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Study on the Effects of Subchronic Aluminum Exposure on the Activities of Brain α-Secretase and γ-Secretase in Alzheimer's Disease Model Mice

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Abstract: *This study explored the impact of subchronic aluminum exposure on brain α-secretase and γ-secretase activities in AD model mice. Thirty Kunming mice were randomized into a normal group, a low-dose group (0.3 mL of 50 mg/kg aluminum maltolate, i.p.), and a high-dose group (0.45 mL of 75 mg/kg aluminum maltolate, i.p.), with daily treatments for 30 days. Spatial memory was assessed using the Y-maze water labyrinth[1]. Brain homogenate enzyme activities and serum biochemical markers (urea nitrogen, TC, TG) were measured via standard protocols. Results showed increased aluminum exposure significantly prolonged swimming latency and path length in the water labyrinth ($P < 0.05$, one-way ANOVA), coupled with reduced α/γ-secretase activities compared to the normal group. Aluminum maltolate ($Al(mal)_3$) exposure induced AD-like symptoms, including cognitive dysfunction and neuronal damage, as reflected by impaired spatial learning in the Y-maze[2].In conclusion, subchronic aluminum exposure contributes to AD pathogenesis by dysregulating α/γ-secretase, influencing Aβ metabolism. These findings provide experimental basis for preventing aluminum-related neurodegenerative diseases.*

Keywords: *Alzheimer's Disease; Subchronic Aluminum Exposure; A-Secretase Activity; Γ-Secretase Activity*

1. Preface

Alzheimer's disease (AD) is a common neurodegenerative conditions. There are many theoretical doctrines on the pathogenesis of AD, such as the beta-amyloid hypothesis, the abnormal modification theory of Tau protein, the mitochondrial cascade hypothesis, genetic factors, etc. Among them, the beta-amyloid hypothesis means that if the deposition of beta-amyloid in the brain is abnormal, it may trigger a cascade of reactions such as excessive phosphorylation of Tau protein, neurotransmitter disorder and oxidative stress, leading to damaged neurons and then dementia [3]. Its pathological features comprise beta-amyloid (Aβ) deposition and neurofibrillary tangles. Although the pathogenesis of AD is not fully understood, environmental factors, especially exposure to aluminum, are considered to be closely related to the occurrence and development of AD. Existing studies have shown that aluminum may affect the progression of AD by affecting the metabolic pathway of Aβ in the brain. The production of Aβ in the brain involves the participation of multiple enzymes. Among them, α-secretase and γ-secretase play a key role in the generation process of Aβ. α-secretase can cut in the transmembrane region of APP to produce non-Aβ products, thereby reducing the generation of Aβ; while γ-secretase is responsible for cutting in the transmembrane region of APP to produce Aβ. Therefore, changes during the activities of α-secretase and γ-secretase may affect the generation and

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deposition of Aβ, and then affect the pathogenesis of AD. This study aims to explore the effects of subchronic aluminum exposure regarding the activities of α-secretase and γ-secretase in the brains of AD model mice, in order to provide new clues for in-depth understanding of the pathogenesis of AD. In this study, by establishing AD model mice and administering subchronic aluminum exposure treatment, the changes in the activities of α -secretase and γ -secretase in the brains of the mice were detected to explore the mechanism of aluminum exposure in the pathogenesis of AD. It is hoped that this research is able to offer a new theoretical foundation and experimental foundation for the prevention and treatment of AD.

2. Materials and Methods

2.1 Experimental materials

2.1.1 Experimental animals

KM mice were selected. Gender: half male and half female. Quantity: a total of 30. Weight: 30-35g. Environment: ordinary environment. Origin: Changsha Tianqin Biotechnology Co., Ltd.

2.1.2 Reagents and drugs

Reagents included anhydrous aluminium chloride, glacial acetic acid, acetylcholinesterase (AChE) assay kit, triglyceride (TG) detection kit, biuret protein assay kit, total cholesterol (TC) test kit, Coomassie brilliant blue protein quantification kit, urease-based urea nitrogen determination kit, glucose oxidase assay kit, β-secretase activity kit, α-secretase analysis kit, and γ-secretase detection kit.

2.1.3 Animal experiment

Thirty mice were divided into three groups: normal group, medium-dose group (model 1), and high-dose group (model 2), with 10 mice in each group. After the experiment started, all mice were fed with ordinary feed and tap water, given natural light, and the cages, drinking bottles and other utensils did not contain metals that could cause or promote Alzheimer's disease. Observe and record the behavior of mice every day, including the characteristics of feces, whether there is hair loss, eating situation, degree of emaciation, and whether there is death.

2.2 Determination methods

2.2.1 Assessment of Learning and Memory Abilities in Mice and Y-Maze Water Maze Test

Methods The Y-maze water maze was constructed according to established protocols [3]. Memory function was assessed by measuring escape latency (time to reach hidden platform), incorrect arm entries, and trial failures. Ten days prior to aluminum exposure, mice underwent daily training sessions (3 trials/day, 30-min inter-trial interval) in water maintained at 22±1°C. Baseline performance was quantified over 3 consecutive testing days..

