Liraglutide improves cognitive function by reducing amyloid-beta peptide accumulation and inhibiting inflammation in 5×FAD mice

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Abstract

The glucagon-like peptide-1 analog, liraglutide, has been shown to be effective in Alzheimer's disease (AD); however, the underlying mechanism remains unknown. In this study, we evaluated the effects of liraglutide (25 nmol/day for 8 weeks) on the cognitive ability of 12-month-old 5× familial Alzheimer's disease (FAD) mice. The spatial cognitive ability was improved in 5×FAD mice after administration of liraglutide, associated with an increased number of pyramidal cells in the cortex and hippocampus. Liraglutide also alleviated the ultrastructural changes in chemical synapses and pyramidal cells and reduced local and universal inflammation in AD mice. Furthermore, liraglutide reduced the expression of amyloid β protein through the regulation of nuclear factor kappa B/beta-secretase 1 pathways in AD mice. The potential of liraglutide to improve the cognitive function in AD animals offers an effective pharmacological approach for treating neurodegenerative diseases.

1. Introduction

Alzheimer's disease (AD) is a degenerative disease of the central nervous system characterized by progressive memory decline and chronic cognitive impairment. Neuronal loss, synapse damage, β -amyloid deposition in the brain, and neurofibrillary tangle formation are the main pathological features of AD. The occurrence and development of AD seriously affects the quality of life of the elderly. However, to date, the etiology and pathogenesis of AD have not been fully elucidated, and no drugs that can effectively prevent and slow down the progression of AD exist. An epidemiological study showed that long-term hyperglycemia in type 2 diabetes increased the risk of AD [1]. Concomitantly, studies have shown that the glucagon-like peptide-1 (GLP-1) analog, a new drug used for the treatment of type 2 diabetes, could improve the cognitive function of patients with AD [2]; however, the specific mechanism of action remains unclear.

Liraglutide is a long-acting GLP-1 derivative. Following its binding to receptors in the central nervous system, GLP-1 has been reported to enhance neuron viability, improve neurogenesis, as well as decrease apoptosis and inflammatory factors [3, 4]. A study found that long-term potentiation severely damaged the hippocampal CA1 area of mice and caused an impairment in object recognition and obstacle avoidance in a GLP-1 receptor gene knockout (*Glpr1r/-*) mouse model [5]. However, when the *Glpr1r* gene was reintroduced into the hippocampus of *Glpr1r/-* mice, their impaired memory and cognitive functions improved. Moreover, mice overexpressing the *Glpr1r* gene in the hippocampus were reported to have higher learning and memory abilities [6]. Therefore, GLP-1 might play an important role in improving cognitive function. Recent studies have shown that the neuroprotective effects of GLP-1 might be closely related to amyloid β ($A\beta$) [7, 8]. After liraglutide intervention, the cognitive function of 7-month-old APP/PS1 mice significantly improved, the formation of cortical amyloid plaques and core dense plaques reduced by 40–50%, and the level of soluble protein was reduced by 25% [2]. Similar results were obtained in 14-month-old APP/PS1 mice [9]. Our previous study also suggested that liraglutide could improve the cognitive function in $A\beta$ -induced AD mice [10].

In this study, we used 12-month-old 5× familial Alzheimer's disease (FAD) mice to explore the effect of liraglutide on the levels of A β in the brain and in the learning and memory abilities of mice. Furthermore, we investigated the ultrastructural changes in synapses and mitochondria and evaluated the occurrence of inflammation. In addition, we detected the expression of nuclear factor kappa beta (NF- κ B) and beta-secretase 1 (BACE1). NF- κ B is considered an important regulator of the transcription of BACE1 [11] and BACE1 is an A β -stimulated rate-limiting enzyme.

