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Investigation on the Specific Pathogenesis of Alzheimer's Disease Model Mice Induced by Subchronic Aluminum Exposure

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Abstract: This experiment sought to investigate the pathogenesis of Alzheimer's due to subchronic aluminum exposure by comparing its impact on mouse brain α-, β-, and γ-secretases. Thirty SPF mice were split into a normal group, a medium-dose group (0.30ml aluminum maltol solution per mouse daily), and a high-dose group (0.45ml). They were intraperitoneally injected for 30 days. Utilizing the obvious neurotoxic effect of aluminum maltol [1], different degrees of poisoning were caused by injecting different doses of aluminum maltol, and the memory ability of mice was evaluated using the Y-maze water test[2], The Y-maze water test evaluated memory. Afterward, serum and brain tissue were sampled for biochemical and enzyme assays. Y-maze results showed that higher aluminum exposure led to longer times and distances for mice in water. Group differences post-exposure and within the medium-dose group were significant. TG, TC, TP, and brain protein levels varied among groups. Regarding secretases, β-secretase differed from the normal group ($P < 0.01$), α-secretase from the high-dose group ($P < 0.05$), and γ-secretase from the normal group ($P < 0.05$). In conclusion, aluminum may trigger Alzheimer's via secretase regulation, and γ-secretase's role requires further exploration.

1. Introduction

Alzheimer's disease (AD), as a neurodegenerative disease of the central nervous system induced by multiple factors, is generally regarded as a disease caused by the joint action of genes and environmental factors. Among many environmental influence factors, aluminum element has received increasing attention due to its potential high risk[3].

Alzheimer's disease is mainly manifested by memory impairment, degradation of behavior and

social ability, and has become one of the most common diseases in the world today. When aluminum enters the human body, due to the imperfect absorption mechanism of the human body for it, aluminum will combine with transferrin and then cross the blood-brain barrier and easily deposit in brain tissue. It is worth noting that amyloid protein (β-amyloid, Aβ) can form extracellular senile plaques (SP), while abnormally phosphorylated microtubule-associated protein tau aggregates in neurons to form neurofibrillary tangles (NFT). These two constitute the most crucial pathological features of AD.

Among the many theories on the pathogenesis of AD, the theory of generation and metabolic disorder of Aβ protein is quite persuasive. [4] This theory clearly states that through the catalytic action of β-secretase on the precursor protein of β-amyloid precipitated protein, the β-amyloid precipitated protein fragment (containing 40 or 42 amino acid fragments, and its secondary structure is transformed from the α conformation to the β-shape for unknown reasons and then precipitates) is decomposed and generated. The formed precipitate gradually accumulates to form senile plaques, which has an adverse effect on nerve conduction function. Although experiments have shown that aluminum has an impact on the activities of brain α, β, and γ-secretases, so far there is no conclusive proof. In view of this, this project is dedicated to further exploring this issue. Modeling is carried out using different doses of maltol aluminum, the activities of brain α, β, and γ-secretases are measured, and combined with the results of the water maze behavior experiment, a comprehensive comparative analysis is carried out.

2. Experimental Materials and Methods

2.1. Experimental Materials

2.1.1. Experimental Animals

Thirty SPF-grade mice were provided by Changsha Tianqin Biotechnology Co., Ltd. with license number: SCXK(Xiang)2019-0013. They had similar activity abilities and a body weight of about 30 grams. They were evenly divided into three groups: normal group (10 mice), medium-dose group (10 mice), and high-dose group (10 mice). The mice were weighed and marked. In the medium-dose group (10 mice, each injected with 0.30 ml of maltol aluminum solution per day), and in the high-dose group (10 mice, each injected with 0.45 ml of maltol aluminum solution per day).

2.1.2. Reagents and Drugs

Aluminum chloride, maltol, sodium chloride, 95% ethanol, physiological saline, o-toluidine, thiourea, boric acid, glacial acetic acid, acetylcholinesterase (AchE) test kit, triglyceride (TG) test kit, protein test kit (biuret method), total cholesterol (TC) test kit, protein test kit (Coomassie brilliant blue method), urea nitrogen test kit (urease method), glucose test kit (oxidase method), β-secretase (β-Secretase) test kit, α-secretase (α-Secretase) test kit, γ-secretase (γ-Secretase) test kit, etc.

2.1.3. Main Instruments

SpectraMax M5 microplate reader, micropipette, high-precision dispenser, constant temperature water bath, centrifuge, refrigerator, etc.

