Glucagon-like peptide-1 receptor agonist exendin-4 protects against interleukin-1β-mediated inhibition of glucose-stimulated insulin secretion by mouse insulinoma β cells

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Abstract. The aim of the present study was to investigate the protective effect of the glucagon-like peptide-1 receptor agonist exendin-4 on the interleukin (IL)-1β-induced impairment of glucose-stimulated insulin secretion (GSIS) in β-TC-6 cells. β-TC-6 cells were pretreated with various concentrations of IL-1β (0.15, 1.5 or 15 ng/ml) and exendin-4 (0.1 or 1 mM). Exendin-4 was administered to β-TC-6 cells prior to, during and following pretreatment. Cells were stimulated with various concentrations of glucose (0, 1.38, 5.5 and 11.1 mM), and insulin was measured via radioimmunoassay of the supernatant; furthermore, western blot analysis was used to detect phosphorylated extracellular receptor kinase (ERK)1/2. The insulin levels (151.08±14.34 μIU/ml) and ERK1/2 phosphorylation in β-TC-6 cells peaked in response to 1.38 mM glucose stimulation compared with 0, 5.5 and 11.1 mM glucose stimulation. IL-1β inhibited GSIS in a dose-dependent manner: Insulin levels were 83.76±1.16 μIU/ml when 0.15 ng/ml IL-1β was added under GSIS, 59.46±3.20 μIU/ml when 1.5 ng/ml IL-1β was added under GSIS, and 56.98±1.19 μIU/ml when 15 ng/ml IL-1β was added under GSIS. Exendin-4 exerted a protective effect against IL-1β-induced GSIS inhibition in a dose-dependent manner. The greatest protective effect was observed when exendin-4 was added prior to IL-1β pretreatment, which was statistically significant (P<0.05). These findings suggested that exendin-4 was able to reverse the IL-1β-induced inhibition of ERK1/2 phosphorylation and serves a protective role by impairing GSIS induced by IL-1β in β-TC-6 cells. This mechanism may be associated with the recovery of ERK1/2 activation.

Introduction

Lifestyle changes may result in an increased incidence of diabetes mellitus, which has become increasingly prevalent and poses a serious threat to human health. Diabetes is a metabolic disorder that results from an inadequate mass of functional β cells. Damage to islet β cells is an important pathophysiological mechanism, which may lead to diabetes. A number of previous studies have demonstrated that the dysfunction of islet β cells may serve an important role in the development of diabetes (1). Glucagon like peptide-1 (GLP-1) is a physiological peptide gut hormone secreted by L cells in the terminal ileum, colon and rectum. GLP-1 combines with specific receptors to promote the synthesis and secretion of insulin in islet β cells (2). GLP-1 agonists, including exendin-4 (Ex-4), have been applied in the clinical treatment of type II diabetes, and various studies have demonstrated that exendin-4 is able to repair inflammatory cytokine-induced damage to islet β cells. This protective mechanism may be associated with the mitogen-activated protein kinase (MAPK) signaling pathway (3). A previous study demonstrated that the inflammatory cytokine IL-1β is able to inhibit the insulin secretion of pancreatic β cells;

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however, the exact mechanism has remained elusive (4). It was previously reported that the mechanism may be associated with the inhibition of the extracellular signal-regulated kinase 1/2(ERK1/2) signal transduction pathway (5). In the present study, the protective effect of Ex-4 on the impairment of glucose-stimulated insulin secretion (GSIS) in response to IL-1β in β-TC-6 cells was investigated.

Materials and methods

Source of cell strain and cultivation. The β-TC-6 mouse islet β-cell line was purchased from the Cell Bank of the Shanghai Institutes for Biological Sciences (Chinese Academy of Sciences, Shanghai, China) and cultivated in Dulbecco's modified Eagle's medium (HyClone; GE Healthcare Life Sciences, Logan, UT, USA) containing 15% fetal bovine serum (Hyclone; GE Healthcare Life Sciences), 100 U/l streptomycin, 100 U/l penicillin, and with a high concentration of glucose (25 mM; Hyclone; GE Healthcare Life Sciences). Cells were cultured in an incubator at 37˚C in an atmosphere containing 5% CO_2, and the medium was changed every three days. The cells were passaged in a 1:2 ratio every 7-10 days. Cells were stained with Trypan blue and the survival rate was found to be >90%.

