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Comparison and Exploration of Aluminum Toxicity and β-Amyloid Protein Theories

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Abstract: This study explored the link between aluminum (Al) toxicity and beta-amyloid pathology in 52 SPF mice divided into control (saline), low- (2 mg/kg), medium- (4 mg/kg), and high-dose (8 mg/kg) maltol aluminum groups. After 60 days of intraperitoneal injections, Y-maze tests revealed significant learning and memory impairments in medium/high-dose groups ($P<0.05$). Serum analysis showed elevated TC, TP, and ALT but reduced BUN and Hb in Al-exposed groups ($P<0.05$). Brain tissue analysis indicated higher Al^{3+} levels in all treated groups ($P<0.05$), with reduced acetylcholinesterase (AchE) activity in medium/high doses ($P<0.05$). BACE1 protein (a key enzyme in amyloid production) increased significantly in the high-dose group ($P<0.05$). Glutathione peroxidase (GSH-PX) levels varied, with serum GSH-PX elevated in the medium-dose group ($P<0.05$) but brain GSH-PX reduced in medium and elevated in high doses ($P<0.05$). Aluminum exposure may promote beta-amyloid generation via BACE1 upregulation, impairing cognitive function, supporting a potential mechanistic link between Al toxicity and Alzheimer's-like pathology.

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative condition and the leading cause of dementia in the elderly[1].AD is characterized by two hallmark pathological features: neurofibrillary tangles (NFT) and amyloid plaques (SP, also known as senile plaques)[2].

The amyloid-beta (Aβ) hypothesis suggests that amyloid precursor protein (APP), when cleaved by β-secretase and γ-secretase, generates amyloid-beta (Aβ), which constitutes the primary component of senile plaques and serves as the biochemical foundation for cerebral amyloid angiopathy and neurofibrillary tangles (NFT)[3]. The key enzyme involved is β-secretase, which under normal conditions exhibits low activity, resulting in minimal amyloid-beta (Aβ) deposition and fewer senile plaques. Conversely, heightened β-secretase activity leads to excessive Aβ accumulation, an increased number of senile plaques, and subsequent functional impairments.

The aluminum toxicity hypothesis posits that aluminum, as a chronic neurotoxin, plays a significant role in the development of Alzheimer's disease. In certain brain regions affected by AD, aluminum concentrations can reach 10 to 30 times the levels found in normal brains. Aluminum

deposits are present in the cores of senile plaques (SP) and neurofibrillary tangles (NFT). Moreover, aluminum binds to nuclear chromosomes, potentially disrupting gene expression, and contributes to the formation of both SP and NFT[4].

The aluminum toxicity hypothesis and the amyloid-beta (Aβ) hypothesis highlight distinct perspectives on Alzheimer's disease. The former emphasizes the detrimental effects of aluminum on the brain, while the latter focuses on the accumulation of Aβ as the primary cause of the disease. Despite their differences, both hypotheses are linked to the formation of the two major pathological features of AD, neurofibrillary tangles (NFT) and senile plaques (SP). This connection suggests that subchronic aluminum exposure may facilitate the formation of amyloid-beta peptides, thereby contributing to the progression of Alzheimer's disease.

This study aims to establish a neurological injury animal model by administering subchronic intraperitoneal injections of maltol-aluminum in SPF mice, exploring the contrasts and connections between the aluminum toxicity hypothesis and the amyloid-beta (Aβ) hypothesis.

2. Materials and Methods Reagents

Crystalline aluminum chloride ($AlCl_3 \cdot 6H_2O$), maltol, α-secretase assay kit, β-secretase assay kit, γ-secretase assay kit, hemoglobin (Hb) assay kit, triglycerides, total cholesterol, protein assay kit, acetylcholinesterase (AChE) assay kit, phosphate-buffered saline (PBS, pH 7.4, 0.01 mol/L), and saline.Instruments: SpectraMax M5 microplate reader, imported pipette, thermostatic water bath, centrifuge, refrigerator, visible light spectrophotometer, among others.