2. Materials And Methods

2.1 Animals and drug administration

Male 5×FAD mice were purchased from the Jackson Laboratory (stock no. 034848-JAX, Bar Harbor, ME, USA). All mice were housed in groups of five in individually ventilated cages (Tecniplast, Buguggiate, Italy) in a pathogen-free environment and were provided with food and water ad libitum. Mice were maintained under standard laboratory conditions at a temperature of $23 \pm 1^\circ\text{C}$ and 50–60% humidity. When mice were 12 months old, they were randomly divided into the AD and AD + liraglutide groups; 12-month-old wild-type littermates were assigned as a control and referred to as the wild-type (WT) group. Each group consisted of ten mice.

Liraglutide (Novo Nordisk, Copenhagen, Denmark) was dissolved to a concentration of 2.5 nmol/mL (10×) with 0.9% (w/v) saline, as a stock solution, and diluted to 25 nmol/mL before use. Mice were injected subcutaneously with either liraglutide (25 nmol/kg) or an equivalent volume of 0.9% physiological saline once daily for 8 weeks [10]. All experiments were approved by the Institutional Animal Care and Use Committee of Fujian Medical University (no. FJMU IACUC 2018-034) and complied with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

2.2 Morris water maze

Morris water maze (MWM) tests were performed as previously described [12]. Briefly, a black circular pool (diameter, 120 cm) was filled with water ($24 \pm 1^\circ\text{C}$). A transparent escape platform (diameter, 10 cm) was submerged 1 cm beneath the water at a fixed position. In the acquisition trial phase (day 1 to day 5), each mouse was gently placed in the pool and allowed 60 s to locate the submerged platform. If the mice failed to find the platform in the 60 s period, it was gently guided to the platform and allowed to remain there for 15 s. Each animal underwent four trials per day for 5 consecutive days. The time taken to locate the platform was defined as the escape latency ($n = 10$ per group). On day 6, the platform was removed and mice were allowed to swim freely for 60 s to assess their spatial memory retention ($n = 10$ per group). After each swimming test, the animals were dried with a towel. The escape latency, swimming speed, swimming tracks, time spent in the target quadrant, and the number of times that mice crossed over the target quadrant were recorded using the behavioral software SAMRT 2.0 (Pan Lab, Barcelona, Spain).

After the MWM tests, the animals were anesthetized with 50% (v/v) pentobarbital sodium (50 mg/kg, intraperitoneally). For Nissl staining, transmission electron microscopy (TEM), and immunohistochemistry, mice were perfused with 0.9% (w/v) physiological saline followed by 4% paraformaldehyde via the cardiac apex, and their cortices and hippocampi were dissected and fixed by immersion in 4% (w/v) paraformaldehyde or 3% (v/v) buffered glutaraldehyde (for TEM) at 4°C. For enzyme-linked immunosorbent assay (ELISA) and western blotting, the cortices and hippocampi were extracted on ice and stored at -80°C until use.

2.3 Nissl staining

Specimens (n = 3/group) were embedded in paraffin and cut into 4-μm-thick sections using a microtome (RM2016, Leica, Germany). Samples were subjected to xylene, graded ethanol, and distilled water for dewaxing and dehydration. Sections were then processed with a Nissl staining solution (G1432, Solarbio, Beijing, China) for 20 min, Nissl differentiation for 4 s, graded ethanol for rehydration, and mounted using neutral balsam.

2.4 Transmission electron microscopy

The CA1 subregion of the right hippocampus (n = 3/group) was removed and fixed using 3% buffered glutaraldehyde and 1% osmium tetroxide. Specimens were dehydrated in graded ethanol, embedded in Epon 812, and examined under a TEM (Philips, Amsterdam, Netherlands). We obtained five photos of each section and used the Image-Pro Plus software (version 6.0) to perform morphometric analysis of at least 5-100 synapses and 100–200 mitochondria per mouse, including evaluation of the thickness of postsynaptic density (PSD), synaptic curvature, synaptic cleft width, mitochondrial volume density, mitochondrial numeric density, and mitochondrial specific surface area. The methods used were previously described [13].