GSIS. β-TC-6 cells were initially digested with 0.25% trypsase, mixed with 0.01% ethylenediaminetetraacetic acid (Hyclone; GE Healthcare Life Sciences) and prepared in a $4x10^4$/ml single-cell suspension. Cells were seeded into 24-well plates, allowed to adhere to the plate wall for 48 h. The cells were subsequently washed with PBS, placed into serum- and sugar-free Krebs-Ringer bicarbonate HEPES buffer (KRBH; 129 mM NaCl, 4.8 mM KCl, 5 mM $NaHCO_3$, 1.2 mM $MgSO_4$, 2 M $CaCl_2$), and incubated for 30 min at 37˚C. Cells were subsequently washed with KRBH and incubated for 60 min at 37˚C in KRBH with glucose concentrations of 0, 1.38, 5.5 or 11.1 mM. Supernatants were collected and the insulin concentration was measured via radioimmunoassay (RIA).

Intervention of interleukin (IL)-1β. βTC-6 cells were seeded into 6-well plates with KRBH, IL-1β (0.15, 1.5 and 15 ng/ml; Peprotech, Inc., Rocky Hill, NJ, USA) was added and plates were incubated at 37˚C for 24 h. The culture medium was subsequently discarded and βTC-6 cells were washed once with PBS. Glucose-free KRBH containing 1% bovine serum albumin (HyClone; GE Healthcare Life Sciences) was added to the culture medium and incubated for 30 min at 37˚C, following which βTC-6 cells were incubated in KRBH containing 1.38 mM glucose for 5 min at 37˚C. Finally, supernatants were collected and the insulin concentration was measured via RIA.

Influence of Ex-4 on impairment of GSIS. β-TC-6 cells were seeded into 24-well plates and incubated for 24 h in complete culture medium. Ex-4 (0.1 or 1 μM; Eli Lilly and Co., Indianapolis, IN, USA) and IL-1β (0.15, 1.5 or 15 ng/ml) were added to the culture medium, followed by incubation for 24 h. The culture medium was subsequently removed and β-TC-6 cells were washed once with PBS. Serum and sugar-free KRBH was added into the culture medium, followed by incubation for 30 min. β-TC-6 cells were incubated in KRBH containing 1.38 mM glucose for 60 min and supernatants were collected

for insulin determination via RIA. Additional β-TC-6 cells were seeded into 24-well plates and incubated for 24 h in complete culture medium. Ex-4 (1 μM) and IL-1β (0.15 ng/ml) were added to the culture medium for 24 h (Ex-4 was added at 2 h prior to, and 18 and 22 h following the addition of IL-1β to the culture). The medium was subsequently removed and β-TC-6 cells were washed with PBS. Serum and sugar-free KRBH was subsequently added to the culture medium and incubated for 30 min. β-TC-6 cells were incubated in KRBH containing 1.38 mM glucose for 60 min and supernatants were collected for insulin determination via RIA.

Western blot analysis of glucose-stimulated ERK1/2 phosphorylation. β-TC-6 cells were seeded into six-well plates, incubated for 48 h and subsequently cultivated in sugar-free KRBH buffer for 2 h. The cells were incubated in KRBH buffer containing 0, 1.3, 5.5, or 11.1 mM glucose for 5 min. Additionally, to assess the effect of Ex-4, β-TC-6 cells were seeded into six-well plates and incubated for 24 h. Ex-4 (1 μM) and IL-1β (0.15 ng/ml) were then added into the culture medium and stored for 24 h (Ex-4 was added at 2 h prior to, and 0, 18 and 22 h following the addition of IL-1β to the culture). The cells were incubated for 24 h, the medium was subsequently removed and β-TC-6 cells were washed with PBS. Serum and sugar-free KRBH was added and cells were incubated for 2 h, following which β-TC-6 cells were incubated in KRBH containing 1.38 mM glucose for 5 min. The cells were lysed in radioimmunoprecipitation assay buffer mixed with protein phosphatase inhibitor (Fuzhou Maixin Biotech, Co., Ltd., Fuzhou, China), followed by centrifugation at 1,049 x g at 4˚C for 5 min. The supernatants were collected and the same volume of 2x SDS loading buffer (Fuzhou Maixin Biotech, Co., Ltd.) was added. Samples were incubated in a water bath for 5-10 min at 95˚C, stored on ice and subsequently separated by SDS-PAGE (5 μg protein per lane) using a 4% polyacrylamide stacking gel and 12% polyacrylamide separating gel for 10 min at 110 V. Protein bands were transferred from the gel to a nitrocellulose membrane (Sigma-Aldrich; Merck Millipore, Darmstadt, Germany) in transfer buffer for 10 min, electroporated for 90 min at 200 mA and blocked in 5% skimmed milk for 1 h. Primary anti-phospho-p44/p42 MAP kinase (cat. no. sc9101S; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) or anti-β-actin antibodies (cat. no. MX30002; Fuzhou Maixin Biotech, Co., Ltd.) were added at a dilution of 1:1,000, and the samples were incubated overnight at 4˚C. Secondary antibody (horseradish peroxidase-linked anti-rabbit immunoglobulin G; cat. no. MX32002; l:2,000; Fuzhou Maixin Biotech, Co., Ltd.) was added and samples were incubated at room temperature for 1 h. The membrane was subsequently washed with PBS and incubated with nitro-blue tetrazolium and 5-bromo-4-chloro-3'-indolyphosphate solution (Fuzhou Maixin Biotech, Co., Ltd.) at room temperature in the dark for 15 min. Images were captured and analyzed using the LAS3000 imaging system (Fujifilm Co., Tokyo, Japan). β-actin was used an internal control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). The protein concentration was determined by RT-qPCR. RNAiso Plus (Takara Biotechnology Co., Ltd., Dalian, China) was used to isolate total RNA from the β-TC-6

