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Comparative Experimental Study on the Effects of Sweet Tea and Shutangbao on the Activities of α-secretase and γ-secretase in Mice Poisoned by Aluminum Maltol

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Abstract: Comparative Experimental Study on the Effects of Sweet Tea and Shutangbao on the Activities of α-Secretase and γ-Secretase and the Cognitive Memory Function in Mice Exposed to Aluminium Maltol. Sixty KM mice (with each body weight ranging from 30 to 35g) were divided into the control group, the model group, treatment group 1, and treatment group 2. Mice except the control were exposed to aluminium maltol for modeling. Treatment groups were treated with sweet tea and Shutangbao. Water maze tests were done in 3 stages. Subsequently, brain homogenates were prepared to measure enzyme activities. The results showed significant differences in water maze tests between the exposed groups and the normal group. During the modeling process, it was found that the level of α - secretase was reduced. Treatment improved enzyme expressions and cognitive memory of exposed mice. The animal model caused by the exposure of mice to aluminium maltol can affect the expressions of α - secretase and γ - secretase, thereby decreasing the cognitive memory ability. Sweet tea and Shutangbao can promote the increase in the expression levels of α-secretase and γ-secretase in mice exposed to aluminium maltol, and have an improving effect on the cognitive memory ability of the exposed mice.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder primarily characterized by cognitive decline, for which there is currently no effective clinical treatment[1].As modern society continues to develop, environmental pollution has become a growing concern, with aluminum

contamination emerging as a significant public health issue. Aluminum, a prevalent environmental toxin, has been linked to various health issues, including neurodegenerative diseases, when humans are exposed to it over extended periods. Among various studies, aluminum maltol, an aluminum compound, has been shown to impair cognitive function in animal models through multiple mechanisms, including its impact on α-secretase and γ-secretase activities in brain tissues.α-secretase and γ-secretase are key enzymes involved in amyloid precursor protein (APP) metabolism[2], and their dysregulation is closely associated with the development and progression of Alzheimer's disease and other neurodegenerative disorders. In recent years, significant attention has been directed towards discovering natural products or drugs that can regulate these enzymes, particularly in the context of preventing and treating neurodegenerative diseases. Sweet tea and Shuganbao, two traditional Chinese medicinal herbs, have demonstrated various bioactive properties, including antioxidant, anti-inflammatory, and neuroprotective effects. This study explores the effects of sweet tea and Shuganbao on α-secretase and γ-secretase activities in the brain tissues of mice exposed to aluminum maltol, and whether these substances can mitigate the cognitive deficits induced by aluminum maltol exposure.By comparing the therapeutic effects of sweet tea and Shuganbao on aluminum-exposed mice, this research aims to elucidate the underlying mechanisms and potential therapeutic benefits of these two substances in modulating secretase activities in brain tissues.

2. Materials and Methods

2.1. Experimental Animals

Sixty KM strain mice were selected, with an equal distribution of males and females (30 each), weighing 30-35 g, housed in a standard environment. The mice were supplied by Changsha Tianqin Biotechnology Co., Ltd., and the experimental animal production license number is Scxk (Xiang) 2019-0013.

2.2. Reagents and Drugs

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

2.3. Animal Grouping And Processing

The mice were randomly divided into four groups based on body weight: the control group (15 mice), the model group (15 mice), treatment group 1 (15 mice), and treatment group 2 (15 mice). Treatment groups 1 and 2 received treatments with Rubus suavissimus S. Lee (Chinese sweet tea) and Shutangbao (a traditional herbal supplement), respectively. For the model, treatment group 1, and treatment group 2, aluminum maltol was administered via intraperitoneal injection at a dose of 0.1 ml/100g. After 42 days of modeling, treatment groups 1 and 2 were treated by gavage with different concentrations of Guangxi sweet tea and Shutangbao. The control and model groups received the same volume of distilled water via gavage until the end of the experiment.

