Anti-inflammatory Injury Mechanism of Rosuvastatin Calcium on Alzheimer's Disease Model Mice

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Abstract: Alzheimer's disease (AD) is a common age-related degenerative disease of the central nervous system. It is characterized by progressive memory impairment, emotional personality and behavioral changes. The pathogenesis is unknown and the treatment effect is not good. With the development of society, the aging of the population is increasing, and the prevalence of AD is gradually increasing. According to the World Health Organization, by 2020, AD will become the fourth disease in China's disease burden. Therefore, raising awareness of AD has great social significance. A number of studies have shown that a large number of activated microglia and astrocytes can be seen around the core and senile plaques (SP) in the brain. Non-steroidal anti-inflammatory drugs can reduce the activation of glial cells and reduce nerves. The damage of the element plays a neuroprotective role. With the deepening of research, most scholars believe that the inflammatory response induced by Aβ deposition is the main mechanism leading to the pathogenesis of AD. How to reduce inflammatory damage becomes an important way to treat AD.

1. Introduction

Alzheimer's Disease (AD) is a common age-related neurodegenerative disease common in the elderly, with progressive memory and mental retardation, personality changes, and behavioral abnormalities as the main clinical manifestations. As the society ages, its incidence rate increases year by year. The prevalence rate of people over 65 years old is about 5%, and the prevalence rate of people over 85 years old rises to 30%, which brings a heavy burden to society and families. The main pathological changes of AD include the formation of senile plaques (SP), neurofibrillary tangles (NFTs), neuronal reduction, and vacuolar degeneration and vascular amyloidosis in hippocampal pyramidal cell. And the pathogenesis is not very clear. At present, the mechanism of pathogenesis of AD mainly includes Aβ as the core of the inflammatory cascade, cholinergic loss, synaptic dysfunction, etc. In recent years, with the deepening of research on the pathogenesis of AD, most scholars believe that chronic inflammatory reaction in the brain may be one of its important pathologies, and studies have shown that non-steroidal anti-inflammatory drugs can alleviate glial cells in AD rats. Activation to reduce neuronal damage plays a neuroprotective role, so the inflammatory response theory has received more and more attention, and has become a hot spot in AD research. A number of studies have shown that there are inflammatory reactions in the AD brain, Toll like receptors (TLRs) are involved, and Aβ can up-regulate the expression of TLRs. Microglia are mononuclear macrophages with phagocytic defense function in brain tissue, and their activation is involved in the development of inflammatory reactions. Studies have confirmed that Aβ activates microglia by recognizing CD14, TLR4, and TLR2. Activation of Toll like receptors (TLRs)/nuclear factor kappa B (NF-κB) signaling pathway may aggravate inflammatory response and brain damage. Therefore, the study of drugs to inhibit the activation of TLRs signaling pathway is an important target for clinical treatment of AD.

2. Materials and methods

Healthy adult clean grade SD male rats, aged 4 months and weighing 250±50g, were provided by

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Experimental Animal Center of Hebei Medical University. All rats were adapted to ventilation, light, free feeding and water intake. Feed for 1 week. According to the random number table method, 32 healthy male Sprague-Dawley rats were randomly divided into AD model group (AD group), sham group (Sham group), atorvastatin calcium low dose group (Ator-L group), Ato The high dose of statin calcium (Ator-H group), 8 in each group.

Atorvastatin calcium was provided by Pfizer. It was certified by the Department of Pharmacology of Hebei Medical University to meet the requirements of the Pharmacopoeia. The quality was qualified and dissolved in 0.9% physiological saline at a concentration of 3 mg/ml.

The equivalent dose is converted according to the "comparative experimental animal to human body surface area ratio (R) table". The high dose atorvastatin calcium group was administered at a dose of 20 mg/kg, and the low dose group was administered at a dose of 5 mg/kg. The AD model group and the Sham group were given only the corresponding volume of normal saline, and the drug intervention was performed for 3 weeks, once a day.

Aβ1-42 (Sigma-Aldrich, St Louis, MO, USA) was diluted to a solution with a solubility of 2 μg/μl using 0.9% sterile physiological saline, and incubated in a 37 ° C incubator for 1 week to become condensed. Neurotoxic Aβ1-42. After 10% chloral hydrate (0.3ml/100mg) was anesthetized by intraperitoneal injection, the rats were fixed on the brain stereotaxic instrument, and referenced by Bao Xinmin's "Cone Stereotactic Map of Rat Brain": 3mm after the anterior iliac crest, 2mm in the median sagittal suture, 3.5mm in the subdural, ie the hippocampus of the SD rat. The AD model group, the low-dose group and the high-dose group were injected into the bilateral hippocampus of rats with 2.5 μl of condensed Aβ1-42, and the Sham group was injected with the same amount of normal saline. The injection time of each side was not less than 5 min. 5 min, slowly withdraw the needle to prevent the drug from overflowing through the pinhole. After the injection, the paraffin blocks block the pinholes, suture the scalp, and disinfect the local iodophor to prevent infection. The Morris water maze is an experimental device designed by British psychologist Morris in the early 1980s and applied to the study of brain learning and memory mechanisms. It consists of a circular pool and an automatic video analysis system, which is widely used in AD research. The test procedure mainly includes two parts: positioning navigation test and space exploration test. The water maze is a circular pool with a diameter of about 200 cm and a height of 50 cm. The inner wall of the pool is black with a water depth of about 25 cm and a water temperature of about 20 °C. In order to ensure the validity and accuracy of the experiment, the illumination in the room is kept constant, and the sound and light are not stimulated in the pool. The rat platform (diameter 12 cm, height 23 cm) was placed approximately 35 cm from the wall of the pool and 2 cm below the surface of the water.