cells according to the manufacturer's protocol. Recombinant DNase I (Takara Biotechnology Co., Ltd.) was used to remove genomic DNA. Total RNA was subjected to a RT reaction that used the PrimeScript RT reagent kit (Takara Biotechnology Co., Ltd.). The reverse transcription was performed in a 10 μl volume containing 2 μl 5xPrimeScript Buffer, 0.5 μl PrimeScript RT Enzyme Mix I, 0.5 μl Oligo dT Primer, 0.5 μl random hexamers, 1 μl total RNA, 5.5 μl RNase free dH$_2$O. The expression of the target gene was determined by SYBR Premix Ex Taq II (Takara Biotechnology Co., Ltd.) using the ABI 7500 Real-time System as previously described (6) (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The primers for ERK1/2 were: Forward, 5-GGACAAGTTTTTGACGTTGC-3 and reverse, 5-CAG TTTCCATAAGATCTTGAAC-3. The control primers (β-actin) were: Forward, 5'-ATTTTGAATGATCAGCCA CC-3' and reverse, 5'-AGGTAAGCCCTGGCTGCCTC-3'. The final reaction volume was 20 μl, containing 10 μl SYBR Premix Ex Taq II, 0.8 μl each primer, 0.4 μl ROX II, 1 μl cDNA, 7 μl RNase free dH$_2$O. Amplification was performed under the following conditions: 95°C for 30 sec, followed by 40 two-step cycles of 95°C for 5 sec and 60°C for 5 sec, and a final dissociation stage. The expression levels of target gene was normalized to the expression level of β-actin using the $2^{-\Delta\Delta Cq}$ method (7). SPSS software v.17.0 (SPSS, Inc., Chicago, IL, USA) was was used to analyze the data. PCR was repeated 3 times.

RIA. RIA was performed using a gamma radiation immune technology instrument (GC-1200) from USTC Chuangxin Co., Ltd. (Hetei, China) to evaluate the concentration of insulin in the supernatant. Experiments were performed in triplicate following the manufacturer's protocol. Intra-group differences were <5%, while inter-group differences were <10%.

Statistical analysis. SPSS software v.17.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. Values are expressed as the mean ± standard deviation. One-way analysis of variance was used for analysis of differences among groups. Independent-sample Student's t-tests were used for comparison between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

GSIS. β-TC-6 cells were incubated in glucose-free KRBH and then stimulated with various concentrations of glucose. The insulin levels in the supernatant were 119.77±3.89 μIU/ml in the absence of glucose, 151.08±14.34 μIU/ml with 1.38 mM glucose, 130.67±11.35 μIU/ml with 5.5 mM glucose and 129.05±4.71 μIU/ml with 11.1 mM glucose. There was a significant difference among the different groups (F=7.50, P=0.004). Compared with the glucose-free group, the insulin levels were 26% higher in the 1.38 mM glucose stimulation group (t=-4.215, P=0.006), 9% higher in the 5.5 mM glucose stimulation group (P=0.136) and 7.7% higher in the 11.1 mM glucose stimulation group (P=0.198).

Glucose-stimulated ERK1/2 phosphorylation. Compared with the glucose-free group, the levels of ERK1/2 phosphorylation

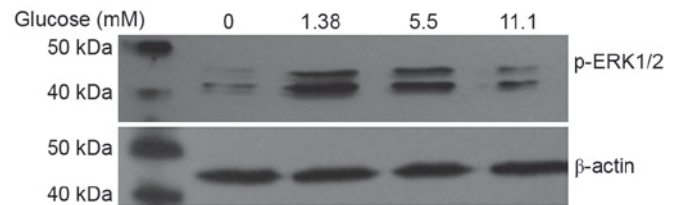Figure 1. Stimulation of ERK1/2 phosphorylation under various glucose concentrations. β-actin was used an internal control. p-, phosphorylated; ERK, extracellular signal-regulated kinase.

were markedly increased in the glucose stimulation groups. In addition, the level of ERK1/2 phosphorylation was highest in the 1.38 mM glucose stimulation group compared with that in the 5.5 and 11.1 mM glucose stimulation groups (Fig. 1).

