Different MAPK signal transduction pathways play different roles in the impairment of glucose-stimulated insulin secretion in response to IL-1β

YANG OU[1,2], ZHONGXIONG ZHENG[2], BEN NIU[2], JIAN SU[2] and HENG SU[2]

[1]Faculty of Environmental Science and Engineering, Kunming University of Science and Technology, Kunming, Yunnan 650500; [2]Department of Endocrinology and Metabolism, First People's Hospital of Yunnan Province, Kunming, Yunnan 650032, P.R. China

Received January 8, 2020; Accepted July 10, 2020

DOI: 10.3892/mmr.2020.11366

Abstract. Mitogen-activated protein kinase (MAPK) signal transduction pathways may be involved in the destruction of pancreatic islet β cells induced by inflammatory cytokines. The present study aimed to investigate the role of different MAPK signal transduction pathways in the interleukin-1β (IL-1β)-induced inhibition of glucose-stimulated insulin secretion (GSIS) in Min6 mouse pancreatic cells. Min6 cells were stimulated with different concentrations of glucose (0.0, 5.5, 11.1 and 22.2 mmol/l), or different concentrations of IL-1β (0.00, 0.25 and 2.50 ng/ml) in combination with high glucose (22.2 mmol/l) and the culture supernatant was collected. The concentration of insulin was measured by enzyme-linked immunosorbent assay and the activation of different MAPK pathways was assessed by measuring the phosphorylation levels of extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-jun N-terminal kinase (JNK) via western blotting. The production of reactive oxygen species (ROS) was determined via flow cytometry, and cell viability was detected by Cell Counting Kit-8 assay. Reverse transcription-quantitative PCR was used to detect the insulin 1 gene. The results revealed that glucose activated ERK1/2 phosphorylation, but inhibited JNK and p38 phosphorylation in a concentration-dependent manner. Furthermore, IL-1β inhibited glucose-stimulated insulin secretion in a dose-dependent manner. Western blotting revealed that IL-1β inhibited the activation of ERK1/2 phosphorylation and attenuated the inhibition of p38 phosphorylation induced by glucose stimulation. JNK was neither activated nor inhibited by IL-1β. These results suggest that MAPK signal transduction pathways participated in the IL-1β-induced GSIS inhibition in Min6 cells, with the ERK1/2, JNK and p38 signaling pathways playing different roles.

Correspondence to: Professor Heng Su, Department of Endocrinology and Metabolism, First People's Hospital of Yunnan Province, 157 Jinbi Road, Xishan, Kunming, Yunnan 650032, P.R. China
E-mail: su_hen@hotmail.com

Abbreviations: MAPK, mitogen-activated protein kinase; GSIS, glucose-stimulated insulin secretion: IL-1β, interleukin-1β; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-jun N-terminal kinase; DM, diabetes mellitus; T1DM, type 1 DM; T2DM, type 2 DM; IFN-γ interferon-γ; TNF-α, tumor necrosis factor-α; PBS, phosphate-buffered saline; KRBH, Krebs buffered HEPES; ELISA, enzyme-linked immunosorbent assay; PF, paeoniflorin; Sfrp5, secreted frizzled-related protein-5; ER, endoplasmic reticulum; ROS, reactive oxygen species; CCK-8, Cell Counting Kit-8; DMEM, Dulbecco's modified Eagle's medium

Key words: MAPK, ERK1/2, JNK, p38, IL-1β, GSIS, Min6 cell line

Introduction

Diabetes mellitus (DM) is a chronic endocrine and metabolic disease that affects human health and is a heavy burden on individuals, their families and society (1). An epidemiological survey conducted in China estimated that the overall prevalence of type I and type II diabetes in Chinese adults in 2010 was 11.6% (1).

The destruction of islet β cells plays an important role in the occurrence and development of DM. The inflammation theory of islet β-cell destruction has drawn considerable attention (2-5). As important regulators in the process of inflammation, inflammatory cytokines directly or indirectly damage islet β cells in different ways. The destruction of pancreatic islet β cells in type 1 DM (T1DM) is associated with inflammatory cytokines, including interleukin-1β (IL-1β), interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) (2). The potential mechanisms of islet β-cell damage in type 2 DM (T2DM) include the induction of inflammatory cytokines, oxidative stress induced by high glucose and lipids, and amyloid deposition in islets (3). Although some progress has been made in the study of the destruction of pancreatic islet β cells induced by inflammatory cytokines in recent years (4,5), the molecular mechanisms by which these factors cause damage remain unclear.

It has been reported that the mitogen-activated protein kinase (MAPK) pathway may be involved in the inflammatory

cytokine-induced destruction of pancreatic islet β cells (1,6), but the specific roles of different MAPK signaling pathways, namely the extracellular signal-regulated kinase 1/2 (ERK1/2), p38 and c-jun N-terminal kinase (JNK) pathways, remain unclear, and their mutual regulation requires further clarification. Previous studies have focused on only one or two pathways (7-13) and the role of the MAPK signaling system is not yet fully understood.

In our previous studies, it was found that IL-1β inhibited the secretion of insulin under glucose stimulation in βTC-6 cells, and the mechanism of insulin secretion was associated with the inhibition of ERK1/2 (14,15). However, in those studies, only the effect of IL-1β on the ERK1/2 pathway was examined and the roles of JNK and p38 signaling pathways in the insulin secretory function of pancreatic β cells remain unclear. In addition, the response of βTC-6 cells to glucose stimulation is relatively weak.

Our previous studies showed that the optimal concentration of glucose for the stimulation of βTC-6 cells was 1.38 mmol/l, but the peak value of insulin secretion after stimulation was only 26% higher than the base value (14,15). Min6 pancreatic β cells are more sensitive to glucose than βTC-6 cells in the study of insulin secretion (16). Therefore, the present study aimed to further investigate the role of the three MAPK signaling pathways in the IL-1β-induced inhibition of insulin-secretion response in Min6 cells under glucose stimulation.

Materials and methods

Cell culture and treatment. Min6 cells (The Cell Bank of Type Culture Collection of the Chinese Academy of Sciences) were cultured in Dulbecco's modified Eagle's medium (DMEM, Hyclone; GE Healthcare life Sciences) containing 25 mmol/l glucose supplemented with 15% fetal bovine serum (M), 10 U/l penicillin and 10 U/l streptomycin (both Shanghai Jingtian Biotechnology Co., Ltd.). Cells were cultured at 37˚C in a 5% CO_2 incubator, and the culture medium was changed every 2 days. Cells were passaged at a 1:3 ratio for 4-7 days.

