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Liraglutide improves pancreatic islet β cell apoptosis in rats with type 2 diabetes mellitus by inhibiting the IKKε/NF-κB pathway

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Abstract. – OBJECTIVE: **The purpose of this study was to explore the effect of liraglutide on pancreatic islet β cell apoptosis in rats with type 2 diabetes mellitus (T2DM) and the potential mechanisms.**

MATERIALS AND METHODS: SD rats were randomly divided into control group, model group, and liraglutide groups (200 and 100 μg/(kg·d)). Rats were fed with high sugar and high-fat diet for 8 weeks, and then streptozotocin (STZ) 40 mg/kg was intraperitoneally injected to establish T2DM model. After successful modeling, rats in the intervention group were given liraglutide through subcutaneous injection for 6 weeks. The indexes of glucose metabolism and lipid metabolism were measured. Apoptosis of islet β cells was detected by TUNEL. Western blot and RT-PCR were used to detect the protein and mRNA expression levels of IKK ε, NF-κ B, Bcl-2, Bax, IL-6, and Gal-3 in pancreatic tissue.

RESULTS: Compared with the control group, the serum FPG, INS, HOMA-IR, TC, TG, LDL-C, IL-6, islet apoptosis rate, glucagon, the positive expression rate of Gal-3, and body weight in the T2DM group were all significantly increased (*p*<0.05). However, the levels of insulin, SOD, HDL, and HOMA-β were notably decreased in the T2DM group in comparison with the control group (*p*<0.05). Moreover, the mRNA and protein expression levels of IKKε, NF-κB, Bax, IL-6, and Bax/Bcl-2 were markedly increased in pancreatic tissue (*p*<0.05). After liraglutide treatment, these changes were reversed in a dose-dependent manner.

CONCLUSIONS: Liraglutide improves pancreatic islet β cell apoptosis in rats with type 2 diabetes mellitus by inhibiting the IKKε/NF-κB pathway.

Key Words:
Liraglutide, Pancreatic islet β cell, Apoptosis, Type 2 diabetes mellitus, IKK-ε/NF-κB.

Introduction

Diabetes mellitus (DM) is one of the most important public health problems in the world. According to the International Diabetes Federation (IDF) latest data in 2019, there are about 463 million adults aged 20-79 years old in the world suffering from diabetes. China is the country with the largest number of DM patients in the world, with 116.4 million adults. DM is a complex metabolic disorder characterized by insulin deficiency (type 1 DM) and relative insufficiencies of insulin secretion/insulin resistance (IR) (type 2 DM) or insulin deficiency caused by other pathological pathways. T2DM is associated with many factors, including hypertension, chronic hyperglycemia, and hyperlipidemia.

T2DM is a multifactorial disease, including genetic and lifestyle, and other environmental factors, especially high-calorie diet. Therefore, it has become an important issue to prevent and treat T2DM by changing diet and lifestyle. People have made a lot of efforts to improve the treatment of DM. Although there are many hypoglycemic drugs that can be used to control hyperglycemia, they still have potential side effects. The analysis of clinical adverse reactions of oral drugs showed that oral hypoglycemic drugs such as glucosidase inhibitors can cause gastrointestinal side effects, liver and kidney dysfunction, lactic acidosis, etc.

IKK-ε, as a newly discovered member of the IKK family, can regulate inflammatory response through the NF-κB signal transduction pathway[1]. At the same time, IKK-ε and NF-κB are also involved in the occurrence of oxidative stress[2,3]. Moreover, inflammation and oxidative stress are

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also important factors to promote islet β function apoptosis. IKK-ε and NF-κB have been found to be closely related to apoptosis[4,5]. It has been reported that inhibiting the IKK-ε expression can significantly inhibit the infiltration of inflammatory macrophages, the activity of matrix metalloproteinase (MMP), reduce oxidative stress, and inhibit the apoptosis of vascular smooth muscle cells[6].

Liraglutide has the effects of anti-inflammation, anti-oxidative stress, and improving the function of islet β cells[7,8]. Previous studies[9,10] have found that liraglutide can reduce inflammation, improve oxidative stress and reduce insulin resistance by inhibiting the levels of IKK-α, IKK-β, and NF-κB. Therefore, the purpose of this study was to explore the effect of liraglutide on pancreatic islet β cell apoptosis in T2DM rats and the potential mechanisms.

Materials and Methods

Reagents

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Liraglutide was obtained from Zhejiang Pai peptide biological Co., Ltd., (Hangzhou, China). The common diet for rats consisted of sorghum flour, soybean flour, corn flour, cod liver oil, wheat bran, multiple vitamins, multiple trace elements, etc., with 4% fat content (Beijing Huafukang Biotechnology Co., Ltd., Wuhan, China). The high sugar and high-fat diet for rats were composed of basic diet, lard, cholesterol, egg yolk powder, deoxycholate, etc. (carbohydrate 30.01%, protein 16.24%, fat 53.75%, total calorie 486 kcal/100 g) (Wuhan bode Bioengineering Co., Ltd., Wuhan, China). Anti-IKKε, anti-NF-κB, anti-Bcl-2, anti-Bax, and anti-Gal-3 antibodies were purchased from Abcam Technology (Cambridge, UK). Anti-GAPDH antibody and Goat anti-rabbit and -mouse IgG and mouse anti-goat secondary antibodies were obtained from Wuhan bode Bioengineering Co., Ltd. (Wuhan, China). Unless specified, all other reagents are obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