IL-1β inhibits GSIS. The insulin levels in the supernatant were 62.30±2.24 μIU/ml without glucose stimulation and 108.13±3.71 μIU/ml under stimulation with 1.38 mM glucose. Under high glucose, the insulin levels were 83.76±1.16 μIU/ml when 0.15 ng/ml IL-1β was added, 59.46±3.20 μIU/ml when 1.5 ng/ml IL-1β was added and 56.98±1.19 μIU/ml when 15 ng/ml IL-1β was added. There was a significant difference among these groups (F=714.573; P<0.05). Compared with the 1.38 mM glucose stimulation group, the GSIS of β-TC-6 cells in the 15 ng/ml IL-1β group decreased by 47% (t=8.591, P<0.05), by 45% in the 1.5 ng/ml IL-1β group (t=14.066; P<0.05) and by 23% in the 0.15 ng/ml Il-1β group (t=8.877; P<0.05) (Fig. 2).

Influence of various Ex-4 concentrations on GSIS. β-TC-6 cells were incubated in sugar- and serum-free KRBH medium for 60 min. The insulin levels in the supernatant were 56.97±1.20 μIU/ml in the absence of glucose stimulation and 94.22±4.09 μIU/ml under stimulation with 1.38 mM glucose. Under high glucose, the insulin levels was 70.16±2.56 μIU/ml when 0.15 ng/ml IL-1β was added, 68.32±0.94 μIU/ml when 1.5 ng/ml IL-1β was added, 55.09±1.46 μIU/ml when 15 ng/ml IL-1β was added, 70.65±1.20 μIU/ml when 0.1 μM Ex-4 and 0.15 ng/ml IL-1β were added, 64.25±1.44 μIU/ml when 0.1 mM Ex-4 and 1.5 ng/ml IL-1β were added, 61.99±0.66 μIU/ml when 0.1 μM Ex-4 and 15 ng/ml IL-1β were added, 77.23±0.69 μIU/ml when 1.0 μM Ex-4 and 0.15 ng/ml IL-1β were added, 60.34±3.66 μIU/m when 1.0 μM Ex-4 and 1.5 ng/ml IL-1β were added, and 59.48±1.94 μIU/ml when 1.0 μM Ex-4 and 15 ng/ml IL-1β were added. The insulin concentration in group 2 (1.38 mM glucose) was significantly higher than in group 1 (absence of glucose stimulation; P<0.05; Fig. 3). Furthermore, the insulin concentrations in groups 3 (1.38 mM glucose + 0.15 ng/ml IL-1β), 4 (1.38 mM glucose + 1.5 ng/ml IL-1β) and 5 (1.38 mM glucose + 15 ng/ml IL-1β) were significantly decreased compared with group 2 (P<0.05; Fig. 3). In group 9 (1.38 mM glucose + 0.15 ng/ml IL-1β +1 nM Ex-4), the insulin concentration was significantly increased compared with groups 3, 4 and 5 (P<0.05; Fig. 3).

These results suggested that IL-1β was able to inhibit GSIS by β-TC-6 cells, with the greatest inhibition observed at 15 ng/ml IL-1β. Compared with the 1.38 mM glucose
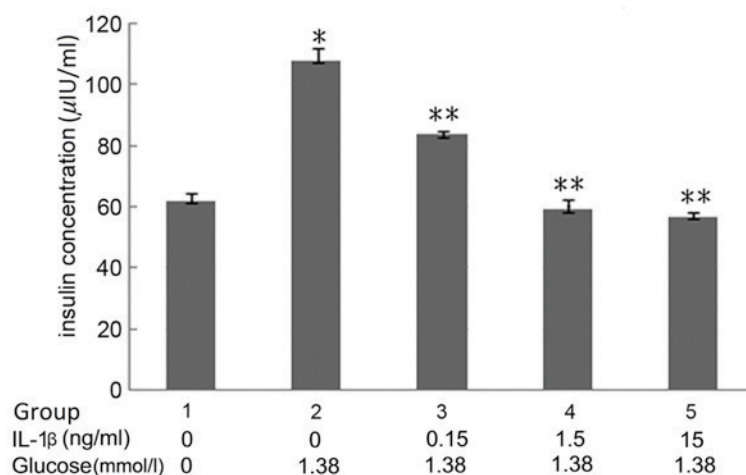
Figure 2. Effect of IL-1β on glucose-stimulated insulin secretion by βTC-6 cells. *P<0.05 vs. group 1 and **P<0.05 vs. group 2. IL-1β, interleukin 1β.

stimulation group, the GSIS by β-TC-6 cells in the 15 ng/ml IL-1β group decreased by 42% (t=39.130, P<0.05), whereas the GSIS by β-TC-6 cells in the 0.15 ng/ml IL-1β group decreased by 26% (t=24.060, P<0.05).