The survival rate of the Min6 cells in the cell culture was measured using an MTT assay and the survival rate was found to be >90%. MTT solution (Beyotime Institute of Biotechnology, China) was added to the cells, which were then incubated in the dark at 37˚C. After 4 h, the liquid was absorbed and discarded, the formazan crystals were dissolved by the addition of 150 µl DMSO (Beyotime Institute of Biotechnology) and the absorbance was measured at 490 nm.

Cells were digested by 0.25% trypsin (Hyclone; Cytiva) for 30-60 sec at 37˚C, which was seeded into 12-well plates at $1x10^5$ cells/well. After 72 h, the cells were adherent to the plate walls and were used for glucose-stimulated insulin secretion (GSIS) assays.

GSIS with and without IL-1β. Min6 cells were washed twice with phosphate-buffered saline (PBS) and the medium was changed to glucose-free Krebs buffered HEPES (KRBH; NaCl 119 mmol/l, KCl 4.74 mmol/l, $NaHCO_3$ 25 mmol/l, $MgSO_4$ 1.19 mmol/l, $CaCl_2$ 2.54 mmol/l, HEPES 10 mmol/l, pH 7.4). The cells were then cultured for 60 min at 37˚C in KRBH with glucose concentrations of 0.0, 5.5, 11.1 and 22.2 mmol/l. The conditioned culture medium was collected and used for the measurement of insulin concentration via enzyme-linked immunosorbent assay (ELISA).

To evaluate the effect of IL-1β on GSIS, Min6 cells were incubated in DMEM at 37˚C for 72 h and then IL-1β (Peprotech, Inc.) at concentrations of 0.00, 0.25 and 2.50 ng/ml was added to the culture medium. After 24 h at 37˚C, the culture medium was removed, and the cells were washed twice with PBS. KRBH buffer containing 0.1% bovine serum albumin (Sigma-Aldrich; Merck KGaA) without glucose was then added and the cells were incubated for 60 min at 37˚C, prior to culture in KRBH medium containing 22.2 mmol/l glucose for 60 min at 37˚C. The procedures for the untreated group (without glucose or IL-1β treatment) were the same as for the intervention group. The conditioned culture medium was collected and its insulin concentration was measured by ELISA.

Western blotting. Cells were collected, lysed with RIPA buffer (ProteinTech Group, Inc.) containing phosphatase inhibitor and protease inhibitors, and then centrifuged at 1,049 x g for 10 min at 4˚C. The cell lysate was collected and an equivalent volume (50 µl) of SDS loading buffer (Beyotime Institute of Biotechnology) was added. BCA protein assay was used to determine the protein concentration. The mixture was then heated in a water bath for 5 min at 95˚C, and then refrigerated with ice immediately afterwards. Using 10% polyacrylamide gel as a separating gel and 4% polyacrylamide gel as stacking gel, the samples (30 µg protein per lane) were electrophoresed for 1 h at 110 V. Transfer buffer was used to balance the gel and nitrocellulose membrane for 10 min, after which electrophoretic protein transfer was conducted for 1.5 h at 200 mV and the membrane was blocked in 5% skimmed milk at room temperature for 1 h. The following primary antibodies were then added: Anti-Erk1 (pT202/pY204) + Erk2 (pT185/pY187; 1:10.000, cat. no. ab50011, Abcam), anti-p38 (phosphorylated (p) T180 + Y182; 1:1,000, cat. no. ab195049, Abcam), anti-JNK1 + JNK2 + JNK3 (p-T183 + T183 + T221; 1:1,000, cat. no. ab124956, Abcam), anti-ERK1 + ERK2 (1:1,000, ab17942, Abcam), anti-p38 (1:1,000, cat. no. ab31828, Abcam), anti-JNK1 + JNK2 + JNK3 (1:2000, cat. no. ab208035, Abcam), or β-actin antibody (1:1,000, cat. no. ab8226, Abcam), and samples were incubated overnight at 4˚C. Secondary antibodies (anti-rabbit IgG, HRP-linked antibody; cat. no. ab7074, Abcam) were then added at a dilution of 1:2,000 and samples were incubated at room temperature for 1 h. Finally, visualization was achieved using an enhanced chemiluminescence analysis system (Merck KGaA) and blots were quantified by densitometry using ImageJ v1.8.0 software (National Institutes of Health). Each experiment was performed in triplicate.

ELISA. An ELISA kit (cat. no. ml001983-1, Mlbio) was used to detect the concentration of insulin in the conditioned culture medium, and each sample was assessed in triplicate. The mean optical density (OD) value for each sample was used to calculate the insulin concentration. The OD values were determined using a microplate reader (Sigma960; Metertech Inc.). All experiments were performed strictly in accordance with the manufacturer's protocol. The standard curves

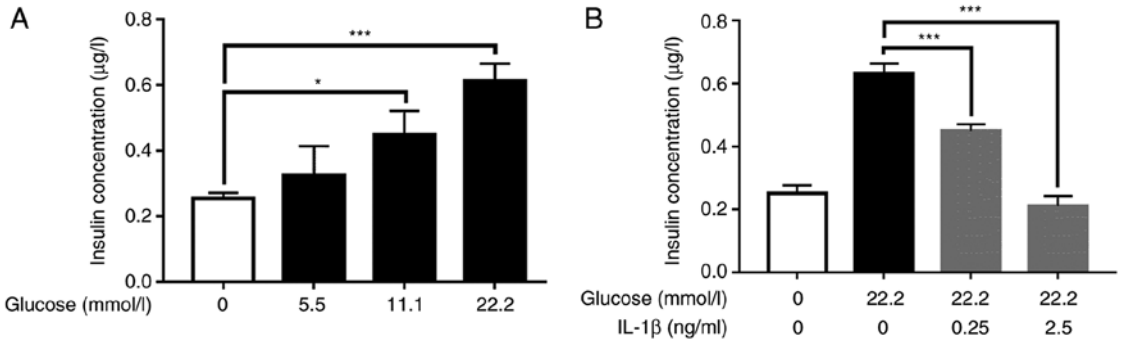

Figure 1. Effect of glucose stimulation on insulin secretion in Min6 cells. (A) Insulin secretion by Min6 cells was stimulated by different concentrations of glucose (0.0, 5.5, 11.1 and 22.2 mmol/l) for 1 h. (B) Effect of pretreatment with IL-1β (0.25 and 2.50 ng/ml) for 24 h on glucose-stimulated insulin secretion in Min6 cells. Data are presented as the mean ± SD. *P<0.05, ***P<0.001. IL-1β, interleukin-1β.

were constructed using CurveExpert v1.3 software (Hyams Development). The correlation coefficients were ≥0.999.

