Regulatory Effects of Radix Salviae Miltiorrhizae Water Extract and Semen Ziziphi Spinosae Compound on β-secretase Activity in Mice Exposed to Aluminum Maltolate

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Abstract [**Objectives**] To explore the effects of Radix Salviae Miltiorrhizae water extract and Semen Ziziphi Spinosae compound on the β-secretase activity in mice exposed to aluminum maltolate. [**Methods**] A total of 60 healthy, clean-grade SPF mice were randomly assigned to four groups based on their body weight, with each group consisting of 15 mice. The groups included a control group, a model group, treatment group 1, and treatment group 2. The control group received an equivalent dose of normal saline, while the model group and treatment groups 1 and 2 were intraperitoneally injected with 0.3 mg/kg of aluminum maltolate solution for 60 d. Additionally, treatment groups 1 and 2 were injected with 0.3 mg/kg of Radix Salviae Miltiorrhizae water extract and 0.3 mg/kg of Semen Ziziphi Spinosae compound, respectively, starting from the 31st day for a total of 30 d. The cognitive functions of mice, specifically their learning and memory capabilities, were assessed using the Y-shaped water maze test at three distinct time points: prior to, during, and following the experimental procedure. Serum samples were collected for the analysis of various biochemical markers, including hemoglobin (Hb), total cholesterol (TC), triglycerides (TG), total protein (TP), alanine aminotransferase (ALT), and blood urea nitrogen (BUN). Additionally, brain tissues were harvested to evaluate the levels of glutathione peroxidase (GSH-PX) and acetylcholinesterase (AChE) in both serum and brain samples. The expression levels of α-secretase, β-secretase, and γ-secretase in mouse serum were quantified using enzyme-linked immunosorbent assay (ELISA). [**Results**] According to the final results of the Y-shaped water maze test, the administration of therapeutic drugs to mice resulted in a gradual reduction in both the swimming time and the distance traveled to reach the platform in treatment groups 1 and 2. Additionally, the number of errors made by these treatment groups was significantly greater than that observed in the control group, with statistically significant differences ($P<0.01$). Among the three groups subjected to subchronic aluminum exposure, statistically significant differences were observed in the levels of Hb, TC, TG, TP, ALT, BUN, brain GSH-PX, and brain AChE ($P<0.05$). Furthermore, with the increasing duration of therapeutic drug administration, the levels of β-secretase in the brains of mice in both the treatment groups and the model group exhibited a significant decrease, while the levels of α-secretase showed a significant increase. Additionally, the differences were statistically significant when compared to the control group ($P<0.05$). [**Conclusions**] Radix Salviae Miltiorrhizae water extract and Semen Ziziphi Spinosae compound will decrease the expression level of β-secretase activity in mice exposed to aluminum maltolate.

Key words Radix Salviae Miltiorrhizae water extract, Semen Ziziphi Spinosae compound, Aluminum, β-secretase, Mice, Learning and memory capability

1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by an insidious onset and a gradual, irreversible progression. Patients primarily exhibit impairments in memory, cognition, and overall intellectual function. Pathological changes associated with AD include a significant reduction in neuronal populations and diffuse cortical atrophy. Additionally, the presence of senile plaques (SP) and neurofibrillary tangles (NFT) is commonly observed in the brains of individuals affected by this condition. Current studies confirm that there is a significant association between aluminum exposure and the development of AD. Aluminum, recognized as a chronic neurotoxicant with a high potential for accumulation, may contribute to cognitive dysfunction through multiple mechanisms[1]. This investigation evaluated the effects of Radix Salviae Miltiorrhizae water extract and Semen Ziziphi Spinosae compound on the cognitive and memory capabilities of AD mice exposed to aluminum maltolate.

2 Materials and methods

2.1 Reagents The reagents utilized in the test included Radix Salviae Miltiorrhizae water extract, Semen Ziziphi Spinosae compound, aluminum maltolate solution, normal saline, phosphate buffer, β-secretase test kit, hemoglobin (Hb) test kit, triglycerides, total cholesterol, protein test kit, acetylcholinesterase (AChE) test kit.