Animals and Modeling

All experimental procedures were conducted in accordance with the Chinese legislation, and the US National Institutes of Health guidelines for the use and care of experimental animals. Animal experiments were approved by the institutional ethical committee of the Renhe Hospital Affiliated with Three Gorges University. Forty-five SPF male SD rats, weighing 200-220 g, aged 7-8 weeks, were purchased from Beijing Basco Biomedical Technology Co., Ltd. (Beijing, China) (No. SCXK (Jing) 2019-0010). Rats were fed adaptively for 1 week, maintained at (22 ± 2)°C, (50-60)% relative humidity, and a 12 h light/dark cycle. All rats were allowed free access to a diet. According to the random number table, rats were divided into the control group (n = 10) and experimental group (n = 35). Rats in the control group were given a common diet, and those in the experimental group were given high sugar and high-fat diet. After eight weeks, streptozotocin (STZ) 40 mg/kg was intraperitoneally injected to establish T2DM model, while rats in the control group were given 0.9% normal saline. After 72 h, the tail vein blood was collected to test blood glucose, and the model was successfully established with blood glucose ≥ 16.7 mmol/L. Rats in the experimental group were randomized into T2DM and liraglutide (200 and 100 μg/(kg·d)) groups (10 rats/group). Rats in the liraglutide groups were given liraglutide through subcutaneous injection for 6 weeks, while those in control and T2DM groups were given 0.9% normal saline.

After successful modeling, the tail vein blood of the rats in the control group and the experimental group were collected and stored at -80°C for later use. At the end of the experiment, all rats were fasted for 12 h, then anesthetized with pentobarbital, and blood was taken from the tail vein again. After blood collection, the pancreatic tissue was immediately taken out, labeled and stored at -80°C.

Measurement of Serum Variables

Fasting blood glucose (FBG) was measured by glucose assay kit (CheKine™) using a blood glucose meter (Johnson & Johnson, USA). Total triglyceride (TG), total cholesterol (TC), HDL cholesterol (HDL-C), and LDL cholesterol (LDL-C) in serum were measured by using C8000 automatic biochemical analyzer (Abbott laboratory, Chicago, USA) and a commercially available diagnostic kit (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China). Fasting insulin (FIN) was detected by Cobase411 automatic electrochemiluminescence analyzer (Roche, Mannheim, Germany) and ultra-sensitive insulin immunoassay kit (Huamei Biotech Co. Ltd., Wuhan China). IL-6 was detected by ELISA with a microplate reader and commercially available diagnostic

Table I. Primer sequences used for determination of IKKε, NF-κB, Bcl-2, Bax, and Gal-3 gene expression.

Name	Primer	Sequence	Size
IKKε	Forward	5'-TGTACAAGGCCCGGAATAAG-3'	280bp
	Reverse	5'-CCTCCACTGCGAATAGCTTC-3'	
NF-κB	Forward	5'-ACTATGAGGTCTCTGGGGGA-3'	250bp
	Reverse	5'-GAAGCTGAGTTTGCGGAAGG-3'	
Gal-3	Forward	5′-CAAGCTTATGGCAGACGGCTTCTCACTTAATG-3'	260bp
	Reverse	5′-CTCTAGACTTAGATCATGGCGTGGGAAGCGCT-3'	
IL-6	Forward	5'-TTCGGTCCAGTTGCCTTCT-3'	256bp
	Reverse	5'-GTACTCATCTGGACAGCTC-3'	
Bax	Forward	5'-AGACACCTGAGCTGACCTTGGA-3'	270
	Reverse	5'-TTGAAGTTGCCATCAGCAAACA-3'	
Bcl-2	Forward	5'-GACTGAGTACCTGAACCGGCATC-3'	255
	Reverse	5'-CTGAGCAGCGTCTTCAGAGACA-3'	

kit (Hangzhou KangZhi Biomedical Co., LTD., Hangzhou, China). Superoxide dismutase (SOD) was determined by the hydroxylamine method with a commercially available diagnostic kit (Nanjing Jiangcheng Bioengineering Institute, Nanjing, China). Homeostasis model assessment -insulin resistance (HOMA-IR) = (FBG × FIN / 22.5).

Histopathological Analysis

Hematoxylin and eosin staining (HE) was performed as previously reported[11]. The pancreatic tissue was fixed with 10% phosphate-buffered formalin for 24 h. After routine histological treatment, 5 μm tissue sections were cut and stained with hematoxylin and eosin. Histological analysis was performed under an optical microscope (OLYMPUS BX41, OLYMPUS, Tokyo, Japan) and photographed at 400 × magnification.

TdT-Mediated Nick end Labeling (TUNEL) Assay

After fixed in 4% paraformaldehyde for 24 h, the pancreatic tissue was routinely embedded in paraffin and sectioned. Then, it was washed with xylene for dewaxing, soaked with gradient ethanol for 3 min, and incubated with protease K at 37°C for 20 min. 50 μl TUNEL solution was added and incubated at 37°C for 1 h in the dark. Then, 50 μl working solution was dripped and incubated at 37°C for 30 min. Finally, DAB staining and hematoxylin staining were added, followed by gradient ethanol dehydration, transparency, and sealing. The nuclei of normal cells were stained blue, and the nuclei of apoptotic cells were stained dark brown. The apoptosis of pancreatic cells was observed under a microscope. The number of TUNEL positive cells and the total number of cells were counted in three fields randomly, and the positive rate of TUNEL staining was calculated. The positive rate of TUNEL staining (%) = the number of positive cells / the total number of cells × 100%.