2.2. Experimental Methods

2.2.1. Toxicity Exposure Method

Except for the normal group, the other groups were injected intraperitoneally with maltol aluminum solution. In the medium-dose group (10 mice, each injected with 0.30 ml of maltol aluminum solution per day), and in the high-dose group (10 mice, each injected with 0.45 ml of maltol aluminum solution per day). The injection was continued for 6 days, followed by a 1-day rest. The modeling injection ended after 30 days. All utensils, such as mouse cages and water bottles, do not contain aluminum products.

2.2.2. Observation of the basic state of animals and collection of materials

During the experiment, the physical signs of mice were observed daily before administration, including abnormal behaviors, deaths, feces, hair loss, food intake, emaciation and other conditions. In the early, middle and late stages of the experiment, the Y-shaped water maze experiment was conducted to measure and record the swimming time. At the same time, the hemoglobin Hb value was measured in the early, middle and late stages of the experiment. At the end of the experiment, blood was taken from the eyeballs for serum separation, and indicators such as ALT, GPT, BUN, TC, TG, and TP in the serum were measured. The mice were sacrificed and the brain was removed.

2.2.3. Test of learning and memory ability of mice and Y-shaped water maze experiment

The construction of the Y-shaped water maze model and the specific experimental method refer to the literature. The purpose is to test the memory function of mice through this experiment. Ten days before establishing the aluminum poisoning model in mice, train the mice's memory of the water maze first. Each mouse is trained three times a day, and then measured continuously for three days. Record the landing time, number of errors, and number of failures of the mice, and calculate the overtime rate, error rate, and failure rate of the mice. If the mouse fails to land correctly within 10 seconds, it is overtime. If the mouse swims in the wrong direction, it is an error. If the mouse fails to land correctly within 40 seconds, it is a failure. Test respectively in the early, middle and late stages of establishing the aluminum poisoning model in mice. Measure continuously for three days in each period. Test each mouse three times a day and record the test data.

2.2.4. Determination of various biochemical indexes in mouse serum

TC is determined by COD-CE-PAP; TG is determined by GPO-OAO method; urea nitrogen is determined by urease Boer's method; AchE is determined by colorimetry. According to the fact that acetylcholine produces acetic acid and choline under the catalysis of AchE, and choline then reacts with sulfhydryl chromogenic agent to produce yellow compound TNB (sym-trinitrobenzene). The specific operations are in accordance with the kit instructions.

2.2.5. Preparation of 10% brain homogenate of mice and determination of secretase

The mice were sacrificed by cervical dislocation. The brain tissue was taken, and the surface blood was washed away with 0.01M, pH7.4 phosphate buffer solution. Then, the excess water was gently absorbed with filter paper. After weighing on a balance (g), the brain was placed in a glass homogenizer, and an appropriate amount of phosphate buffer (0.01M, pH = 7.4) was added. The grinding was carried out for 10 minutes under ice bath to prepare 10% brain homogenate. The activities of β-secretase, α-secretase and γ-secretase in mouse brain homogenate were determined by double antibody sandwich method (ELISA detection method). The detailed operation was in

accordance with the instructions.

2.3. Statistical processing: spss13 software.

Perform analysis of variance on the detected data. The results are expressed as (±s). The data between groups are compared with each other. If the analysis result is P<0.05 or P<0.01, the difference is statistically significant.

3. Results

3.1. Determination results of brain β-secretase, α-secretase, and γ-secretase (u/L) in each group of mice (see Table 1).

Table 1: Comparison of brain β-secretase, α-secretase, and γ-secretase (u/L) in each group of mice (±S).

Group	Brain β-secretase 1	Brain α-secretase	Brain γ-secretase
Normal group	10.11±1.69	21.62±2.03▲	11.60±2.46
Medium-dose group	12.55±1.43▲▲	20.68±1.09	10.83±1.70
High-dose group	11.52±1.32	19.71±1.10	9.18±1.86▲

Note: Analysis of variance: Comparison between groups: For brain β-secretase: F = 4.880, P = 0.021. Compared with the normal group, ▲▲P < 0.01, and the difference is statistically significant. For brain α-secretase, F = 2.582, P = 0.099. Compared with the high-dose group, ▲P < 0.05, and the difference is statistically significant. For γ-secretase: F = 2.227, P = 0.133. Compared with the normal group, ▲P < 0.05, and the difference is statistically significant.

3.2. Determination results of water maze test time (s) of each group of mice before, during and after exposure

The results showed that before exposure, there was no statistically significant difference between groups. During exposure (s), there was no statistically significant difference. After exposure (s), there was statistically significant difference. Comparison within groups in the early, middle and late stages: For the medium-dose group, there was statistically significant difference. See Table 2.

