Experimental Investigation into the Impact of Aluminium on Secretase Activity

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Abstract [**Objectives**] To investigate the impact of aluminium on secretase activity. [**Methods**] A total of 60 Kunming mice were divided into three groups: a control group, a medium-dose model group, and a high-dose model group, with 20 mice in each group. All groups, except for the control group, received sequential injections of aluminum maltolate. The dosage for each injection was 0.3 and 0.45 mL per mouse, administered once daily. An injection pause of 2 d was observed every 5 d throughout the duration of the experiment, which lasted for a total of 40 d. At the conclusion of the study, blood samples were collected to isolate serum, and the brains were taken to prepare a 10% brain homogenate for subsequent analysis. [**Results**] The activities of brain β-secretase were 11.08 ± 1.65, 11.94 ± 1.37$^{▲}$, and 12.32 ± 0.93$^{▲}$ u/L, respectively, with an *F*-value of 4.290 and a *P*-value of 0.018. When compared to the control group, the differences were statistically significant, as indicated by $^{▲}P < 0.05$. The activities of α-secretase were 23.02 ± 3.52, 19.04 ± 1.10$^{▲}$, and 18.44 ± 1.40$^{▲▲}$ u/L, respectively, yielding an *F*-value of 5.972 and a *P*-value of 0.016. Comparisons with the control group revealed statistically significant differences, as denoted by $^{▲}P < 0.05$ and $^{▲▲}P < 0.01$. The activities of γ-secretase were 11.01 ± 2.05$^{▲▲}$, 10.38 ± 1.94$^{▲}$, and 9.00 ± 1.62 u/L, respectively, with an *F*-value of 5.780 and a *P*-value of 0.005. When compared to the high-dose group, the differences were statistically significant, with $^{▲}P < 0.05$ and $^{▲▲}P < 0.01$. [**Conclusions**] Aluminium exerts a considerable influence on the activities of brain β, α, and γ-secretases.

Key words β-secretase, α-secretase, γ-secretase, Secretase activity

1 Introduction

Alzheimer's disease (AD), commonly referred to as senile dementia, is a neurodegenerative disorder with unknown etiology[1]. The primary clinical manifestations of this condition include memory deterioration, cognitive impairment, and alterations in personality, *etc.* The academic community has put forth several theories regarding the underlying mechanisms of AD, including the aluminum poisoning theory, the genetic theory, the cholinergic theory, the free radical theory, the β-amyloid protein theory, *etc.* While each theory is supported by experimental evidence, they also exhibit certain limitations. One of the pathological characteristics of AD is the cleavage of amyloid protein precursors by β-secretase, resulting in the formation of β-amyloid (Aβ). This peptide subsequently undergoes folding to create insoluble fiber deposits, as soluble α-helical second-order structures transition into insoluble β-type lamellar structures. Throughout the onset and progression of AD, Aβ assumes a critical role that warrants significant attention. Soluble Aβ does not exhibit neurotoxicity in its native form. However, upon folding into insoluble fibrillar deposits, which contribute to the formation of senile plaques, it induces neurotoxicity within the brain, ultimately leading to the apoptosis of neuronal cells and accelerating the pathological progression of AD. Under typical conditions, the production and degradation of Aβ are maintained in a state of equilibrium. However, when this balance is disrupted, it can accelerate the pathological progression of AD. The objective of this experiment is to investigate the effects of elevated aluminum levels on the activities of various secretases and the maintenance of this equilibrium.

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2 Materials and methods

2.1 Reagents Aluminum chloride, maltol, β-secretase test kit, α-secretase test kit, γ-secretase test kit, acetylcholinesterase (AChE) test kit, triglyceride (TG) test kit, protein test kit (biuret method), total cholesterol test kit, total protein test kit (Coomassie brilliant blue method), and urea nitrogen test kit (urease method) were procured from Nanjing Jiancheng Reagent Company.

2.2 Experimental animals A total of 60 KM mice, half male and half female, each weighing 30 - 35 g, were acquired from Changsha Tianqin Biotechnology Co., Ltd.

