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The neuroprotection of liraglutide on diabetic cognitive deficits is associated with improved hippocampal synapses and inhibited neuronal apoptosis

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ABSTRACT

Aims: Diabetes mellitus can cause cognitive impairments, a state between normal aging and dementia. Effective clinical interventions are urgently needed to prevent or treat this complication. Liraglutide as a glucagon–like peptide 1 analog has been shown to exert memory-enhancing and neuroprotective effects on neurodegenerative diseases. This study aims to investigate the neuroprotective effects of liraglutide in streptozotocin (STZ)-induced diabetic mice with cognitive deficits.

Methods: Male C57BL/6J mice were intraperitoneal injected with STZ (65 mg/kg body weight daily for 5 days) to induce type 1 diabetes model. Then the mice were treated with liraglutide (250 mg/kg/day, for 6 weeks) or saline. Weekly changes of body weight and fasting blood glucose were measured. Cognitive performance was evaluated by Morris water maze test. The ultrastructure of hippocampus was observed by transmission electron microscope. The superoxide dismutase activities and malondialdehyde levels in the hippocampus were detected by biochemistry assay. Apoptosis-related proteins and phosphoinositide 3-kinase (PI3K)/protein kinase-B (Akt) signaling were detected by Western blotting.

Key findings: We found that STZ-induced diabetic mice exhibited impaired learning and memory, ultrastructure damage of hippocampal neurons and synapses, exacerbated oxidative stress and neuronal apoptosis, as compared to the control mice. These effects were attenuated by the treatment with liraglutide. Furthermore, liraglutide reversed diabetes-induced alterations in PI3K/Akt signaling pathway that plays an essential role in modulating neuronal survival, apoptosis and plasticity.

Significance: These data suggest that the neuroprotective effects of liraglutide on diabetes-induced cognitive impairments are associated with the improvements of hippocampal synapses and inhibition of neuronal apoptosis.

1. Introduction

Diabetes mellitus (DM) is one of the most common metabolic disorders whose prevalence is increasing year by year. DM causes a variety of complications, among which diabetes-associated cognitive dysfunction has gained widespread focus for its negative effect on the cognitive performance. About 60–70% patients with DM has mild or moderate cognitive impairments [1], manifest in slower in information processing, learning and memory deficits, impaired execution function etc. [2]. The cognitive decline in DM is closely related to the impaired structure and function of brain neurons. The possible mechanisms affecting the diabetic brain include lack of nerve growth factor, oxidative stress, apoptosis of nerve cells, etc. [3–5]. Currently, the pathogenesis of diabetic cognitive dysfunction has not been completely understood, and the effective clinical drug therapy is very limited.

Glucagon-like peptide-1 (GLP-1) is a endogenous incretin hormones secreted by L-cells in the gut epithelium in response to nutrients and modulates the glucose homeostasis by potentiating glucose-dependent

Abbreviations: DM, diabetes mellitus; T1DM, type 1 diabetes mellitus; T2DM, type 1 diabetes mellitus; GLP-1, glucagon-like peptide-1; GLP-1R, glucagon-like peptide-1 receptors; AD, Alzheimer's disease; PD, Parkinson's disease; STZ, streptozotocin; TEM, transmission electron microscope; SOD, superoxide dismutase; MDA, malondialdehyde; FBG, fasting blood glucose; PSD, post-synaptic density; PI3K, phosphoinositide 3-kinase; Akt, protein kinase-B; GSK-3β, glycogen synthase kinase -3β

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insulin secretion. GLP-1 exerts various physiological actions by binding to the GLP-1 receptors (GLP-1R). Apart from in the pancreas, the GLP-1R is widely expressed in the neural circuits which are responsible for cognition [6]. GLP-1 also acts as a growth factor and is able to cross the blood brain barrier [7]. Liraglutide as a GLP-1 analog has been approved for treatment for type 2 diabetes mellitus (T2DM). Several studies demonstrated that liraglutide exerted neurotrophic and neuroprotective effects on some neurological disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD) [8]. In the mouse models of AD, liraglutide was found to attenuate the impaired learning and memory, synaptic plasticity, decrease hippocampal neuronal loss, reduce beta-amyloid oligomer levels and tau hyperphosphorylation, and promote neurogenesis [9–11]. Liraglutide has also shown beneficial effects on cognitive impairments in the rodents' models of type 1 diabetes mellitus (T1DM) and T2DM by activating autophagy and inhibiting apoptosis [12,13].

In this study, we explored the effects and underlying mechanisms of liraglutide on cognitive deficits, neuronal and synaptic loss, and neuronal apoptosis in streptozotocin (STZ)-induced type 1 diabetic mice.

2. Materials and methods

2.1. The establishment of type 1 diabetes (T1DM) model and drug administration

The male C57BL/6J mice weighing 16–20 g were purchased from Medical Experimental Animal Center of Xi'an Jiaotong University Health Science Center. All the mice were housed in a temperature-controlled environment under dark/light cycle with free access to water. The mice were intraperitoneal injected with 65 mg/kg body weight STZ (Sigma-Aldrich, St Louis, USA) for consecutive 5 days to establish T1DM model after fasting for 12 h. Control mice ($n = 10$) were injected with equivalent volume of citrate buffer. In the 7th day after STZ administration, the fasting blood glucose levels were determined with a blood glucometer by snipping the tails. The standard of successful model was that the fasting blood glucose level was above 16.7 mmol/L. Then the model mice were randomized into the groups as follows: (1) model group ($n = 10$) which received subcutaneous injection of normal saline; (2) liraglutide group ($n = 10$) which received subcutaneous injection of liraglutide (Victoza, Novo Nordisk, Beijing, China) at a dose of 250 μg/kg body weight for 6 weeks. Correspondingly, the mice in the control group were subcutaneous injected with normal saline. The weekly change of body weight and fasting blood glucose level were measured. All animal experimental procedures were approved by Institutional Animal Care and Use Committee of Xi'an Jiaotong University and in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals.

