Exploration of neuroprotective mechanism of GLP-1 in animal models with Alzheimer’s disease

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Abstract: Objective: To explore the protective mechanism of glucagon-like peptide-1 (GLP-1) in the neurological function of rats with Alzheimer’s disease (AD). Methods: A total of 48 pure inbred SD rats were divided into group A, group B and group C according to the principle of similar body weight, with 16 rats in each group. Streptozotocin (STZ) was used for establishing AD rat models. Group A was injected with 10 μL of saline, group B with 10 μL of STZ solution, and group C with 5 μL of (Val8) GLP-1 30 min after injected with 10 μL of STZ solution. Morris water maze was performed on three groups of rats on the 1st, 2nd, 3rd, 4th, 5th and 6th days after modeling. Radioimmunoassay was used for detecting the concentration of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD) in the hippocampus tissue of three groups of rats, Western blot for detecting the expressions of protein kinase B (AKT) and extracellular signal-regulated protein kinase (ERK) proteins. Results: There were statistical differences in the escape latency of rats in different periods. The escape latency of rats was significantly lower in group A and group C than that in group B (both P<0.0001). That of rats was significantly lower on the 2nd to 6th days than that on the 1st day in three groups of rats after modeling (all P<0.0001). That of rats was significantly lower in group A and group C than that in group B on the 1st to 6th days after modeling (all P<0.0001). The ratio in the rat platform quadrant was significantly lower in group B and group C than that in group A (P<0.0001), and was significantly higher in group C than that in group B (P<0.0001). The concentration of MDA in the hippocampus tissue of rats was significantly lower in group A and group C than that in group B (both P<0.0001). The activity of SOD in the hippocampus tissue of rats was significantly lower in group B and group C than that in group A (both P<0.0001). The SOD activity, AKT and ERK proteins in the hippocampus tissue of rats were significantly higher in group C than those in group B (all P<0.0001). Conclusion: Having certain protective effects on the learning and memory abilities of rats after injection of STZ, GLP-1 can protect the neurological function of rats from injury. The mechanism may be achieved by activating AKT/ERK signal transduction pathway through strengthening the antioxidant activity of the brain tissue and reducing oxidative stress injury.

Keywords: GLP-1, Alzheimer’s disease, animal model, learning-memory, neuroprotective mechanism

Introduction

Alzheimer’s disease (AD), an unexplained central nervous system degenerative disease, is the most common cause of dementia in the elderly [1]. Clinically, AD patients are mainly characterized by progressive decline in memory, cognitive dysfunction, behavioral ability changes in patients, decline in learning and memory function and loss of basic self-care ability. Patients with severe AD even suffer brain tissue atrophy and nerve cell necrosis, finally coming to death [2, 3]. AD, as the fourth cause of death in elderly patients, is highly prevalent in them with a 50% incidence [4]. Physiological and pathological changes can occur in the brain of AD patients, with many biochemical substances. The pathological changes of the disease are mainly the extracellularly deposited β-amyloid protein in the hippocampus and cerebral cortex, and neurofibrillary tangles caused by the hyperphosphorylation of Tau protein in nerve cells of the brain [5]. Studies show that as an early manifestation of AD neuronal injury, oxidative stress response plays an important role in the pathogenesis of AD. Amyloid-like peptide, by activating extracellular signal-regulated protein kinase (ERK)/mitogen-activated protein kinase (MAPK) signal transduction pathway, can lead to memory dis-
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order in mice. The activated phosphatidylinositol 3-kinase (PI3K)/AKT/glycogen synthase kinase-3β pathway can reduce the hyperphosphorylation of Tau protein [6-8]. At present, the pathogenesis of AD has not been elucidated, and safe and effective drugs still cannot fit in the prevention and treatment of AD in clinical practice. Therefore, it’s of great significance to solve both of the problems above.