Reverse transcription-quantitative PCR. Total RNA was extracted from Min6 cells using Eastep® Super Total RNA reagent (Promega Corporation) according to the manufacturer's protocols. The purity of the total RNA was examined and RNA quantification performed by detecting the absorbance at 260 and 280 nm using a spectrophotometer (Biophotometer; Eppendorf). The cDNA was synthesized by reverse transcription using the GoScript™ Reverse Transcription System (Promega Corporation) and corresponding genes were amplified by employing SYBR-Green Master Mix (cat. no. KK4601, KAPA; Sigma-Aldrich; Merck KGaA). The thermocycling program was: 95˚C for 5 min, followed by 35 cycles of denaturization at 95˚C for 30 sec, subsequent annealing at 50˚C for 1 min and extension at 72˚C for 1 min, followed by a final extension at 72˚C for 10 min. The PCR primers were synthesized by Takara Bio, Inc. and their sequences were as follows: Insulin 1 forward, 5'-CGTTGA AATGCCACTGAAGCTACT-3' and reverse, 5'-TTGCTGTG ACTCCCCTGC T-3'; GAPDH forward, 5'-TTTGTCAAGCA GCACCTTTGT-3' and reverse, 5'-CTCCACCCAGCTCCAG TTGT-3'. The mRNA level of insulin 1 was normalized to that of GAPDH and was calculated using the $2^{-\Delta\Delta Cq}$ method (17). The experiments were performed in triplicate.

Cytotoxicity assays. Cell Counting Kit-8 (CCK-8; Dojindo Molecular Technologies, Inc.) assay was used to measure cell viability. Following treatment, 10 μl CCK-8 reagent was added to the cells in each well and incubated for 3 h at 37˚C. The absorbance was then measured using a microplate reader at 450 nm. The experiment was performed in triplicate.

Measurement of reactive oxygen species (ROS). Intracellular ROS production was measured using a DCFH-DA probe (Beyotime Institute of Biotechnology). Following treatment, the medium was removed from the cells, which were then incubated with 10 μM DCFH-DA for 30 min at 37˚C. The cell fluorescence was detected by flow cytometry (FlowMax v2.8.2, Sysmex).

Statistical analysis. Technical triplicates were performed for each experiment, with a minimum of three biological replicates for each study. Data are expressed as the mean ± standard deviation. One-way ANOVA was used to analyze differences among groups followed by Bonferroni's post hoc test to analyze differences between two groups. Statistical analysis was performed using GraphPad Prism 6 software (GraphPad Software, Inc.). P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of glucose stimulation on insulin secretion in Min6 cells. The insulin level in the conditioned culture medium was 0.25±0.02 μg/l without glucose stimulation, and 0.33±0.09, 0.45±0.07 and 0.61±0.05 μg/l with 5.5, 11.1 and 22.2 mmol/l glucose stimulation, respectively. There was a significant difference in insulin level among the groups (F=18.38, P=0.0006). The insulin level in the conditioned culture medium was highest following stimulation with 22.2 mmol/l glucose. The insulin level in the 22.2 mmol/l glucose stimulation group was 241% of that in the glucose-free group (0.61±0.05 vs. 0.25±0.02 μg/l, P=0.0003) (Fig. 1A).

Effect of glucose stimulation on ERK1/2, JNK and p38 phosphorylation in Min6 cells. Following stimulation with glucose, the level of ERK1/2 phosphorylation was increased compared with that in the glucose-free group and appeared to peak in the 11.1 mmol/l glucose stimulation group. However, glucose stimulation inhibited JNK and p38 phosphorylation in Min6 cells. As the glucose concentration increased the phosphorylation levels of JNK decreased in a concentration-dependent manner. By contrast, the level of p38 phosphorylation was only reduced in the 22.2 mmol/l glucose stimulation group (Fig. 2).

Effect of IL-1β on GSIS in Min6 cells. When no IL-1β pretreatment was performed, the insulin level in the conditioned culture medium was 0.25±0.03 μg/l without glucose stimulation and 0.63±0.03 μg/l under 22.2 mmol/l glucose stimulation. The insulin level was reduced to 0.45±0.02 μg/l when 0.25 ng/ml IL-1β was added prior to GSIS and 0.21±0.03 μg/l when 2.5 ng/ml IL-1β was added prior to GSIS. There was a significant difference in insulin level among these groups (F=142.1, P<0.001). The 2.5 ng/ml IL-1β group exhibited the highest inhibitory effect on the GSIS of Min6 cells, with a