2.2 Animals A total of 60 standard, clean, and healthy mice, comprising 30 males and 30 females, with weights ranging from 32 to 40 g and aged between 2 to 3 months, were obtained from the Experimental Animal Center of Youjiang Medical University for Nationalities, located in Baise City, Guangxi Zhuang Autonomous Region.

2.3 Experimental methods In the intergroup control experiment, mice were randomly assigned to four groups: the control

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group, the model group (aluminum poisoning untreated group), treatment group 1 (Radix Salviae Miltiorrhizae water extract), and treatment group 2 (Semen Ziziphi Spinosae compound), with each group consisting of 15 mice. With the exception of the control group, all other groups received treatment with an aluminum maltolate solution. Specifically, aluminum was administered via intraperitoneal injection at a dosage of 2 mg/(kg · day) (0.15 mL per mouse, with each mouse weighing approximately 37 g). This treatment was conducted once daily for consecutive 5 d, followed by a 2 d rest period. The modeling injections were completed after a total of 60 d. Thirty days following the modeling phase, the treatment group commenced a treatment of traditional Chinese medicine for 30 d. On the 31st day, treatment group 1 received an administration of 0.3 mg/kg of Radix Salviae Miltiorrhizae water extract, while treatment group 2 was administered 0.3 mg/kg of Semen Ziziphi Spinosae compound. In contrast, both model group and the control group were provided with an equivalent volume of normal saline every day for a total of 60 d.

2.4 Measurement methods At the end of the experiment, ocular blood samples were collected from the mice to assess biochemical indicators, specifically urea nitrogen, serum total cholesterol (TC), and triglyceride (TG) levels. The mice were subsequently dissected, and various organs, including the hearts, livers, kidneys, and brains, were extracted. Some of these organs were processed into pathological sections to examine morphological changes, while others were prepared as 10% brain homogenates to evaluate the activities of β-secretase, AChE, and to quantify protein content. Additionally, the aluminium content in both serum and brain homogenates was measured using an atomic spectrophotometer, and the results were subjected to comparative analysis.

2.5 Statistical methods The data adhered to a normal distribution and were presented as mean ± standard deviation ($\bar{x} \pm s$). Statistical analysis was conducted using SPSS 13 software. The least significant difference (LSD) t-test was employed for pairwise comparisons, while one-way analysis of variance (ANOVA) was utilized for comparisons among multiple groups. A P-value of less than 0.01 was considered indicative of a statistically significant difference.

3 Results and analysis

3.1 Contents of α-secretase, β-secretase, and γ-secretase in the brain of mice As illustrated in Table 1, a comparison among the groups revealed statistically significant differences in the levels of α-secretase, β-secretase, and γ-secretase within the brain.

Table 1 Comparison of the levels of α-secretase, β-secretase, and γ-secretase within the brain across various groups ($\bar{x} \pm s$) u/L

Group	Number of animals	β-secretase	α-secretase	γ-secretase
Control	14	14.91 ± 2.37	74.06 ± 16.97▲▲	14.62 ± 3.94
Model	6	11.41 ± 1.84▲●●	78.46 ± 7.79▲	11.74 ± 4.60
Treatment 1	8	16.16 ± 2.87	82.41 ± 4.82	9.33 ± 2.38▲▲
Treatment 2	7	11.89 ± 2.59▲●●	93.50 ± 4.49	8.89 ± 1.85▲▲

NOTE β-secretase: $F = 5.693$, $P = 0.005$; compared to the control group, ▲$P < 0.05$; compared to treatment group 1, ●●$P < 0.01$. α-secretase: $F = 4.057$, $P = 0.170$; compared to treatment group 2, ▲$P < 0.05$, ▲▲$P < 0.01$. γ-secretase: $F = 6.167$, $P = 0.002$; compared to the control group, ▲▲$P < 0.01$.

3.2 Water maze duration The intergroup comparison indicated that prior to modeling, the results were $F = 1.095$, $P = 0.362$, suggesting that the difference was not statistically significant. Prior to treatment (after modeling), the results were $F = 0.546$, $P = 0.654$, which also indicated that the difference was not statistically significant. Following treatment, the learning and memory capabilities of mice in treatment group 2 were significantly enhanced compared to those in treatment group 1 and the model group, with $F = 8.488$, $P = 0.000$. In comparison to treatment group 2, the differences were statistically significant, with ▲▲$P < 0.01$. Furthermore, the learning and memory capabilities of mice in the model group were markedly inferior to those in the normal group as well as in treatment groups 1 and 2. When compared to the model group, the differences were statistically significant, with ●$P < 0.05$ and ●●$P < 0.01$.