2.2. Morris water maze

The spatial learning and memory ability was evaluated by Morris water maze (Taimeng Technology, Chengdu, China). The swimming behavior of mice was detected in a circular pool, 120 cm in diameter and 50 cm in height, in which the water temperature was controlled at 23–25 °C. The circular pool was divided into four quadrants. A hidden platform (10 cm in diameter) was located in 2 cm below the water surface. In the place navigation test, every mouse was forced to finish a swimming test to find the hidden platform within 90 s. If the mice found the platform and remained on it over 3 s, the test was terminated, and the time spent by each mouse was defined as escape latency. If the mice failed to find the platform within 90 s, the mice were guided to the platform and keep them stay on it for 10 s, and the escape latency were recorded as 90 s. The place navigation test consisted of four trials per day for 5 consecutive days. The spatial probe test was conducted on 6th day. The platform was removed and each mouse was allowed to swim freely in the pool within 90 s. The number of times across the platform was recorded [14,15].

2.3. Transmission electron microscope (TEM)

The preparation of samples was described as previously [16]. Briefly, the hippocampus were isolated and cut into $1mm^3$ cubes, fixed with 2.5% glutaraldehyde and 2% osmic acid, then dehydrated and embedded in epoxy resin. Ultrathin sections were collected onto 200-mesh copper grids, double stained with uranyl acetate and lead acetate, and then observed under Hitachi H-7650 TEM (Hitachi, Tokyo, Japan).

2.4. Detection of superoxide dismutase (SOD) activities and malondialdehyde (MDA) levels in the hippocampus

The SOD activities and MDA levels in the hippocampus were detected. The SOD activities were detected in Hydroxylamine method. The MDA levels were detected in thiobarbituric acid method. All procedures were in accordance with manufacturer's instructions (Nanjingjiancheng, China).

2.5. Western blot

Western blot analysis was performed as previously described [17]. The isolated hippocampus were homogenized in RIPA lysate containing protease inhibitor (Roche, Basel, Switzerland) with an electric homogenizer (Kinematica, Swiss), then processed by ultrasonic disruptor (Sonics, Newtown, USA). The lysate were centrifuged at 12000 rpm ∗ 10 min at 4 °C, then transfer supernatant to a fresh tube cautiously. The protein concentration was tested using a bicinchoninic acid (BCA) protein assay kit (Heart, Xi'an, China). 30–50 μg of protein was loaded into 10%–15% SDS/PAGE gels. Following electrophoresis, the separated proteins were transferred to PVDF membranes (Millipore, MA, USA). The PVDF membranes were blocked in 5% non-fat milk (5 g non-fat milk powder was dissolved in 100 mL Tris-buffered saline supplement with 0.01% tween-20 (TBS-T)) for 2 h. The membranes were incubated with primary antibodies against Bcl-2 (Sigma-Aldrich, St Louis, USA), Bax (Cell Signaling Technology, Boston, USA), cleaved caspase 3 (Cell Signaling Technology, Boston, USA), PI3K (Abcam, Cambridge, UK), Akt2 (Epitomics, USA), GSK-3β (Cell Signaling Technology, Boston, USA) and β-actin (Sigma-Aldrich, St Louis, USA) at 4 °C overnight and washed thrice with TBS-T for 10 min each. After incubation with secondary antibodies for 2 h, the membranes were washed with TBS-T for 3 times. The bands were detected in the chemiluminescence imaging system (UVP, Upland, USA). The band intensity was quantified by using ImageJ.

2.6. Data analysis

The data were processed by IBM SPSS 20.0 software. Data values were expressed as mean ± SEM. Differences between the groups were analyzed by one way ANOVA followed by least significant difference (LSD) test. The escape latency in the Morris water maze was analyzed by using a multivariate analysis of variance (MANOVA) of repeated measures for comparisons among trials. Statistical significance was defined as a two-sided P value < 0.05.

3. Results

3.1. The effects of liraglutide on the body weight and fasting blood glucose (FBG) of STZ-induced diabetic mice

The experimental design is shown in Fig. 1. Significantly elevated FBG levels (over 16.7 mmol/L) and typical syndromes (polydipsia, polyuria, and more food intake) in the STZ-injected mice confirm our successful establishment of T1DM mice model. To evaluate whether