3. Results

In the Morris water maze experiment, the escape latency of the four groups of rats was compared with $F=2.183$, $P<0.05$, and the number of crossing platforms was $F=12.908$, $P<0.05$, which were statistically significant. Compared with the Sham group, the escape latency of the AD group was significantly prolonged, and the number of crossing platforms was significantly reduced ($P < 0.05$). Compared with the AD model group, the escape latency of the high-dose Atorva group was significantly shortened, and the number of crossing platforms increased significantly, and there was statistical significance ($P<0.05$). The low-dose Atorva group improved compared with the AD model group, but the difference was not statistically significant ($P>0.05$).

In the Sham group, the number of pyramidal cells in the hippocampal CA1 region was large, neatly arranged, normal in morphology, dense in structure and evenly distributed. The number of pyramidal cells in the hippocampal CA1 region of the AD model group was significantly reduced, the arrangement was sparse, the structure was disordered, and the morphology of the nucleus was irregular. Compared with the AD model group, the number and morphology of hippocampus in the rats in the Avalanche intervention group recovered to different degrees. The high dose group was the most obvious, the number of pyramidal cells increased, the cells were arranged neatly, and the morphology was normal. Evenly.

The immunohistochemical results showed that the number of immunoreactive cells of Iba-1, TLR4, TRAF6 and NF-κB in the hippocampus CA1 region of the four groups was $P<0.05$, and the difference was statistically significant. Compared with the Sham group, the number of immunopositive cells in the AD model group was significantly increased ($P<0.05$); the number of Iba-1, TLR4, TRAF6 and NF-κB immunoreactive cells in the high dose of atorvastatin calcium was significantly lower than that in the AD model group. The difference was statistically significant ($P<0.05$). The number of immunopositive cells in the low-dose group was lower than that in the AD group, but there was no statistical difference ($P>0.05$).

Western blot results were semi-quantitatively analyzed using Quantity One image analysis software in the Gel Doc gel imaging system. The gray values of GAPDH, TLR4, TRAF6 and NF-κB were analyzed by Quantity One image analysis software, and TLR4 was calculated. , TRAF6 and NF-κB and the corresponding GAPDH band gray value ratio, and statistical analysis. Western Blot results showed that the protein expressions of TLR4, TRAF6 and NF-κB in AD group were higher than those in Sham group ($P<0.05$). The protein expression of TLR4, TRAF6 and NF-κB was decreased after Atorva intervention. The difference between the group and the AD group was statistically significant ($P<0.05$). There was no significant difference between the low dose group and the AD group ($P>0.05$).

4. Discussion

AD is one of the diseases that seriously endanger the health of the elderly, and its pathogenesis is still unclear. It is essential to establish an animal model during the research of AD, and it is also the prerequisite and key to the success of the experiment. Therefore, the ideal animal model of Alzheimer's disease is of great significance for studying the pathogenesis and treatment of the disease. At present, several influential modeling methods at home and abroad include: (1) animal models of natural aging cognitive impairment, which can appear similar to cognitive impairment in AD patients, but the model The rat is poor in body quality and easy to die. It is not suitable for long-term experiments, and the model is expensive, so its application is limited. (2) Damage model, including the model of breaking the hippocampus parasitic pathway, the aluminum poisoning model, the chronic ischemic dementia model, etc., such models can not appear pathological changes such as SP and NFT during the pathogenesis of AD. (3) AD transgenic animal model, which can have pathological features such as SP and NFT, but it can not fully simulate the pathological and behavioral changes of AD, and it is expensive and difficult to breed in large quantities. (4) AD animal model was injected into the bilateral hippocampus by injection of Aβ. This modeling method is based on the important role of Aβ in AD to simulate the pathological and behavioral changes of AD. At present, more attention is paid in the research field of AD. And adoption. Its advantages are: simple and convenient operation, less damage to experimental animals, good experimental repeatability and controllability. In recent years, more and more studies have shown that Aβ1-42, as the main component of senile plaques (SP) in AD, plays a key role in the activation of microglia. Aβ is a peptide of β-precursor protein (APP) which is hydrolyzed by β-secretase and γ-secretase, and mainly includes two molecules, Aβ1-40 and Aβ1-42. Among them, Aβ1-42 is considered to be in the inflammatory reaction and amyloid. It plays an important role in the formation of plaques. The specific mechanism may be: excessive Aβ aggregation eventually forms insoluble fibrous filaments and amyloid deposits, and Aβ may be an stimulator of inflammatory response in AD, leading to activation of glial cells, releasing a large amount of inflammatory mediators, the latter It also acts on neurons, activates the corresponding receptors, and undergoes a series of extracellular intracellular signaling, which ultimately leads to neuronal degeneration and necrosis. Therefore, the injection of Aβ1-42 into the bilateral hippocampus is a scientific and effective method for making animal models of AD. It can comprehensively simulate the pathological and behavioral changes of AD, and it is easy to operate.

5. Conclusion

The AD model was made by injecting condensed Aβ1-42 into the hippocampus of bilateral rats. The rats experienced learning and memory loss and corresponding pathological changes, indicating that the AD model was successfully produced. The expression of TLR4, TRAF6 and NF-κB in the AD model group increased significantly, indicating the presence of inflammatory response in the AD model, and the TLR4/NF-κB signaling pathway is involved in the pathogenesis of AD. Atorvastatin calcium can improve the learning and memory ability of AD rats and reduce the death of neurons, indicating that it has neuroprotective effect on AD.

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