The intragroup comparison indicated that the time taken by mice in the normal group to complete the water maze test during the late stage of the experiment was significantly longer than that observed in the early and middle stages. The statistical analysis yielded an F-value of 17.919 and a P-value of 0.000. When comparing the early stage to the middle and late stages, the results demonstrated a statistically significant difference, with [a]$P < 0.01$. The duration of the water maze test for mice in the model group during the late stages of the experiment was significantly longer than that observed in the early and middle stages. Furthermore, this duration was shorter than that of the mice in the control group, with an F-value of 21.841 and a P-value of 0.000. Compared to the results following treatment, [b]$P < 0.01$; compared to the results prior to treatment, [c]$P < 0.01$. All observed differences were statistically significant. In treatment group 1, the analysis yielded $F = 10.071$, $P = 0.001$. When compared to the results obtained prior to modeling, [d]$P < 0.01$ indicated a statistically significant difference. Mice in the middle stage of the experiment in treatment group 2 exhibited an increased duration on the water maze test relative to their performance before the experiment, although this duration was less than that observed in the late stage of the experiment. This suggests a significant enhancement in the learning and memory capabilities of the mice in the late stages of the experiment in this group, as indicated by $F = 3.876$, $P = 0.035$. Compared to the results following modeling, [e]$P < 0.01$; compared to the results following treatment, [f]$P < 0.01$. Both observed differences were statistically significant (Table 2).

Table 2 Comparison of water maze duration of mice across various groups ($n=15$, $\bar{x}\pm s$) sec

Group	Prior to modeling	Prior to treatment (following modeling)	Following treatment
Control	4.27 ± 0.99	6.09 ± 1.09a	6.80 ± 1.01▲▲●[a]
Model	3.94 ± 0.77[bc]	5.72 ± 1.84b	8.10 ± 1.12▲▲[c]
Treatment 1	4.51 ± 0.85	6.59 ± 1.81d	7.24 ± 1.41▲▲[d]
Treatment 2	4.44 ± 0.67[e]	5.82 ± 1.91	4.88 ± 1.20●●[e]

NOTE ① Intergroup comparison: compared to treatment group 2, ▲▲$P<0.01$; compared to the model group, ●$P<0.05$, ●●$P<0.01$. ② Intragroup comparison: control group: compared to the early stage, [a]$P<0.01$. Model group: compared to following treatment, [b]$P<0.01$; compared to prior to treatment, [c]$P<0.01$. Treatment group 1: compared to prior to modeling, [d]$P<0.01$. Treatment group 2: compared to following modeling, [e]$P<0.01$; compared to following treatment, [f]$P<0.01$.

3.3 Hb concentration For Hb concentration, comparison across various groups before modeling revealed an F-value of 13.963 with a P-value of 0.000; compared to treatment group 2, ▲$P<0.05$, ▲▲$P<0.01$; compared to treatment group 1, ●$P<0.05$ (Table 3). Comparison across various groups prior to treatment (following modeling) revealed an F-value of 22.964 with a P-value of 0.000; compared to the control group, ▲▲$P<0.01$. Comparison across various groups following treatment revealed an F-value of 11.652 with a P-value of 0.000; compared to the control group, ▲▲$P<0.01$; compared to treatment group 2, ●$P<0.05$.

The statistical analysis of the control group showed an F-value of 14.675 with a P-value of 0.000; compared to following modeling, [a]$P<0.01$. The statistical analysis of the model group revealed an F-value of 7.745 with a P-value of 0.002; compared to prior to modeling, [b]$P<0.05$, [a]$P<0.01$. The statistical analysis of treatment group 1 yielded an F-value of 25.321 with a P-value of 0.000; compared to prior to modeling, [c]$P<0.01$. The statistical analysis of treatment group 2 indicated an F-value of 26.177 with a P-value of 0.000; compared to prior to modeling, [d]$P<0.01$.