Furthermore, Ex-4 reduced the IL-1β-mediated inhibition of GSIS by β-TC-6 cells, with the greatest protective effect observed at 1.0 μM Ex-4. The GSIS by β-TC-6 cells in the 1.0 μM Ex-4 and 0.15 ng/ml IL-1β group increased by 10.1% compared with the 0.15 ng/ml IL-1β group (t=-7.065, P<0.05).

Inhibition of IL-1β-mediated GSIS in β-TC-6 cells by Ex-4 added at various time-points. β-TC-6 cells were incubated in serum- and sugar-free KRBH medium for 60 min. The insulin levels in the supernatant were 43.61±1.68 μIU/ml without glucose stimulation and 83.95±0.17 μIU/ml under stimulation with 1.38 mM glucose. Under high glucose, the insulin levels were 49.04±0.21 μIU/ml when 0.15 ng/ml IL-1β was added, 74.45±5.98 μIU/ml when 1.0 μM Ex-4 was added 2 h prior to the addition of 0.15 ng/ml IL-1β, and 60.71±4.35 μIU/ml when 1.0 μM Ex-4 and 0.15 ng/ml IL-1β were added simultaneously, 60.21±2.39 μIU/ml when 1.0 μM Ex-4 was added 18 h following the addition of 0.15 ng/ml IL-1β and 56.70±0.78 μIU/ml when 1.0 μM Ex-4 was added 22 h following the addition of 0.15 ng/ml IL-1β. There was a significant difference among these groups (F=42.806, P<0.05; Fig. 4). These results indicated that Ex-4 attenuated the inhibition of GSIS by IL-1β in β-TC-6 cells, with its efficacy being highest if added 2 h prior to IL-1β and decreasing with the delay in the time of its addition. The GSIS by β-TC-6 cells following the addition of 1.0 μM Ex-4 2 h prior to the addition of 0.15 ng/ml IL-1β increased by 52% compared with that in the 0.15 ng/ml IL-1β only group (t=25.415, P<0.05). The GSIS by β-TC-6 cells treated with 1.0 μM Ex-4 and 0.15 ng/ml IL-1β added simultaneously increased by 24% compared with that in the 0.15 ng/ml IL-1β only group (t=11.670, P<0.05). The GSIS by β-TC-6 cells increased by 23% when 1.0 μM Ex-4 was added at 18 h following the addition of 0.15 ng/ml IL-1β compared with that in the 0.15 ng/ml IL-1β only group (t=11.175, P<0.05). The GSIS by β-TC-6 cells treated with 1.0 μM Ex-4 added at 22 h following the addition of 0.15 ng/ml IL-1β increased by 16% compared with that in the 0.15 ng/ml IL-1β only group (t=7.665, P<0.05).

Influence of Ex-4 on ERK1/2 phosphorylation. As presented in Fig. 5, 1.38 mM glucose stimulated ERK1/2 phosphorylation in β-TC-6 cells and 0.15 ng/ml IL-1β was able to inhibit this, which was in turn attenuated by Ex-4. However, there was no marked difference observed in the protein levels among different samples within each group.

Discussion

The β-TC cell line is derived from insulinoma cells, which are produced by SV40 t-antigen transgenic mice under the control of the insulin operon; β-TC-6 cells produce insulin I and II, secreting insulin and a small amount of glucagon. The maximum threshold of glucose stimulation in β-TC-6 cells to induce insulin secretion is lower than in normal β cells, and GSIS peaks at 1.3-3.0 mM, which is 1.6-fold higher than under sugar-free stimulation (stimulation index, 1.6) (8). In the present study, it was demonstrated that GSIS peaks at 1.38 mM, which was 1.26-fold higher than under sugar-free stimulation (stimulation index, 1.26) In the present study, levels of insulin secretion at higher concentrations of glucose (5.5 and 11.1 mM) were lower than those obtained with 1.38 mM glucose, indicating that 1.38 mM was the most suitable glucose concentration for GSIS in β-TC-6 cells.