2.3 Methods Thirty KM mice were divided into three distinct groups: the control group, model group 1 (medium-dose group), and model group 2 (high-dose group). Mice were administered aluminum maltolate via intraperitoneal injection[2]. The formulation of the injected drug consisted of 7.15 g/L of aluminum trichloride and 7.56 g/L of maltol, which were mixed in equal proportions and sterilized through filtration. Each injection was delivered at a dosage of 48 mg of aluminum per kg of body weight per day. For a mouse weighing 30 g, a single injection volume of 0.3 mL was classified as a medium dose, while an injection volume of 0.45 mL was classified as a high dose. Following continuous injections over a period of 5 d (administered once daily), the injections were suspended for 2 d. The medium-dose group received a total of 34 injections, whereas the high-dose group received 22 injections, followed by an additional 12 injections at the medium dose. The control group did not receive any injections.

2.4 Y-shaped water maze test The construction of the Y-shaped

water maze model and the specific experimental methods were based on existing literature, with the objective of assessing the memory function of mice through this test. Ten days prior to the establishment of the mouse aluminum poisoning model, the mice underwent training in the water maze to assess their memory capabilities. Each mouse was subjected to three training sessions per day, followed by continuous measurement over a period of 3 d. The parameters recorded included the landing time, the number of errors, and the number of failures exhibited by the mice. Subsequently, the timeout rate, error rate, and failure rate were calculated. A timeout was defined as an instance where a mouse failed to land correctly within 10 sec. An incorrect direction of swimming was classified as an error, while a failure was noted if a mouse did not successfully land within 40 sec[3].

2.5 Measurement methods At the end of the test, the blood vessels located behind the ocular region of the mice were severed to facilitate the collection of whole blood. This blood was subsequently subjected to centrifugation, and the supernatant serum was extracted. The pertinent parameters assessed included total cholesterol (TC), urea nitrogen (BUN), triglycerides (TG), and total protein (TP), in accordance with the provided protocols.

The mice were euthanized using the cervical dislocation method. Following euthanasia, the mice were promptly dissected to obtain their brain tissues. Excess moisture was carefully removed using filter paper, and the tissues were subsequently weighed on a balance (g). The brains were then transferred to a glass homogenizer, to which an appropriate volume of phosphate buffer solution (0.01 M, pH 7.4) was added. The mixture was homogenized in an ice bath for 7 min. Subsequently, the homogenate was transferred to a separate graduated test tube. A small volume of buffer solution was added to rinse the glass homogenizer multiple times, after which the solutions were combined and adjusted to a constant volume, resulting in a 10% brain homogenate. The homogenate was then placed in a centrifuge, where the rotational speed was set between 3 000 and 3 500 rpm, and centrifuged for 10 min. The supernatant obtained following centrifugation was transferred to a separate test tube, sealed with a rubber stopper, and stored at a low temperature. 30 μL of the supernatant from each mouse brain homogenate was collected and analyzed for AChE activity using an AChE test kit. The specific method followed the instructions provided in the test kit. The microplate reader was set up in advance to determine the activities of β-secretase, α-secretase and γ-secretase in the mouse brain homogenate by the double antibody sandwich method (ELISA assay). The detailed operation followed the manual.

2.6 Statistical methods The variance analysis of the test data was conducted utilizing SPSS 24 software. The results were presented as ($\bar{x} \pm s$). Comparisons were made between the data across different groups. A P-value of less than 0.05 or 0.01 was considered indicative of a statistically significant difference.

3 Results and analysis

3.1 Mortality of mice A total of 20 mice were included in the control group, which exhibited no fatalities, resulting in a mortality rate of 0%. In the medium-dose group, which also comprised 20 mice, 2 individuals died, yielding a mortality rate of 10%. The high-dose group, consisting of 20 mice, experienced 10 deaths, leading to a mortality rate of 50%.

3.2 β-secretase, α-secretase and γ-secretase activities in the mouse brain The data presented in Table 1 indicate the results of the intergroup comparisons for the activity of various secretases in the brain. For β-secretase activity, the analysis yielded an F-value of 4.290 with a P-value of 0.018, demonstrating a statistically significant difference when compared to the control group ($^{▲}P < 0.05$). For α-secretase activity, the F-value was 5.972 with a P-value of 0.016, indicating statistically significant differences relative to the control group ($^{▲}P < 0.05$, $^{▲▲}P < 0.01$). Lastly, for γ-secretase activity, the F-value was 5.780 with a P-value of 0.005, revealing statistically significant differences when compared to the high-dose group ($^{▲}P < 0.05$, $^{▲▲}P < 0.01$).