Table 3 Comparison of Hb concentration across various groups ($\bar{x}\pm s$) g/L

Group	Prior to modeling	Prior to treatment (following modeling)	Following treatment
Control	68.92 ± 13.08▲▲●●[a]	92.07 ± 15.20	75.46 ± 5.72[a]
Model	75.69 ± 10.42▲▲●	66.07 ± 6.96▲▲[b]	59.41 ± 12.22▲▲[a]
Treatment 1	86.08 ± 13.62▲	58.71 ± 8.23▲▲[d]	57.91 ± 7.04▲▲[d]
Treatment 2	99.45 ± 17.19●	61.96 ± 12.13▲▲[d]	63.71 ± 7.92▲▲

3.4 TG, TC and urea nitrogen levels When comparing various groups, the differences in TG levels were found to be statistically significant. Additionally, the comparison of TC levels among the groups revealed statistically significant differences. Furthermore, urea nitrogen levels also exhibited statistically significant differences when the groups were compared (Table 4).

Table 4 Comparison of TG, TC and urea nitrogen levels across various groups ($\bar{x}\pm s$) mmol

Group	TG	TC	Urea nitrogen
Control	2.49 ± 1.73	2.74 ± 0.57	8.44 ± 2.47
Model	2.66 ± 1.81	2.32 ± 0.91	8.48 ± 4.14
Treatment 1	3.17 ± 2.18	2.42 ± 0.88	7.20 ± 2.40
Treatment 2	3.93 ± 2.18	2.30 ± 0.37	5.65 ± 1.45▲

NOTE Urea nitrogen: $F=2.007$, $P=0.133$; compared to the control group, ▲$P<0.05$.

3.5 GSH-PX, TP and AChE levels As presented in Table 5, the statistical analysis of GSH-PX levels across different groups yielded an F-value of 1.858 with a P-value of 0.157. Additionally, the comparison of TP levels among the various groups revealed an F-value of 0.100 and a P-value of 0.364. Furthermore, the statistical analysis of AChE levels across the groups resulted in an F-value of 0.581 and a P-value of 0.632.

Table 5 Comparison of GSH-PX, TP and AChE levels across various groups ($\bar{x}\pm s$)

Group	GSH-PX//mmol	TP//mmol	AChE//U/mg
Control	2.62 ± 0.89	17.99 ± 1.52	0.48 ± 0.14
Model	1.99 ± 0.51	16.04 ± 4.17	0.43 ± 0.12
Treatment 1	2.04 ± 0.59	17.18 ± 2.32	0.41 ± 0.11
Treatment 2	2.57 ± 0.55	17.81 ± 1.25	0.46 ± 0.11

3.6 Error rate, timeout rate and failure rate of water maze It can be observed from Table 6 that prior to modeling, each group experienced errors, timeouts, and failures. Specifically, a timeout is defined as failing to reach the finish line within 10 sec, which is recorded as 10 sec. Conversely, if the finish line is not reached within 40 sec, it is classified as a failure and is recorded as 40 sec. Notably, the treatment group 1 exhibited the highest incidence of these issues. Following the modeling process, treatment group 1 demonstrated the highest error rate, whereas only the model group experienced failures. Following treatment, the model group exhibited an increase in errors, timeouts, and failures compared to pre-treatment levels, while the other groups showed a decrease in these metrics.

Table 6 Error rate, timeout rate and failure rate of water maze test %

Group	Prior to modeling			Prior to treatment (following modeling)			Following treatment		
	Error rate	Timeout rate	Failure rate	Error rate	Timeout rate	Failure rate	Error rate	Timeout rate	Failure rate
Control	16.30	13.33	1.48	23.80	30.95	0	13.33	25.93	0
Model	14.07	14.81	2.96	19.44	25.00	1.59	22.22	44.40	7.94
Treatment 1	20.74	28.89	9.60	40.00	32.00	0	18.06	23.61	6.94
Treatment 2	14.81	18.52	3.70	16.67	16.67	0	5.56	11.11	1.85