2.4. Measurement Method

Assessment of Learning and Memory in Mice using the Y-maze Water Test.The Y-maze water model was constructed, and the experimental procedures were performed as described in the literature to evaluate the memory function of the mice. Ten days before the induction of the aluminum toxicity model, mice underwent daily training on the water maze, with 3 training sessions per day. Following the training phase, memory function was assessed over 3 consecutive days by recording the time to reach the platform, the number of errors, and the number of failures. Timeout was defined as failure to reach the platform within 10 seconds, an error was recorded if the mouse swam in the wrong direction, and failure was defined as not reaching the platform within 40 seconds. Assessments were conducted at the early, middle, and late stages of aluminum toxicity, with each stage consisting of 3 consecutive days of testing, during which each mouse was tested 3 times daily. All data were recorded for further analysis.

Preparation of 10% Mouse Brain Homogenate and Secretase Activity Assay The mice were sacrificed via cervical dislocation, and their brain tissues were immediately collected. The brain surfaces were washed with 0.01 M phosphate-buffered saline (PBS, pH 7.4) to remove any residual blood. Excess moisture was gently blotted with filter paper, and the brains were weighed using an analytical balance. Subsequently, the brain tissues were placed in a glass homogenizer, and an appropriate volume of 0.01 M PBS (pH 7.4) was added. The tissues were then homogenized for 10 minutes in an ice bath to prepare a 10% brain homogenate. The activity of β-secretase, α-secretase, and γ-secretase in the mouse brain homogenate was determined using a double-antibody sandwich ELISA assay, following the manufacturer's protocol in detail.

Statistical Analysis Statistical analysis was performed using SPSS 13.0 software. The data were expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was conducted to compare the data between groups.

Statistical significance was considered when P-values were less than 0.05 or 0.01. Additionally, a Q-test (Student-Newman-Keuls test) was used to further analyze the data.

3. Results

3.1. Survival Status of Mice in Each Group

In the normal group (n=15), no mice died, while in the model group (n=15), 1 mouse died. In treatment group 1 (n=15), 2 mice died, whereas no mice died in treatment group 2 (n=15).

3.2. Comparing the Related Data of Mice in Each Group, The Results Show That

3.2.1. The activities of β-secretase, α-secretase, and γ-secretase (u/L) in the mouse brain were measured

The results indicated that β-secretase activity increased in the model group but decreased in the treatment groups. Meanwhile, α-secretase activity was higher in the normal group compared to other groups, and γ-secretase activity in the brain was similarly higher in the normal group than in the other groups.Refer to Tables 1, 2, and 3 for details.

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

group	β-secretase	α-secretase	γ-secretase
Normal group	11.95±1.36▲	11.29±3.51▲▲	14.87±2.09
Model group	13.00±1.10	10.07±2.42▲	15.50±1.55
Treatment group 1	11.64±1.09▲▲	9.49±2.50	15.70±1.16
Treatment group 2	11.90±0.90▲▲	8.08±1.38	15.83±0.68

ANOVA among groups: Brain β-secretase: F=4.286, P=0.009, compared to the model group, ▲P<0.05, ▲▲P<0.01, indicating statistically significant differences; Brain α-secretase: F=3.362, P=0.026, compared to treatment group 2, ▲P<0.05, ▲▲P<0.01, indicating statistically significant differences; γ-secretase: F=1.33, P=0.385, indicating no statistically significant differences.

3.2.2. Comparison of water maze test time (s) of mice in each group before, during and after exposure

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

group	Number of animals	Before exposure (s)	Poisoning (s)	After exposure (s)
Normal group	15	3.64±0.37	3.68±0.63	3.71±0.60▲▲
Model group	14	3.82±0.44	4.82±1.15▲▲	5.55±0.73a
Treatment group 1	13	3.56±0.79b	5.16±1.14▲▲	3.61±0.66▲▲b
Treatment group 2	15	3.77±0.38c	4.58±0.55▲▲	3.65±0.49▲▲c

ANOVA results showed that before exposure, F=0.589, P=0.626, P>0.05, indicating no significant statistical differences between the groups. During exposure, F=7.402, P<0.001, comparison with the normal group showed statistically significant differences (▲▲P<0.01). After exposure, F=26.796, P<0.001, comparison with the model group also indicated statistically significant differences (▲▲P<0.01).