Immunohistochemistry Assay

The tissue sections were prepared by the above method. They were baked at 60°C for 30 min, and then dewaxed with xylene and dehydrated in gradient ethanol. Normal sheep serum (Bio-Technology) was added and incubated at 37°C for 10 min. The slices were incubated with anti-IKKε, anti-NF-κB, anti-Bcl-2, anti-Bax, and anti-Gal-3 antibodies at 4°C overnight and washed with PBS for 3 min. The Goat anti-rabbit and -mouse IgG and mouse anti-goat secondary antibodies were added and incubated at room temperature for 30 min. DAB solution (Suzhou Industrial Park Yake Chemical Reagent Co., Ltd.) was used for dyeing for 3 min, and distilled water was used for cleaning for another 3 min. They were then dyed with hematoxylin, dehydrated and sealed. The slices were placed under the optical microscope to observe and collect images.

Quantitative Real-Time PCR

The total RNA of the pancreatic tissue was extracted and revised to cDNA as previously described[12]. Target genes were amplified using the MJ PTC-200 PCR system (Bio-Rad, Hercules, CA, USA) and the RT-PCR kit (2× Taq PCR Master Mix, Aidlab Biotech Co., Ltd., Beijing, China). Specific primers for the target genes used in this study are listed in Table I. The reaction parameters of PCR are as follows: Pre-denaturation for 5 min at 95°C, 1 cycle; denaturation for 30 s at 95°C, annealing for 30 sec. at (51-62)°C, extend-

ing for 1 min at 72°C, 35 cycles; and extra-extension for 5 min at 72°C.

Western Blot

The total protein of the pancreatic tissue was extracted and quantified as previously described[12]. The protein samples were separated by SDS-PAGE and transferred to a nitrocellulose membrane. The membrane was blocked with 5% skimmed milk powder at room temperature for 1 h, and combined with anti-IKKε, anti-NF-κB, anti-Bcl-2, anti-Bax, and anti-Gal-3 antibodies at 4°C overnight. Goat anti-rabbit antibody labeled with horseradish peroxidase (HRP) was added and incubated at 37°C for 1 h. The protein bands were developed by ECL kit and recorded in a UVP gel imager. The gray value of protein bands was analyzed by Image J. The ratio of the gray value of the target band and internal reference band was used as the relative expression of the target protein.

Statistical Analysis

SPSS 22.0 (IBM, Armonk, NY, USA) was used for statistical analysis, and the data were expressed as means ± standard deviation (SD). The values between the two groups were compared by non-paired *t*-test, and the values among multiple groups were compared by one-way ANOVA analysis. $p < 0.05$ was considered as statistically significant.

Results

Liraglutide Improved Insulin Resistance in T2DM Rats

As shown in Figure 1Aand 1B, there were significant differences in the levels of FBG and FIN between control rats and T2DM rats ($p<0.05$). Furthermore, the levels of FBG and FIN were significantly decreased after liraglutide treatment ($p<0.05$). Correspondingly, HOMA-IR was significantly increased in the T2DM group compared to the control group, which was notably decreased by liraglutide treatment in a dose-dependent manner ($p<0.05$, Figure 1C). Moreover, the imaging of immunohistochemistry indicated that compared with the control group, the glucagon expression in the T2DM group was significantly lower ($p<0.05$). However, the insulin expression in the liraglutide group was notably higher than that in the T2DM group, with a dose-dependent manner ($p<0.05$, Figure 2A). On the contrary, the imaging of immunohistochemistry indicated that compared with the control group, the glucagon expression in the T2DM group was significantly higher ($p<0.05$). However, the glucagon expression in the liraglutide group was notably lower than that in the T2DM group in a dose-dependent manner ($p<0.05$, Figure 2B). Taken together, these results indicated that liraglutide had a protective effect against insulin resistance in T2DM rats.

Liraglutide Improved Pancreatic β Cell Apoptosis in T2DM Rats

Compared with the control group, the weight in the T2DM group was significantly increased ($p<0.05$). However, the treatment with liraglutide could notably improve the increased weight in the T2DM rats ($p<0.05$, Figure 3A). Moreover, the apoptosis of pancreatic β cells in the T2DM rats was also significantly increased compared to the control rats. This effect was reversed by liraglutide treatment ($p<0.05$, Figure 3B). Furthermore, the IL-6 level was markedly increased, and the SOD level was notably decreased in the T2DM group in comparison with the control group

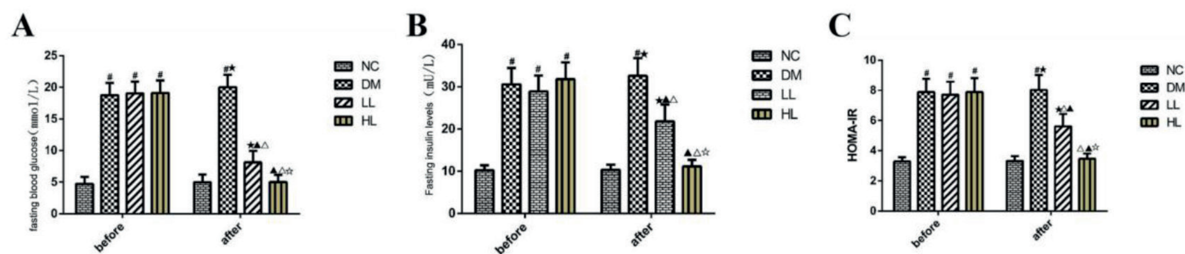

Figure 1. Effects of Liraglutide on insulin resistance in T2DM rats. **(A)** FBG level; **(B)** FIN level; **(C)** HOMA-IR. Data are mean ± SD of at least three separate experiments (n = 10). Before the intervention of liraglutide, the experimental group was compared with the normal control group: #$p<0.05$. After the intervention of liraglutide, compared with the T2DM group: △$p<0.05$; compared with the LL group: ☆$p<0.05$; compared with the NC group, ★$p<0.05$. Comparison before and after liraglutide intervention: ▲$p<0.05$. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