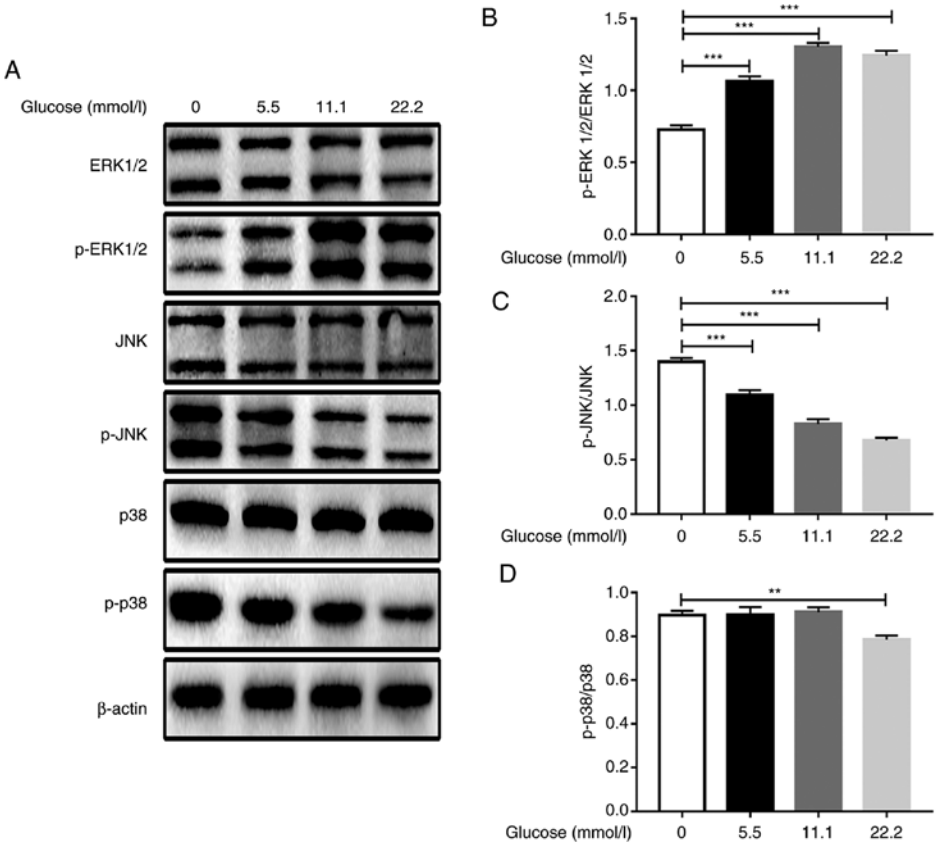

Figure 2. Effect of glucose stimulation on ERK1/2, JNK and p38 phosphorylation in Min6 cells. (A-D) The expression and phosphorylation levels of ERK1/2, JNK and p38 following stimulation with different concentrations of glucose (0.0, 5.5, 11.1 and 22.2 mmol/l) for 1 h were analyzed by western blotting. (A) Representative blots and quantified results for (B) ERK1/2, (C) JNK and (D) p38 phosphorylation. Data are presented as the mean ± SD. **P<0.01, ***P<0.001. ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-jun N-terminal kinase; p, phosphorylated.

reduction in the insulin level of 66% compared with the glucose stimulation only group (0.21±0.03 vs. 0.63±0.03 μg/l, P=0.0001); the insulin level of Min6 cells in the 0.25 ng/ml IL-1β group was decreased by 28% compared with that in the glucose stimulation only group (0.45±0.02 vs. 0.63±0.03 μg/l, P=0.0001) (Fig. 1B).

Effect of IL-1β on ERK1/2, JNK, p38 phosphorylation induced by glucose stimulation in Min6 cells. As presented in Fig. 3, 22.2 mmol/l glucose stimulated ERK1/2 phosphorylation in Min6 cells and IL-1β inhibited the glucose-induced phosphorylation. Pretreatment with 2.5 ng/ml IL-1β significantly reduced the level of ERK1/2 phosphorylation compared with that in the cells only stimulated with glucose. However, 22.2 mmol/l glucose inhibited p38 phosphorylation in Min6 cells, and IL-1β attenuated the glucose-induced inhibition of p38 phosphorylation. The p38 phosphorylation levels of the 0.25 and 2.5 ng/ml IL-1β pretreatment groups were increased compared with those in the cells only stimulated with glucose, and the highest p38 phosphorylation level was observed in the 2.5 ng/ml IL-1β group. However, IL-1β exhibited no effect on the JNK signaling pathway as no significant changes in JNK phosphorylation levels were induced by IL-1β following glucose stimulation (Fig. 3).

Level of intracellular oxidative stress. The levels of ROS were measured to evaluate the oxidative stress of the cells. The hyperglycemic condition (22.2 mmol/l glucose) caused intracellular oxidative stress to the Min6 cells. When hyperglycemia was combined with IL-1β pretreatment, the level of intracellular oxidative stress was elevated further and increased with IL-1β concentration (Fig. 4).

Effect of high glucose and IL-1β on cell viability and insulin gene expression. The CCK8 assay was used to evaluate cell viability. The results revealed that the viability of Min6 cells was decreased following treatment with 22.2 mmol/l glucose alone or in combination with different concentrations of IL-1β (Fig. 5A).

In order to determine whether the changes in the levels of insulin secretion were caused by pretreatment with IL-1β and not due to changes of cell viability, insulin mRNA levels in the Min6 cells were further examined. The results revealed that the changes in insulin mRNA levels were consistent with those of insulin concentration (Fig. 5B). These findings indicate that the decrease of insulin secretion was caused by IL-1β.

Discussion

The present study aimed to investigate the roles of different MAPK signal transduction pathways in the IL-1β-induced inhibition of GSIS in Min6 mouse pancreatic cells. The results revealed that insulin secretion was stimulated by various concentrations of glucose in Min6 cells. Glucose stimulation