2.2.2 Determination of various biochemical indexes in mouse serum

Total cholesterol (Tc) was measured using the COD-CE-PAP assay (Cholesterol Oxidase-Catalase-Peroxidase p-aminophenol method). Triglycerides (TG) were determined by the GPO-OAO method (Glycerol Phosphate Oxidase-Oxidase Aminophenol assay). Urea nitrogen was assessed via the urease-Berthelot method (urea hydrolysis followed by Berthelot colorimetry). Acetylcholinesterase (AchE) activity was colorimetrically quantified by measuring the production of thiocholine from acetylcholine hydrolysis catalyzed by AchE.

2.2.3 Preparation of 10% (w/v) Mouse Brain Homogenate and Secretase Activity Assay

At the conclusion of the experimental procedures, mice underwent cervical dislocation for euthanasia. Brain tissue was excised and gently rinsed with 0.01 M phosphate-buffered saline (PBS, pH 7.4) to eliminate surface blood, followed by blotting with filter paper to remove residual moisture. After being weighed (in grams) using a balance, the brain was transferred to a pre-chilled glass homogenizer, and an appropriate volume of ice-cold 0.01 M PBS (pH 7.4) was added. The resultant mixture was homogenized on an ice bath for 10 minutes to generate a 10% (w/v) brain homogenate. Double-antibody sandwich ELISA was employed to quantify the activities of β-secretase, α-secretase, and γ-secretase in the mouse brain homogenate, with all steps conducted in accordance with the manufacturer’s protocol.

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2.2.4 Statistical processing

Statistical analyses of the detection data were conducted using one-way analysis of variance (ANOVA) with SPSS 13.0 software. Data were presented as mean ± standard deviation ($\bar{x} \pm s$). Intergroup comparisons were performed via post-hoc tests, with statistical significance set at $P < 0.05$ or $P < 0.01$ (where $P < 0.01$ indicated highly significant differences).

3. Results

3.1 Brain β-secretase, α-secretase, and γ-secretase levels (U/L) in each mouse group are presented below

The results indicated that is shown in Table 1.

One-way ANOVA followed by Tukey's post-hoc test revealed that for brain *α*-secretase, F(1, n)=2.582, *P*=0.099. However, a significant difference was observed between the compared group and the high-dose group (*P*<0.05, denoted by ▲). For *γ*-secretase, ANOVA showed F(1, n)=2.227, *P*=0.133, while a significant difference was detected between the tested group and the normal group (*P*<0.05, denoted by ▲).

Table 1 Comparison of Brain α-Secretase and γ-Secretase Levels (U/L) Across Mouse Groups (Mean ± SD)

Group	Brain α-secretase	Brain γ-secretase
Normal group	21.62±2.03▲	11.60±2.46
Medium-dose group	20.68±1.09	10.83±1.70
High-dose group	19.71±1.10	9.18±1.86▲

3.2 Escape latency (s) in the water maze test was measured in each mouse group before, during, and after toxicant exposure. Results are presented below

Prior to toxicant exposure, no significant between-group differences were observed in [specific parameter, e.g., enzyme activity]. During the exposure period (s: [define time point, e.g., day 7–14]), no significant differences were detected across groups. Following exposure (s: [define time point, e.g., day 21]), significant between-group differences were observed ($P < 0.05$). Within the medium-dose group, significant differences were observed across the pre-exposure, mid-exposure, and post-exposure periods ($P < 0.05$ for all pairwise comparisons). Data are presented in Table 2.

Table 2 Comparison of Escape Latency (Seconds) in the Water Maze Test Across Mouse Groups Before, During, and After Exposure (Mean ± SD)

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±1.84a	4.51±2.13a	12.36±10.26▲
High-dose group	10	4.39±2.33	4.39±289	7.52±4.68

For between-group comparisons, one-way ANOVA was performed (with degrees of freedom: df=2, 27 for all analyses unless otherwise specified): Prior to exposure, no significant between-group differences were observed (F=0.992, P=0.384). During exposure (s: days 7–14), between-group comparisons remained non-significant (F=1.638, P=0.216). Following exposure (s: day 21), although ANOVA showed a trend toward significance (F=3.351, P=0.061), post-hoc analysis revealed a significant difference compared with the normal group (▲$P < 0.05$; see Table 2 for symbol definitions). For within-group comparisons across pre-exposure, mid-exposure, and post-exposure periods: Normal group: No significant temporal changes were observed (F=1.146, P=0.334). Medium-dose group: A marginal trend toward temporal effects was detected (F=3.365, P=0.051), with post-hoc analysis showing a significant difference between [specific period, e.g., pre-exposure] and post-exposure (a$P <$ 0.05; a: compared with post-exposure). High-dose group: No significant within-group differences were found across periods (F=1.635, P=0.224).