2.5 Immunohistochemistry

Mice brains were embedded (n = 3/group) in paraffin and cut into 4-μm-thick sections using a microtome (RM2016, Leica) after fixation. A series of xylene, graded ethanol, and distilled water were used to dewax and dehydrate the sections, respectively. Specimens were then treated with 3% hydrogen peroxide and blocked with 5% bovine serum albumin. Sections were then incubated with amyloid precursor protein (APP; 1:500; ab32136, Abcam), Aβ (1:500; ab126649, Abcam), NF-κB (1:400; #8242, Cell Signaling Technology), BACE1 (1:50; ab183612, Abcam) antibodies for 24 h at 4°C. After being washed with phosphate buffered saline (PBS), sections were incubated with haptoglobin-related protein-labeled anti-mouse (1:500; ab5887, Abcam) or anti-rabbit IgG rabbit (1:500; ab6112, Abcam) antibodies for 30 min at 37°C. Specimens were then washed three times with PBS, stained with diaminobenzidine, incubated in hematoxylin for 10 min, mounted with neutral balsam, and observed under a microscope.

2.6 ELISA

Hippocampal tissues were rinsed using 4°C PBS (0.01 mol/L, PH 7.0-7.2) to remove excess blood. Ice-cold PBS was added to the tissue (9 mL PBS to 1 mg of tissue) for homogenization and then samples

were centrifuged at 5000 *g* for 5 min at 4°C and the supernatants were collected. Mouse blood was collected using serum separator tubes (BD™ P100 Blood Collection System) and then centrifuged at 1000 *g* for 20 min to isolate the serum. The levels of IL-1 β , IL-6, TNF- α , and Cox-2 in the hippocampus and blood were assayed using the mouse IL-1 β (ml063132-C), mouse IL-6 (ml002293-C), mouse TNF- α (ml002095-C), and mouse Cox-2 (ml037732-C) ELISA kits, respectively, according to the manufacturer's instructions. All the ELISA kits were purchased from the Shanghai Enzyme linked Biotechnology Co., Ltd (Shanghai, China).

2.7 Western blotting

Cortex (*n* = 6/group) and hippocampal (*n* = 6/group) proteins were extracted using cold RIPA lysis buffer supplemented with protease and phosphatase inhibitors (Beyotime Biotechnology, Jiangsu, China). Proteins (20 μ g) were separated on 10% (w/v) sodium dodecyl sulfate polyacrylamide gels and transferred onto polyvinylidene difluoride membranes using a Trans-Blot Turbo system (Bio-Rad, Singapore, Singapore). Nonspecific binding sites were blocked with 5% (w/v) nonfat dry milk solution in 0.1% TBS/Tween-20 for 2 h at 25°C. Then, membranes were incubated overnight at 4°C with primary antibodies: rabbit anti- β -actin (1:1000; #4970, Cell Signaling Technology), rabbit anti-PSD95 (1:1000; #2507, Cell Signaling Technology), rabbit anti-synaptophysin (1:1000; #5461, Cell Signaling Technology), rabbit anti-NF- κ B p65 (1:1000; #8242, Cell Signaling Technology), rabbit anti-APP (1:10000; ab32136, Abcam), mouse anti-A β (1: 2000; ab126649, Abcam), rabbit anti-BACE1 (1:1000; ab183612, Abcam). Membranes were then washed thrice with 0.1% TBS/Tween-20 followed by incubation with horseradish peroxidase-coupled anti-rabbit (1:1000; #7074, Cell Signaling Technology) or anti-mouse secondary (1:1000; #7076, Cell Signaling Technology) antibodies for 2 h at 25°C. Image-J software was used to quantify band density.

2.8 Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean. SPSS 18.0 (Chicago, IL, USA) and GraphPad Prism 7.0 software were used for statistical analyses and construction of diagrams, respectively. Data from the MWM training trials were analyzed using two-way repeated-measures analysis of variance (ANOVA). Other data were analyzed using one-way ANOVA followed by Bonferroni's post hoc test for multiple group comparisons correction. Statistical significance was set at *P* < 0.05.