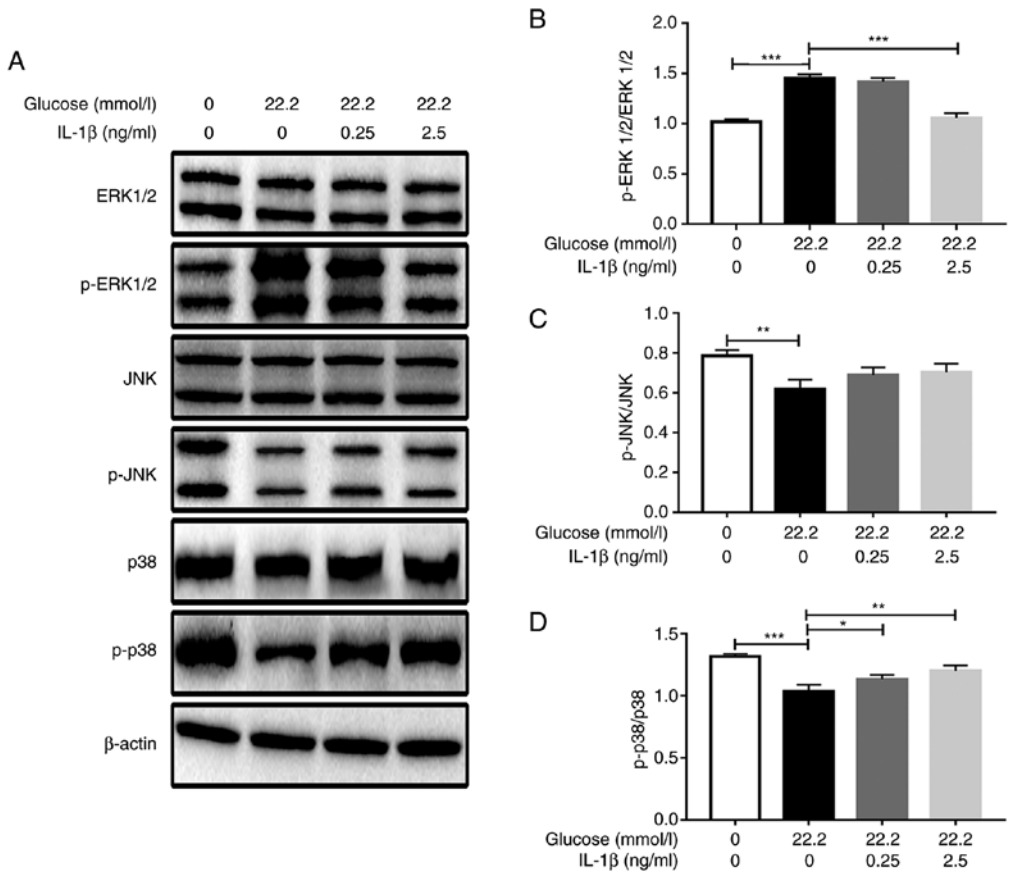

Figure 3. Effect of IL-1β on ERK1/2, JNK and p38 phosphorylation induced by glucose in Min6 cells. (A-D) The expression and phosphorylation levels of ERK1/2, JNK and p38 stimulated by glucose (22.2 mmol/l) with or without IL-1β (0.25 and 2.50 ng/ml) were analyzed by western blotting. (A) Representative blots and quantified results for (B) ERK1/2, (C) JNK and (D) p38 phosphorylation. *P<0.05, **P<0.01, ***P<0.001. IL-1β, interleukin-1β; ERK1/2, extracellular signal-regulated kinase 1/2; JNK, c-jun N-terminal kinase; p, phosphorylated.

activated ERK1/2 phosphorylation and inhibited JNK and p38 phosphorylation in a concentration-dependent manner. The inflammatory cytokine IL-1β inhibited GSIS and the GSIS-induced activation of ERK1/2 phosphorylation but attenuated the GSIS-induced inhibition of p38 phosphorylation. However, JNK phosphorylation was neither activated nor inhibited by IL-1β.

MAPK signal transduction pathways comprise serine/threonine protein kinases that exist in the majority of cells and transduce extracellular stimuli to cells and their nuclei (18). Numerous kinds of extracellular stresses, including ultraviolet radiation, heat shock, proinflammatory factors, specific antigens and other stressors activate MAPK pathways and cause cell proliferation, differentiation, transformation and apoptosis (19). MAPK signal transduction pathways are highly conserved in cells, with prokaryotic cells and mammalian cells having multiple parallel MAPK signaling pathways (20). At present, three MAPK signaling pathways, namely the ERK1/2, JNK and p38 pathways, have been clearly studied (20), but their specific roles remain unclear.

Recent studies have shown that MAPK signaling pathways may play an important role in the pathogenesis of diabetes, especially in insulin secretion. For example, Liu *et al* (21) revealed that paeoniflorin (PF), a natural glycoside, attenuated the inhibitory effect of streptozotocin (STZ) on the insulin secretion ability of INS-1 cells. Furthermore, PF inhibited the STZ-induced phosphorylation of p38 and JNK in INS-1 cells (21). Wei *et al* (22) reported that the single nucleotide polymorphism rs2076878 of p38 was associated with insulin secretion in the Chinese Han population, and revealed that the plasma insulin levels of db/db mice were increased following administration of the p38 MAPK inhibitor SB203580 for 9 weeks. A study of α-mangostin revealed that it stimulated insulin secretion in INS-1 cells by increasing phosphorylation in the phospho-phosphatidylinositol-3 kinase and ERK signaling cascades (23). In another study, secreted frizzled-related protein-5 (Sfrp5) dose-dependently increased glucose-stimulated insulin secretion but not basal insulin secretion in INS-1E cells. In addition, Sfrp5 decreased JNK signaling activity in INS-1E cells, suggesting that decreased JNK activity may associated with the increased insulin secretion induced by Sfrp5 (24). Youl *et al* (25) demonstrated that an MEK inhibitor completely abolished glucose-induced ERK1/2 phosphorylation and significantly decreased glucose-induced insulin secretion in INS-1 pancreatic β-cells. The aforementioned studies indicate that ERK1/2 phosphorylation promotes insulin secretion while the phosphorylation of JNK and p38 inhibits insulin secretion, and the results of the present study are in agreement with the previous findings.

The inflammation theory of islet β cell destruction has been widely researched, and inflammatory factors are known

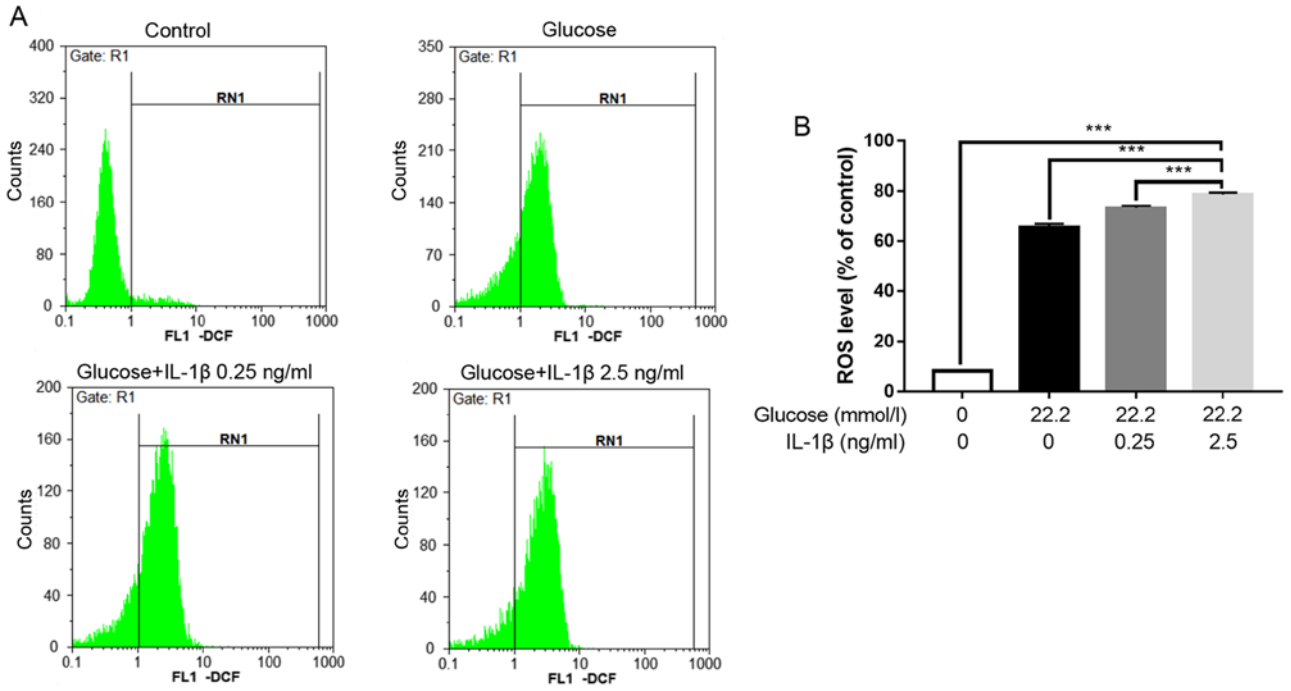

Figure 4. Determination of intracellular oxidative stress in Min6 cells. ROS were measured as oxidative stress-associated markers. (A) Representative flow cytometry plots and (B) quantified ROS levels. ***P<0.001. ROS, reactive oxygen species; IL-1β, interleukin-1β.