Glucagon-like peptide-1 (GLP-1), an insulin-like intestinal peptide composed of multiple amino acids, is secreted by intestinal endocrine L cells, playing a role in regulating glucose homeostasis in the body [9]. It can inhibit glucagon secretion directly by binding to receptors on islet α cell as well as indirectly by acting on islet δ cell to stimulate the secretion of somatostatin [10]. It can protect neurons by mediating receptors [11]. GLP-1 specifically binding to its receptors activates adenylate cyclase, and then produces cyclic adenosine monophosphate. The receptor agonist coupled with the latter can regulate and improve calcium homeostasis in neurons, so neurons are allowed to resist excitatory apoptosis as a result of intracellular calcium overload [12]. Previous studies have shown that GLP-1 has certain protective effects on the nervous system, which may become a novel drug for prevention and treatment of AD [13].

In this study, AD rat models were established through Streptozotocin (STZ) and GLP-1 was given at the same time for treatment. The protective effects and mechanism of GLP-1 in the neurological function of AD rat models were explored, in order to find a safe and effective drug for prevention and treatment of AD patients.

Materials and methods

Experimental animals

There were 48 pure inbred SD rats, aged from 20 to 24 months, with a body mass of 320-400 g. They were purchased from Hunan Slack Jingda Experimental Animal Co., Ltd. (license No. SCXK (Xiang 2014-0010)), kept in a clean environment with good ventilation, half light and dark, at an indoor temperature of 21-26°C and a relative humidity of 51-57%. Given normal drinking water and food intake, they were fasted for 6h before the experiment. The animal experiment was approved by the Ethics Committee of the Heilongjiang Provincial Hospital. Experimental procedures were performed in accordance with the principles of the protection and use of experimental animals [14].

Animal model preparation

Rats were divided into group A, group B and group C based on the principle of similar body weight, with 16 rats in each group. AD rat models were established according to the study of Huang et al. [15]. Modeling methods: 10.00% 0.5 mL/kg chloral hydrate (Shanghai Enzyme-linked Biotechnology Co., Ltd., China) was used by intraperitoneal injection for anesthesia, brain stereotactic apparatus (Anhui Zhenghua Biological Instrument Equipment Co., Ltd., China) was used for fixing the head of the rat, the skin of which was disinfected with iodophor, with a 1-cm sagittal incision along the cranial midline. Anterior fontanelle as the starting point, 1.5 mm backward, the hole was drilled at 2.0 mm around the sagittal incision. A needle was inserted vertically 5 mm into the hole, and 3 mg/kg STZ solution (Beijing Coolaber Technology Co., Ltd., China) was slowly injected into the bilateral ventricles. Group A was injected with 10 μL of saline (Nantong Kaiheng Biotechnology Development Co., Ltd., China), group B with 10 μL of STZ solution, and group C with 5 μL of (Val8) GLP-1 (AdooQ BioScience Company, USA) 30 min after injected with 10 μL of STZ solution. After that, the needle was retained for 2 min, and then the incision was sutured. The injection was performed once again at the original injection site after 48 h.

Morris water maze

Morris water maze was performed on three groups of rats after modeling on the 1st, 2nd, 3rd, 4th, 5th and 6th days [16]. The Morris water maze devices (Kilton Biotechnology (Shanghai) Co., Ltd., China) consisted of a pool, a mobile platform and an image acquisition and analysis system. The platform was 20 cm in diameter and 50 cm in height, and the pool was 160 cm in diameter and 80 cm in height. The experiment was divided into place navigation task and spatial probe tests. The former was conducted twice a day in the morning and afternoon, for five consecutive days. The escape ability of rats for finding platform in the
water and their latency were observed and recorded, and their ability to remember and learn the water maze was measured. The latter was conducted on the 6th day, once in the morning and afternoon. After the platform was removed, the swimming time of rats to the original platform position was observed and recorded. The ratio of the total swimming distance to the swimming time was the ratio in the rat platform quadrant. The ability of rats to remember the spatial position of the platform was measured.

**Experimental setting:** The maximum place navigation time was 110 s. If rats could find the platform within 110 s, they can stay for 15 s. The space exploration time was 110 s.