Table 1 Comparison of β-secretase, α-secretase and γ-secretase activities in the mouse brain across various groups ($n = 20$, $\bar{x} \pm s$)

u/L

Group	β-secretase	α-secretase	γ-secretase
Control	11.08 ± 1.65	23.02 ± 3.52	11.01 ± 2.05$^{▲▲}$
Medium-dose	11.94 ± 1.37$^{▲}$	19.04 ± 1.10$^{▲}$	10.38 ± 1.94$^{▲}$
High-dose	12.32 ± 0.93$^{▲}$	18.44 ± 1.40$^{▲▲}$	9.00 ± 1.62

NOTE $^{▲}P < 0.05$ signifies a statistically significant difference in intergroup comparisons; $^{▲▲}P < 0.01$ denotes an extremely significant difference in intergroup comparisons.

3.3 Duration of water maze test For intergroup comparisons, prior to exposure to toxic substances, the analysis yielded $F = 1.498$ and $P = 0.247$, which is greater than 0.05, indicating that the difference was not statistically significant (Table 2). During the exposure, the results showed $F = 2.258$ and $P = 0.135$, also exceeding 0.05, thus confirming that the difference was not statistically significant. However, after exposure to toxic substances, the analysis revealed $F = 3.242$ and $P = 0.061$. In comparison to the control group, a significance level of $^{▲}P < 0.05$ was observed, suggesting that the difference was statistically significant.

Table 2 Comparison of water maze test durations in mice across various groups before, during and after exposure to toxic substances ($n = 20$, $\bar{x} \pm s$)

sec

Group	Before exposure	During exposure	After exposure
Control	3.47 ± 0.31	3.17 ± 0.29^{a}	3.80 ± 0.61
Medium-dose	4.00 ± 0.55^{a}	4.00 ± 0.55^{a}	11.13 ± 9.07$^{▲}$
High-dose	3.66 ± 0.88	5.34 ± 2.98^{a}	7.52 ± 4.68

NOTE $^{▲}P < 0.05$ signifies a statistically significant difference in intergroup comparisons; $^{a}P < 0.05$ denotes a statistically significant difference in intragroup comparisons.

For intragroup comparisons, the analysis of control group yielded an F-value of 3.311 and a P-value of 0.052. When comparing the results following exposure to toxic substances, a significance level of $^{a}P < 0.05$ was observed, indicating a statistically significant difference. In the medium-dose group, the analysis produced an F-value of 3.473 and a P-value of 0.047, with a significance level of $^{a}P < 0.05$, confirming a statistically significant difference. Conversely, in the high-dose group, the F-value was

2.678 and the *P*-value was 0.104, with a significance level of $^{a}P<0.05$, indicating the difference was statistically significant.

3.4 Serum BUN, TP and TC levels of mice Intergroup comparisons presented in Table 3 indicated that the analysis of BUN levels resulted in an *F*-value of 0.805 and a *P*-value of 0.463, suggesting that the difference was not statistically significant. In contrast, the analysis of TP levels yielded an *F*-value of 7.449 and a *P*-value of 0.004, indicating a statistically significant difference when compared to the control group ($^{▲▲}P<0.01$). Additionally, the analysis of serum TC levels produced an *F*-value of 4.045 and a *P*-value of 0.038, demonstrating a statistically significant difference when compared to the high-dose group ($^{▲}P<0.05$).

Table 3 Comparison of BUN, TP and TC levels of mice across various groups ($n=20$, $\bar{x}\pm s$)

Group	BUN // mmol/L	TP // g/L	TC // mmol/L
Control	2.65 ± 0.36	64.58 ± 5.36	2.42 ± 0.08$^{▲}$
Medium-dose	2.90 ± 0.55	58.69 ± 2.29$^{▲▲}$	2.51 ± 0.32$^{▲}$
High-dose	2.91 ± 0.40	57.04 ± 2.87$^{▲▲}$	3.48 ± 1.39

NOTE $^{▲}P<0.05$ signifies a statistically significant difference in intergroup comparisons; $^{▲▲}P<0.01$ denotes an extremely significant difference in intergroup comparisons. The same below.