3.2.3. Comparison of body weights within and between groups across time points

In the normal group, F=71.207, P<0.001, body weight significantly increased compared to both the early stage (P<0.01) and the mid stage (P<0.01). In the model group, F=12.994, P<0.001, a significant weight increase was observed from the early stage (P<0.01), with a pronounced rise from early to mid stage but no significant change from mid to late stage. For treatment group 1, F=2.720, P=0.078, the weight change was significant compared to the early stage (P<0.05), with minimal increase from early to mid stage but a slight rise from mid to late stage. In treatment group 2, F=16.590, P<0.001, weight increases were significant in both the early and mid stages (P<0.01).

ANOVA results for intergroup comparisons: In the early stage, F=2.654, P=0.063, a statistically significant difference was observed between the groups and treatment group 2 (P<0.05). In the mid stage, F=20.404, P<0.001, a highly significant difference was found compared to treatment group 1 (P<0.01). By the end of the late stage, F=21.023, P<0.001, the difference between the groups and treatment group 1 remained highly significant (P<0.01).

Table 3: Comparison of body weight of mice in each group ($\bar{x}$ s) (g)

group	Number of animals	in the early	middle	late
Normal group	15	32.23±1.61▲b	42.05±3.69▲▲a	46.46±4.68▲▲ab
Model group	14	31.77±1.19	42.84±3.38▲▲a	44.29±4.16▲▲a
Treatment group 1	13	31.99±1.63▲	31.62±2.83	32.39±3.45c
Treatment group 2	15	30.56±1.27	41.89±4.77▲▲a	45.09±4.75▲▲a

4. Discussion

Alzheimer's disease (AD) involves various mechanisms, such as abnormal production and metabolism of Aβ, abnormal phosphorylation of Tau protein, the cholinergic deficit hypothesis, genetic theories, and oxidative stress hypothesis[3]. However, no single hypothesis can fully explain the pathogenesis of Alzheimer's disease. It is widely believed to be the result of multiple mechanisms acting together[4]. However, one clear point is that the metabolic disorder of α-secretase and γ-secretase is one of the most extensively studied hypotheses. Amyloid precursor protein (APP) is a molecular chaperone protein expressed in the nervous system. Under normal conditions, APP acts as a protective substance, stabilizing DNA and maintaining normal cellular functions without neurotoxicity [5]. However, in the development of AD, the α-secretase pathway of APP is inhibited, and the β-secretase pathway becomes the main route of APP metabolism [6]. Aβ generated by the cleavage of β-secretase and γ-secretase is insoluble and prone to precipitate in the brain. When large amounts of Aβ are produced and cannot be cleared in time, Alzheimer's disease can be triggered[7].In this experiment, by treating aluminum-exposed mice with sweet tea and Shutangbao, the activities of α-secretase and γ-secretase were compared between the treatment group and the control group, indirectly reflecting whether sweet tea and Shutangbao have therapeutic effects on Alzheimer's disease.

Through comparative analysis of experimental data, the time required for the water maze test significantly increased post-modeling compared to pre-modeling, confirming the successful establishment of the Alzheimer's disease model. A notable reduction in α-secretase and γ-secretase activities was observed after modeling, which further supports the hypothesis that Alzheimer's disease leads to decreased enzymatic activity. Following treatment, the time spent in the maze was significantly lower than in the post-modeling phase, indicating a recovery in memory function. Additionally, the levels of α-secretase and γ-secretase in the treatment group were higher than those in the model group, suggesting that the recovery of memory is associated with increased levels of these enzymes. These results demonstrate that both Sweet Tea and Shutangbao exhibit therapeutic effects on Alzheimer's disease.

5. Conclusions

In summary, this study demonstrates that Suanzaoren (Ziziphus jujuba seed) improves cognitive function and delays brain aging, showing potential as a therapeutic agent for Alzheimer's disease. One of the mechanisms through which it achieves these effects is by enhancing the activities of α-secretase and γ-secretase. These findings establish a solid experimental foundation for further investigation into the mechanisms of Sweet Tea and Shutangbao, providing new directions for

Alzheimer's disease treatment. However, the current research is limited to animal models, and there is a lack of robust clinical data, with most existing studies being single-center trials. To validate these findings, there is a pressing need for multi-center, large-sample randomized controlled clinical trials to assess the clinical efficacy and underlying mechanisms of Sweet Tea and Shutangbao in Alzheimer's treatment. Future studies should prioritize such trials to generate stronger evidence from an evidence-based medicine perspective, supporting their therapeutic potential.

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