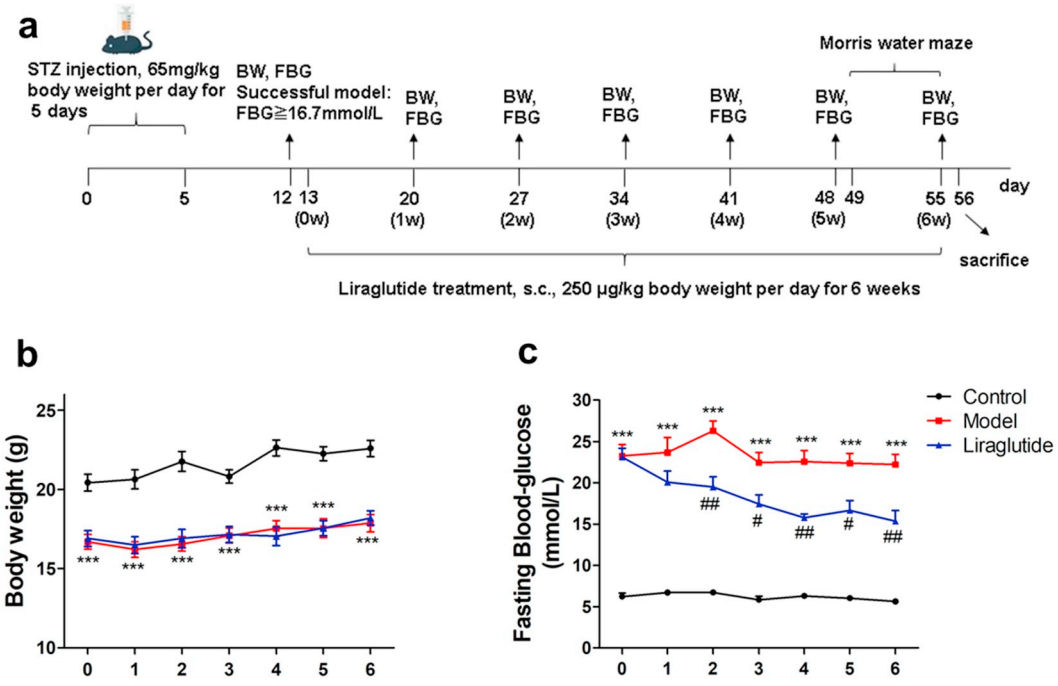

Fig. 1. The summary of experimental design and effects of liraglutide on the metabolic parameters of STZ-induced diabetic mice. (a) Experimental design: Five days of STZ injections were performed to induce T1DM model. Then diabetic mice were received 6 weeks of liraglutide treatment at a dose of 250 μg/kg body weight. Body weight (b) and FBG levels (c) were measured weekly during 6 weeks of liraglutide treatment. At the 6th week following treatment, Morris water maze test was used to evaluate the cognitive performance of C57BL/6J mice. Then the mice were sacrificed to isolate hippocampus for further experiments. Data were expressed as mean ± SEM. $^{***}P < 0.001$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus model group. $n = 10$ per group.

liraglutide had effects on the metabolic parameters of STZ-induced diabetic mice, we measured the body weight and FBG levels weekly during 6 weeks of drug administration. As shown in Fig. 1, the STZ administration resulted in lower body weight and elevated FBG levels (seen from the data in 0 week). During 6 weeks of treatments, the control mice showed gradually increased body weight and maintained steady FBG levels. Compared with the control group, the model group witnessed a smaller change in body weight and kept significantly higher FBG levels (Fig. 1c, $P < 0.001$). Compared with the model group, liraglutide had no effect on body weight. However, the mice which received liraglutide treatment exhibited significantly decreased FBG levels (Fig. 1c, $P < 0.01$).

3.2. Liraglutide ameliorated the impaired spatial learning and memory ability in STZ-induced diabetic mice

We next examined the effects of liraglutide on the spatial learning and memory by using Morris water maze test. As the results revealed, the escape latency of the mice in the model group was markedly increased compared with that of the control group (Fig. 2a, $P < 0.001$), which meant that the model group spent more time to find the hidden platform in the place navigation test. In the spatial probe test, the model group showed significantly decreased number of times across the platform (Fig. 2b, $P < 0.01$). Whereas, the mice treated with liraglutide found the hidden platform more quickly (Fig. 2a, $P < 0.001$) and made more crossing of the platform (Fig. 2b, $P < 0.05$). The results suggested that the STZ-induced diabetic mice showed a cognitive deficit, while Liraglutide ameliorated the impaired spatial learning and memory ability in STZ-induced diabetic mice.

3.3. Liraglutide relieved hippocampus and synapses injuries in STZ-induced diabetic mice

As many researchers reported hippocampus and synapse loss in

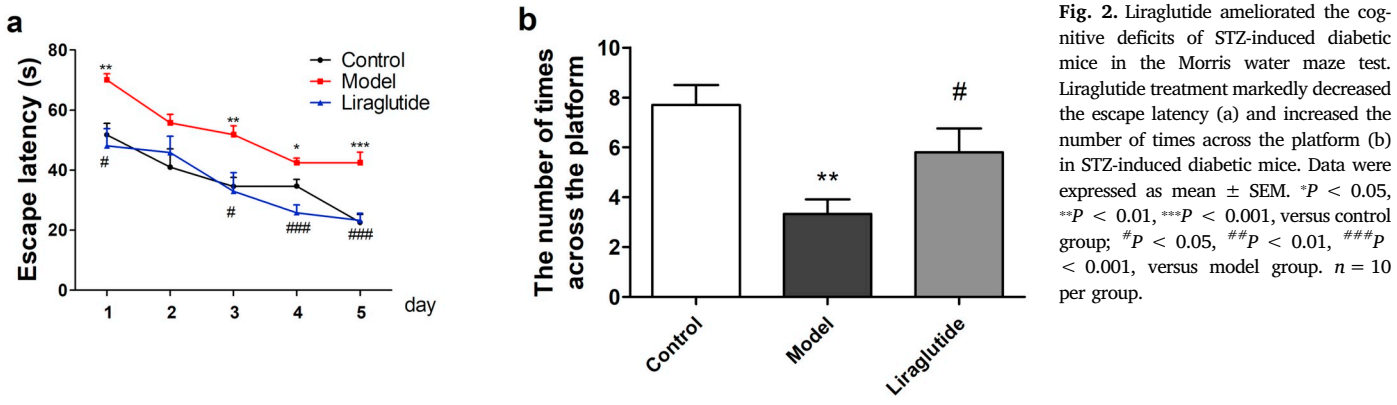

Fig. 2. Liraglutide ameliorated the cognitive deficits of STZ-induced diabetic mice in the Morris water maze test. Liraglutide treatment markedly decreased the escape latency (a) and increased the number of times across the platform (b) in STZ-induced diabetic mice. Data were expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus model group. $n = 10$ per group.