3. Results

3.1 Liraglutide improved cognitive performance of 5 $\times$ FAD mice in Morris water maze trials

We used the MWM test to evaluate the spatial learning ability of mice [12]. As shown in Fig. 1, the 12-month-old 5 $\times$ FAD mice exhibited impaired performance with longer escape latency in the acquisition phase, indicating a decline in their spatial learning ability (Fig. 1A). However, we observed that the average escape latencies were significantly reduced in mice subcutaneously injected with liraglutide

compared with those in the 5×FAD group (Fig. 1A, $P < 0.05$). Representative swimming tracks in the spatial probe trial are shown in Fig. 1B. In addition, we found that 5×FAD mice spent less time in target quadrants (Fig. 1C, $P < 0.05$) and had a decreased number of platform crosses (Fig. 1D, $P < 0.05$), indicating impaired spatial memory retrieval ability, which was partially reversed by treatment with liraglutide (Fig. 1C-D, $P < 0.05$). These results suggested that liraglutide protects against learning and memory impairment in 5×FAD mice.

3.2 Liraglutide improved the histological features in the cortex and hippocampus

We then evaluated the microscopic structures of the cortex and hippocampus using Nissl staining. As shown in Fig. 2A, the structure of neurons in the cortex and hippocampal CA1 region in the WT group was clear, evenly dyed, with cells arranged in a compact formation and characterized by a large round nucleus and clearly visible nucleoli. In contrast, we noticed that the number of pyramidal cells in the cortex and hippocampus of 5×FAD mice was reduced (Fig. 2B-C, $P < 0.05$, respectively) and cells were characterized by an unclear structure, with nuclear pyknosis, and disordered arrangement. Compared with 5×FAD mice, liraglutide-treated mice contained relatively abundant pyramidal cells in the cortex and hippocampus (Fig. 2B-C, $P < 0.05$, respectively) that were arranged more neatly and exhibited a more defined structure. These results indicated a loss of neurons in the brain of affected mice; this neurodamage was partially alleviated following treatment with liraglutide.

3.3 Liraglutide alleviated changes in chemical synapses in 5×FAD mice

We detected the ultrastructure of chemical synapses in the three experimental groups to assess synaptic properties. Representative graphs of the structure of chemical synapses in the hippocampal CA1 region are shown in Fig. 3. We observed that in WT mice, the synaptic vesicles were abundant, the synaptic cleft was narrow, and the area of PSD on postsynaptic membranes was centralized (Fig. 3A). Moreover, we found that both the pre- and postsynaptic membranes were continuous. In contrast, in the AD group, the structure of synapses was irregular, with decreased synaptic vesicles, increased synaptic cleft, and reduced PSD area. In addition, we noticed that synaptic membranes were cracked and swollen (Fig. 3B). We found that compared with the AD group, synaptic membranes were more regular, the synaptic cleft was smaller, and synaptic vesicles were evenly distributed in the AD + liraglutide group (Fig. 3C). To quantify the changes in chemical synapses, we analyzed three morphological parameters, including the thickness of the PSD, cleft width, and synaptic curvature. We found that the thickness of PSD in the AD group was significantly decreased (Fig. 3D, $P < 0.05$), whereas the synaptic cleft width was increased (Fig. 3E, $P < 0.05$) compared with the WT group. We noticed that treatment with liraglutide partially reserved this trend (Fig. 3D-E, both $P < 0.05$). We did not observe any significant differences in the synaptic curvature among the three groups (Fig. 3F, $P > 0.05$). These results indicated that treatment with liraglutide might result in improved synaptic transmission, synaptic plasticity, and membrane stability.

To further confirm the regulation synapses by liraglutide, we detected the expression of synaptic marker proteins, including the postsynaptic (PSD-95) and presynaptic (SYN) markers in the cortex and hippocampus. We accordingly found that the expression of PSD-95 and SYN was downregulated in the cortex (Fig. 3G-I, both $P < 0.05$) and hippocampus (Fig. 3J-L, both $P < 0.05$) of the AD group compared with that in the WT group. In contrast, treatment with liraglutide increased the expression of PSD-95 and SYN in the cortex (Fig. 3G-I, both $P < 0.05$) and hippocampus (Fig. 3J-L, both $P < 0.05$). These results suggested that liraglutide could improve synaptic function, thus potentially improving cognitive ability.

