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A Comparative Study on the Pathogenesis of Alzheimer's Disease Induced by Subchronic Aluminum Exposure Using Tremella and Its Compound Polysaccharides

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Abstract: *This study compared the effects of tremella and its compound polysaccharides on subchronic aluminum-induced Alzheimer's disease (AD) in mice. Sixty mice were divided into normal, model (aluminum maltol-induced), tremella preparation (Treatment 1), and tremella compound polysaccharide (Treatment 2) groups. After modeling, treatments were administered via gavage while maintaining aluminum exposure. Memory was assessed using water maze tests, and brain enzymes (β-secretase 1, α/γ-secretase, AchE) and serum biochemical markers were analyzed. Model mice showed significantly reduced brain α-secretase ($P<0.05$ vs. normal), validating AD modeling. Treatment 2 demonstrated stronger reduction in γ-secretase than Treatment 1 ($P=0.008$). Both treatments normalized blood indices (e.g., urea nitrogen), suggesting mechanisms via regulating enzyme activities (e.g., β-amyloid cleaving enzyme) and biochemical pathways. While sharing similar anti-AD mechanisms, the two preparations exhibited distinct advantages in modulating specific markers. Results highlight tremella-based traditional Chinese medicine treatments as potential interventions for aluminum poisoning-related AD, warranting further study.*

Keywords: *Alzheimer's Disease; Aluminum Poisoning; Beta-Amyloid Cleaving Enzyme; Traditional Chinese Medicine Treatment*

1. Introduction

Alzheimer's disease (AD) is a relatively common degenerative brain disease that occurs in old age. With the development of the times, it has become a common clinical neurological activity disorder disease that affects activities. A large number of studies have shown that aluminum is related to Alzheimer's disease. Aluminum intake in animals can produce symptoms and brain damage similar to Alzheimer's disease. Therefore, aluminum poisoning is often used as a method to establish an animal model of Alzheimer's disease. There are many hypotheses about the pathogenesis of AD. Currently, the most accepted ones are the oxygen free radical theory, the cholinergic theory, and the aluminum poisoning theory. In order to seek better drug treatment for AD, this article uses aluminum maltol to poison mice to establish an animal model experiment of subchronic aluminum exposure. Conduct in-depth research and comparative exploration on the pathogenesis of Alzheimer's disease induced by subchronic aluminum exposure using tremella and its compound polysaccharides by combining changes in biochemical indicators of mouse brains and serum with changes in brain morphology. The

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results are reported as follows.

2. Experimental materials and methods

2.1 Experimental animals

Sixty healthy standard clean-grade mice, half male and half female, are produced and supplied by Changsha Tianqin Biotechnology Co., Ltd. for the following animal varieties. The production license numbers for experimental animals are Scxk (Xiang) 2019-0013, Scxk (Xiang) 2019-0014, and Scxk (Xiang) 2019-0015.

Animal grouping: The mice are divided into a normal group, a model group, treatment group 1 (tremella preparation group), and treatment group 2 (tremella compound polysaccharide preparation group). A total of 45 mice in the model group and treatment groups 1 and 2 are first modeled by intraperitoneal injection of aluminum maltol. After the aluminum maltol solution is prepared, it is sealed and stored at low temperature in a refrigerator for later use. Each mouse is intraperitoneally injected with 0.3 ml per day for 30 days. After 30 days of modeling, in treatment group 1, 2.5 ml of tremella preparation is taken daily and mixed with an equal amount of distilled water, and 0.3 ml of the mixed solution is administered to each mouse by gavage. In treatment group 2, 2.5 ml of tremella compound polysaccharide preparation ("Good Wakefulness") is mixed with an equal amount of distilled water, and 0.3 ml of the mixed solution is administered to each mouse by gavage. The dose of aluminum maltol injection remains unchanged after treatment until the end of the experiment.

2.2 Reagents and drugs

Aluminum chloride, maltol, sodium chloride, 95% ethanol, physiological saline, tremella, Good Wakefulness, 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 test kit, α-Secretase test kit, γ-Secretase test kit, etc.

2.3 Determination methods

The Y-shaped water maze is used to measure the memory of mice. The test method is in accordance with reference [1]. Pre-training is started for 10 days (d), and swimming training is conducted three times a day to make them familiar with the landing place and establish memory. After the training is completed and after one day of rest, the test is conducted: using a stopwatch to time, continuous testing is conducted for three days, three times a day. Calculate the time (s) from when the mice enter the water to reaching the landing place and the number of errors (taking the wrong route, calculate the error rate%). More than 10 seconds/time is considered overtime, (calculate the overtime rate%).