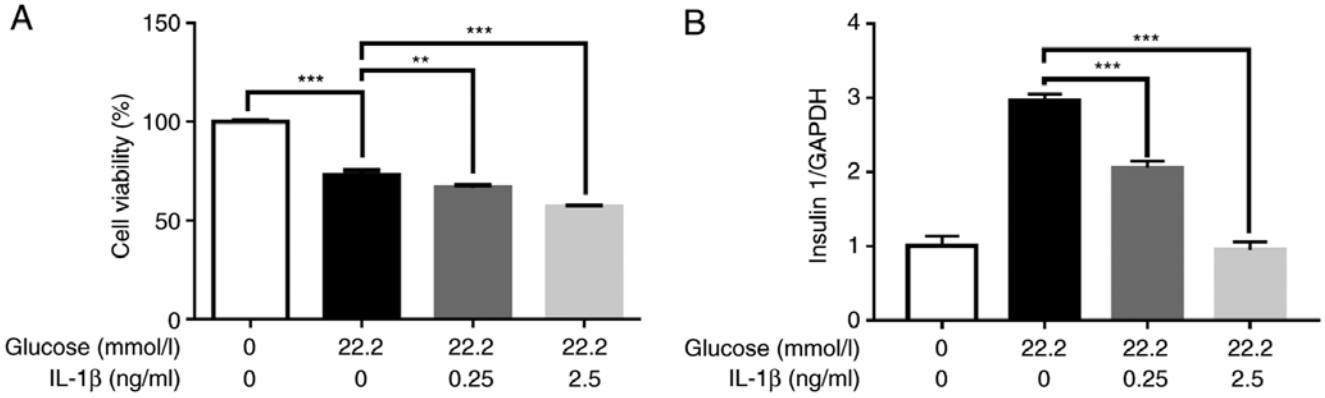

Figure 5. Effect of glucose and IL-1β on cell viability and insulin gene expression. (A) Effect of glucose and IL-1β on cell activity determined by Cell Counting Kit-8 assays. (B) Effect of glucose and IL-1β on insulin gene expression. **P<0.01, ***P<0.001. IL-1β, interleukin-1β.

to play an important role in dysfunctional insulin secretion and the destruction of islet β cells (2,26). Inflammatory cytokines, including IL-1β, TNF-α and IFN-γ, have been shown to contribute to long term functional suppression and β-cell apoptosis in T1DM and T2DM (27-29). Notably, IL-1β contributes to β-cell failure and decreases insulin secretion (30,31). β cells appear to be sensitive to short pulses of cytokine exposure, as the incubation of rat islets with IL-1β for 1 h resulted in the nitric oxide-dependent inhibition of insulin secretion 18 h after cytokine removal (32). IL-1β also inhibited GSIS in Cohen diabetic rat islets through nitric oxide-induced mitochondrial cytochrome c oxidase inhibition (33). Furthermore, when elevated serum levels of IL-1β in diabetic rats were decreased by nitrite administration, significantly increased insulin secretion was observed (34). In a clinical trial, a trend towards improved insulin secretion was observed in patients treated with the anti-IL-1β antibody canakinumab, supporting the hypothesis that insulin secretion is improved by blocking IL-1β (35). Weaver *et al* (36) revealed that GSIS was attenuated by the inflammatory cytokines TNF-α, IL-1β and IFN-γ, but protected by the NADPH-1 oxidase-1 inhibitor ML171 in isolated mouse islets and murine β cell lines. Our previous study found that IL-1β and/or IFN-γ inhibited insulin secretion by βTC-6 cells in a glucose stimulation test with a synergistic effect, and the inhibitory effect of IL-1β on GSIS was dose-dependent (15). The present study revealed similar results in Min6 cells.

It has been reported that insulin secretion *in vivo* is associated with intracellular calcium (Ca2+) (37). In pancreatic β cells, proinflammatory cytokines affect insulin secretion by regulating Ca^{2+}; they induce changes in intracellular Ca^{2+} levels by depleting Ca^{2+} stores in the endoplasmic reticulum

(ER) and increasing extracellular Ca^{2+} influx (38). In mouse islets, exposure to TNF-α, IL-1β and IFN-γ has been shown to disrupt the regulation of intracellular Ca^{2+} (39). Cytokine signaling has also been demonstrated to disrupt β-cell glucose-stimulated Ca^{2+} influx and Ca^{2+} ER handling, leading to diminished insulin secretion in response to glucose stimulation (40). Furthermore, the analysis of islets from normal mice that underwent overnight exposure to IL-1β and IL-6 via a cytokine-pump revealed deficiencies in Ca^{2+} handling and insulin secretion that were similar to observations with islets exposed to cytokines *in vitro* (41).

Kim *et al* (7) demonstrated that TNF-α reduced glucose-stimulated Ca^{2+} influx in INS-1 cells and decreased GSIS, potentially by the activation of JNK and p38 MAPK signaling. Similar findings have been reported for IL-1β, with Ca^{2+} being indicated to participate in the IL-1β-mediated activation of the JNK signaling pathway in insulin-secreting cells (8). In other studies, treatment with IL-1β increased the phosphorylation of JNK in islets and Min-6 β cells (9), and elevated IL-1β induced apoptosis through JNK1/2 activation-induced cellular Ca^{2+} movement in human primary β-cells (10). Furthermore, in a study of primary rat β cells and Min6 cells, IL-1β promoted ER Ca^{2+} release by activating JNK and the decreased activation of JNK provided protection against IL-1β-mediated apoptosis via ER stress (11). Comparable results were not observed in the present study when the JNK signaling pathway was assessed. The potential reasons may be that human islet cells or higher IL-1β concentrations were used in the other studies. However, the present study suggested that IL-1β has the potential to activate the glucose-stimulated JNK signaling pathway, although no significant activation was detected.