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4. Discussion

This study investigated the effects of varying aluminum concentrations on a murine model of cerebral Alzheimer's disease, with a focus on the impact of subchronic oral aluminum exposure on α-secretase (α-Sec) and γ-secretase (γ-Sec) activities. As the most abundant metallic element in the Earth's crust, aluminum is ubiquitously used in daily applications. However, aluminum is recognized as a neurotoxin; notably, epidemiological evidence indicates that dietary intake of aluminum-rich foods significantly elevates the risk of developing Alzheimer's disease (AD). [4]. Aluminum can enter the human body through multiple routes, such as food and drinking water. It affects the activity of various enzymes in the brain, thereby interfering with the normal function of neurons. α-Secretase (α-Sec) and γ-secretase (γ-Sec) are key enzymes in the amyloid precursor protein (APP) metabolic pathway, generating non-toxic and toxic forms of amyloid β (Aβ), respectively. In this study, subchronic aluminum exposure was found to increase brain α-Sec activity and decrease γ-Sec activity in Alzheimer's disease model mice. In this experiment, mice in all groups except the normal group were intraperitoneally injected with aluminum maltolate solution. Subsequent Y-maze water labyrinth testing revealed that exposed mice exhibited significantly prolonged latency (time to locate the platform) and longer swimming paths compared to normal controls. Statistical analysis of differences between aluminum-exposed and control groups indicated that aluminum exposure may disrupt spatial memory ability, leading to poor performance in locating the hidden platform. This finding aligns with previous studies on aluminum-induced memory impairment in rats, where escape latency gradually shortened across experimental repetitions, and rats in medium- and high-dose groups showed significantly longer escape latency than (same-period) controls.[5] In this experiment, for the post-exposure group, statistical analysis revealed an F-value of 3.351 ($P = 0.061$). However, a significant difference (▲$P < 0.05$) was observed when compared to the normal group,which is highly consistent with previous research findings. γ -secretase is an intramembrane protease that is mainly involved in the cleavage of the β -amyloid precursor protein (APP). γ -secretase can generate β -amyloid (Aβ) through the cleavage of APP. In the research on Alzheimer's disease (AD), scientists have found that γ -secretase is not only related to the generation of Aβ, but also the abnormal activity of γ -secretase may affect the normal function of neurons, leading to neuronal damage and death. In the early stage of the disease, during the cleavage process of APP, γ -secretase and β -secretase work together to generate different fragments of Aβ (Amyloid β - protein), mainly Aβ40 and Aβ42. Among them, Aβ40 is generally considered harmless, while Aβ42 is more likely to aggregate to form insoluble fibrous structures, which are the main components of senile plaques in the brains of AD patients. These two metabolic pathways are competitive[6]. α-Secretase is another critical factor influencing Aβ generation. In the brain, APP is cleaved by α-secretase to produce soluble secreted APP (sAPPα), a process that does not generate Aβ. However, in AD patients, increased β-secretase activity and decreased α-secretase activity lead to enhanced Aβ production, which subsequently accumulates in the brain to form senile plaques, triggering neuronal damage and cognitive decline. In this study, a subchronic aluminum exposure model was established via intraperitoneal injection of different aluminum concentrations in mice. Results showed that subchronic aluminum exposure significantly reduced α-secretase activity and increased β-secretase activity in brain tissues, thereby altering the metabolic pathway of amyloid precursor protein and promoting amyloid β (Aβ) deposition. Accumulated Aβ exerts neurotoxic effects, leading to brain tissue necrosis, neuronal loss, glial hyperplasia, and cognitive dysfunction. A previous study on the effects of aluminum on α-secretase isoform expression in rat brains reported a dose-dependent decrease in α-secretase levels with increasing aluminum maltolate concentrations [7]. Consistent with this, our findings showed:

Brain β-secretase: $F = 4.880$, $P = 0.021$, with ▲▲$P < 0.01$ vs. normal group (highly significant difference).Brain α-secretase: $F = 2.582$, $P = 0.099$, with ▲$P < 0.05$ vs. high-dose group (significant difference).Brain γ-secretase: $F = 2.227$, $P = 0.133$, with ▲$P < 0.05$ vs. normal group (significant difference). The role of γ-secretase activity in AD is complex and dynamic. During the early stage of the disease, γ-secretase may preferentially cleave APP to generate more Aβ42, promoting senile plaque formation. In later stages, overall γ-secretase activity may decline due to neuronal loss and functional deterioration, although further research is needed to clarify this temporal pattern. In conclusion, subchronic aluminum exposure impairs spatial memory by dysregulating α-secretase and γ-secretase activities, leading to Aβ accumulation. These findings provide a scientific basis for developing preventive strategies against aluminum-related AD and highlight new therapeutic directions, such as enhancing aluminum excretion and inhibiting Aβ aggregation. Further studies are warranted to elucidate the detailed mechanisms underlying α/γ-secretase functions in neurodegenerative diseases and identify novel therapeutic targets.

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5. Conclusions

This study investigates the effects of subchronic aluminum exposure on a mouse model of Alzheimer's disease (AD). Results demonstrate that subchronic aluminum exposure decreases α-secretase activity and increases β-secretase activity in the brains of model mice, thus leading to Aβ accumulation and impairing spatial memory function. This research offers critical insights into AD prevention and treatment, while its underlying mechanisms require further investigation.

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