2.4 Determination of various enzymes in mice and various biochemical indexes of serum

At the end of the experiment, whole blood is taken from behind the eyeballs of mice, and serum is separated to determine blood biochemical indexes: total cholesterol (TC), triglyceride (TG), urea nitrogen, and total cholesterol (TC). The mice are sacrificed, and the brain tissue is ground with a glass homogenizer under ice bath. A 10% brain homogenate is made with physiological saline. Centrifuge at 3000 rpm for 10 minutes. Take the supernatant and store it in a refrigerator at 4-8°C for later use. Determine acetylcholinesterase (AchE), mouse brain secretory enzymes and the content of Al3+ in the mouse brain. TC is determined by CHOD-PAP; TG is determined by GPO-PAP method; urea nitrogen is determined by urease Berthelot method; serum total protein (TP) is determined by Coomassie brilliant blue method.

2.5 Statistical processing

The results were processed and analyzed by SPSS13.0 statistical software. The data were expressed as ($\overline{x}$ ±S), and analysis of variance and Q test were performed.

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3. Results

3.1 Survival status of mice in each group

General situation: The experiment started on April 15, 2024 and ended on June 23, 2024, lasting for 70 days. There were a total of 60 mice divided into four groups: 15 in the normal group, and no mice died; 15 in the model group, and two mice died; 15 in treatment group 1 (tremella preparation), and one mouse died; 15 in treatment group 2 (tremella compound polysaccharide preparation), and two mice died. Trained mice were used instead.

3.2 Comparison of biochemical determination results in each group shows that

Result analysis of brain secretory enzymes: The determination result of brain beta-secretase in the model group was significantly higher than that in the treatment group, and the difference was statistically significant (P<0.05). For brain alpha-secretase: F=6.7683, P=0.014. Compared with the normal group, the difference was statistically significant (P<0.05), indicating successful modeling. For gamma-secretase: F=4.461, P=0.008. There was a statistically significant difference between treatment group 1 and treatment group 2. See Table 1.

3.3 Comparison of biochemical determination results of each group shows

Analysis of brain secretory enzyme results: The determination result of brain β-secretase in the model group was significantly higher than that in the treatment groups (P < 0.05), and the difference was statistically significant. For brain α-secretase: F = 6.7683.958, P = 0.014. Compared with the normal group, the difference was statistically significant (P < 0.05), indicating that the modeling was successful. For γ-secretase: F = 4.461, P = 0.008. There was a statistically significant difference between treatment group 1 and treatment group 2, as shown in Table 1.

Table 1 Comparison of brain β-secretase, α-secretase, and γ-secretase (u/L) in mice of each group ($\overline{x}$ ±S)

Group	Brain β-secretase 1	Brain α-secretase	Brain γ-secretase
Normal group	11.76 ± 1.78	9.90 ± 2.80	10.03 ± 1.04
Model group	12.25 ± 1.06	8.32 ± 1.42[2)]	10.94 ± 1.17
Treatment group 1	11.21 ± 0.62[1)]	8,58 ± 0.92[2)]	10.85 ± 1.03[3)]
Treatment group 2	11.07 ± 1.01[1)]	7.68 ± 0.72[2)]	9.96 ± 0.86

Note: Analysis of variance 1) Compared with the model group, P < 0.05; 2) Compared with the normal group, P < 0.2.2.2 Comparison of brain aluminum Al3+ among groups, F = 3.408, p = 0.061. Compared with the model group: P < 0.05, and the difference was statistically significant; as shown in Table 2.

Table 2 Comparison of brain aluminum Al3+ content (μg/ml) in white mice ($\overline{x}$ ±S)

Group	Al3+ content (μg/ml)
Normal group	0.22 ± 0.01★
Model group	0.30 ± 0.07
Treatment group 1	0.24 ± 0.02★
Treatment group 2	0.23 ± 0.02★

Note: Analysis of variance ★Compared with the model group, P < 0.05.

Table 3 Comparison of water maze test times (s) of mice in each group before, during, and after poisoning ($\overline{x}$ ±S)

Group	Number of animals	Before poisoning (s)	During poisoning (s)	After poisoning (s)
Normal group	15	4.14 ± 0.64	3.73 ± 0.79	5.12 ± 1.37
Model group	15	3.96 ± 0.54	5.78± 1.97[1) a]	5.47 ± 1.13[a]
Treatment group 1	15	3.90 ± 0.58	5.41 ± 1.51[1)]	4.78 ± 1.57
Treatment group 2	15	4.52 ± 0.88[b]	6.68 ± 1.73[1)]	5.17 ± 1.16[b]

Determination results of water maze test times (s) of mice in each group before, during, and after

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poisoning. The results showed that during poisoning (s), F = 9.296, P = 0.000, 1) Compared with the normal group, P < 0.01, and the difference was statistically significant; comparison within the group before, during, and after: for the model group: F = 7.599, P = 0.008, aCompared with before poisoning: p < 0.01, and the difference was statistically significant; for treatment group 2, F = 10.727, P = 0.000, bCompared with during poisoning: p < 0.01, and the difference was statistically significant; as shown in Table 3.