However, the effects of IL-1β on ERK1/2 are inconsistent. High glucose and IL-1β can lead to the apoptosis of islet β cells and impairment of GSIS secretion, which are associated with Ca^{2+} influx and activation of the ERK signaling pathway (12). Burke *et al* (13) revealed that JNK and p38 were rapidly phosphorylated 15 min following the exposure of pancreatic β-cells to IL-1β. By contrast, ERK was not activated within 60 min. The present study revealed that IL-1β inhibited the glucose-induced activation of ERK1/2 phosphorylation. The reasons for these inconsistencies may be due to the different concentrations and action times of IL-1β in the various studies. The present study revealed that the phosphorylation of p38 was activated by IL-1β in a concentration-dependent manner.

The present study has certain limitations. The results only indicate that the mechanism by which inflammatory cytokines impair insulin secretion in pancreatic β cells is associated with ERK1/2 and p38 pathways. However, the changes of upstream kinases such as MEK, Raf and Ras and their downstream transcription factors remain to be elucidated. Furthermore, a cell line rather than primary cells was used. Future studies will aim to confirm the findings of the current study in rat primary cells.

In summary, the present study indicates that MAPK signal transduction pathways participate in IL-1β-induced GSIS inhibition in Min6 cells, with the ERK1/2 and p38 signaling pathways appearing to have different effects. Activation of the three MAPK pathways following glucose stimulation differs in Min6 cells and the effects of IL-1β on the three MAPK pathways also differ, suggesting that these MAPK pathways play different roles in the secretion of insulin by islet β cells, and that mutual regulatory mechanisms may exist among them. The results are valuable for elucidating the mechanism of islet β-cell destruction and may aid the investigation of new intervention targets for the protection of islet β-cells function in patients with DM.

Acknowledgements

Not applicable.

Funding

The present study was funded by the National Natural Science Foundation of China (grant no. 81560135).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

HS designed and analyzed the experiments. YO read the literature, analyzed data and wrote the manuscript. JS performed the experiments. BN performed the supplementary experiments (RT-qPCR, ROS detection and CCK-8 assays) and revised the manuscript. ZZ searched the literature and performed statistical analysis All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Wang L, Jiang Y, Dai M, Lu J, *et al*; 2010 China Noncommunicable Disease Surveillance Group: Prevalence and control of diabetes in Chinese adults. JAMA 310: 948-959, 2013.
2. Kim KA and Lee MS: Recent progress in research on β-cell apoptosis by cytokines. Front Biosci 14: 657-664, 2009.
3. Ehses JA, Ellingsgaard H, Böni-Schnetzler M and Donath MY: Pancreatic islet inflammation in type 2 diabetes: From alpha and β cell compensation to dysfunction. Arch Physiol Biochem 115: 240-247, 2009.
4. Lambelet M, Terra LF, Fukaya M, Meyerovich K, Labriola L, Cardozo AK and Allagnat F: Dysfunctional autophagy following exposure to pro-inflammatory cytokines contributes to pancreatic β-cell apoptosis. Cell Death Dis 9: 96, 2018.
5. Berchtold LA, Prause M, Størling J and Mandrup-Poulsen T: Cytokines and Pancreatic β-Cell Apoptosis. Adv Clin Chem 75: 99-158, 2016.
6. Ammendrup A, Maillard A, Nielsen K, Aabenhus Andersen N, Serup P, Dragsbaek Madsen O, Mandrup-Poulsen T and Bonny C: The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic β-cells. Diabetes 49: 1468-1476, 2000.

