Exogenous Adiponectin Protects Neurological Function in Mice with Alzheimer's Disease by Affecting NOD Receptor/ NF-κB/TNF Signal Pathway

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To explore the protective effect of exogenous adiponectin on neurological function of Alzheimer's disease mice by affecting nucleotide-binding oligomerization domain receptor/nuclear factor-kappa B/tumor necrosis factor signal pathway. 60 healthy male mice with C57BL/6J were divided into 5 groups; control (group A) (n=12), sham operation (group B) (n=12), model (group C) (n=12) and high (group D) and low dose adiponectin groups (group E) (n=12). Normal saline was given to the group A, B, C, D and E group, once a day. Adiponectin high and low dose group was given adiponectin (10 mg/kg and 2.5 mg/kg) intragastric administration. The Morris water maze test of mice in each group was analyzed, the expressions of nucleotide-binding oligomerization domain 2, nuclear factor-kappa B, toll-like receptor-4, tumor necrosis factor-alpha and interleukin-1 beta in each group were compared, and the expressions of caspase-3, c-caspase-3 and B-cell lymphoma 2 proteins in each group were studied. Compared with the group C, the latent period of the experiment was decreased and the number of diving was increased in the high and low dose groups of adiponectin. Compared with the group C, the nucleotide-binding oligomerization domain 2, nuclear factor-kappa B and toll-like receptor-4 in group D and E were lower. Compared with the group C, the levels of the above factors in the group D and E decreased. Compared with the group C, the caspase-3 and cleaved caspase-3 in the group D and E decreased, while the B-cell lymphoma 2 increased. Exogenous adiponectin can enhance the learning and memory ability of patients with Alzheimer's disease, affect the expression of nucleotide-binding oligomerization domain 2, nuclear factor-kappa B, toll-like receptor-4 and other pathways, and reduce the levels of tumor necrosis factor-alpha and interleukin-1 beta, so as to inhibit the inflammatory response of nerves in the brain, reduce the damage to nerve function, and provide new therapeutic ideas for the prevention and treatment of Alzheimer's disease.

Key words: Exogenous adiponectin, Alzheimer's disease, nucleotide-binding oligomerization domain receptor, nuclear factor-kappa B, tumor necrosis factor, signal pathway

Alzheimer's Disease (AD) is characterized by cognitive impairment, memory impairment and personality changes[1]. The main pathological features of AD are senile plaque, neuronal reduction and neurofibrillary tangles, but the related mechanism is still unclear[2]. Studies have found that the inflammatory response caused by senile plaque deposition is the main mechanism of the pathogenesis of AD, and non-steroidal anti-inflammatory drugs can activate glial cells in AD mice, thus reducing neuronal damage, and finally play a neuroprotective effect[3]. Nucleotide-binding Oligomerization Domain (NOD receptor) is mainly expressed in the cytoplasm and is a pattern recognition receptor, which plays an important role in infection and autoimmunity[4]. Through the stimulation of different effector molecules, NOD receptors activate transcription Nuclear Factors Kappa-B (NF-kB) and Tumor Necrosis Factor (TNF), thus participate in the expression of inflammatory mediators, and finally aggravate the