Analysis of variance: 1) Compared with the normal group, P < 0.01; a compared with before poisoning, p < 0.01; b compared with during poisoning, p < 0.01.

Comparison of water maze error rate %, timeout rate % before, after, and during modeling Modeling before: There were no errors or timeouts in each group (if it exceeded 10 seconds and did not reach the end point, it was considered a timeout, counted as 10 seconds). Modeling after: There were errors and timeouts in each group, and they increased compared to before modeling. The model group and treatment group 2 increased the most, and the normal group was the lowest. After treatment: The treatment group 1, treatment group 2, and model group all decreased, but the normal group also increased(Table 4).

Table 4 Comparison of water maze error rate % and timeout rate % before, after, and during modeling

	Before modeling		After modeling		After treatment	
Group	Error rate %	Timeout rate %	Error rate %	Timeout rate %	Error rate %	Timeout rate %
Normal group	0	0	0.74	0.74	17.76	22.94
Mode group	0	0	20.72	24.42	16.22	17.08
Treatment group 1	0	0	22.2	14.8	16.65	18.24
Treatment group 2	0	0	34.04	37.00	12.58	18.5

Determination results of mouse brain AchE, serum urea nitrogen, and total protein. The results showed that for serum urea nitrogen, F = 2.729, P = 0.055. Compared with the normal group, 1) P < 0.01, and the difference was statistically significant. For serum total protein: F = 4.830, P = 0.005. 2)Compared with treatment group 1, P < 0.05, and the difference was statistically significant. 3)Compared with treatment group 1, P < 0.01, and the difference was statistically significant; see Table 5.

Table 5 Comparison of mouse brain AchE, serum urea nitrogen, and total protein in each group ($\overline{x}$ ±S)

Group	Number of animals	AchE (u/mg.prot)	Urea nitrogen (mmol/L)	Total protein (g/L)
Normal group	15	1.18±1.83	8.10±0.89	151.21±10.05[3)]
Mode group	15	2.32±8.52	7.44±1.06	142.59±15.45[2)]
Treatment group 1	13	0.64±0.38	6.49±1.26[1)]	130.28±17.31
Treament group 2	15	2.04±3.54	7.09±2.07	142.11±14.87[2)]

Analysis of variance: 1) Compared with the normal group, P < 0.01; 2)Compared with treatment group 1, P < 0.05; 3)Compared with treatment group 1, P < 0.01.

Determination results of serum total cholesterol (TC) and triglyceride (TG) in mice of each group. The results showed that for serum total cholesterol (TC): F = 6.928, P = 0.000. ▲▲Compared with the model group, P < 0.01, and the difference was statistically significant. See Table 6 for details.

Table 6 Comparison of serum TC and TG in mice of each group (±S)

Group	Number of animals	TC(mmol/L)	TG(mmol/L)
Normal group	15	2.75±1.72▲▲	1.04±0.44
Mode group	14	5.60±3.20	1.17±0.50
Treatment group 1	13	3.30±2.05▲▲	0.84±0.28
Treatment group 2	15	2.58±0.78▲▲	1.05±0.51

Analysis of variance: ▲▲Compared with the model group, P < 0.01

4. Discussion

Senile dementia, namely Alzheimer's disease (AD), is a degenerative disease of the central nervous system. Clinically, it first presents with memory impairment in the early stage, and then various cognitive functions are gradually impaired. In the late stage, the intelligence is severely declined, and

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the patient is completely unable to take care of himself/herself [2]. Looking at the experimental studies on the relationship between aluminum and the onset of AD, it can be basically affirmed that the aluminum content in the brain of AD patients significantly exceeds the normal value. Aluminum has a toxic effect on the central nervous system, causing neurons to degenerate or die and producing NFT pathological changes, which then manifest as atrophy of the cerebral cortex, reduction of brain weight, and memory and cognitive dysfunction[3]. The latest research has found that aluminum is a chronic accumulative toxin and one of the inducers of neurodegenerative diseases. Animal experiments have confirmed that when aluminum compounds are given, the aluminum content in the animal brain is significantly increased, triggering neurological diseases, and its toxic effects in diseases such as AD and dialysis encephalopathy (ED) have been affirmed by many scholars [4-5].