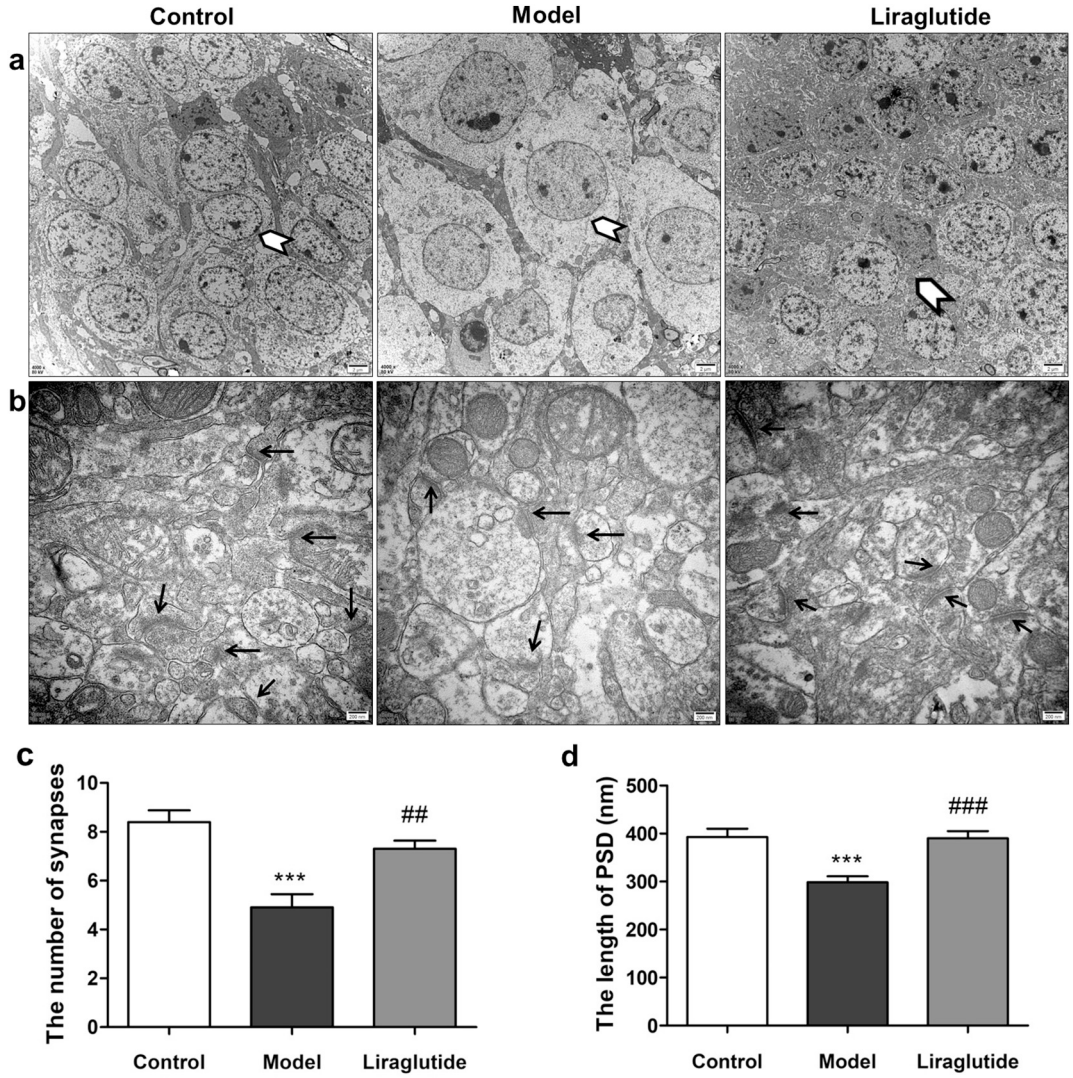

Fig. 3. Liraglutide relieved hippocampus and synapses injuries in STZ-induced diabetic mice. The ultrastructure of hippocampal neurons (4000×) and synapses (40, 000×) were observed under TEM. (a) Representative images of hippocampal neurons in control, model, and liraglutide group. The white arrowheads represented hippocampal neurons. Scale bar, 2 μm. (b) Representative images of synapses in control, model, and liraglutide group. The black arrows represented synapses. Scale bar, 200 nm. (c) The number of synapses in each group. (d) The length of PSD in each group. The hippocampus of three mice in each group was collected. The number of synapses and the length of PSD were analyzed in 10 images per group. Data were expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, versus model group.

many neurodegeneration [18,19], we observed the ultrastructure of hippocampal neurons and synapses by using TEM. The length of post-synaptic density (PSD) was measured by Image-Pro Plus 6.0 software. As shown in Fig. 3, the hippocampus of control mice contained abundant neurons and synapses. Compared with the control group, the hippocampal neurons and synapses in the model group exhibited marked changes, as indicated by the reduction in the density of hippocampal neurons (Fig. 3a) and synapses (Fig. 3b and c, $P < 0.001$), and unclear front and back membranes of the synapses. Additionally, the length of PSD was significantly decreased in the model group (Fig. 3d, $P < 0.001$). Liraglutide treatment evidently increased the density of hippocampal neurons and synapses (Fig. 3c, $P < 0.01$), and increased the length of PSD (Fig. 3d, $P < 0.001$). These results suggested that liraglutide restored the injuries of hippocampal neurons and synapses in the STZ-induced diabetic mice.