MAPKs are a class of serine/threonine protein kinases that exist in most cells. The ERK1/2 pathway is an important MAPK signaling pathway and ERK1/2 may be activated by extracellular stimuli, such as ultraviolet rays, high osmotic pressure, heat shock, as well as cytokines to regulate cell growth, proliferation, differentiation and death (5). Certain studies have demonstrated that glucose stimulation may activate the ERK1/2 signal transduction pathway in islet β cells. Longuet *et al* (9) demonstrated that glucose stimulation is able to activate the ERK1/2 signal transduction pathway in rat islets, with the degree of activation being directly proportional to the glucose concentration used for stimulation. Furthermore, the ERK1/2 signal transduction pathway is also activated by glucose stimulation in the INS-1 rat pancreatic β-cell line and the MIN6 mouse pancreatic β-cell line (5). The present study also demonstrated that glucose stimulation is able to

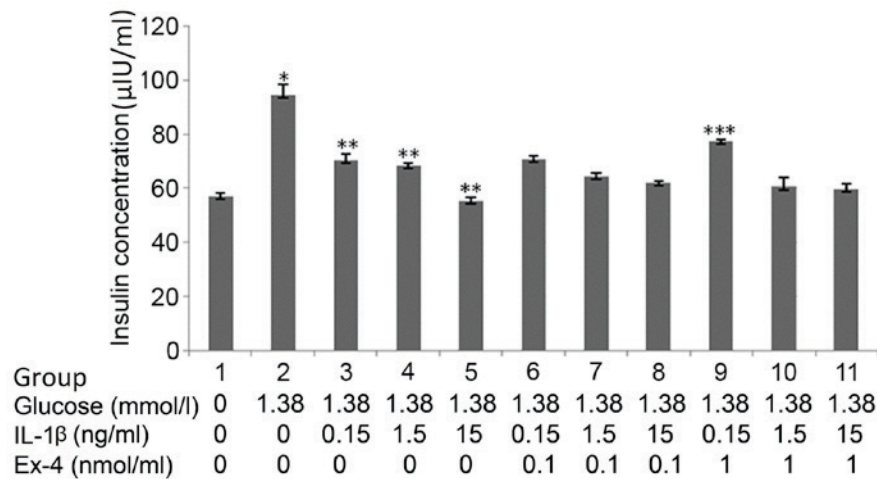Figure 3. Effect of various concentrations of Ex-4 on IL-1β-mediated inhibition of glucose-stimulated insulin secretion by β-TC-6 cells. [*]P<0.05 vs. group 1, [**]P<0.05 vs. group 2 and [***]P<0.05 vs. groups 3, 4 and 5. Ex-4, Exendin-4; IL-1β, interleukin 1β.
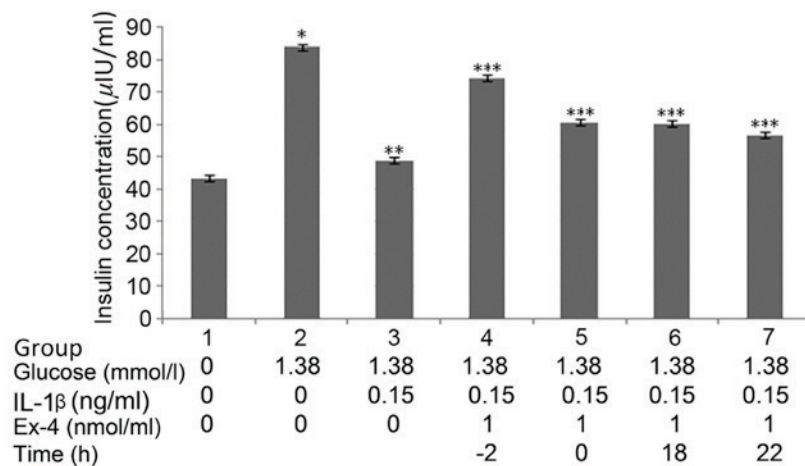

Figure 4. Time-dependent effects of Ex-4 on IL-1β-mediated inhibition of glucose-stimulated insulin secretion by β-TC-6 cells. [*]P<0.05 vs. group 1, [**]P<0.05 vs. group 2 and [***]P<0.05 vs. group 3. Ex-4, Exendin-4; IL-1β, interleukin-1β.
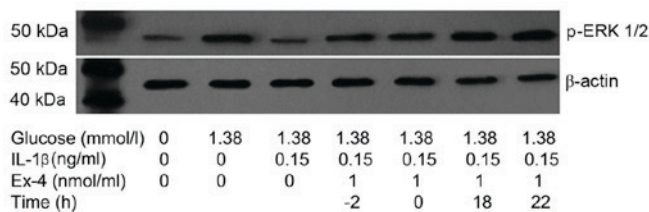

Figure 5. Effects of Ex-4 added at different time-points on IL-1β-mediated inhibition of ERK1/2 phosphorylation in β-TC-6 cells under high glucose conditions. β-actin was used an internal control. Ex-4, Exendin-4; IL-1β, interleukin-1β; ERK, extracellular signal-regulated kinase.

activate the ERK1/2 signal transduction pathway and that insulin secretion peaked at stimulation with 1.38 mM glucose, which was 1.26-fold higher than that under sugar-free conditions (stimulation index, 1.26). These findings suggested that ERK1/2 activation following glucose stimulation is associated with insulin secretion in β-TC-6 cells.