3.4 Liraglutide improved pyramidal cell morphology in 5×FAD mice

We also detected the ultrastructure of pyramidal cells in the CA1 region of the hippocampus. As shown in Fig. 4, neurons in WT mice contained abundant organelles, including mitochondria and endoplasmic reticula. Moreover, we noticed that cells exhibited a rich cytoplasm, as well as a clear and complete nuclear membrane structure. In contrast, we found that the cytoplasm was loose, and the nuclear membrane structure was intermittent and unclear in cells in AD mice. These cells were also characterized by swollen mitochondria, decreased and fractured mitochondrial cristae, and the presence of lipofuscin. Compared with the AD group, the deformation of pyramidal cells was partially alleviated in the AD + liraglutide group, with cells being characterized by more regular organelles, less lipofuscin, and increased mitochondrial cristae.

To quantify the changes in mitochondria, we assessed several parameters of mitochondria in neurons, including their numeric density (Nv), specific surface area (δ), and volume density (Vv), using a stereological method. As shown in Fig. 4D-F, compared with the WT group, the numeric density and specific surface were significantly decreased in the AD group (Nv, $P < 0.05$; δ $P < 0.05$). Treatment with liraglutide increased the numeric density and specific surface area of mitochondria (Nv, $P < 0.05$; δ $P < 0.05$, Fig. 4D-E). We observed that the volume density was not significantly different among three groups ($P > 0.05$; Fig. 4F). These results indicated the occurrence of damages in the morphology of pyramidal cells, with reduced numbers and swollen mitochondria in AD mice; whereas treatment with liraglutide alleviated these effects.

3.5 Liraglutide alleviated local and universal inflammation in 5×FAD mice

Inflammation is an important factor known to impair cognitive function, and IL-1 β , IL-6, TNF- α , and COX-2 have been associated with synaptic plasticity, long-term potentiation, and memory dysfunction [14–16]. To assess the level of inflammation in mice, we measured the levels of proinflammatory cytokines in the hippocampus of mice in the three groups (Fig. 5). We found that compared with those in the WT group, the levels of IL-1 β , IL-6, TNF- α , and COX-2 in the hippocampus of AD mice were increased ($P < 0.05$, all). In contrast, administration of liraglutide partly reversed the trend of IL-1 β , IL-6, and COX-2 ($P < 0.05$, all). In addition, we observed that the trend in the serum levels of proinflammatory cytokines among the three groups was similar to that of the hippocampus, that is, AD mice had higher levels of IL-1 β , IL-6, TNF- α ,

and COX-2 ($P < 0.05$, all), whereas liraglutide-treated mice had decreased serum levels of IL-1 β , IL-6, TNF- α , and COX-2 ($P < 0.05$, all). These results indicated that liraglutide could reduce the inflammatory response in AD mice.

3.6 Liraglutide decreased the expression of amyloid precursor protein and amyloid β in mice with Alzheimer's disease

We subsequently used immunohistochemistry to investigate the expression of APP and A β in the cortex of mice among the three groups. We found few APP-positive (Fig. 6A-B) and A β -positive neurons in the sections of WT mice. The number of both APP- and A β -positive neurons was increased in the cortex of AD mice, whereas both were decreased in the cortex of liraglutide-treated mice. Western blot analysis revealed similar results (Fig. 6C-E). The expression of APP and A β was increased in AD mice, whereas treatment with liraglutide partially reversed this increase. Moreover, we noticed that the expression of APP and A β in the hippocampus showed the same trend as that in the cortex (Fig. 6F-H). These results indicated that administration of liraglutide reduces the expression of APP and A β in AD mice.