3.4. Liraglutide restored oxidative stress in STZ-induced diabetic mice

As the imbalance of oxidation-antioxidation system is implicated in the process of diabetes-associated cognitive dysfunction [20], we investigated whether liraglutide restore the oxidative stress in the STZ-induced diabetic mice. Compared with the control group, the hippocampus of STZ-induced diabetic mice showed significantly increased oxidative stress, as revealed by decreased SOD activities (Fig. 4a, $P < 0.01$) and increased MDA levels (Fig. 4b, $P < 0.05$). Contrarily, the treatments with liraglutide significantly increased SOD activities (Fig. 4a, $P < 0.01$) and decreased MDA levels (Fig. 4b, $P < 0.05$), as compared to the model group. The results suggested that liraglutide alleviated oxidative stress induced by STZ.

3.5. Liraglutide decreased apoptosis by regulating apoptosis-related proteins in STZ-induced diabetic mice

As illustrated in Fig. 5, the model group showed significantly decreased Bcl-2 expression (Fig. 5b, $P < 0.05$) and the ratio of Bcl-2/Bax (Fig. 5d), and increased expression of Bax (Fig. 5c, $P < 0.01$) and cleaved caspase 3 (Fig. 5e, $P < 0.001$), as compared to the control group. Compared with the model group, the liraglutide reversed the changes of apoptosis-related proteins in STZ-induced diabetic mice. Specifically, liraglutide treatment markedly upregulated Bcl-2

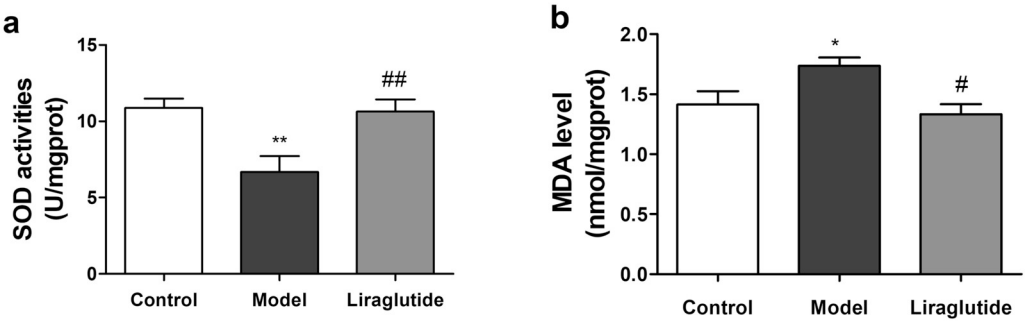

Fig. 4. Liraglutide restored oxidative stress in STZ-induced diabetic mice. Liraglutide significantly enhanced SOD activities (a) and decreased MDA levels (b) in the hippocampus of STZ-induced diabetic mice. Data were expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus model group. $n = 5$.

expression (Fig. 5b, $P < 0.01$) and the ratio of Bcl-2/Bax (Fig. 5d, $P < 0.05$), and decreased the expression of Bax (Fig. 5c, $P < 0.05$) and cleaved caspase 3 (Fig. 5e, $P < 0.01$). The results suggested that STZ-induced diabetic mice exhibited significantly increased apoptosis in hippocampus, and liraglutide decreased apoptosis by regulating apoptosis-related proteins in STZ-induced diabetic mice.

3.6. The effects of Liraglutide were involved in phosphoinositide 3-kinase (PI3K)/protein kinase-B (Akt) signaling pathway

The PI3K/Akt signaling pathway plays significant role in the regulation of proliferation, cell growth and survival, and hippocampal synaptic plasticity [21]. The downstream substrates include Bad (Bcl-2-