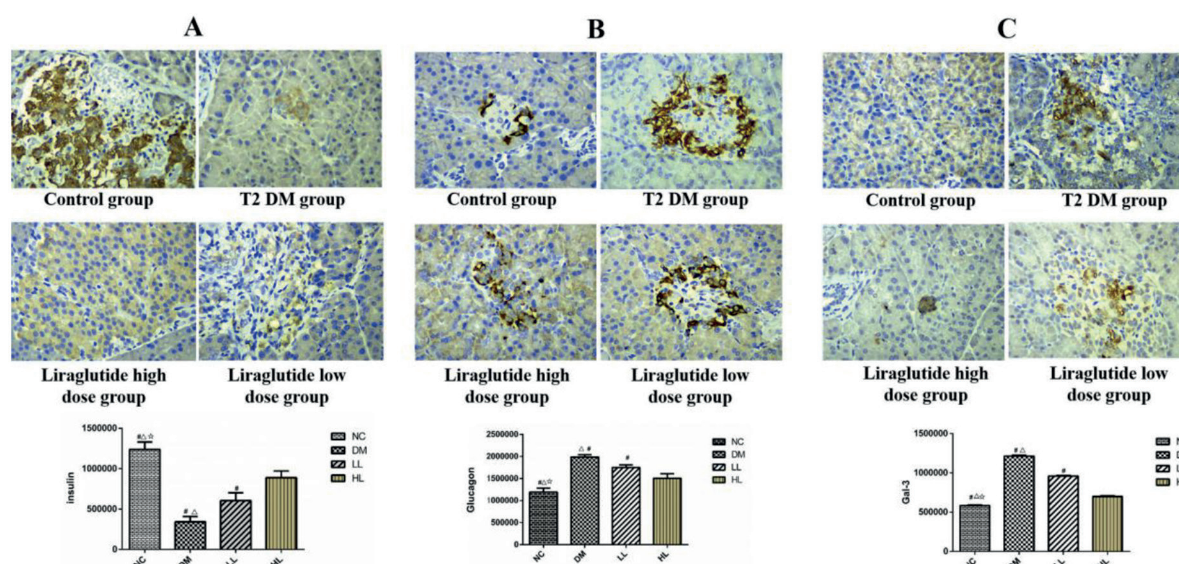

Figure 2. Effects of Liraglutide on the levels of insulin, glucagon and Gal-3 in T2DM rats by Immunohistochemistry. **(A)** Insulin; **(B)** Glucagon; **(C)** Gal-3. Data are means ± SD of at least three separate experiments (n = 3). Magnification was × 400. Compared with HL group, $^{\#}p<0.05$ compared with LL group $^{\triangle}p<0.05$ compared with DM group $^{☆}p<0.05$. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

($p<0.05$, Figure 3C and 3D). Those effects also were reversed by liraglutide treatment ($p<0.05$, Figure 3C and 3D). In addition, HE staining showed that the islets of control rats were round or oval in shape, with regular shape, uniform distribution of β cells, and clear boundary. Compared with the control group, the number of pancreatic islet β cells in the model group was decreased, the distribution was uneven, the arrangement was disordered, and the shape of islet was irregular. In the liraglutide treatment groups, the nucleus of pancreatic islet β-cells was enlarged, the chromatin was thickened, and the nucleolus was obvious. Moreover, the number of pancreatic islet β-cells was increased, and the nucleus was enlarged (Figure 3E). Taken together, these results indicated that liraglutide had a protective effect against pancreatic β cell apoptosis in T2DM rats.

Liraglutide Improved Serum Lipid Deposition in T2DM Rats

As shown in Figure 4, the levels of serum TG, TC, and LDL were significantly increased in the T2DM rats in comparison with the control rats ($p<0.05$, Figure 4). After treatment with liraglutide, the levels of serum TG, TC, and LDL were significantly decreased in the T2DM rats ($p<0.05$, Figure 4). Moreover, the serum HDL level was markedly lower in the T2DM group in comparison with the control group, whereas that in the liraglutide group was significantly higher than those in the T2DM group ($p<0.05$, Figure 4). In short, those results suggested that liraglutide improved serum lipid deposition in T2DM rats.