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inflammatory response[5]. The pathogenesis of AD is a multi-factor, multi-stage process, so the treatment of AD cannot only rely on a single treatment, but need multi-faceted, multi-level, multi-angle progressive research, so as to completely change the neurodegenerative diseases. Exogenous Adiponectin (ADPN) is secreted by fat and belongs to an endogenous bioactive peptide[6]. ADPN plays an important role in metabolic diseases such as diabetes, cardiovascular disease and obesity, and can play an important role in anti-inflammation, improving insulin resistance and anti-atherosclerosis[7]. It has been found that ADPN plays a regulatory role in the central nervous system, but there are few reports in AD, so this study aimed to explore the effects of ADPN on the neurological function of AD mice through NOD receptor/NF-κB/TNF signal pathway. 60 healthy male mice from C57BL/6J were selected. The average age was (32.63±2.41) w and the body weight was (156.37±12.45) g. The mice were fed under the condition of clean grade, and the mice were given indoor light off and avoiding light for 12 h respectively, and were fed adaptively for 7 d. Keep the room temperature at 22°-25°, indoor humidity 55 %-60 %, to ensure that mice are free to eat and water. All animal experiments were approved by the Ethics Committee of our hospital. The feeding environment, body weight and week age of mice were no difference ($p>0.05$). The electronic balance (Mettler-Toledo International Co., Ltd.,); high temperature and low speed centrifuge (Shanghai Beckman Kurt International Trading Co., Ltd.,); automatic biochemical analyzer (Shanghai Jumu Medical Devices Co., Ltd.,); optical microscope (Shanghai Tielun Optical instrument Co., Ltd.,); Stereo Positioner (Beijing Youcheng Jiaye Biotechnology Co., Ltd.,) and automatic biological tissue slicer (Beijing Jiayuan Xingye Technology Co., Ltd.,). Experimental reagents are Fetal Bovine Albumin (FBA) serum (Shanghai Wenren Biotechnology Co., Ltd.,); NOD receptor kit (Shanghai Future Industrial Co., Ltd.,) and NF-κB, TNF detection kit (Shanghai Jingkang Bioengineering Co., Ltd.,). All mice were screened by water maze before the experiment, and the learning and memory ability of mice was tested by Morris water maze. In positioning navigation experiment, the mice were put into the water in four quadrants head down, and the escape latency, i.e. the time it took the mice to find the platform within 2 min, was recorded. When the mice find the platform within 2 min, the mice should be given traction by the experimenter and stay on the platform for 10 s. Record the escape lurking for 2 min. This was done for twice a day for 5 d, pay attention to the warmth of mice. In space exploration experiment, the platform was removed on the 6th d of the experiment, and the mice were put into the water in four quadrants, and the number of times the mice crossed the original platform in 2 min was recorded. After screening, the mice with congenital dementia and poor swimming posture were excluded. Treatment of Aβ including the 500 g of Aβ1-42 in powder state was dissolved with aseptic normal saline, shaken well by centrifuge and incubated in an incubator at 37° for 7 d. All mice were anesthetized by intraperitoneal injection of 10 % chloral hydrate, the needle was slowly inserted into the abdominal cavity, the medicine was pulled back, and attention was paid to whether it was mistakenly into the intestine. When the resistance became small, there was no return of blood and stomach contents, and the anesthetic was injected. 300 s later, the mice showed paralysis and apathy, and the tail of the mice was pulled with hemostatic forceps and fixed on the brain stereotactic instrument when the mice did not resist. In the course of the experiment, the body temperature of mice was maintained at 37° and the laboratory temperature was maintained at 25°, which reduced the mortality of mice. The AD mouse model was established by localized injection of Aβ1-42 into the bilateral hippocampus of mice. The mice were fixed on the brain stereotactic instrument, and the hair on the top of the mice was removed. The mice were disinfected with iodine, and their skin was cut open. The bilateral hippocampal CA1 area was used as the injection area, and the anterior halogen was used as the starting point. The skull group was drilled with a thick needle to expose the dura mater, and 1 μg/μl Aβ solution was injected into the hippocampus for 10 min to make it fully dispersed in the hippocampus. After the needle was removed, the skull wound was closed with bone wax and sutured, and gentamicin sulfate was used for disinfection in the surgical area. After the modeling was completed, the mice were put into a warm environment to revive them. Mice were raised in separate cages and injected with penicillin once a

day for 5 d to prevent infection. Again, the mice were tested by Morris water maze to judge the short-term memory ability of mice. Mice were randomly divided into 5 groups including the control group (group A), sham operation group (group B), model group (group C) and high (group D) and low dose (group E) ADPN groups with 12 mice in each group. Normal saline was given to the group A, B, C, D and E group, once a day. Group D and E were given ADPN (10 mg/kg, 2.5 mg/kg) intragastric administration. After 21 d of intragastric administration, the mice were treated with Morris water maze test for 6 d. The shorter the incubation period, the stronger the learning ability, and the longer the incubation period means the weaker the learning ability. The experimental latency and jumping times of different groups of mice were recorded; the expressions of NOD2, NF-κB and TRL-4 related pathways in different groups of mice were compared; the levels of TNF-α, IL-1β and other related inflammatory factors in each group of mice were detected and the apoptosis of related proteins was detected by Western blotting, and the expression of B Cell Lymphoma-2 (Bcl-2), caspase-3 and cleaved (c) caspase-3 was detected. In this study, Statistical Package for the Social Sciences (SPSS) 20.0 statistical software was used for statistical analysis, and the measurement data were expressed in the form of mean±standard deviation (variance x±s) with t-test. The experimental latency and jumping times between the group A and B were no difference. Compared with the group B, the experimental latency of the group C increased and the number of diving decreased. Compared with the group C, the experimental latency of the D and E groups decreased, and the number of diving increased (Table 1). The NOD2, NF-κB and TRL-4 between the group A and B were no difference. Compared with the group B, these in the group B was increased, and these pathways in the group D and E was lower than that in the group C (Table 2). The TNF-α and IL-1β between the group A and B were no difference. Compared with the group B, these in the group C were raised, and in the groups D and E were reduced than the group C (Table 3). The caspase-3, c-caspase-3 and Bcl-2 between the group A and B were no difference. Compared with the group B, the caspase-3 and c-caspase-3 in the group C was raised and the Bcl-2 was reduced, while the caspase-3 and c-caspase-3 in the group D and E were decreased than that in the group C, and the Bcl-2 was increased than that in the group C (Table 4). AD is a common polygenetic disease in the elderly, which involves many aspects of participation, and its pathogenesis is phased. Senile plaque is the most characteristic pathological change of AD, and Aβ constitutes the most important component of senile plaque, and the abnormal accumulation of Aβ is the main pathological feature of AD[8]. Aβ is the core pathogenic substance of AD, which exists in amyloid precursor protein and is mainly expressed in neurons and microglia of the central nervous system[9]. Recent studies have found that there are a variety of activated neurokeratinocytes and inflammatory factors near the senile plaque in the brain of patients with AD, so it is judged that there is persistent chronic inflammation in the brain of patients with AD. The above inflammatory reaction is the main influencing factor of neurodegenerative disease in patients with AD[10]. NOD2 is mainly expressed in mammalian macrophages, monocytes and granulocytes. It cannot only recognize foreign infections and invasions, but also activate cellular defense function and inflammatory response[11]. NF-κB plays an important role in immune response and inflammatory stimulation. The increase of TNF-α will lead to a series of intracellular responses that affect the expression of NF-κB[12]. NF-κB and IκB bind to each other and exist in the cytoplasm in an inactive state, but due to the activation of inflammation, IκB presents a state of phosphorylation, which makes NF-κB free and transferred to the nucleus, affecting the binding of κB finally, the feedback loop aggravates the inflammatory process[13]. It has been found that the presence of cerebellar glial cells plays critical role in the progression of AD patients[14]. Cerebellar glial cells are the main immune cells in the brain, which have both advantages and disadvantages in the development of AD. In the early stage of the disease, cerebellar glial cells can secrete neurotrophic factors, which can clear and phagocytize Aβ, but after the aggravation of AD, cerebellar glial cells near senile plaques are over-activated, resulting in a large number of inflammatory factors[15]. The release of a large number of inflammatory factors will have a toxic effect on neurons, resulting in neuronal necrosis and injury; it can also further activate cerebellar glial cells, trigger a vicious circle, aggravate