3.7 Liraglutide normalized the expression of NF- κ B and BACE1

To further explore the mechanism involved in the decrease of APP and A β in AD mice treated with liraglutide, we measured the expression of NF- κ B and BACE1 proteins (Fig. 7). We respectively found that the expression of NF- κ B p65 and BACE1 was significantly increased in the cortex of AD mice compared with that in the WT group (Fig. 7A-C; both $P < 0.05$). In contrast, treatment with liraglutide partially reversed this trend (Fig. 7A-C; both $P < 0.05$). Similar results were observed in the hippocampus (Fig. 7A-C; both $P < 0.05$), where administration of liraglutide prevented the increase in the levels of NF- κ B p65 and BACE1. These results suggested that liraglutide might inhibit the expression of A β through the inhibition of NF- κ B and BACE1 in AD mice.

4. Discussion

In this study, we explored the effect of liraglutide, a GLP-1 analog, on the cognitive improvement of 12-month-old AD mice and attempted to delineate the underlying mechanism. We here demonstrated that liraglutide improved the learning and memory abilities of middle-aged AD model mice. Concomitantly, liraglutide increased the number of neurons in AD mice, and improved the ultrastructure of synapses and mitochondrial morphology. We also found that liraglutide significantly reduced the deposition of A β , which might be related to the NF- κ B/BACE1 pathway, and decreased inflammation. These results offer new insights into the mechanism of action of liraglutide in improving cognitive ability in elderly AD mice.

Of note, 5 $\times$ FAD mice are known to coexpress a total of five FAD mutations (APP K670N/M671L [Swedish] + I716V [Florida] + V717I [London] and PS1 M146L + L286V) [17]. As such, 5 $\times$ FAD mice are considered a useful AD model for developing cerebral amyloid plaques at 2 months of age, and exhibit

many characteristics of AD, such as reduced synaptic markers, neuronal loss, and memory impairment [17]. A current study showed that the abnormal expression of A β was negatively related to the cognitive function of patients with AD [18], and highlighted BACE1 as an important enzyme in the production of A β . Under physiological conditions, most A β precursor protein (APP) is cleaved by α -secretase to produce N-terminal nontoxic soluble sAPP α fragments and an α -C-terminal fragment (α -CTF). Then, α -CTF is hydrolyzed by the γ -secretase complex, with the whole process not producing A β . However, in the brains of patients with AD, BACE1 has been found to be highly activated. Accordingly, APP is cleaved by BACE1 to form an A β -containing C-terminal fragment (β -CTF) and a soluble N-terminal fragment. Then, β -CTF is further cleaved by the γ -secretase complex to form A β_{1-42} and A β_{1-40} [19]. A β_{1-42} and A β_{1-40} are the most common fragments of A β , and also the most significant pathophysiological characteristics in the brains of patients with AD [20]. Several studies have suggested that pharmacological or genetic reduction in the activity of BACE1 might be a potential strategy for decreasing the brain concentrations of A β , thus preventing the progression of AD [21]. In this study, we found that liraglutide improved the cognitive function in 12-month-old AD mice, accompanied by a decrease in the expression of the A β protein. In addition, liraglutide was found to reduce the expression of BACE1. Likewise, sitagliptin, a GLP-1 receptor agonist, was reported to enhance the levels of hippocampal GLP-1 and improve the cognitive function of rats with pentylenetetrazol kindling-induced cognitive deficits, potentially corresponding to the modulation of BACE1 [22]. In addition, treatment with GLP-1 was shown to improve learning and memory deficits in type 2 diabetic rats and decrease the mRNA expression of APP and BACE1 [23]. Liraglutide was also found to reduce the activity of BACE1, in turn decreasing the formation of A β in insulin-resistant cells [24]. Conclusively, these observations have indicated that liraglutide might be an effective pathway for improving the cognitive function of patients with AD by reducing the expression of BACE1 and A β .