Liraglutide Improved Pancreatic Islet β-Cell Apoptosis by Inhibiting the IKKε/NF-κB Pathway

As shown in Figures 5 and 6, the protein and mRNA levels of IKKε, NF-κB, Bax, IL-6, Gal-3, and Bax/Bcl-2 were significantly increased in the T2DM group in comparison with the control group. After treatment with liraglutide, the protein and mRNA levels of IKKε, NF-κB, Bax, IL-6 and Gal-3 and Bax/Bcl-2 were significantly decreased in a dose-dependent manner ($p<0.05$). However, the Bcl-2 level was notably decreased in the T2DM rats compared to the control rats, while this was reversed by liraglutide treatment ($p<0.05$). Moreover, the imaging of immunohistochemistry indicated that compared with the control group, the Gal-3 expression in the T2DM group was significantly lower ($p<0.05$). However, the Gal-3 expression in the liraglutide group was notably higher than that in the T2DM group ($p<0.05$, Figure 2C). All in all, those results indicated that liraglutide treatment could significantly IKKε/NF-κB pathway in T2DM rats.

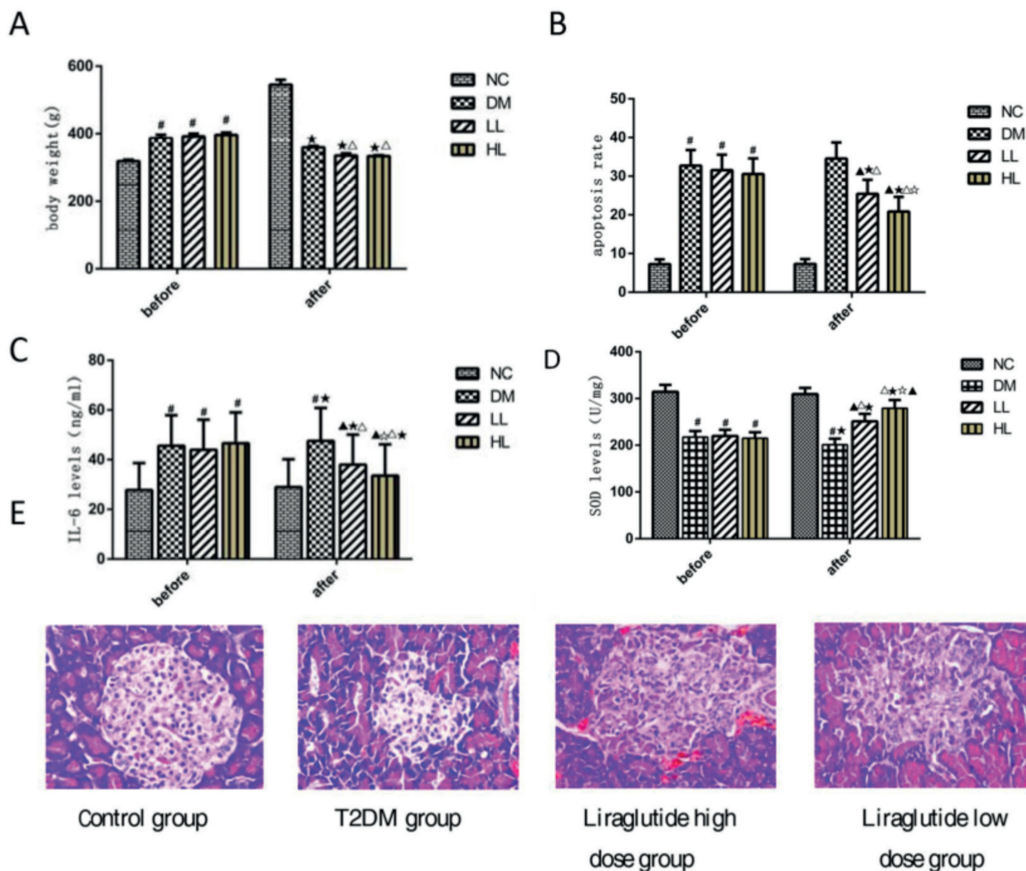

Figure 3. Effects of Liraglutide on apoptosis in T2DM rats. **(A)** Weight; **(B)** apoptosis; **(C)** IL-6 level; **(D)** SOD level; **(E)** Histological analysis of pancreatic tissue sections in each group. H&E staining was performed (magnification, × 400). Data are means ± SD of at least three separate experiments (n = 10). Before the intervention of liraglutide, the experimental group was compared with the normal control group: #*p*<0.05. After the intervention of liraglutide, compared with the T2DM group: △*p*<0.05; compared with the LL group: ☆*p*<0.05; compared with the NC group, ★*p*<0.05. Comparison before and after liraglutide intervention: ▲*p*<0.05. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

Discussion

Our study suggested that liraglutide treatment can inhibit the apoptosis of pancreatic β-cells in a dose-dependent manner, which was significantly related to the IKKε/NF-κB pathway.

Inflammation is an important promoter of apoptosis. Inflammatory factors can increase the expression of IKK-ε. A previous study has found that tumor necrosis factor-alpha (TNF-α), an inflammatory factor, can increase the IKK-ε expression level in a dose-dependent manner[13]. Péant et al[14] found that exogenous TNF-α can increase the IKK-ε expression in prostate cancer cells. We believe that the increased IKK-ε expression in the pancreas of diabetic rats may be related to the high IL-6 expression. At the same time, another previous study also found that IKK-ε can promote inflammation itself, and overexpression of IKK-ε could promote the secretion of IL-6[15]. Therefore, there is a vicious circle between inflammation and IKK-ε expression.