2.1. Experimental Animals

A total of 52 SPF-grade mice were obtained from Changsha Tianqin Biotechnology Co., Ltd. (License No. SCXK (Xiang) 2022-0011), including 25 females and 27 males with comparable activity levels and body weights ranging from 25 to 30 grams. After one week of acclimatization in the animal facility, the mice were randomly divided into four groups: Normal group (saline group): 11 mice, Low-dose maltol-aluminum group (0.4 mg/kg): 15 mice, Medium-dose group (0.8 mg/kg): 16 mice, High-dose group (1.2 mg/kg): 10 mice.

2.2. Toxicity Induction Method

Prior to toxicity induction, maltol solution and aluminum chloride solution of equal volumes were prepared into low-dose maltol-aluminum (2 mg/kg), medium-dose maltol-aluminum (4 mg/kg), and high-dose maltol-aluminum (8 mg/kg), and the pH was adjusted to 7.1–7.4. The solutions were filtered and set aside. The mice were administered intraperitoneal injections for five consecutive days, followed by a 2-day interval, for a total of 60 days. All animals were fed standard diet with free access to drinking water and food. No aluminum-containing products were used for cages or drinking bottles.

2.3. Observation of Animal Basic Condition and Sample Collection

Before each administration, animal signs were observed for abnormal behaviors, death, changes in feces, hair loss, feeding, and weight loss. Hemoglobin (Hb) levels were measured before, during, and after the experiment. At the end of the experiment, blood was collected from the eyeball, serum was separated, and triglycerides, total cholesterol, and total protein in the serum were measured. The mice were then euthanized, and their brains were removed for 10% brain homogenates.

2.4. Preparation of Mouse Brain Tissue Homogenates

The mouse brains were first frozen, then removed and washed with PBS (pH 7.4, 0.01 M) to remove blood. Excess liquid was blotted dry with filter paper, and the brains were weighed. Under ice bath conditions, the brains were homogenized using a glass homogenizer for 10 minutes with PBS (pH 7.4, 0.01 M) to prepare a 10% brain homogenate. The supernatant was collected and stored in the refrigerator for later use. The protein content, acetylcholinesterase (AChE) activity, and secretase levels in the homogenate were measured.

2.5. Detection of Brain Homogenate

The β-secretase activity was measured using a microplate reader, with comparison and analysis conducted among groups. The operation followed the manufacturer's instructions, and for each sample, duplicate wells were set up. The optical density was measured at 450 nm using the microplate reader, and the enzyme activity for each sample was calculated.

2.6. Observation of Mouse Learning and Memory Ability

The learning and memory abilities of the mice were assessed according to the method outlined in the literatur [5]. The learning and memory abilities of the mice were assessed using the Y-maze water maze test. The test was conducted at the beginning, middle, and end of the experiment. The error rate and swimming time of the mice were recorded during each trial.

2.7. Statistical Analysis

The experimental data were expressed as mean ± standard deviation (SD) and analyzed using the SPSS 13.0 statistical software package. Comparisons among multiple groups were performed using one-way analysis of variance (ANOVA), with a threshold of $P < 0.05$ considered statistically significant.

3. Results

3.1. Survival Status of Mice at the End of the Experiment

At the end of the experiment, the survival rates were as follows: 11 mice in the normal group, 15 in the low-dose group, 16 in the medium-dose group, and 8 in the high-dose group.