neuronal damage and eventually lead to the deterioration of the disease[16]. This study found that, compared with the group C, the experimental latency of group D and E decreased, the number of diving increased, indicating that ADPN can enhance the learning ability and memory ability of mice. Compared with the group C, the NOD2, NF-κB and TRL-4 in the group D and E of ADPN decreased, indicating that the use of ADPN can block the expression of NOD2, NF-κB and TRL-4, and slow down the aggravation of the disease. Compared with the group C, the TNF-α and IL-1β in the group D and E decreased, suggesting that ADPN can inhibit the release of inflammatory factors and reduce the level of inflammatory factors in patients with AD. Compared with the group C, the caspase-3 and c-caspase-3 in the group D and E decreased, while the Bcl-2 increased, indicating that ADPN can enhance the expression of Bcl-2, thus inhibit the expression of caspase-3 and c-caspase-3. To sum up, exogenous ADPN can enhance the learning and memory ability of patients with AD, affect the expression of NOD2, NF-κB, TRL-4 and other pathways, and reduce the levels of TNF-α and IL-1β, so as to inhibit the inflammatory response of nerves in the brain, reduce the damage to nerve function, and provide new therapeutic ideas for the prevention and treatment of AD.

TABLE 1: COMPARISON OF MORRIS WATER MAZE TEST (x±s)

Group	n	Experimental incubation period	Number of jumping platform
A	12	25.22±15.42	17.45±8.61
B	12	28.74±12.47	13.47±9.24
C	12	55.78±18.64#	4.35±3.15#
D	12	37.15±5.95*	10.02±5.68*
E	12	41.62±6.32*	8.23±4.21*

Note: *p>0.01 and #p>0.05

TABLE 2: COMPARISON OF SIGNAL PATHWAYS (x̄±s)

Group	n	NOD2	NF-κB	TRL-4
A	12	0.58±0.05	0.38±0.05	0.67±0.07
B	12	0.61±0.04	0.41±0.04	0.62±0.06
C	12	1.22±0.12#	0.88±0.06#	1.16±0.11#
D	12	0.79±0.06*	0.52±0.03*	0.71±0.02*
E	12	0.91±0.05*	0.63±0.04*	0.82±0.04*

Note: *p>0.01 and #p>0.05

TABLE 3: COMPARISON OF INFLAMMATORY FACTORS ((x±s), ng/l)

Group	n	TNF-α	IL-1β
A	12	20.14±3.56	103.18±9.45
B	12	19.45±5.34	110.65±11.35
C	12	108.78±13.47#	318.78±44.35#
D	12	59.48±8.63*	153.07±26.15*
E	12	75.44±9.41*	254.36±26.78*

Note: *p>0.01 and #p>0.05

TABLE 4: THE EXPRESSION OF RELATED PROTEINS (x±s)

Group	n	Caspase-3	c-Caspase-3	Bcl-2
A	12	1.02±0.08	1.01±0.12	1.03±0.08
B	12	1.05±0.03	1.03±0.07	1.01±0.10
C	12	1.56±0.19#	1.68±0.21#	0.45±0.02#
D	12	1.15±0.02*	1.18±0.04*	0.85±0.05*
E	12	1.35±0.08*	1.35±0.05*	0.62±0.08*

Note: *p>0.01 and #p>0.05

Conflict of interests:

The authors declared no conflict of interests.

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