IKKε phosphorylates targets in the NF-κB signaling pathway, thereby activating the NF-κB pathway[16]. A recent study found that human cells transfected with IKK-ε analogs can promote NF-κB expression through phosphorylation, while transfection knocking out IKK-ε analogs can make NF-κB activity disappear[17]. NF-κB is an important nuclear transcription factor, which promotes inflammation regulation and apoptosis[18,19]. A clinical study found that activation of the IKK/NF-κB pathway is also involved in the occurrence of inflammation in patients with ulcerative colitis[20]. Therefore, IKK-ε also plays a pro-inflammatory effect through NF-κB. This study found that

the expression levels of IL-6, IKK-ε, and NF-κB in pancreatic cells in the T2DM group were increased, accompanied by a significant increase in Bax expression, decreased Bcl-2 expression and decreased pancreatic β-cell function. We speculated that the interaction between IKK-ε/NF-κB and inflammation may be involved in the occurrence of pancreatic β-cell apoptosis.

Liraglutide has the effect of inhibiting inflammation[21]. We found that the IL-6 expression was decreased in the liraglutide group. Inflammation can promote the expression of IKK-ε/NF-κB. Therefore, the inhibitory effect of liraglutide on inflammation may be involved in the low expression of IKK-ε/NF-κB in the liraglutide group. This study found that the expression of IKK-ε and NF-κB in the high-dose liraglutide group was more evident than that in the low-dose group, which may be related to the decrease of IL-6 expression in the high-dose liraglutide group. It was found that the levels of TNF-α, IL-1β, and IL-6 in the foot tissue of IKK=ε gene knockout rats with rheumatoid arthritis were significantly lower than those in the wild-type control group[22]. Choi et al[23] also found that inhibiting the expression of NF-κB can reduce the expression levels of IL-6, TNF-α, IL-1β, and other inflammatory factors. However, this study found that the expression of IKK-ε/NF-κB was significantly decreased after liraglutide treatment, accompanied by the decreased IL-6 expression. This suggests that liraglutide may partially inhibit inflammation by reducing IKK-ε/NF-κB. In other words, liraglutide may reduce the expression of IKK-ε/NF-κB by inhibiting inflammation, and the decrease of IKK-ε/NF-κB expression can improve inflammation at the same time.

In the liraglutide group, the Bax expression was decreased, the Bcl-2 expression was increased, the tissue structure and HOMA-β of pancreatic islets were improved, the positive expression rate of insulin was increased, the positive expression rate of glucagon was decreased, and the apoptosis rate of β cells was decreased, especially in the liraglutide high dose group. These results suggest that

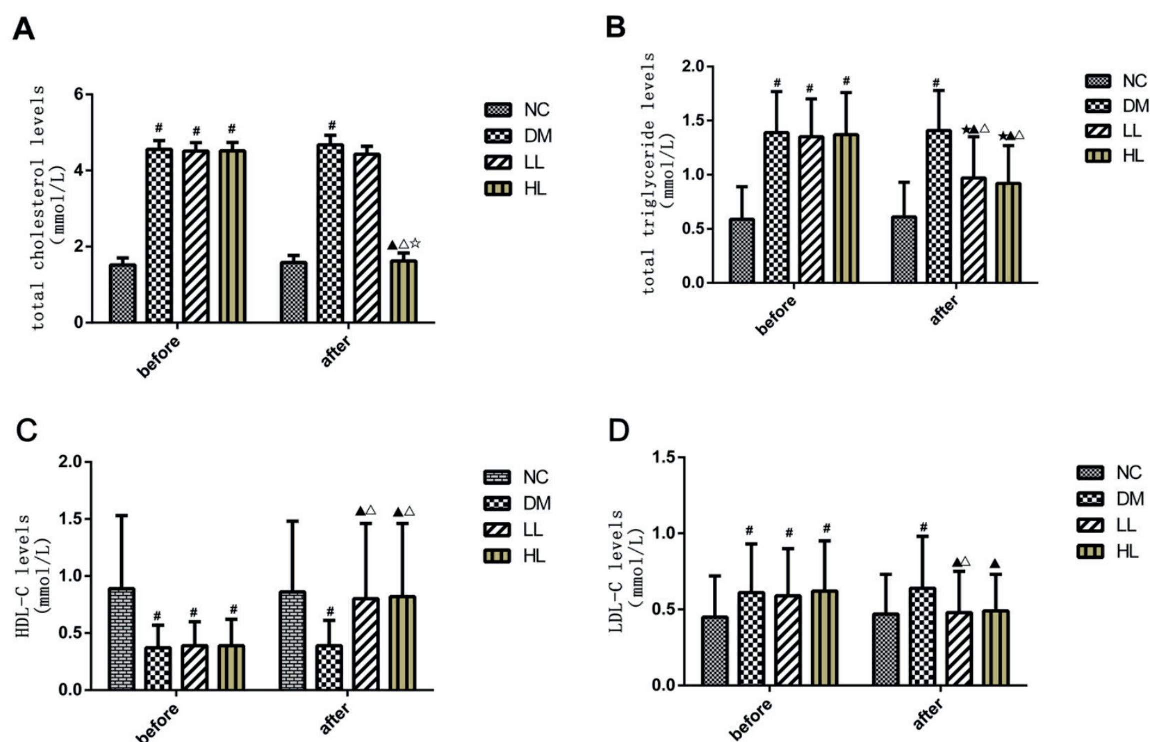

Figure 4. Effects of Liraglutide on serum lipid metabolism in T2DM rats. **(A)** TG level; **(B)** TC level; **(C)** HDL level; **(D)** LDL level. Data are means ± SD of at least three separate experiments (n = 10). Before the intervention of liraglutide, the experimental group was compared with the normal control group: #*p*<0.05. After the intervention of liraglutide, compared with the T2DM group: △*p*<0.05; compared with the LL group: ☆*p*<0.05; compared with the NC group, ★*p*<0.05. Comparison before and after liraglutide intervention: ▲*p*<0.05. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