Islet β-cell damage caused by a lack of insulin has an important role in the occurrence and development of diabetes.

Dogan et al (10) demonstrated that islet β-cell damage is associated with the inflammatory process in type II diabetes. Recently, incretins, particularly the effect of GLP-1 protection of islet β cells, have received increasing attention (11). The GLP-1 agonist Ex-4 has been applied in the clinical treatment of type II diabetes. GLP-1 is able to combine with GLP-1 receptors in islet β cells, thereby improving the sensitivity of islet β cells to glucose and stimulating transcription and translation of insulin genes in the β cells, which in turn promotes the synthesis and secretion of insulin (12). Arakawa et al (13) reported that Ex-4 is able to increase the survival rate of β cells in rodents under stimulation with inflammatory cytokines such as IL-1β. The protective effect of GLP-1 on islet-cells is affected by Ex-4 in a dose-dependent manner. The proliferation of islet-cells exposed to high levels of Ex-4 increases compared with those exposed to low levels of Ex-4 (14). Arakawa et al (13) found that in C57BL/6J mice, a high dose of Ex-4 (24 nmol/kg) was able to increase β-cell proliferation and amplify the effects of a long-term high-fat diet, leading to weight gain, abnormal glucose tolerance and decreased insulin secretion. The findings of the present study indicated that Ex-4

had an inhibitory effect on IL-1β-induced impairment of GSIS by β-TC-6 cells. High-dose Ex-4 (1 μM) led to a high inhibition of GSIS impairment induced by IL-1β, compared with low-dose Ex-4 (0.1 μM).

The protective effect of GLP-1 on islet β cells may be associated with its onset of action. Pérez-Arana et al (15) found that the proliferation of islet β cells ceased before they underwent apoptosis and diabetes was induced in BB/Wistar rats, and earlier administration of Ex-4 effectively delayed this cessation. The results of the present study demonstrated that the inhibitory effect of Ex-4 on GSIS impairment induced by IL-1β was associated with the order in which Ex-4 and IL-1β were added. Addition of Ex-4 to β-TC-6 cells prior to the addition of IL-1β induced a strong protective effect, which suggested that Ex-4 should be administered as early as possible to protect islet β cells.

In vitro studies on cells and animal experiments have shown that GLP-1 serves an important role in the process of proliferation, differentiation and apoptosis of islet β cells, and the underlying mechanisms may be associated with ERK1/2 pathway regulation. Blandino-Rosano et al (16) demonstrated that IL-1β, interferon-γ and tumor necrosis factor α were able to inhibit ERK1/2 activation in rat islet β cells under glucose stimulation, and that GLP-1 was able to completely prevent this suppression, suggesting that GLP-1 may protect islet β cells damaged by inflammatory cytokines via ERK1/2. The present study demonstrated that IL-1β was able to inhibit ERK1/2 activation and GSIS in β-TC-6 cells, which was attenuated by Ex-4, the underlying mechanisms of which may be associated with the restoration of ERK1/2 activation. Ex-4 is able to increase calcium transport via L-type calcium voltage-gated ion channels and activate calcium/calmodulin protein kinase II, which in turn activates protein kinase A, which acts on Ras, MAPK kinase and Raf-1/2, and mediates the phosphorylation of ERK1/2 (17). Whether Ex-4 restores ERK1/2 activation through this transduction pathway remains to be determined. In addition, Ex-4 may protect islet β cells by interfering with the c-Jun amino terminal kinase pathway (18), inhibiting nuclear factor-κB activation (19) and activating the c-Jun N-terminal kinase pathway (20), which suggests that Ex-4 may protect islet β-cell function by regulating various MAPK-associated pathways. The findings of the present study suggest that Ex-4 may be able to protect islet cells from IL-1β-induced damage.