**Indicator detection**

After successful modeling, rats were sacrificed by decapitation, whose hippocampus tissue was taken, homogenized and centrifuged. The supernatant was taken to tissue lysate (1 mL of RIPA lysate, 10 μL of Na3VO4, 10 μL of PMSF, 10 μL of Protease Inhibitor Cocktail and 10 μL of Phosphatase Inhibitor Cocktail 2, mixed), ground at low temperature to prepare tissue homogenate; it was centrifuged at 10,000 rpm for 30 min at 4°C. The supernatant was taken and the BCA method was used for determining the protein concentration. The protein sample was diluted by Lysis buffer, mixed well and inactivated at 95°C for 5 min. 8.00% separation gel and 5.00% laminating gel were prepared, and then SDS-PAGE electrophoresis and PVDF membrane transfer were performed. The PVDF membrane was removed and blocked with 5.00% skim milk for 1 h. Referring to the antibody instructions of rabbit anti-rat AKT monoclonal antibody (Shanghai Hufeng Chemical Co., Ltd., China) and rabbit anti-rat ERK monoclonal antibody (Beijing TideRader Technology Co., Ltd., China), diluted AKT (1:1,000) and ERK (1:1,000) primary antibodies and internal reference β-actin (1:3,000) (Hefei Lai Er Biotechnology Co., Ltd., China) were added, placed at 4°C overnight, and washed 3 times with TBST. Then, the diluted horseradish peroxidase (1:5,000) goat anti-rabbit IgG secondary antibody (Beijing Solarbio Technology Co., Ltd., China) was added. The mixture was incubated for 1 h, and washed 3 times with TBST. Next, ECL chemiluminescence reagent (Dalian Meilun Biotechnology Co., Ltd., China) was added, so as to develop and photograph for analyzing and processing. Gel imaging analysis software Gel-Pro_analyzer 4.0 was used for determining the expressions of AKT and ERK proteins in the hippocampus tissue.

**Statistical methods**

SPSS18.0 was used for statistical analysis. Measurement data are expressed as mean ± standard deviation (X ± sd). One-way ANOVA was used for comparison of the means among multiple groups, repeated-measures ANOVA for
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Table 2. Comparison of escape latency of rats in place navigation experiment in different periods (X ± sd)

<table>
<thead>
<tr>
<th>Group</th>
<th>Group A (n=16)</th>
<th>Group B (n=15)</th>
<th>Group C (n=15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 d</td>
<td>72.8±4.9****</td>
<td>81.6±8.4</td>
<td>73.2±3.5*****</td>
<td>10.6100</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>2 d</td>
<td>53.6±3.8****</td>
<td>73.5±7.9</td>
<td>56.7±4.1*****</td>
<td>56.7100</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>3 d</td>
<td>39.27±2.6******</td>
<td>64.2±6.2</td>
<td>41.3±3.2*****</td>
<td>160.1000</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>4 d</td>
<td>30.14±3.1*****</td>
<td>50.32±6.7</td>
<td>32.5±3.0*****</td>
<td>88.6900</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>5 d</td>
<td>24.08±2.5******</td>
<td>41.7±5.2</td>
<td>26.1±2.8*****</td>
<td>104.6000</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6 d</td>
<td>16.01±1.4******</td>
<td>29.4±3.5</td>
<td>17.2±1.5******</td>
<td>154.6000</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>F</td>
<td>665.500</td>
<td>138.700</td>
<td>660.900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Compared with group B, ****P<0.0001.

Results

General information of three groups of rats

One rat in group B and one in group C failed to be modeled, with a success rate of 93.75% (15/16). There were no differences in gender, month of age, body mass, indoor temperature and indoor humidity among group A, group B and group C of rats, with no statistical significance (all \( P > 0.05 \)). See Table 1.

Changes in learning ability of three groups of rats in different periods

Repeated-measures ANOVA showed that there were statistical differences in the escape latency of rats in different periods. The escape latency was significantly lower on the 2nd to 6th days than that on the 1st day after modeling in three groups of rats (all \( P < 0.0001 \)). The rats in group A and group C show significantly lower escape latency than group B on the 1st to 6th days after modeling (all \( P < 0.0001 \)). There was no significant difference in the escape latency between group A and group C in different periods (all \( P > 0.05 \)). See Table 2 and Figure 1.

Ratio in the rat platform quadrant among three groups

The ratio in the rat platform quadrant was (48.52±5.07)% in group A, (27.52±4.83)% in group B and (34.37±4.62)% in group C. And it was significantly lower in group B and group C show significantly lower escape latency than group B on the 1st to 6th days after modeling (all \( P < 0.0001 \)). There was no significant difference in the escape latency between group A and group C in different periods (all \( P > 0.05 \)). See Table 2 and Figure 1.