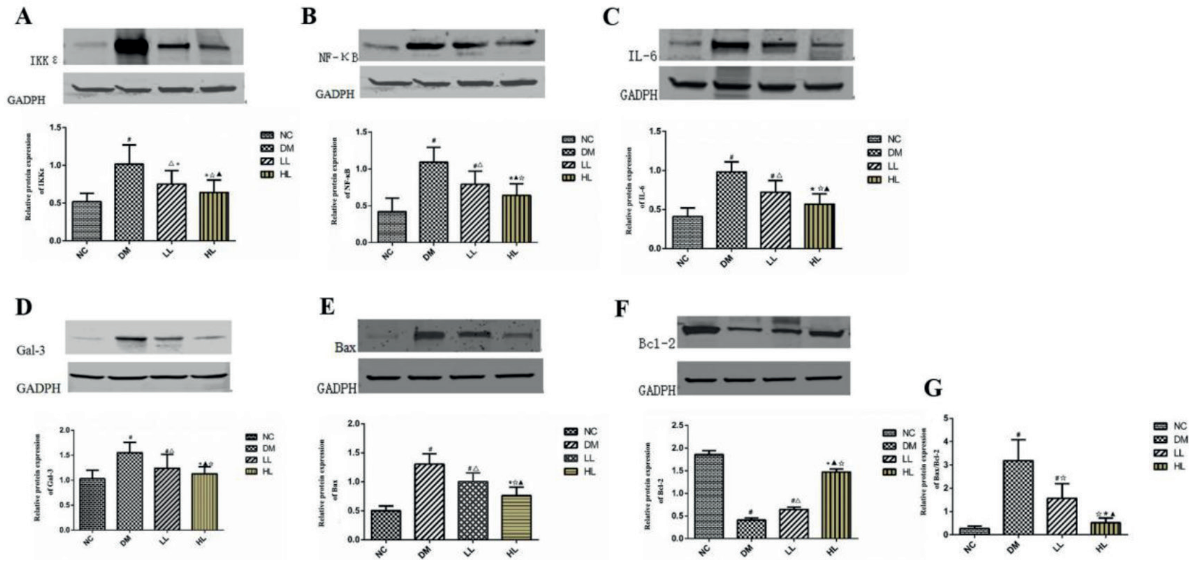

Figure 5. Effects of Liraglutide on the protein expression levels of IKKε, NF-κB, Bcl-2, Bax, IL-6, Gal-3 in T2DM rats. **(A)** IKKε; **(B)** NF-κB; **(C)** IL-6; **(D)** Gal-3; **(E)** Bcl-2; **(F)** Bax; **(G)** Bax/Bcl-2. Data are means ± SD of at least three separate experiments (n = 3). Compared with NC group, $^{*}p<0.05$, $^{\#}p<0.01$; compared with DM group, $^{\triangle}p<0.05$, $^{☆}p<0.01$; compared with LL group, $^{▲}p<0.05$. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

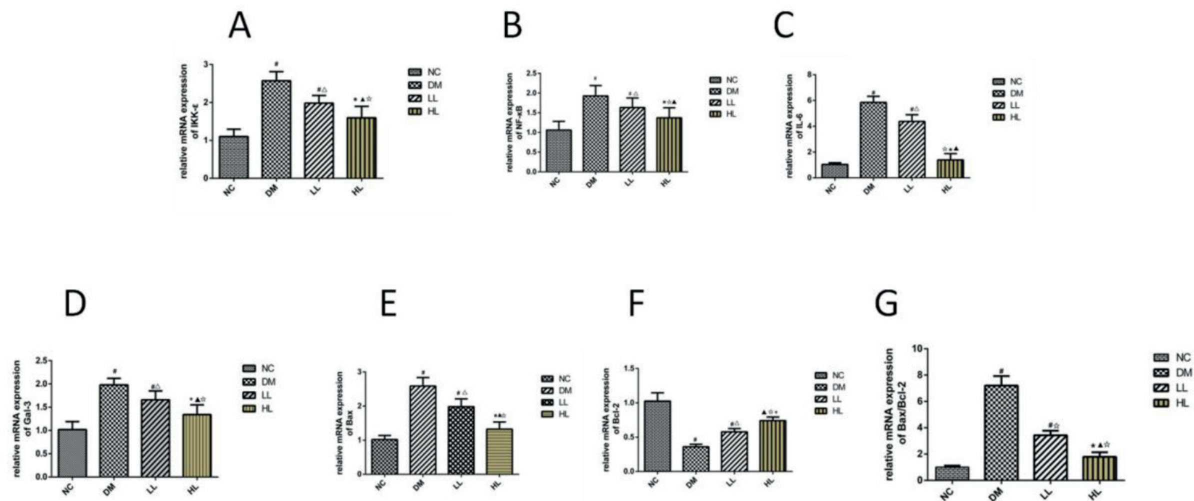

Figure 6. Effects of Liraglutide on the mRNA expression levels of IKKε, NF-κB, Bcl-2, Bax, IL-6, Gal-3 in T2DM rats. **(A)** IKKε; **(B)** NF-κB; **(C)** IL-6; **(D)** Gal-3; **(E)** Bcl-2; **(F)** Bax; **(G)** Bax/Bcl-2. Data are means ± SD of at least three separate experiments (n = 3). Compared with NC group, $^{*}p<0.05$, $^{\#}p<0.01$; compared with DM group, $^{\triangle}p<0.05$, $^{☆}p<0.01$; compared with LL group, $^{▲}p<0.05$. NC: control group; DM: T2DM group; HL: high-dose liraglutide group; LL: low-dose liraglutide group.

liraglutide may improve inflammation by inhibiting IKK-ε/NF-κB, thereby reducing the apoptosis of islet β cells and improving the function of islet β cells in a dose-dependent manner.