7. Kim HE, Choi SE, Lee SJ, Lee JH, Lee YJ, Kang SS, Chun J and Kang Y: Tumour necrosis factor-alpha-induced glucose-stimulated insulin secretion inhibition in INS-1 cells is ascribed to a reduction of the glucose-stimulated Ca^{2+} influx. J Endocrinol 198: 549-560, 2008.
8. Størling J, Zaitsev SV, Kapelioukh IL, Karlsen AE, Billestrup N, Berggren PO and Mandrup-Poulsen T: Calcium has a permissive role in interleukin-1β-induced c-jun N-terminal kinase activation in insulin-secreting cells. Endocrinology 146: 3026-3036, 2005.
9. Edén D, Siegbahn A and Mokhtari D: Tissue factor/factor VIIa signalling promotes cytokine-induced β cell death and impairs glucose-stimulated insulin secretion from human pancreatic islets. Diabetologia 58: 2563-2572, 2015.
10. Verma G, Bhatia H and Datta M: JNK1/2 regulates ER-mitochondrial Ca^{2+} cross-talk during IL-1β-mediated cell death in RINm5F and human primary β-cells. Mol Biol Cell 24: 2058-2071, 2013.
11. Wang Q, Zhang H, Zhao B and Fei H: IL-1β caused pancreatic β-cells apoptosis is mediated in part by endoplasmic reticulum stress via the induction of endoplasmic reticulum Ca^{2+} release through the c-Jun N-terminal kinase pathway. Mol Cell Biochem 324: 183-190, 2009.
12. Fei H, Zhao B, Zhao S and Wang Q: Requirements of calcium fluxes and ERK kinase activation for glucose- and interleukin-1β-induced β-cell apoptosis. Mol Cell Biochem 315: 75-84, 2008.
13. Burke SJ, Goff MR, Updegraff BL, Lu D, Brown PL, Minkin SC Jr, Biggerstaff JP, Zhao L, Karlstad MD and Collier JJ: Regulation of the CCL2 gene in pancreatic β-cells by IL-1β and glucocorticoids: Role of MKP-1. PLoS One 7: e46986, 2012.
14. Niu B, Liu L, Su H, Xia X, He Q, Feng Y, Xue Y and Yan X: Role of extracellular signal regulated kinase 1/2 signal transduction pathway in insulin secretion by β TC6 cells. Mol Med Rep 13: 4451-4454, 2016.
15. Niu B, Su H, Xia XS, He Q, Xue YM and Yan XM: The role of interleukin-1β and extracellular signal-regulated kinase 1/2 in glucose-stimulated insulin secretion. Kaohsiung J Med Sci 33: 224-228, 2017.
16. Nie Y, Li J, Jin Y, Nyomba BLG, Cattini PA and Vakili H: Negative effects of cyclic palmitate treatment on glucose responsiveness and insulin production in mouse insulinoma Min6 cells Are Reversible. DNA Cell Biol 38: 395-403, 2019.
17. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the $2(-\Delta\Delta C(T))$ Method. Methods 25: 402-408, 2001.
18. Anbazhagan K, Rabbind Singh A, Isabelle P, Stella I, Céline AD, Bissac E, Bertrand B, Rémy N, Naomi T, Vincent F, *et al*: Human pre-B cell receptor signal transduction: Evidence for distinct roles of PI3kinase and MAP-kinase signalling pathways. Immun Inflamm Dis 1: 26-36, 2013.
19. Kyriakis JM and Avruch J: Mammalian MAPK signal transduction pathways activated by stress and inflammation: A 10-year update. Physiol Rev 92: 689-737, 2012.
20. Singh R and Jwa NS: The rice MAPKK-MAPK interactome: The biological significance of MAPK components in hormone signal transduction. Plant Cell Rep 32: 923-931, 2013.
21. Liu Y, Han J, Zhou Z and Li D: Paeoniflorin protects pancreatic β cells from STZ-induced damage through inhibition of the p38 MAPK and JNK signaling pathways. Eur J Pharmacol 853: 18-24, 2019.
22. Wei X, Gu N, Feng N, Guo X and Ma X: Inhibition of p38 mitogen-activated protein kinase exerts a hypoglycemic effect by improving β cell function via inhibition of β cell apoptosis in db/db mice. J Enzyme Inhib Med Chem 33: 1494-1500, 2018.
23. Lee D, Kim YM, Jung K, Chin YW and Kang KS: Alpha-mangostin improves insulin secretion and protects INS-1 cells from streptozotocin-induced damage. Int J Mol Sci 19: 19, 2018.
24. Carstensen-Kirberg M, Röhrig K, Niersmann C, Ouwens DM, Belgardt BF, Roden M and Herder C: Sfrp5 increases glucose-stimulated insulin secretion in the rat pancreatic β cell line INS-1E. PLoS One 14: e0213650, 2019.
25. Youl E, Bardy G, Magous R, Cros G, Sejalon F, Virsolvy A, Richard S, Quignard JF, Gross R, Petit P, *et al*: Quercetin potentiates insulin secretion and protects INS-1 pancreatic β-cells against oxidative damage via the ERK1/2 pathway. Br J Pharmacol 161: 799-814, 2010.
26. Pham MN, Hawa MI, Pfleger C, Roden M, Schernthaner G, Pozzilli P, Buzzetti R, Scherbaum WA, Seissler J, Kolb H, *et al*; Action LADA Study Group: Pro- and anti-inflammatory cytokines in latent autoimmune diabetes in adults, type 1 and type 2 diabetes patients: Action LADA 4. Diabetologia 54: 1630-1638, 2011.
27. Eizirik DL, Colli ML and Ortis F: The role of inflammation in insulitis and β-cell loss in type 1 diabetes. Nat Rev Endocrinol 5: 219-226, 2009.
28. Alexandraki K, Piperi C, Kalofoutis C, Singh J, Alaveras A and Kalofoutis A: Inflammatory process in type 2 diabetes: The role of cytokines. Ann N Y Acad Sci 1084: 89-117, 2006.
29. Padgett LE, Broniowska KA, Hansen PA, Corbett JA and Tse HM: The role of reactive oxygen species and proinflammatory cytokines in type 1 diabetes pathogenesis. Ann N Y Acad Sci 1281: 16-35, 2013.
30. Burke SJ, Stadler K, Lu D, Gleason E, Han A, Donohoe DR, Rogers RC, Hermann GE, Karlstad MD and Collier JJ: IL-1β reciprocally regulates chemokine and insulin secretion in pancreatic β-cells via NF-κB. Am J Physiol Endocrinol Metab 309: E715-E726, 2015.
31. Zhao G, Dharmadhikari G, Maedler K and Meyer-Hermann M: Possible role of interleukin-1β in type 2 diabetes onset and implications for anti-inflammatory therapy strategies. PLOS Comput Biol 10: e1003798, 2014.
32. Corbett JA, Sweetland MA, Lancaster JR Jr and McDaniel ML: A 1-hour pulse with IL-1β induces formation of nitric oxide and inhibits insulin secretion by rat islets of Langerhans: Evidence for a tyrosine kinase signaling mechanism. FASEB J 7: 369-374, 1993.
33. Weksler-Zangen S, Aharon-Hananel G, Mantzur C, Aouizerat T, Gurgul-Convey E, Raz I and Saada A: IL-1β hampers glucose-stimulated insulin secretion in Cohen diabetic rat islets through mitochondrial cytochrome c oxidase inhibition by nitric oxide. Am J Physiol Endocrinol Metab 306: E648-E657, 2014.
34. Gheibi S, Bakhtiarzadeh F, Jeddi S, Farrokhfall K, Zardooz H and Ghasemi A: Nitrite increases glucose-stimulated insulin secretion and islet insulin content in obese type 2 diabetic male rats. Nitric Oxide 64: 39-51, 2017.
35. Rissanen A, Howard CP, Botha J and Thuren T; Global Investigators: Effect of anti-IL-1β antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: Results of a randomized, placebo-controlled trial. Diabetes Obes Metab 14: 1088-1096, 2012.
36. Weaver JR, Grzesik W and Taylor-Fishwick DA: Inhibition of NADPH oxidase-1 preserves β cell function. Diabetologia 58: 113-121, 2015.
37. Sabatini PV, Speckmann T and Lynn FC: Friend and foe: B-cell Ca^{2+} signaling and the development of diabetes. Mol Metab 21: 1-12, 2019.
38. Ramadan JW, Steiner SR, O'Neill CM and Nunemaker CS: The central role of calcium in the effects of cytokines on β-cell function: Implications for type 1 and type 2 diabetes. Cell Calcium 50: 481-490, 2011.
39. Dula SB, Jecmenica M, Wu R, Jahanshahi P, Verrilli GM, Carter JD, Brayman KL and Nunemaker CS: Evidence that low-grade systemic inflammation can induce islet dysfunction as measured by impaired calcium handling. Cell Calcium 48: 133-142, 2010.
40. Dickerson MT, Bogart AM, Altman MK, Milian SC, Jordan KL, Dadi PK and Jacobson DA: Cytokine-mediated changes in K+ channel activity promotes an adaptive Ca^{2+} response that sustains β-cell insulin secretion during inflammation. Sci Rep 8: 1158, 2018.
41. O'Neill CM, Lu C, Corbin KL, Sharma PR, Dula SB, Carter JD, Ramadan JW, Xin W, Lee JK and Nunemaker CS: Circulating levels of IL-1B+IL-6 cause ER stress and dysfunction in islets from prediabetic male mice. Endocrinology 154: 3077-3088, 2013.