF membranes. Sealed in the 5% skim milk and gently shaken with p65 and p50 primary antibodies at 4°C overnight. Then, the proteins were incubated with the HRP-labeled second antibodies. Proteins to be detected were exposed using the ECL reagent. The β-actin detected via the same blotting assay was taken as the control.

Data statistics

Used SPSS 20.0 for data processing. Different treatment groups data were expressed by mean ± standard deviation ($\bar{x}$ ±s). p<0.05 meant that the difference was statistically significant.

Results

The function of the high-glucose environment on PDLC proliferation

After the PDLCs were cultured using 5.5- and 24.0-mM glucose for 1, 3, 5 and 7 d, the CCK-8 assay results indicated that 24.0 mM glucose significantly reduced the PDLC proliferation (p<0.01), but the cells still presented the proliferative tendency overall (Figure 1).

The function of the high-glucose environment on PDLC apoptosis

After the PDLCs were cultured at 5.5 and 24.0 mM glucose for 24 h, the TUNEL assay results indicated that the PDLC apoptosis level at the glucose concentration of 24.0 mM was higher than that at the glucose concentration of 5.5 mM (Figure 2).

Effect of high-glucose environment on inflammatory reaction inPDLCs

At 24 h after the culture of PDLCs with 5.5 mM and

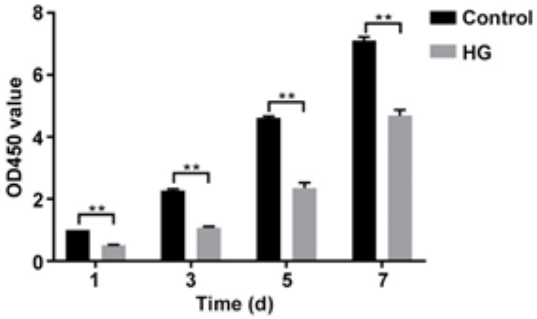

Figure 1. OD valuesof PDLCs at 1, 3, 5 and 7 d after culture with 5.5 mM and 24.0 mM of glucose detected via CCK-8 assay. The proliferative capacity of the PDLCs cultured at the glucose concentration of 24.0 mM is significantly lower than that at the glucose concentrationof5.5 mM(p<0.01). ** p<0.01.

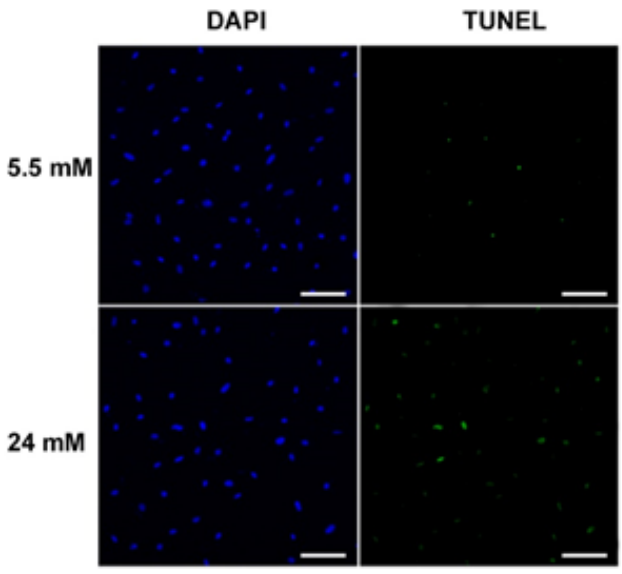

Figure 2. Cell apoptosis was detected via TUNEL assay. The green fluorescence intensity at the glucose concentration of 24 mM is stronger than that at the glucose concentration of 5.5 mM. The high-concentration glucose can facilitate cell apoptosis.