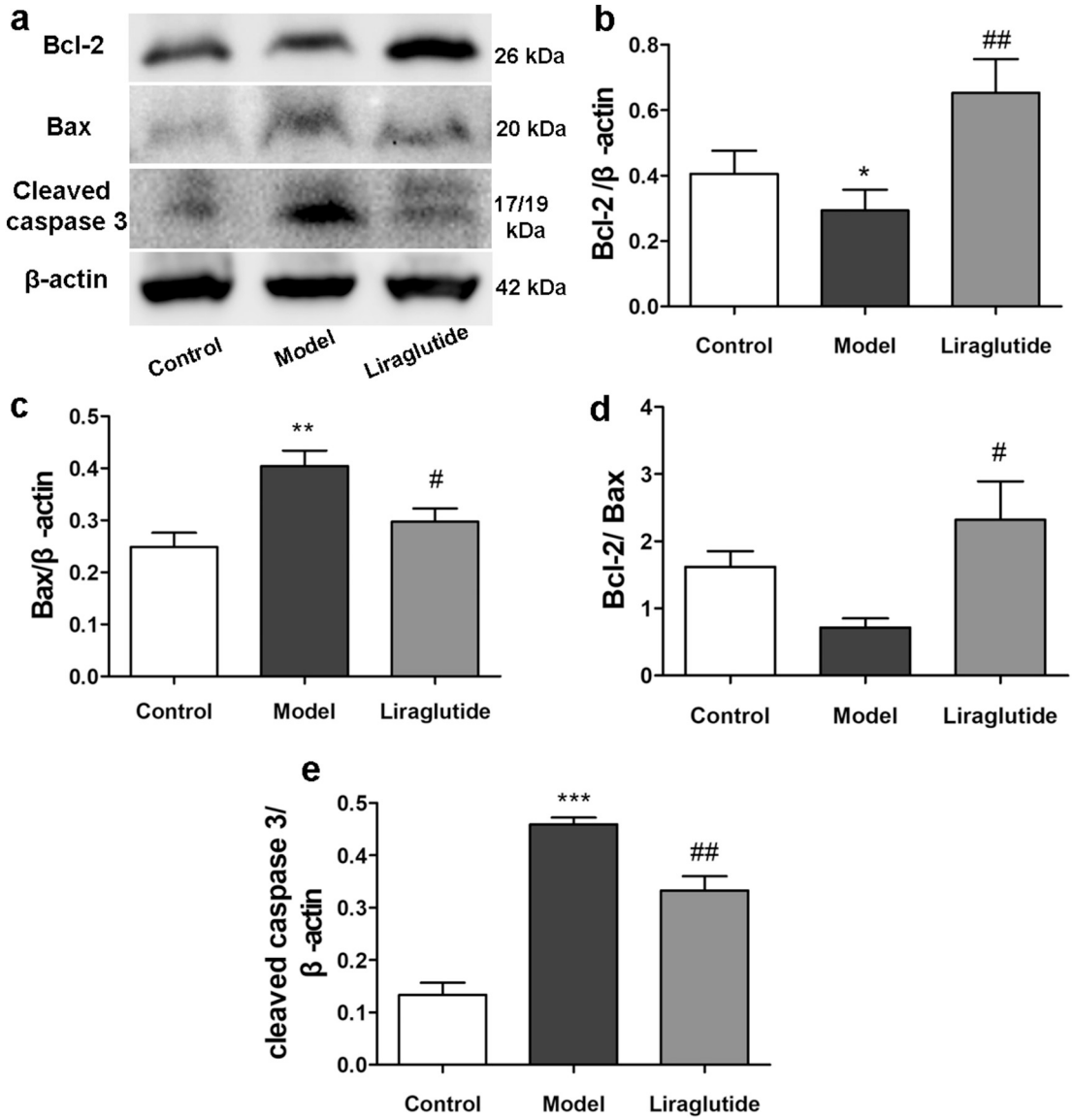

Fig. 5. Liraglutide decreased apoptosis by regulating apoptosis-related proteins in STZ-induced diabetic mice. (a) Representative protein bands and quantification of (b)Bcl-2, (c) Bax, (d) Bcl-2/Bax and (e) cleaved caspase 3 in the hippocampus of STZ-induced diabetic mice. Data were expressed as mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus model group. $n = 4$.

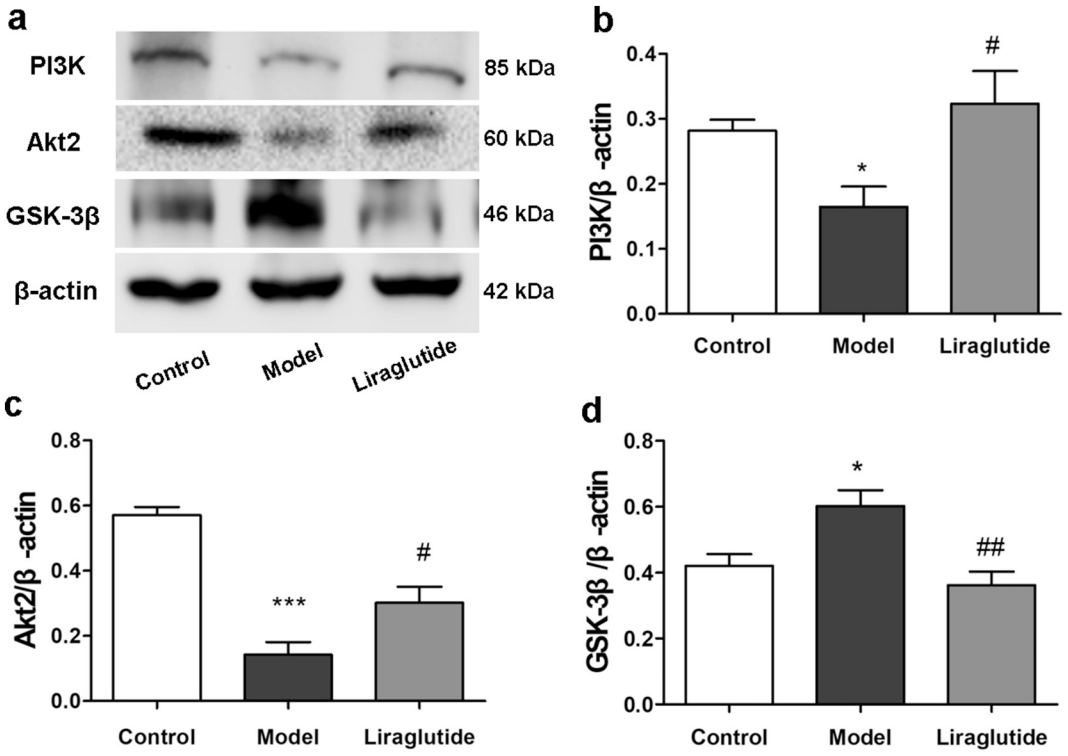

Fig. 6. Liraglutide reversed the alterations in the PI3K/Akt signaling pathway induced by STZ. (a) Representative protein bands and quantifications of (b) PI3K, (c) Akt2 and (d) GSK-3β. Data were expressed as mean ± SEM $^{*}P < 0.05$, $^{**}P < 0.01$, versus control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, versus model group. $n = 4$.

assocaited death promoter) and glycogen synthase kinase-3β (GSK-3β) [22]. Activation of GSK-3β has been implicated in destabilization of microtubules, therefore promoting impaired synaptic plasticity [23]. Targeting PI3K/Akt pathway may act as a therapeutic approach to prevent the progression of neurodegeneration. To gain further insight into the mechanisms by which liraglutide exerted neuroprotective effects in STZ-induced diabetic mice, we determined the expression of PI3K, Akt2, and downstream GSK-3β. As exhibited in Fig. 6, the model group showed significantly decreased expression of PI3K (Fig. 6b, $P < 0.05$) and Akt2 (Fig. 6c, $P < 0.001$), and enhanced expression of GSK-3β (Fig. 6d, $P < 0.05$), as compared to the control group. Compared with the model group, the liraglutide treatment notably upregulated the expression of PI3K (Fig. 6b, $P < 0.05$) and Akt2 (Fig. 6c, $P < 0.05$), and suppressed the expression of GSK-3β (Fig. 6d, $P < 0.01$). These data indicated that the neuroprotective effects of liraglutide might be attributed to regulating PI3K/Akt signaling pathway.