4 Discussion

AD is a progressive neurodegenerative disorder primarily characterized by a gradual decline in cognitive functions, which adversely affects memory, reasoning, analytical abilities, visuospatial recognition, emotional regulation, and other cognitive domains. Numerous factors contribute to the onset and progression of this disease. As AD advances, patients exhibit impairments in recent memory, diminished learning capabilities, various psychological symptoms, atypical behaviors, and alterations in the levels of α-secretase and β-secretase[2]. Research has demonstrated that the administration of β-secretase inhibitors yields comparable therapeutic effects and enhances both the AD-like pathology and the memory learning capabilities of AD model mice. In this study, a mouse model of dementia was established through the intraperitoneal injection of aluminum maltolate solution to replicate the pathological characteristics associated with AD. The AD mice that were exposed to aluminum maltolate subsequently received treatment and intervention with Radix Salviae Miltiorrhizae water extract and Semen Ziziphi Spinosae compound. A blank control group was set to facilitate analysis of the research methods. Through the application of β-secretase and α-secretase reagent tests, along with statistical data analysis utilizing SPSS 13 software, we conducted measurements of Y-shaped maze learning and training across the early, middle, and late stages. The levels of α-secretase and β-secretase in the mice were subsequently extracted and quantified. The results indicated that the activities of α-secretase in treatment groups 1 and 2 were significantly higher than those observed in the model group ($P<0.05$)[3]. This suggests that the Radix Salviae Miltiorrhizae water extract and Semen Ziziphi Spinosae compound can effectively inhibit β-secretase and promote the synthesis of α-secretase to varying degrees[4].

The learning and memory capabilities of mice were assessed through training in a Y-shaped maze. Comparative analyses, both intergroup and intragroup, revealed that treatment groups 1 and 2 exhibited significantly improved performance relative to the model group. Following the intraperitoneal administration of Radix Salviae Miltiorrhizae[5] and Semen Ziziphi Spinosae[6] solutions during the model establishment, the data indicated a progressive linear improvement over time. Consequently, the Radix Salviae Miltiorrhizae and Semen Ziziphi Spinosae solutions demonstrated a notable efficacy in enhancing the learning and memory capabilities of mice exhibiting AD pathological characteristics.

The findings of this experiment indicated that, in comparison to the control group, the serum urea nitrogen levels of mice in the model group were significantly elevated ($P<0.05$). An analysis of Hb levels across the various groups of mice, conducted prior to modeling, prior to treatment, and following treatment, suggested that the pathology associated with AD may result in abnormal Hb levels. Specifically, low hemoglobin levels can directly impair neuronal function, while elevated hemoglobin levels may increase the risk of stroke. Consequently, both conditions could contribute to the development of dementia. The fluctuations in Hb levels observed in AD patients may be associated with the underlying pathophysiological mechanisms of the condition. Specifically, neuronal damage and inflammatory responses occurring within the brain may induce modifications in the hematological system, including variations in Hb levels. Furthermore, individuals with AD frequently present with comorbid vascular conditions, such as cerebrovascular and cardiovascular diseases, which may also influence Hb concentrations.

As the condition of patients with AD deteriorates, alterations in the levels of α-secretase, β-secretase, serum urea nitrogen, and Hb are associated with the patients' memory and learning capabilities, as well as their mental and behavioral health. It is advisable to closely monitor these biomarkers in clinical settings and implement targeted interventions aimed at enhancing the well-being of individuals with AD[7].

In this experiment, the levels of α-secretase and β-secretase in the brain of mice were extracted and quantified. The results indicated that the concentration of β-secretase in treatment group 2 was marginally lower than that in treatment group 1, while the concentration of α-secretase in treatment group 2 was significantly higher than that in treatment group 1 ($P>0.05$). Consequently, the therapeutic effect of treatment group 2, which received Radix Salviae Miltiorrhizae solution, was found to be more pronounced than that of treatment group 1, which was administered Semen Ziziphi Spinosae solution, in the context of an AD pathological model in mice. This experiment provides a foundation for further investigation into the mechanisms of action of Radix Salviae Miltiorrhizae and Semen Ziziphi Spinosae solutions concerning the pathological symptoms of AD and their potential influence on the activity of β-secretase.

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