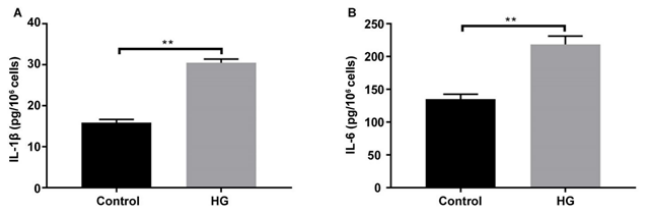

Figure 3. Levels of proinflammatory factors IL-1βand IL-6 secreted by PDLCs detected via ELISA assay. A: Level of proinflammatory factor IL-1β secreted after the PDLC culture with 5.5 mM and 24.0 mM of glucose for 24 h. In comparison with that in the control group, the secretion of IL-1β by the PDLCs is enhanced remarkably after the high-glucose culture (p<0.01). B: Level of proinflammatory factor IL-6 secreted after the PDLC culture with 5.5 mM and 24.0 mM of glucose for 24 h. In comparison with that in the control group, the secretion of IL-6 by the PDLCs is improved evidently after the high-glucose culture (p<0.01).

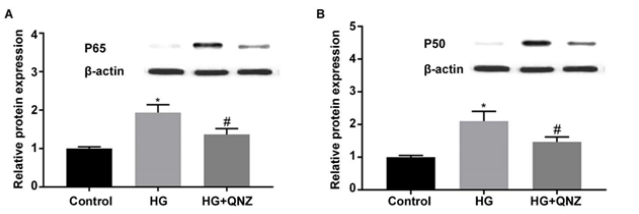

Figure 4. Expressions of proteins p65 and p50 in the NF-κB signaling pathway under different conditions were detected via WB assay. A: Compared with the control group, the high-glucose culture significantly facilitates the expression of p65 in the PDLCs (p<0.05). QNZ can obviously lower the expression of p65 under the high-glucose environment (p<0.05). B: In comparison with the control group, the high-glucose culture remarkedly promotes the expression of p50 in the PDLCs (p<0.05). The QNZ inhibitor can prominently reduce the expression of p50 under a high-glucose environment (p<0.05). * There are significant differences between the HG group and Control group and HZ + QNZ group (p<0.05). # There are significant differences between HG + QNZ group and Control group and HG group (p<0.05).

24.0 mM of glucose, the ELISA assay results manifested that in comparison with that in the control group, the secretion of the proinflammatory factors IL-1β (Figure 3A) and IL-6 (Figure 3B) by the PDLCs was enhanced remarkably after the high-glucose culture (p<0.01).

High-glucose environment effect on proteins in the NF-κB signaling pathway

Compared with those cultured at the glucose concentration of 5.5 mM, the expressions of p65 and p50 were up-regulated after the PDLCs were cultured at the glucose concentration of 24.0 mM (p<0.05) (Figure 4). QNZ could exert a significant inhibitory effect on the up-regulated expressions of p65 and p50 triggered by the culture using 24.0 mM of glucose (p<0.05) (Figure 4).

Effects of the NF-κB signaling pathway inhibition on PDLC

The cells were cultured under the effect of the NF-κB inhibitorQNZfor 24 h, which obviously suppressed the adverse effect of the high-glucose environment on the PDLC proliferation and elevated the cell proliferative capacity (p<0.05) (Figure 5A). Moreover, it also mitigated the PDLC apoptosis induced by the high-glucose environment (p<0.05) (Figure 5B).

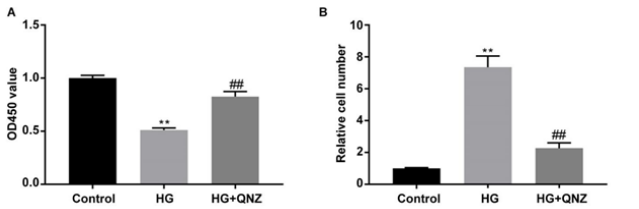

Figure 4. Effects of inhibition of the NF-κB signaling pathway on PDLC proliferation and apoptosis. A: QNZ can significantly inhibit the adverse effect of the high-glucose environment on PDLC proliferation and improve the cell proliferative capacity (p<0.05). B: QNZ can remarkedly relieve the PDLC apoptosis caused by the high-glucose environment (p<0.05). ** HG group compared with Control group and HG + QNZ group, with significant differences (p<0.01). ## indicates that HG + QNZ group compared with the Control group and HG group, with significant differences (p<0.01).