3.5 Serum TG, AChE, and brain protein of mice The intergroup comparisons presented in Table 4 revealed that the analysis of TG levels yielded an *F*-value of 16.801 with a *P*-value of 0.000. When compared to the control group, the results indicated a statistically significant difference ($^{▲▲}P<0.01$). The analysis of brain AChE levels produced an *F*-value of 12.010 and a *P*-value of 0.001. In comparison to the control group, the differences were statistically significant ($^{▲▲}P<0.01$, $^{▲}P<0.05$). Additionally, the analysis of brain protein levels resulted in an *F*-value of 3.507 with a *P*-value of 0.049, indicating a statistically significant difference when compared to the control group ($^{▲}P<0.05$).

Table 4 Comparison of TG, AChE, and brain protein levels in the serum of mice across various groups ($n=20$, $\bar{x}\pm s$)

Group	TG // mmol/L	AChE // U/mg	Brain protein // mg/mL
Control	3.28 ± 0.78	0.20 ± 0.02	26.47 ± 1.80
Medium-dose	2.00 ± 0.37$^{▲▲}$	0.16 ± 0.02$^{▲▲}$	27.75 ± 2.56
High-dose	1.48 ± 0.33$^{▲▲}$	0.18 ± 0.01$^{▲}$	29.34 ± 0.82$^{▲}$

4 Conclusions and discussion

The study revealed that the levels of brain β-secretase were lowest in the control group, increased in the medium-dose group, and were highest in the high-dose group. Furthermore, as the dosage of aluminum injection increased, the aluminum concentration in the body also rose, which was associated with a corresponding increase in β-secretase activity. Under the catalysis of β-secretase, amyloid protein precursors are cleaved to produce Aβ, which subsequently undergoes folding to form insoluble fibrillar deposits. Specifically, soluble α-helical secondary structures are transformed into insoluble β-lamellar structures. Aβ plays a critical and indispensable role in the onset and progression of AD. Soluble Aβ does not exhibit neurotoxicity in its native form. However, upon folding into insoluble fibrillar deposits and subsequently forming senile plaques, it induces neurotoxicity within the brain[4]. This process contributes to the apoptosis of neuronal cells and accelerates the pathological progression of AD. The activity of α-secretase was found to be highest in the control group, decreased in the medium-dose group, and was lowest in the high-dose group. Under normal physiological conditions, α-secretase catalyzed the catabolism of precursor proteins and exhibited maximal activity. However, an increase in aluminum concentration correlated with a reduction in α-secretase activity, which was diminished in the medium-dose group and reached its lowest level in the high-dose group. Conversely, the activity of β-secretase demonstrated an increasing trend in response to aluminum exposure. The activity of brain γ-secretase was observed to be highest in the control group, decreased in the medium-dose group, and reached its lowest level in the high-dose group. Additionally, the activity of γ-secretase, in conjunction with the catalytic activity of the aforementioned secretase, exhibited variations[5].

The activity of brain AChE in the control group was significantly higher than that observed in the other two experimental groups. This finding suggests that aluminum exposure adversely affects the activity of brain AChE, leading to a reduction in its enzymatic function. AChE is an enzyme responsible for catalyzing the hydrolysis of the neurotransmitter acetylcholine. A decrease in the activity of this enzyme results in a slowed breakdown of acetylcholine, consequently leading to an increase in its concentration. This elevation in acetylcholine levels may be advantageous for sustaining neural activity, albeit potentially in a temporary or compensatory manner.

The findings regarding the impact of aluminum on various biochemical components in serum, including BUN, TP, TC, and TG, revealed no significant change in BUN levels. However, a decrease in TP and TG levels was observed, while TC levels exhibited an increase. Further investigation is warranted to elucidate the significance of these changes.

The limitation of this experiment is the relatively high mortality rate observed in the high-dose group. Additionally, some data indicate that there are no significant differences or a linear relationship among the control group, the medium-dose group, and the high-dose group, necessitating further investigation.

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