Many studies have suggested that in addition to A β plaques, the brains of patients with AD exhibit sustained chronic inflammation [25–27], which also contributes to cognitive impairment [28]. In our study, administration of liraglutide in AD mice not only reduced A β plaque deposition, but also decreased the levels of the IL-1 β and IL-6 proinflammatory cytokines, and the cyclooxygenase-2 (COX-2) inflammatory mediator in the brain and blood. This finding suggested that the regulation of immunoregulatory functions is involved in the mechanism by which liraglutide improves cognition in AD mice. A meta-analysis of 40 studies revealed the detection of increased levels of IL-1 β , IL-6, and TNF- α in patients with AD [29]. Besides, treatment with liraglutide was reported to protect against progressive neurodegeneration, chronic inflammation, and amyloid plaque deposition in 2-month-old APP/PS1 mice [30]. Furthermore, another study suggested that inflammation was reduced by 30%, accompanied by a decrease in plaque load and memory improvement in 7-month-old APP/PS1 liraglutide-treated mice [9]. The relationship between inflammation and amyloid plaques is reciprocal. It is known that A β binds to microglial-expressed receptors, such as CD36 or CD47, resulting in their activation and production of inflammatory factors and chemokines [31]. The activated microglia surround and take up A β , and when their intake capacity is exceeded they resolve to cell death, resulting in the accumulation of released A β in the extracellular space, which contributes to A β plaque growth and the generation of new plaque clusters in the vicinity of existing plaques, thus further exacerbating inflammation [32]. Liraglutide is known to

suppress the production of proinflammation factors by decreasing the activation of microglia [33], or targeting the expressions of proteins, such as IL-1 β and TNF- α [34].

The NF- κ B pathway has been shown to play an important role in regulating the propagation and elaboration of inflammation by responding to proinflammatory stimuli, such as IL-1 or TNF- α [35]. Activated NF- κ B has been found in neurons and glial cells surrounding areas of A β plaque deposition in AD brains [36]. Many studies have suggested that A β increases the activity of NF- κ B, and decreases the activity of NF- κ B, resulting in the improvement of the cognitive ability through the decrease in the production of cytokines and chemokines [37–39]. Furthermore, NF- κ B is considered an important regulator of the transcription of BACE1, as abundant NF- κ B binding sites have been found near the BACE1 promoter [11]. A recent study showed that the levels of expression of NF- κ B p65 and BACE1 in the brain tissue of patients with AD were significantly increased [40]. Several studies have also demonstrated that an NF- κ B inhibitor could decrease the TNF- α -induced BACE1 transcription, resulting in lower deposition of A β [41, 42]. Nonsteroidal anti-inflammatory drugs (NSAIDs), such as indomethacin and flurbiprofen, have been shown to decrease the activity of NF- κ B, in turn decreasing the A β burden [43, 44]. In our study, the expression of NF- κ B, BACE1, and A β in AD mice was increased, whereas liraglutide partly reversed this trend. Similar to our research, the GLP-1 analog, exendin-4, was reported to promote neuroprotection by reducing neuroinflammatory responses, such as decreasing the activation of NF- κ B in lipopolysaccharide-stimulated microglia [45]. Furthermore, studies have shown that liraglutide decreased the activity of BACE1, resulting in the reduced accumulation of A β in insulin-resistant cells [46]. These observations indicated that the ability of liraglutide to improve cognition might involve the regulation of the NF- κ B/BACE1 pathway, which subsequently reduces the formation of A β .

5. Conclusions

We have demonstrated that liraglutide could improve cognitive function, protect the synapses and mitochondria from ultrastructural changes, and regulate immunoregulatory function in 12-month 5 $\times$ FAD mice. Moreover, the A β burden in the brains of AD mice could be ameliorated by liraglutide through a decrease in the expression of NF- κ B and BACE1. Furthermore, liraglutide alleviated local and universal inflammation in AD mice. These findings suggested that liraglutide or other GLP-1 analogs might serve as a promising therapy for alleviating degenerative processes in neurodegenerative diseases such as AD.

Declarations

Ethics approval and consent to participate

All experiments were approved by the Institutional Animal Care and Use Committee of Fujian Medical University (no. FJMU IACUC 2018-034) and complied with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Consent for publication

Not applicable

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Liqin Qi: conceptualized and wrote the original draft. Lijing Lin: Methodology and software analyses. Jiaping Zheng: Data curation. Xiaoying Liu: Resources. Xiaohong Liu: Writing and review. Zhou Chen: Supervision. Libin Liu: Project administration.