MDA concentration and SOD activity in hippocampus tissue of three groups of rats

The concentrations of MDA in the hippocampus tissue of rats were significantly lower in group A and group C than in group B (t=6.9060, \( P < 0.0001 \); t=6.6490, \( P < 0.0001 \)). There was no
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Table 3. Comparison of MDA concentration and SOD activity in hippocampus tissue (X ± sd)

<table>
<thead>
<tr>
<th>Index</th>
<th>Group A (n=16)</th>
<th>Group B (n=15)</th>
<th>Group C (n=15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mL)</td>
<td>1.267±0.204</td>
<td>2.104±0.437 ****</td>
<td>1.253±0.234</td>
<td>38.0800</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>SOD (U/mL)</td>
<td>166.547±19.145</td>
<td>140.547±7.158 ####</td>
<td>151.637±6.247##</td>
<td>16.8200</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: MDA, malondialdehyde; SOD, superoxide dismutase. Compared with group A or C, ****P<0.0001; compared with group A or C, ####P<0.0001; compared with group A, ##P<0.01.

Figure 3. Comparison of MDA concentrations and SOD activity in hippocampus tissues. (A) Compared with group A and C, ****P<0.0001; (B) Compared with group A, ****P<0.001, **P<0.01; compared with group B, ####P<0.0001. MDA, malondialdehyde; SOD, superoxide dismutase.

Expression of AKT and ERK proteins in hippocampus tissue of three groups of rats

AKT protein in the hippocampus tissue of rats in group B was significantly lower than that in group A (t=4.9420, P<0.0001; t=2.8740, P=0.0075) while that was significantly higher in group C than in group B (t=4.5210, P<0.0001). See Table 3 and Figure 3.

Discussion

AD is one of the main causes of human dementia, the major clinical manifestations of which are cognitive dysfunction and memory loss [18]. Its pathogenesis is closely related to neurofibrillary tangles, the pathological deposition of Aβ in the brain and hyperphosphorylation of Tau protein [19]. In this study, AD rat models were established by injecting 3 mg/kg STZ into the lateral ventricle of SD rats. Similar to the pathological features of AD, this model can damage ability metabolism and glucose metabolism functions in the brain of rats, and learning and memory function, as the most popular animal model to explore pathogenesis and treatment of AD [20]. GLP-1, a modified product of glucagon protogene after transcription, can enter the human brain by blood circulation via the peptide transporter. GLP-1 receptors, which are widely distributed in the human brain, are expressed in the brain stem, cerebral cortex and hippocampus of the human body [21]. Studies show that GLP-1 protects neurons through its receptors [22].

In this study, AD rat models were established by injecting 3 mg/kg STZ into the lateral ventricle of SD rats with the rat behavior observed. The results showed that the escape latency of rats was significantly lower on the 2nd to 6th days than that on the 1st day after modeling in three groups of rats. The escape latency of rats in group A and group C was significantly lower than that in group B on the 1st to 6th days after modeling. There was no significant difference in the escape latency between group A and group C in different periods. The ratio in the rat platform quadrant was significantly lower in group B and group C than that in group A. And the ratio was significantly higher in group C than that in group B. After injection of STZ, the escape latency of SD rats increased significantly, but after injection of GLP-1, it shortened.
GLP-1 has a certain protective ability to the learning function of rats after injection of STZ. In a spatial probe experiment, the memory ability of rats in group B reduced. However, after injection of GLP-1 it was higher. So GLP-1 has certain protective effects on the memory ability of rats. A study by Hansen et al. showed that GLP-1 receptor agonist-type hypoglycemic agents improved memory function in accelerated aging AD mouse models, similar to this study [23].