Oxidative stress plays an important role in islet β apoptosis and DM. It was found that in the lung tissue of obese asthmatic rats, oxidative stress significantly increased compared with the control rats, which was related to the activation of the NF-κB signaling pathway[24]. The activation of NF-κB can increase the production of ROS, which can aggravate myocardial injury in rats[25]. Wang et

al[26] found that in the mouse model of iron accumulation, iron accumulation can promote the occurrence of oxidative stress by activating NF-κB, leading to the differentiation of osteoclasts. Studies have found that benzo[a]pyrene induces oxidative stress and dysfunction of human endothelial progenitor cells by activating NF-κB[27]. All these suggest that NF-κB is involved in the occurrence of oxidative stress. In this study, the increased NF-κB expression in the pancreas of T2DM rats may be one of the reasons for the decrease of SOD level in diabetic rats. NF-κB may participate in the apoptosis of islet β cells through the effect of oxidative stress. Studies have found that in the rat model of acute lung injury, inhibiting the NF-κB expression can reduce the levels of MDA and ROS in lung tissue to improve oxidative stress[28].

An *in vitro* study found that PDTC, an inhibitor of NF-κB, can reduce the oxidative stress of ovarian granulosa cells induced by lipopolysaccharide[29]. NF-κB is also involved in the oxidative stress injury of neural stem cells induced by hydrogen peroxide, and inhibition of NF-κB can reduce the injury of neural stem cells induced by oxidative stress[30]. These results suggest that inhibition of NF-κB can improve oxidative stress. It has been reported that liraglutide can inhibit oxidative stress[31]. Our investigation found that the NF-κB level in the liraglutide group was significantly decreased, while the SOD level was increased. We think that inhibiting the NF-κB expression is one of the mechanisms of liraglutide improving oxidative stress. The SOD level in the high-dose group was higher than that in the low-dose group, which may be related to the decrease of NF-κB expression in the high-dose group.

Galectin 3 (Gal-3) is a member of the galectin family. Gal-3 is involved in insulin resistance (IR), inflammation, and abnormal glucose and lipid metabolism[32,33]. At the same time, Gal-3 is also involved in the occurrence of apoptosis. It has been found that Gal-3 can aggravate the apoptosis of human umbilical vein endothelial cells induced by ox LDL, and this effect is related to the activation of the integrin β1-RhoA-JNK pathway by Gal-3[34]. 35) Petrovic et al[35] also found that overexpression of Gal-3 in pancreatic islet β cells of T2DM rats can aggravate the apoptosis of β cells induced by palmitic acid, IL-1β, and TNF-α. In this study, increased Gal-3 expression was involved in the apoptosis of islet cells in T2DM rats. It was found that NF-κB could up-regulate the Gal-3 expression in glial cells of Huntington disease rats[36]. Weinmann et al[37] also have found that NF-κB is involved in the increased Gal-3 expression in Osteoarthritis Chondrocytes. These results suggest that NF-κB can regulate the expression of Gal-3 and increase the Gal-3 expression. This study also found that the expression levels of NF-κB and Gal-3 were increased in the T2DM group. This suggests that NF-κB may also participate in the apoptosis of islet β cells through Gal-3.

In vivo and *in vitro* experiments showed that the expression of Caspase-3, Bax, and other apoptotic factors was decreased after administration of Gal-3 inhibitor[38]. Ticagrelor, an inhibitor of NF-κB, decreased the expression of Gal-3[39]. Wang et al[40] have found that administration of NF-κB inhibitor (PDTC) can significantly reduce the Gal-3 level secreted by mononuclear macrophages under hypoxic conditions. These further indicate that NF-κB is closely related to Gal-3, and the inhibition of NF-κB can reduce the Gal-3 expression. In this study, we found that the expression levels of NF-κB and Gal-3 were decreased in the liraglutide group. After liraglutide treatment, the Gal-3 expression was decreased, and the apoptosis of islet β cells was improved compared with the T2DM group. We speculate that liraglutide can reduce Gal-3 level by inhibiting NF-κB, which is one of the mechanisms of liraglutide improving the apoptosis of islet β cells.

Conclusions

In the present investigation, we first explored the effect of liraglutide on T2DM *in vivo*. We found that liraglutide improved pancreatic islet β cell apoptosis in T2DM rats by inhibiting the IKKε/NF-κB pathway. In conclusion, IKK-ε/NF-κB is involved in the inflammation, oxidative stress, and apoptosis of islet β cells. Liraglutide may inhibit inflammation, oxidative stress, islet β cell apoptosis, improve islet β cell function and glucose metabolism by inhibiting IKK-ε/NF-κB. Further study of IKK-ε/NF-κB on the prevention and treatment of DM was needed.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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