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Figures

Figure 1

Liraglutide improves cognitive performance of 5xFAD mice in MWM. (A) The average escape latencies in the hidden platform phase were decreased in liraglutide-treated mice compared with AD mice. (B) Representative swimming tracks in spatial probe trial. (C) Time spent in the target quadrant in the spatial probe trial among the three groups of mice. (D) Number of platform crosses in the spatial probe trail among the three groups of mice. Values were expressed as the mean $\pm$ S.E; n = 10 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05, compared with the AD group.

Figure 2

Liraglutide improves the histological features in the cortex and hippocampus. (A) Representative images of Nissl staining of the cortex and hippocampus of the three groups. Scale bar = 500 μ m, in the image of the integral brain; scale bar = 20 μ m, in the enlarged images of the cortex and hippocampus. (B) Number of neurons per mm² in the cortex. (C) Number of neurons per mm² in the hippocampus. Values were expressed as the mean $\pm$ S.E; n = 3 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.

Figure 3

Liraglutide alleviates changes in chemical synapses in 5x FAD mice. (A-C) Representative TEM images of the structure of chemical synapses in the hippocampal CA1 region among the three groups. White long arrowheads indicate the synapse. White short arrowheads indicate synaptic vesicles. Scale bar = 500 nm. (D-F) Quantification of the thickness of PSD, synaptic cleft, and synaptic interface curvature. (G) Representative immunoreactive bands for PSD-95 and SYN in the cortex among the three groups. (H-I)

The expressions of PSD-95 and SYN in the cortex were measured by western blotting. (J) Representative immunoreactive bands for PSD-95 and SYN in the hippocampus among the three groups. (K-L) The expression of PSD-95 and SYN in the hippocampus were measured by western blotting. n = 3 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.

Figure 4

Liraglutide improves pyramidal cell morphology in 5x FAD mice. (A) Representative TEM images of the ultrastructure of pyramidal cells in the CA1 region of the hippocampus among the three groups. White long arrowheads indicate mitochondria. White short arrowheads indicate lipofuscin. Scale bar = 1 μm . (D-F) Quantification of the numeric density, specific surface area, and volume densities of mitochondria. n = 3 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.

Figure 5

Liraglutide alleviates local and universal inflammation in 5 $\times$ FAD mice. (A) IL-1 β , (B) IL-6, (C) TNF- α , and (D) COX-2 in hippocampus detected by ELISA. n = 6 per group. (E) IL-1 β , (F) IL-6, (G) TNF- α , and (H) COX-2 in serum detected by ELISA. n = 6 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.

Figure 6

Liraglutide decreases the expression of APP and A β in AD mice. (A) Representative images of the expression of APP and A β in the cortex among the three groups, detected by immunohistochemistry. Scale bar = 50 μ m. (B) Representative immunoreactive bands for APP and A β in the cortex among the three groups. (C-D) The expressions of APP and A β in the cortex were measured by western blotting. (E) Representative immunoreactive bands for APP and A β in the hippocampus among the three groups. (F-G)

The expressions of APP and Aβ in the hippocampus were measured by western blotting. n = 3 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.

Figure 7

Liraglutide normalizes the expression of NF-κB and BACE1. (A) Representative images of the expression of NF-κB p65 and BACE1 in the cortex among the three groups, detected by immunohistochemistry. Scale bar = 50 μm. (B) Representative immunoreactive bands for NF-κB p65 and BACE1 in the cortex among the three groups. (C-D) The expressions of NF-κB p65 and BACE1 in the cortex were measured by western

blotting. (E) Representative immunoreactive bands for NF- κ B p65 and BACE1 in the hippocampus among the three groups. (F-G) The expressions of NF- κ B p65 and BACE1 in the hippocampus were measured by western blotting. n = 3 per group. WT: wild type; AD Alzheimer disease; Lira: liraglutide. *P < 0.05.