3.2. Y-Maze Water Maze Experimental Results Before, During, and After Toxicity Induction

The results of the Y-maze water maze experiment for each group before, during, and after toxicity induction are shown in Table 1 and Table 2. Between-Group Comparison: Before toxicity induction, there were no significant differences in water maze time or memory error rate among the groups ($P > 0.05$). During and after toxicity induction, the medium- and high-dose groups showed significantly longer water maze times and higher memory error rates compared to the normal group ($P < 0.05$), while the low-dose group did not show significant differences compared to the normal group ($P>0.05$).Within-Group Comparison: Compared to before toxicity induction, there were no significant differences in water maze time for the normal and low-dose groups during toxicity induction ($P>0.05$). However, after toxicity induction, the water maze times were significantly higher than before ($P < 0.05$). In the medium- and high-dose groups, water maze times were significantly longer during and after toxicity induction compared to before ($P < 0.05$). Compared to

during toxicity induction, water maze times after toxicity induction were significantly longer for the low, medium, and high-dose groups (P<0.05), while the normal group showed no significant difference between before and after toxicity induction (P > 0.05).

Table 1: Comparison of Water Maze Times (s) for Each Group of Mice Before, during, and After Toxicity Induction"($\bar{x}$ ±S)

Constituencies	count	Prepoisoning(s)	Poisoned(s)	Postpoisoning(s)
Normal group	11	4.89±2.26	5.01±2.36	6.88±2.00$^{\bullet}$
Low-dose group	15	5.00±2.17	5.12±4.35	7.75±3.60$^{\bullet※}$
Medium-dose group	16	5.45±2.46$^{※}$	8.30±3.02$^{\bullet▲}$	9.25±6.97$^{\bullet▲※}$
High-dose group	8	5.06±1.68$^{※}$	9.55±3.04$^{\bullet▲}$	11.03±7.04$^{\bullet▲※}$

Note: Within-group comparison: Compared to before toxicity induction, $^{\bullet}P$ < 0.05. Compared to during toxicity induction, $^{※}P$ < 0.05; Between-group comparison: Compared to the normal group, $^{▲}P$ < 0.05.

Table 2: Comparison of Water Maze Memory Error Rates (%) for Each Group of Mice Before, During, and After Toxicity Induction($\bar{x}$ ±S)

Constituencies	count	Before poisoning (%)	Poisoned (%)	Post-poisoning (%).
Normal group	11	4.04%±1.38%	14.15%±3.77%	10.09%±3.68%
Low-dose group	15	5.41%±1.04%	15.37%±3.35%	12.89%±4.23%
Medium-dose group	16	4.67%±1.11%	22.31%±5.47%$^{▲}$	23.47%±5.69%$^{▲}$
High-dose group	8	5.06%±1.28%	28.10%±6.88%$^{▲}$	32.32%±7.01%$^{▲}$

Note: Between-group comparison: Compared to the normal group, $^{▲}P$ < 0.05.

3.3. Serum Total Cholesterol (TC), Triglycerides (TG), Alanine Aminotransferase (ALT), Blood Urea Nitrogen (BUN), and Total Protein (TP) Measurements for Each Group of Mice

The results are shown in Table 3. In the low-, medium-, and high-dose groups, serum TC, TP, and ALT levels were significantly higher than those in the normal group (P < 0.05), while serum BUN levels were significantly lower compared to the normal group (P < 0.05). There were no statistically significant differences in serum TG levels between the groups (P > 0.05).

Table 3: Comparison of Serum TC, TG, BUN, ALT, and TP Levels in Each Group of Mice($\bar{x}$ ±S)

Constituencies	count	TC/(mmol/L)	TG/(mmol/L)	BUN/(mmol/L)	ALT/(umol/L)	TP/(g/L)
Normal group	11	2.06±0.34	0.76±0.23	34.52±2.26	1.94±0.60	38.18±24.13
Low-dose group	15	2.52±0.61$^{▲}$	1.27±0.65	26.29±7.66$^{▲}$	3.30±1.59$^{▲}$	39.08±16.06$^{▲}$
Medium-dose group	16	2.56±0.66$^{▲}$	0.81±0.27	22.87±7.20$^{▲}$	2.49±1.06$^{▲※}$	56.83±47.94$^{▲}$
High-dose group	8	2.38±0.38$^{▲}$	0.62±0.14	18.84±3.22$^{▲}$	4.27±2.70$^{▲}$	77.12±61.67$^{▲}$

Note: Between-group comparison: Compared to the normal group, $^{▲}P$ < 0.05.