Oxidative stress response is an early feature of AD neuronal injury. Oxidative imbalance and neuronal injury are important in occurrence and development of AD. The accumulation of Aβ increases oxidative stress, leading to mitochondrial dysfunction and energy failure [24]. Previous studies have shown that STZ-induced memory impairment in rats is closely related to oxidative stress response [25]. The abnormal increase of MDA concentration indicates strong lipid peroxidation and severe injury in cell mitochondrial membrane and cell membrane. SOD activity reflecting antioxidant capacity is a scavenger of oxygen free radicals. Therefore, MDA concentration and SOD activity reflect the degree of oxidative stress response [26, 27]. The results of this study showed that the concentrations of MDA in the hippocampus tissue of rats were significantly lower in group A and group C than that in group B. The activity of SOD in the hippocampus tissue of rats was significantly lower in group B and group C than that in group A. And group C showed higher activity of SOD than group B. It suggests that GLP-1 may alleviate the damage of learning and memory function in rats by enhancing the antioxidant activity of the brain tissue and reducing the oxidative stress injury of STZ in the brain tissue of SD rats, so as to protect the neurological function of rats.

Breaking the balance between antioxidant capacity and free radical damage in the body, the imbalance of oxidative stress can induce hyperphosphorylation of Tau protein and over-expression of Aβ by activating p38MAPK signal transduction pathway [28]. Aβ can regulate the activity of ERK. Conversely, ERK signal pathway can also regulate production of Aβ. ERK, activated in the early stage of AD, gradually inactivates with the development of AD [29]. Studies by Jiang et al. show that inhibition of MAPK/ERK signal pathway will aggravate hippocampal neuronal apoptosis, reducing neurogenesis [30]. It also impairs the cognitive function of offspring, down-regulating the p-ERK levels.

AKT controls transcription, apoptosis, metabolism and other processes of the cell cycle in the matrix [31]. Studies by Tiwari and Liu et al.

**Table 4. Comparison of the expression of AKT and ERK proteins in hippocampus tissue (X ± sd)**

<table>
<thead>
<tr>
<th>Index</th>
<th>Group A (n=16)</th>
<th>Group B (n=15)</th>
<th>Group C (n=15)</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKT</td>
<td>1.027±0.347</td>
<td>0.347±0.183***</td>
<td>0.927±0.359</td>
<td>21.6600</td>
<td>P&lt;0.0001</td>
</tr>
<tr>
<td>ERK</td>
<td>1.283±0.319</td>
<td>0.435±0.127###</td>
<td>0.857±0.264####</td>
<td>43.9000</td>
<td>P&lt;0.0001</td>
</tr>
</tbody>
</table>

Note: AKT, protein kinase B; ERK, extracellular signal-regulated protein kinase. Compared with group A or C, ***P<0.0001; compared with group A or C, ###P<0.0001; compared with group A, ####P<0.0001.

Figure 4. Comparison of the expression of AKT and ERK proteins. (A) Compared with group B, ****P<0.0001; (B) Compared with group A, ****P<0.0001; compared with group B, ####P<0.0001.
show that P13K/AKT signal pathway significantly down-regulates in the AD brain, the activation of which can alleviate the Aβ-induced neuronal toxicity in the body [32, 33]. The results of this study showed that AKT protein in the hippocampus tissue of rats was significantly higher in group A and group C than that in group B. ERK protein in the hippocampus tissue of rats was significantly higher in group A than that in group B and group C, which was significantly higher in group C than in group B. GLP-1 can inhibit down-regulation of the expression of AKT and ERK in the hippocampus tissue of rats. Therefore, the fact that GLP-1 alleviates the STZ-induced neurological impairment in SD rats may be achieved by activating AKT and ERK.

In this study, the repeatability of animal experiments was considered, and rats purchased were strictly screened. There were no differences in gender, month of age, body mass, indoor temperature and indoor humidity in rats, which ensured the reliability of the experiment. AD rat models were established, with easy operation, low costs and high value. This study provides a new theoretical basis for the neuroprotective mechanism of GLP-1, but the related mechanism and correlation of AKT and ERK in the occurrence and development of AD need further verification.

In summary, having certain protective effects on the learning and memory ability of rats after injection of STZ, GLP-1 can protect the neurological function of rats from injury. The mechanism may be achieved by activating the AKT/ERK signal transduction pathway by strengthening the antioxidant activity of the brain tissue and reducing oxidative stress injury.

Disclosure of conflict of interest

None.

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