Table 2: Comparison of water maze test time (s) before, during and after poisoning in each group of mice ($\bar{x}$ ±S)

Group	Number of animals.	Before exposure (s)	During exposure (s)	After exposure (s)
Normal group	10	3.80±1.10	3.35±0.47	3.66±0.89
Medium-dose group	10	4.94±1.84[a]	4.51±2.13[a]	12.36±10.26▲
High-dose group	10	4.39±2.33	4.39±289	7.52±4.68

Note: Analysis of variance: Comparison between groups: Before exposure, comparison between groups showed F = 0.992, P = 0.384, P > 0.05, and there was no statistically significant difference. During exposure (s), F = 1.638, P = 0.216, P > 0.05, and there was no statistically significant difference. After exposure (s), F = 3.351, P = 0.061. Compared with the normal group, ▲ < 0.05, and the difference is statistically significant.

Comparison within groups in the early, middle and late stages: For the normal group: F = 1.146, P = 0.334, P > 0.05. There is no statistically significant difference in the comparison between the early, middle and late stages. For the medium-dose group: F = 3.365, P = 0.051. Compared with after

exposure, a < 0.05, and the difference is statistically significant. For the high-dose group: F = 1.635, P = 0.224, P > 0.05. There is no statistically significant difference in the comparison between the early, middle and late stages.

3.3. Determination results of serum urea nitrogen, total protein, and TC in each group of mice

The results show that there is no statistically significant difference in urea nitrogen; there is a statistically significant difference in total protein; there is a statistically significant difference in TC. See Table 3.

Table 3: Comparison of serum urea nitrogen, total protein and TC in each group of mice ($\bar{x} \pm S$)

Group	Urea nitrogen (mmol/L)	Total protein (g/L)	TC (mmol/L).
Normal group	2.72±3.41	64.00±0.06	2.58±0.57
Medium-dose group	3.20±0.63	59.00±0.03▲	2.92±0.94
High-dose group	3.03±0.44	57.00±0.03▲	3.78±1.39▲

Note: Analysis of variance: Comparison between groups: For urea nitrogen: F = 2.188, P = 0.137, there is no statistically significant difference. For total protein: F = 4.530, P = 0.023. Compared with the normal group, ▲P < 0.05, there is a statistically significant difference. For serum TC: F = 2.857, P = 0.080. Compared with the normal group, ▲P < 0.05, there is a statistically significant difference.

3.4. Results of serum TG, brain acetylcholinesterase (AchE), and brain protein measurements in each group of mice.

The results showed that for TG, there was statistically significant difference in comparison between groups. For brain acetylcholinesterase (AchE): there was no statistically significant difference in comparison between groups. For brain protein, there was statistically significant difference in comparison between groups. See Table 4.

Table 4: Comparison of serum TG, brain acetylcholinesterase AchE and brain protein in each group of mice (±S)

Group	TG(mmol/L)	AchE(U/mg.prot	Brain protein (mg/ml).
Normal group	2.95±0.98	0.19±0.02	26.47±1.80
Medium-dose group	2.95±0.98	0.17±0.04	27.75±2.56
High-dose group	1.64±0.46▲▲	0.18±0.02	29.34±0.82▲

Note: Analysis of variance: Comparison between groups: For TG: F = 7.454, P = 0.004. Compared with the normal group, ▲▲P < 0.01, and the difference is statistically significant. For brain acetylcholinesterase (AchE): P > 0.05, and there is no statistically significant difference. For brain protein: F = 3.507, P = 0.049. Compared with the normal group, ▲P < 0.05, and the difference is statistically significant.

4. Discussion

Aluminum, as the metal element with the highest abundance in the earth's crust, is widely used in many fields, which greatly increases people's chances of contact with aluminum. Since aluminum is not an essential element for the human body and has been identified as a key risk factor for inducing neurodegenerative diseases such as Alzheimer's disease (AD)[5], it has long been the focus of academic attention. Alzheimer's disease is a neurodegenerative disease of the central nervous system

characterized by progressive cognitive and memory impairment. As the disease progresses, a series of symptoms of damage to the central nervous system such as executive dysfunction, language impairment, and mental abnormalities will appear one after another[6].

In this study, we observed that after different doses of aluminum maltol were injected intraperitoneally into mice, the time spent by the mice in exploring the Y-shaped water maze subsequently increased significantly, and the swimming distance also became significantly longer. This phenomenon clearly indicates that subchronic intraperitoneal injection of aluminum maltol poisoning has caused severe damage to the spatial memory and learning ability of mice. This result is highly consistent with the conclusions of previous studies. As a classic method recognized by the academic community for measuring the learning and memory ability of animals, the changes in swimming distance, required time, and error frequency of the Y-shaped water maze test can intuitively and effectively reflect the spatial positioning learning ability of experimental animals[7-8].