4. Discussion

T1DM has been shown an important risk factor for cognitive decline. However, the effective clinical drug therapy is very limited. The GLP-1 analog liraglutide has been marketed for the treatments of T2DM. In addition to controlling the blood sugar, liraglutide has also been shown to exert neurotrophic and neuroprotective effects on some neurodegeneration, including AD, PD, depression, epilepsy, ischemia, and traumatic brain injury [24–28]. In our present study, we investigate the effects of liraglutide on learning and memory and explored its underlying mechanism in a STZ-induced mouse model of T1DM. The finding was that treatments with liraglutide alleviated memory impairments, reduced hippocampal and synaptic injuries, protected against oxidative stress, and inhibited neuronal apoptosis. Additionally, these actions may involve in PI3K/Akt signaling pathway.

Liraglutide is mainly used to lower the blood sugar level of T2DM patients and is not recommended for the treatment of T1DM due to its inability to increase insulin secretion from pancreatic β cells in T1DM patients. In the present study, we found that liraglutide had little effect on the body weight, but significantly decreased FBG levels in the STZ-induced diabetic mice. The hypoglycemic effect of liraglutide in the STZ-induced T1DM models has also been confirmed in the previous studies [29,30]. However, some studies showed that GLP-1 and its analogues had no significant improvement in the glucose levels or body weight in the STZ-induced animal models [11,31]. This may be due to the mechanism of action of STZ. Intracellularly, STZ results in DNA fragmentation and eventually cell death of pancreatic β-cells [32]. Different results might result from species difference and differentiated severity of β cells injuries.

Chronic hyperglycemia is considered major cause for diabetic complications, triggering progressive structural and functional abnormalities of brain [33]. It has been proved that cognition dysfunction improves with improved glycemic control in older subjects with non-insulin-dependent diabetes mellitus [34]. These provide the evidence that improved glycemic control is able to slow down the progression of cognitive impairments. Numerous studies have indicated that the neuroprotective effect of GLP-1 agonist is an independent property of the drug independent of glycemic control [35,36]. Whereas, we found that liraglutide significantly decreased the FBG level of STZ-induced diabetic mice in our study. Therefore, lowering blood glucose may influence the spatial learning and memory to some extent. But the average FBG level in the liraglutide-treated group (over 15 mmol/L) was still much higher than the normal standard, which meant that the improvement in FBG level caused by liraglutide was subtle. Based on these data, we tend to believe that the positive role played by liraglutide in this manuscript were mainly associated with the neuroprotective property of the drug, while its hypoglycemic effect played limited role. It would be interesting to learn whether the cognition would be improved to a greater degree if the blood glucose levels were decreased more effectively. This awaits further study.

Mounting studies have reported that liraglutide has positive effects on nerve cells [37–39]. GLP-1 receptors are widely expressed in the

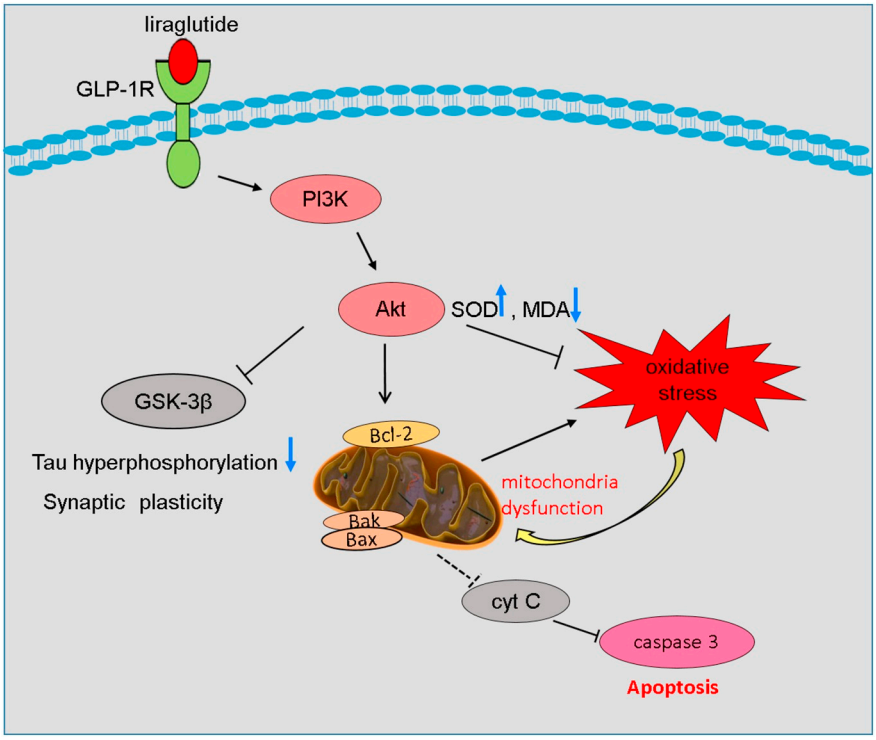

Fig. 7. The underlying mechanisms of liraglutide-mediated neuroprotective effects on diabetes-induced cognitive impairments. Liraglutide binds to GLP-1R, which leads to the activation of PI3K/Akt signaling pathway. The downregulation of GSK-3β facilitates the improved hippocampal synaptic plasticity. The PI3K/Akt signaling pathway also interrupts the mitochondrial pathway of apoptosis. Additionally, the activated Akt enhances the expression of antioxidant enzyme SOD, and decreases the oxidation product MDA, resulting in the relieved oxidative stress. Mitochondrial dysfunction can lead to oxidative stress which in turn aggravates the function of mitochondrial. Cyt C represents for cytochrome C.