Acknowledgements

References

1. Kim KA and Lee MS: Recent progress in research on beta-cell apoptosis by cytokines. Front Biosci 14: 657-664, 2009.
2. Ellenbroek JH, Töns HA, Westerouen van Meeteren MJ, de Graaf N, Hanegraaf MA, Rabelink TJ, Carlotti F and de Koning EJ: Glucagon-like peptide-1 receptor agonist treatment reduces beta cell mass in normoglycaemic mice. Diabetologia 56: 1980-1986, 2013.
3. Kang JH, Chang SY, Jang HJ, Kim DB, Ryu GR, Ko SH, Jeong IK, Jo YH and Kim MJ: Exendin-4 inhibits interleukin-1beta-induced iNOS expression at the protein level, but not at the transcriptional and posttranscriptional levels, in RINm5F beta-cells. J Endocrinol 202: 65-75, 2009.
4. Donath MY, Böni-Schnetzler M, Ellingsgaard H and Ehses JA: Islet inflammation impairs the pancreatic beta-cell in type II diabetes. Physiology 24: 325-331, 2009.
5. Lawrence M, Shao C, Duan L, McGlynn K and Cobb MH: The protein kinases ERK1/2 and their roles in pancreatic beta cells. Acta Physiol (Oxf) 192: 11-17, 2008.
6. Lee YM, Yang IJ, Noh JK, Kim HC, Park CJ, Park JW, Noh GE, Kim WJ and Kim KK: Expression analysis of lily type lectin isotypes in the rock bream, oplegnathus fasciatus: In the tissue, developmental stage and viral infection. Dev Reprod 20: 297-304, 2016.
7. 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.
8. Ohtani M, Oka T, Badyuk M, Xiao Y, Kellar KJ and Daly JW: Mouse beta-TC-6 insulinoma cells: High expression of functional alpha3beta4 nicotinic receptors mediating membrane potential, intracellular calcium, and insulin release. Mol Pharmacol 69: 899-907, 2006.
9. Longuet C, Broca C, Costes S, Hani EH, Bataille D and Dalle S: Extracellularly regulated kinases 1/2 (p44/42 mitogen-activated protein kinases) phosphorylate synapsin I and regulate insulin secretion in the MIN6 beta-cell line and islets of Langerhans. Endocrinology 146: 643-654, 2005.
10. Dogan Y, Akarsu S, Ustundag B, Yilmaz E and Gurgoze MK: SerumIL-1beta, IL-2, and IL-6 in insulin-dependent diabetic children. Mediators Inflamm 2006: 59206, 2006.
11. van Raalte DH and Verchere CB: Glucagon-like peptide-1 receptor agonists: Beta-cell protection or exhaustion? Trends Endocrinol Metab 27: 442-445, 2016.
12. Aulinger BA, Vahl TP, Wilson-Pérez HE, Prigeon RL and D'Alessio DA: β-Cell Sensitivity to GLP-1 in healthy humans is variable and proportional to insulin sensitivity. J Clin Endocrinol Metab 100: 2489-2496, 2015.
13. Arakawa M, Ebato C, Mita T, Hirose T, Kawamori R, Fujitani Y and Watada H: Effects of exendin-4 on glucose tolerance, insulin secretion and, beta-cell proliferation depend on treatment dose, treatment duration and meal contents. Biochem Biophys Res Commun 390: 809-814, 2009.
14. Zhou J, Pineyro MA, Wang X, Doyle ME and Egan JM: Exendin-4 differentiation of a human pancreatic duct cell line into endocrine cells: Involvement of PDX-1 and HNF3beta transcription factors. J Cell Physiol 192: 304-314, 2002.
15. Pérez-Arana G, Blandino-Rosano M, Prada-Oliveira A, Aguilar-Diosdado M and Segundo C: Decrease in {beta}-cell proliferation precedes apoptosis during diabetes development in bio-breeding/worcester rat: Beneficial role of Exendin-4. Endocrinology 151: 2538-2546, 2010.
16. Blandino-Rosano M, Perez-Arana G, Mellado-Gil JM, Segundo C and Aguilar-Diosdado M: Anti-proliferative effect of pro-inflammatory cytokines in cultured beta cells is associated with extracellular signal-regulated kinase 1/2 pathway inhibition: Protective role of glucagon-like peptide-1. J Mol Endocrinol 41: 35-44, 2008.
17. Selway J, Rigatti R, Storey N, Lu J, Willars GB and Herbert TP: Evidence that Ca2+ within the microdomain of the L-type voltage gated Ca2+ channel activates ERK in MIN6 cells in response to glucagon-like peptide-1. PLoS One 7: e33004, 2012.
18. Ferdaoussi M, Abdelli S, Yang JY, Cornu M, Niederhauser G, Favre D, Widmann C, Regazzi R, Thorens B, Waeber G and Abderrahmani A: Exendin-4 protects beta-cells from interleukin-1 beta-induced apoptosis by interfering with the c-Jun NH2-terminal kinase pathway. Diabetes 57: 1205-1215, 2008.
19. Pugazhenthi U, Velmurugan K, Tran A, Mahaffey G and Pugazhenthi S: Anti-inflammatory action of exendin-4 in human islets is enhanced by phosphodiesterase inhibitors: Potential therapeutic benefits in diabetic patients. Diabetologia 53: 2357-2368, 2010.
20. Erdogdu O, Eriksson L, Xu H, Sjöholm A, Zhang Q and Nyström T: Exendin-4 protects endothelial cells from lipoapoptosis by PKA PI3K eNOS p38MAPK and JNK pathways. J Mol Endocrinol 50: 229-241, 2013.