Discussion

PDLCs, which grow between the alveolar bone and cementum, are the principal cellular components of the periodontal tissues. Most collagenous fibers and protein polysaccharides in the periodontal membrane matrix are synthesized and secreted by the PDLCs which also perform the physiological functions of absorbing collagens and phagocytizing foreign matters and play vital roles in protecting and repairing the periodontal tissues. The glucose concentration in the blood of diabetes patients with uncontrolled glucose levels can reach 20.0-30.0 mM (14). Related studies have indicated that the high glucose concentration will inhibit the proliferative capacities of PDCLs and mesenchymal stem cells (15,16) and enhance the lipopolysaccharide-induced inhibitory effect on PDCL proliferation (17). The PDLCs were cultured at the glucose concentration of 24 mM in this experiment. In comparison with the normal glucose concentration, the high glucose concentration suppressed the proliferation ability of the PDLCs, indicating that the cell proliferation ability in the periodontal tissues may be degraded in diabetes patients.

Furthermore, the high glucose concentration effect on cell apoptosis was verified. We manifested that the high-glucose environment can induce the histocytes to generate oxidative stress and lead to myocardial injury resulting from diabetic cardiomyopathy (18). High glucose concentration can also induce podocyte injury and bring about diabetic nephropathy, while berberine facilitates the activation of AMPK, enhances autophagy and protects podocytes from damage induced by high glucose (19). According to the latest study results, high glucose concentration can trigger the oxidative stress reaction in the periodontal tissues. Erythropoietin can alleviate high glucose-induced oxidative stress (20). In the lipopolysaccharide-induced PDLC apoptosis process, the high-glucose environment can enhance cell apoptosis (17). The PDLCs culture at the high-glucose concentrations remarkedly promoted cell apoptosis.

Diabetes is generally considered as a low-grade inflammatory disease. The hyperglycemia state can go through a series of non-enzymatic reactions with proteins to form a type of advanced glycosylation end-products, to which macrophagocytes bind to cause the secretion of TNF-α and

IL in quantity (21). The high-glucose concentration can induce the expressions of proinflammatory cytokines closely related to periodontal destruction (22). Periodontitis has a high correlation with the secretion of various kinds of inflammatory cytokines evoked by diabetes (23-26). It was revealed in this experiment that the high-glucose culture could strengthen the IL-6 and IL-1β by PDLCs.

The NF-κB signaling pathway is capable of promoting cell apoptosis in different stimulating factors and specific cell types (13). In order to further investigate the action mechanisms of the high-glucose environment on the suppression of cell proliferation and the occurrence of cell apoptosis, the expressions of the NF-κB signaling pathway-related proteins were analyzed. Related studies have indicated that the NF-κB signaling pathway-induced bone destruction is closely associated with diabetes (27-30). The high-glucose environment-induced activation of the NF-κB in the PDLCs and the effect of the NF-κB inhibitor QNZ were studied in the experiment. Based on the experimental results, the high-glucose environment could stimulate the activation of the NF-κB in the PDLCs, weaken the cell proliferation ability, promote cell apoptosis and give rise to the cellular inflammatory reaction. After QNZ was utilized to repress the NF-κB of the PDLCs, it was found that the cell proliferation level could be effectively elevated, and meanwhile, the cell apoptosis was reduced.

These study results manifest that the high-glucose environment of diabetes may suppress the periodontal regeneration and promote the expressions of the inflammatory cytokines participating in the periodontal destruction via the NF-κB signaling pathway, thus further resulting in cell apoptosis.

Conflict of interests

The authors declare no conflict of interest.

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