3.4. Hb Content Measurement for Each Group of Mice Before, During, and After Toxicity Induction

The results are shown in Table 4. Between-group comparison: Before toxicity induction, there were no significant differences in Hb content among the groups (P > 0.05). During and after toxicity induction, Hb levels in the medium- and high-dose groups were significantly lower than

those in the normal group ($P < 0.05$), while no significant difference was observed between the low-dose and normal groups ($P > 0.05$).Within-group comparison: Compared to before toxicity induction, Hb content in the normal and low-dose groups showed no significant difference during or after toxicity induction ($P > 0.05$). However, in the medium- and high-dose groups, Hb content during and after toxicity induction was significantly lower than before ($P < 0.05$). Compared to during toxicity induction, no significant differences in Hb content were found between the groups after toxicity induction ($P >0.05$).

Table 4: Hb Content for Each Group of Mice Before, During, and After Toxicity Induction($\bar{x}$ ±S)

Constituencies	count	Before Poisoning (g/L)	Poisoning(g/L)	After poisoning (g/L)
Normal group	11	78.82±11.60	79.94±3.00	81.51±6.73
Low-dose group	15	77.10±12.69	76.24±11.21	77.77±4.69
Medium-dose group	16	79.05±12.51※	67.88±9.04•▲	65.60±8.06•▲
High-dose group	8	78.48±12.88※	65.35±4.97•▲	65.90±7.45•▲

Note: Within-group comparison: Compared to before toxicity induction, •$P < 0.05$. Compared to during toxicity induction, ※$P < 0.05$; Between-group comparison: Compared to the normal group, ▲$P < 0.05$.

3.5. Measurement of Brain BACE1 and Brain Al3+ (μg/ml) Content in Each Group of Mice

The results are shown in Table 5. Brain BACE1: The brain BACE1 levels in the low- and high-dose groups were significantly higher than those in the normal group ($P < 0.05$). However, the brain BACE1 level in the medium-dose group was lower than that in the normal group, but the difference was not statistically significant ($P > 0.05$).Brain Al3+: The brain Al3+ levels in the low-, medium-, and high-dose groups were significantly higher than those in the normal group ($P < 0.05$).

Table 5: Comparison of Brain BACE1 and Brain Al3+ (μg/ml) Content in Each Group of Mice($\bar{x}$ ±S)

Constituencies	count	Brain(BACE1)	Brain Al^{3+}(ug/ml)
Normal group	11	18.89±3.29	0.59±0.02
Low-dose group	15	20.63±5.06▲	0.62±0.02▲
Medium-dose group	16	17.57±2.64	0.63±0.02▲
High-dose group	8	21.51±2.72▲	0.68±0.03▲

Note: Between-group comparison: Compared to the normal group, ▲$P < 0.05$.

3.6. Measurement of Serum GSH-PX, Brain GSH-PX, and Brain AchE Content in Each Group of Mice

The results are shown in Table 6. Serum GSH-PX: The serum GSH-PX levels in the medium-dose group were significantly higher than those in the normal group ($P < 0.05$). There were no statistically significant differences between the low- and high-dose groups and the normal group ($P > 0.05$).Brain GSH-PX: The brain GSH-PX levels in the medium-dose group were significantly lower than those in the normal group ($P < 0.05$). The brain GSH-PX levels in the high-dose group were significantly higher than those in the normal group ($P < 0.05$). No significant difference was observed between the low-dose group and the normal group ($P > 0.05$).Brain AchE: The brain AchE levels in the medium- and high-dose groups were significantly lower than those in the normal group ($P < 0.05$). No significant difference was found between the low-dose group and the normal group ($P > 0.05$).