central nervous system, and when stimulated, they could enhance cell survival and promote neuroprotection through several pathways [40,41]. Our study revealed that STZ-induced diabetic mice showed significantly impaired learning and memory, while Liraglutide treatment markedly decreased the escape latency and increased the number of times across the platform, as determined by Morris water maze. The results indicated that liraglutide showed a potential in ameliorating cognitive deficits in a STZ-induced TIDM mouse model.

Hippocampus is a key brain region which plays significant role in the memory formation and consolidation [42]. Additionally, it is one of the most sensitive areas of the brain to the metabolic disorders including diabetes mellitus [43]. Numerous studies have indicated that experimental diabetes has negative effects and induce apoptosis in hippocampal neurons via multiple known mechanisms [44,45]. The hippocampal neuronal injuries partially reflect the effects of DM on the impaired learning and memory. Neuronal synaptic plasticity can be termed as structural and functional adaptations of neuronal circuits to alterations due to learning and memory, environmental factors and brain damage [46]. Synaptic plasticity is believed to be cellular mechanisms of learning and memory. In the present study, the STZ-induced diabetic mice exhibited significant damage of hippocampal neurons and synapses, as revealed by reduced density of hippocampal neurons and synapses, and decreased length of PSD. Compared with the model group, liraglutide obviously increased the neuronal and synaptic density and extended the length of PSD.

Oxidative stress occurs in the course of AD, PD and other neurodegenerative disorders [47–49], which support its role in the pathogenesis of neurodegenerative disease. In diabetic condition, hyperglycemia, free fatty acids and some cytokines induce excess generation of reactive oxygen species (ROS) [50]. Elevated levels of ROS damage the lipids, proteins and DNA, and it is associated with cancer, cardiovascular diseases, and neurodegenerative diseases [51]. In a cross-sectional study, significantly higher serum MDA levels and lower serum levels of antioxidants, such as vitamin A, E, and C, were observed in T2DM patients, as compared to healthy subjects [52]. Oxidative stress is implicated in mediating cellular activities including inflammation and cell survival [53]. Antioxidant therapies have also been shown to be beneficial in the neurodegeneration [54–56]. In our study, STZ administration induced amplified oxidative stress, as indicated by deceased SOD activities and enhanced MDA levels. However, liraglutide treatment relieved the oxidative stress injuries in the STZ-induced diabetic mice.

It is well known that apoptosis is correlated with the pathogenesis of many neurodegenerative diseases. Apoptosis is mainly modulated by a family of cysteine proteases called caspases [57]. The mitochondrial pathway is the most common mechanism of apoptosis, in which growth/survival factors or other stimuli affect the Bcl-2 family on the outer mitochondrial membrane. Bcl-2 family proteins include anti-apoptotic Bcl-2 and Bcl-xL, and pro-apoptotic Bax and Bak. They control the mitochondrial outer membrane permeabilization by directly localizing to this organelle. Upon apoptosis induction, they translocate from cytosol to the mitochondrial and damage the integrity of mitochondrial outer membrane, controlling the release of cytochrome C into the cytosol [58]. Then cytochrome C complexes with caspase-9 and the adaptor Apaf-1 to form the apoptosome, resulting in the activation of the downstream executioner caspase-3 and caspase-7 [59,60]. In our study, we measured the protein expression of Bcl-2, Bax and cleaved caspase 3. The results revealed that STZ-induced diabetic mice exhibited downregulation of Bcl-2 and the ratio of Bcl-2/Bax, and upregulation of Bax and cleaved caspase 3, while liraglutide was able to augment the expression of Bcl-2 and the ratio of Bcl-2/Bax, and suppressed the expression of Bax and cleaved caspase 3. These data suggested that liraglutide inhibited caspase-dependent apoptosis in the STZ-induced diabetic mice.

The PI3K/Akt signaling pathway is a well-defined pathway which regulates many cellular activities, such as neuronal survival, apoptosis and plasticity [61,62]. PI3K/Akt signaling has also been shown involved in the expression of antioxidant enzyme SOD [63]. The downstream molecule GSK-3β has been proved to be associated with tau hyperphosphorylation and Aβ accumulation [64,65], both of which are the main pathological events in AD. Our study reported reduced expression of PI3K and Akt2, and enhanced expression of GSK-3β in the hippocampus of STZ-induced diabetic mice. However, the alterations in PI3K/Akt signaling were reversed by liraglutide treatment.

5. Conclusions

These above observations suggested that liraglutide ameliorated cognitive impairments by improving hippocampal synaptic plasticity, relieving oxidative stress, and inhibiting neuronal apoptosis in a STZ-induced T1DM mouse model. The neuroprotective effect of liraglutide may be related to the regulation of PI3K/Akt signaling pathway (Fig. 7). The data from the current study provide a further insight into a better understanding of liraglutide-mediated neuroprotective effects on diabetes-induced cognitive impairments.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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