A large number of studies have confirmed that aluminum has significant neurotoxicity. The impairment of learning and memory functions in humans and animals is closely related to the degree of aluminum exposure. Existing studies have also fully demonstrated that aluminum can cause irreversible nerve damage through multiple pathways[9-10]. In recent years, studies have further shown that aluminum can not only interfere with the normal metabolic process of nerve cells but also affect the synthesis, release, and uptake of neurotransmitters. For example, aluminum can inhibit the synthesis and release of acetylcholine in cholinergic neurons, and acetylcholine plays a key role in the learning and memory process. At the same time, aluminum can also induce oxidative stress in nerve cells, leading to the accumulation of reactive oxygen species in cells and damaging important biological molecules such as cell membranes, proteins, and nucleic acids. In terms of cell signal transduction, aluminum has been found to interfere with the normal function of calcium channels and affect intracellular calcium homeostasis, thereby affecting the normal physiological activities of nerve cells.

In addition, among many hypotheses about the pathogenesis, the hypothesis of the generation and metabolic disorder of Aβ protein has received extensive attention and in-depth exploration from researchers. Aluminum can promote the aggregation of β-amyloid protein (Aβ) in the brain, and α-secretase, β-secretase, and γ-secretase in the brain are closely related to the generation process of Aβ protein[4].Aβ is generated from amyloid precursor protein (APP) under the action of enzymes. Under normal circumstances, APP is cleaved by α-secretase between residues 16 and 17, so that most of APP is divided into soluble α-secretions (α-APPs) and the C-terminal residue (C-83) located in the membrane[11], which can effectively prevent the accumulation of a large amount of Aβ in the brain. It has been confirmed that three membrane-fixed zinc ion-dependent metalloproteases, ADAM9, ADAM10, and ADAM17 (TACE), have α-secretase activity[12]. However, in the brains of AD patients, the expression levels of these three α-secretase subtypes all show a downward trend, especially the expression level of ADAM10 decreases the most significantly. β-secretase has both key and rate-limiting dual roles in the biochemical reaction of APP generating Aβ[13]. It competes with α-secretase for the substrate APP. In the brains of AD patients, the content of α-secretase is significantly reduced. This situation makes β-secretase take an advantageous position in the competition, which in turn leads to the accumulation of Aβ in the brain[14].As the only protease with β-secretase activity in the body, β-secretase-1 (BACE1). Relevant studies have shown that in the frontal cortex with Aβ deposition, the expression level of BACE1 reaches three times that of the cerebellum without Aβ deposition. This fully proves that BACE1 plays a crucial role in the generation process of Aβ. γ-secretase is the last shear enzyme for Aβ generation. Its complex is composed of presenilin (PS) heterodimer, transient transmembrane protein nicastrin (NCT), anterior pharynx defective 1 (APH1), and presenilin enhancer-2 (PEN2).

Existing research results have shown that aluminum maltol can cause the expression levels of nicastrin protein and presenilin 1 protein, the main active units of γ-secretase in the cerebral cortex and hippocampus of rats, to increase, thus also increasing the content of Aβ in the cerebral cortex and hippocampus of rats[15].

In summary, subchronic aluminum exposure leads to a decrease in the expression of α-secretase in the brains of Alzheimer's disease model mice, which in turn causes an increase in the expression of β-secretase and an increase in the expression of nicastrin protein and presenilin 1 protein, the main active units of γ-secretase. Moreover, different degrees of subchronic aluminum exposure have different degrees of influence on the expression levels of these three enzymes. However, the exact underlying mechanism still needs to be further explored. The changes in the expression levels of these three enzymes in the brains of aluminum-induced Alzheimer's disease model mice ultimately lead to the accumulation of Aβ in the brain. If we can successfully explore an effective way to change the above changes of these three enzymes and develop an effective aluminum removal method, it will undoubtedly open up a new way for the prevention and treatment of AD and make a significant contribution to human health and well-being. Looking to the future, we still need to carry out more in-depth research work from the following key aspects: further clarify the detailed molecular mechanism of aluminum exposure affecting the expression of these three enzymes; deeply explore how to achieve precise regulation of the activities of these three enzymes to achieve the purpose of preventing and treating Alzheimer's disease; strictly verify the effectiveness and safety of our research results and treatment strategies in larger animal models and clinical trials.

5. Conclusions

In summary, subchronic aluminum exposure reduces α-secretase, increases β- and γ-secretase related proteins, causing Aβ buildup in the brains of Alzheimer's model mice. The exact mechanism needs further study. Future work should clarify how aluminum affects these enzymes at the molecular level, find precise control methods, and validate results and treatment safety in larger animal models and clinical trials. Altering enzyme changes and developing aluminum removal methods could aid AD prevention and treatment.

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