β-secretase is a transmembrane aspartic protease - β-site APP cleaving enzyme (BACE1 and BACE2), and BACE1 and BACE2 are called a new transmembrane aspartic (ASP) protease family[6]. The experimental data showed that subchronic aluminum exposure-induced Alzheimer's disease (AD) would lead to an increase in brain β-secretase 1 (BACE1), and BACE1 may be a visual biochemical indicator for clinically detecting AD [6]. At the same time, the use of α-secretase has a significant effect in the treatment of Alzheimer's disease patients [7]. There is an antagonistic relationship between α-secretase and brain β-secretase. Alzheimer's disease causes an increase in the content of brain β-secretase, resulting in a decrease in the content of α-secretase in the brain, which is lower than the normal value. In this experiment, the mechanism and treatment effect of Tremella and its compound polysaccharides on Alzheimer's disease mice under aluminum exposure were explored. The contents of β-secretase 1 and α-secretase in the mouse brain are the key factors reflecting the success of the experiment modeling. The experiment proved that under the same treatment conditions, after the mice in the treatment group were intragastrically administered with Tremella preparation and Tremella compound polysaccharide Chinese medicine preparation (“Good Wakefulness”), the brain β-secretase content in treatment group 1 decreased to the normal value level, indicating that the Tremella preparation has a certain treatment effect on AD. The decrease in treatment group 2 was even greater, which also reflected that the treatment effect of the Tremella compound polysaccharide Chinese medicine preparation on AD was stronger than that of the single Tremella preparation. For the determination of the memory ability of mice, a large number of experiments have shown that the water maze method for determining the memory ability of mice is reliable. In addition, the data of the water maze can be used to cross-verify the accuracy of the experimental results with biochemical indicators.

Tremella is a fruiting body of a fungus belonging to Basidiomycota, Tremellomycetes, Tremellales, Tremellaceae, and Tremella genus. It is also called white fungus, snow fungus, Tremella sp., etc., and has the reputation of "the king of fungi"[8]. Tremella has been used as a tonic on the table since ancient times. The mechanism of action of Tremella still needs to be explored. In view of the increasing trend of an aging population in China, the disease of Alzheimer's has become an urgent problem to be solved. Therefore, it is very important to explore the relationship between Tremella and Alzheimer's disease.

The experiment showed that acetylcholine (Ach) as a central cholinergic neurotransmitter is closely related to the occurrence of AD. The content of Ach is related to the activities of acetylcholine transferase (ChAT) and acetylcholine esterase (AchE) in the hippocampus. ChAT can promote the synthesis of Ach, while AchE can decompose Ach [9]. Therefore, as long as the contents of ChAT and AchE in the hippocampus are measured, the content of Ach in the hippocampus can be calculated, and then the degree of impairment of the cognitive function and learning ability of AD rats can be inferred [9]. However, this animal experiment data did not show statistical significance. After treatment with Tremella preparation and Tremella compound polysaccharide, the values of blood indexes such as urea nitrogen and total protein TP in mice were relatively normal. It can be speculated that the treatment of Alzheimer's disease by Tremella preparation and Tremella compound polysaccharide may be achieved by regulating the contents of related enzymes and related biochemical indexes. Some studies have also shown that lipid metabolism disorders, liver and kidney function disorders, and other complications, such as diabetic nephropathy, cardiovascular diseases, and eye diseases, can increase the incidence of Alzheimer's disease [10].

Comparing the Tremella preparation and its compound polysaccharide preparation, the mechanisms of the two in treating Alzheimer's disease are roughly the same. The treatment of Alzheimer's disease by Tremella preparation and Tremella compound polysaccharide may be achieved by regulating the contents of related enzymes and related biochemical indexes. At the same time, their influences on different blood biochemical indexes and the contents of secretory enzymes in the brain have their own advantages. In the index of urea nitrogen, the ability of the Tremella preparation to regulate urea nitrogen is significantly stronger. The content of brain γ-secretase in mice treated with the Tremella

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compound polysaccharide preparation is less than that in the Tremella preparation group. Therefore, the treatment effects of the two need to be further explored through more experiments.

5. Conclusions

In summary, aluminum maltolate poisoning affects the expression amount of β-site amyloid precursor protein cleaving enzyme 1 (BACE1), causing β-amyloid protein to accumulate and further destroying the learning and memory abilities. α-secretase also plays an important role in the pathogenesis of Alzheimer's disease. Tremella or its compound polysaccharides treat Alzheimer's disease by inhibiting brain β-secretase 1, changing the content of α-secretase, and regulating related biochemical indexes, making a new attempt for the treatment of Alzheimer's disease with traditional Chinese medicine.

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