Table 6: Comparison of Serum GSH-PX, Brain GSH-PX, and Brain AchE Levels in Each Group of Mice($\bar{x}$ ±S)

Constituencies	count	Serum GSH-PX	Brain GSH-PX	Brain AchE
Normal group	11	171.44±37.89	229.73±31.19	8.29±6.64
Low-dose group	15	208.35±53.18	235.83±22.59	5.68±3.32
Medium-dose group	16	232.83±85.14▲	185.38±43.23▲	3.75±1.16▲
High-dose group	8	210.13±40.09	353.30±135.57▲	3.75±1.18▲

Note: Between-group comparison: Compared to the normal group, ▲$P < 0.05$.

4. Discussion

Aluminum is a chronic neurotoxin with diverse physiological effects on the nervous system, which are closely related to its chemical form[6]. Maltol is an excellent ligand for aluminum, exhibiting a strong affinity for the metal. Compared to aluminum chloride, maltol-aluminum is more readily absorbed, leading to higher concentrations of bioavailable aluminum ions and a lower tendency to form aluminum hydroxide precipitates. When maltol-aluminum is neutralized, its pH is very close to that of body fluids, minimizing disruption to the stability of the organism's internal environment. Maltol is a common food flavoring and enhancer, typically a byproduct of sucrose hydrolysis or starch pyrolysis. Upon oral ingestion, it reacts with aluminum in the gastrointestinal tract to form maltol-aluminum, potentially impacting human health[7]. Therefore, the use of maltol-aluminum to establish Alzheimer's disease (AD) mouse models has gained widespread recognition both domestically and internationally. The results of this experiment indicate that, compared to the normal group, the low-, medium-, and high-dose groups of mice exhibited significantly elevated serum TC, TP, ALT levels, brain Al3+ content, and brain β-secretase activity. In contrast, learning and memory abilities, serum BUN, Hb levels, and brain AchE activity were significantly reduced, confirming the successful establishment of the AD mouse model.

This study found that subchronic intraperitoneal injection of maltol-aluminum led to an increase in swimming time and memory errors in mice. Moreover, both swimming time and memory errors increased significantly with higher doses of aluminum exposure, indicating that subchronic aluminum toxicity can impair learning and memory abilities in mice. These findings are consistent with previous research results [8]. The results of this study revealed that in mice injected with maltol-aluminum, brain Al3+ concentrations increased linearly with the rising concentration of maltol-aluminum. However, brain BACE1 levels followed a nonlinear pattern: the low and high-dose groups exhibited significantly higher BACE1 levels than the normal group ($P < 0.05$), while the medium-dose group showed lower BACE1 levels than the normal group. This phenomenon may be attributed to a synergistic effect between aluminum and Aβ, which promotes an increase in BACE1 levels. The compensatory mechanisms in the medium-dose group may be highly activated, leading to a reduction in brain BACE1 levels, while in the high-dose group, the brain BACE1 levels exceed the compensatory threshold, resulting in decompensation and an increase in BACE1 levels. Additionally, this may be related to cholinergic and free radical theories, though the exact mechanisms require further investigation.

Previous studies exploring the synergistic effect between aluminum (Al) and Aβ suggest that some researchers [9] believe that aluminum, by replacing magnesium in the brain, disrupts the balance between phosphorylation and dephosphorylation, leading to a disturbance in protein metabolism and ultimately resulting in Aβ deposition. Additionally, some researchers[10] believe that aluminum interacts with the acidic groups on Aβ, promoting the aggregation of Aβ. This study, however, suggests that aluminum may affect cognitive abilities by altering the expression of β-secretase 1, thereby facilitating the production of Aβ.

5. Conclusions

In summary, there are both differences and connections between the theories of aluminum toxicity and β-amyloid protein. However, the precise interaction between aluminum toxicity and β-amyloid protein requires further investigation to fully understand and determine their relative contributions to the development of neurodegenerative diseases.

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