Social Medicine and Health Management (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/socmhm.2025.060112
ISSN 2616-2210 Vol. 6 Num. 1

Effects of Suanzaoren on Brain-Related Enzymes and Serum Biochemical Indicators in a Mouse Model of Alzheimer's Disease

Junning Qiu[1], Qingcui Huang[1], Huilin Zhao[1], Yongfu Tang[1], Shuqiu Zhang[2], Qijun Long[3,4,*]

[1]Youjiang Medical University for Nationalities, Baise, Guangxi, 533000, China
[2]Heavy Metal and Fluorine-Arsenic Toxin Research Laboratory, Youjiang Medical University for Nationalities, Baise, Guangxi, 533000, China
[3]College of Humanities and Management, Youjiang Medical University for Nationalities, Baise, 533000, Guangxi, China
[4]College of Innovation and Entrepreneurship, Youjiang Medical University for Nationalities, Baise, 533000, Guangxi, China
[*]Corresponding author

Keywords: Aluminum, Alzheimer's Disease, Brain Biochemical Indicators

Abstract: To investigate the effects of Suanzaoren solution on serum and brain biochemical indicators in an aluminum toxicity-induced Alzheimer's disease (AD) mouse model. The aluminum toxicity-induced AD mouse model was established using maltol aluminum solution. Sixty SPF-grade male and female mice were randomly divided into four groups: normal group, model group, treatment group 1 (high-dose group), and treatment group 2 (low-dose group) [5]. Except for the normal group, all other groups received daily intraperitoneal injections of 30 mmol/L maltol aluminum solution (0.3 mL/mouse) for 60 consecutive days [5]. From day 31 onward, treatment group 1 was orally administered Suanzaoren solution (0.3 mL/mouse), while treatment group 2 received a 1:2 diluted Suanzaoren solution (Suanzaoren solution: distilled water = 1:2). The normal and model groups were given equal volumes of saline via gavage until the end of the experiment (day 60). Memory ability changes were assessed using the Morris water maze test during the experimental period. After sacrifice, serum triglycerides (TG), total cholesterol (TC), total protein (TP), and blood urea nitrogen (BUN) levels were measured. Brain tissues were homogenized in pH 7.4 phosphate buffer (0.01 mmol/L) to prepare 10% (w/v) homogenates, and enzymatic activities of α-secretase, β-secretase, γ-secretase, and acetylcholinesterase (AChE) were determined. Statistical analysis was performed [7]. Intraperitoneal injection of maltol aluminum solution successfully established an AD mouse model, characterized by elevated β-secretase activity and reduced α-secretase activity. Post-treatment with Suanzaoren solution significantly altered serum TG, TC, TP, and BUN levels, decreased β-secretase and AChE activities, and increased α-secretase activity, suggesting potential therapeutic effects against AD. Suanzaoren solution may serve as a promising therapeutic strategy for AD by regulating the balance of β-/α-secretase and modulating cholinergic system function.

1. Introduction

Aluminum, one of the most common metals in nature, is widely present in the environment[9]. With the increasing use of aluminum products in daily life, aluminum toxicity-related diseases have emerged[1]. Among these, research has demonstrated a significant association between aluminum exposure and the pathogenesis of Alzheimer's disease (AD), confirming its role as an inducible risk factor for dementia onset. A degenerative central nervous system disorder primarily affecting the elderly[2]. AD is characterized by irreversibility and degeneration, manifesting as memory impairment, language and behavioral disorders, and cognitive difficulties[8]. While the exact pathogenesis of AD remains unclear, several theories have been proposed, including the aluminum toxicity hypothesis, β-amyloid protein deposition hypothesis, and cholinergic hypothesis. Recent studies have highlighted the role of aluminum in the β-amyloid protein deposition hypothesis, wherein elevated aluminum levels increase β-secretase activity, a key enzyme catalyzing the formation of β-amyloid (Aβ) fragments.

The present study aimed to establish an AD mouse model using maltol aluminum solution and investigate the effects of Suanzaoren solution, administered at different doses, on brain-related enzymes and biochemical indicators in this model. The findings provide insights into the prevention and treatment of AD.

2. Materials and Methods

2.1. Reagents and Instruments

Materials: Maltol, aluminum chloride, sodium chloride, hydrochloric acid, sodium nitrite, disodium hydrogen phosphate, and sodium dihydrogen phosphate.

Reagents: Triglyceride (TG) kit, total protein (TP) kit, acetylcholinesterase (AchE) kit, total cholesterol (TC) kit, α-, β-, γ-secretase kits, etc.

Instruments: Visible spectrophotometer, enzyme-linked immunosorbent assay (ELISA) reader, etc.

2.2. Herbal Medicine Preparation

Suanzaoren was weighed, extracted with water for 1 hour, filtered, and concentrated. Triple the volume of 95% ethanol was added to precipitate the extract, which was then filtered and the ethanol recovered. The remaining liquid was concentrated to 1 g/ml, bottled, and sterilized at 105 ℃.

2.3. Animals

Sixty healthy male and female SPF-grade mice, aged 2-3 months and weighing 32-40 g, were purchased from Tianqin Biotechnology Co., Ltd., Changsha, Hunan Province (Experimental Animal Production License No.: Scxk (Hunan) 2019-0013).

2.4. Experimental Method

2.4.1. Model Establishment

Following a one-week acclimatization period, the experimental mice were randomly divided into four groups: normal control group, model group, high-dose treatment group, and low-dose treatment group, with 15 mice per group. All animals were housed separately by sex in individual cages.[5]

2.4.2. Experimental Procedure

Except for the normal group, the other groups received an intraperitoneal injection of maltol aluminum solution at 2 mg/kg body weight per day for 60 days, with 5 days of injection followed by 2 days of rest. Treatment with Suanzaoren solution commenced on day 31 for 30 days: treatment group 1 received undiluted Suanzaoren solution, while treatment group 2 received a 1:2 dilution. The normal group and model group received saline. Morris water maze tests were conducted at three time points—pre-experiment (day 0), mid-experiment (day 30), and post-experiment (day 60)—to evaluate memory function changes in mice.

2.5. Measurement Methods

2.5.1. Serum Sample Preparation

Blood was collected from the retro-orbital sinus, centrifuged, and the serum was stored at 4℃ for the measurement of TG, TP, TC, and BUN.

2.5.2. Measurement of α-Secretase Activity in the Brain

Brain tissues were immediately harvested, rinsed with phosphate-buffered saline (PBS, 0.01 M, pH 7.4), and weighed. Subsequently, the tissues were homogenized in ice-cold PBS (1:10 w/v) and centrifuged at 12,000 × g for 15 min at 4°C to collect supernatants. α-Secretase activity was quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's standardized protocols.[10]

2.5.3. Measurement of β- and γ-Secretase Activities in the Brain

The methods were similar to those used for α-secretase.

2.6. Statistical Analysis

Data were analyzed using SPSS 13 software and presented as mean ± standard deviation (SD). The Q-test was used for comparison, with $P<0.05$ considered statistically significant.[5]

3. Results

3.1. Brain Enzyme Activities

Table 1 Comparison of β-Secretase, α-Secretase and γ-Secretase Activities in the Brain (mean ± SD)

Group	N	β-Secretase (U/L)	α-Secretase (U/L)	γ-Secretase (U/L)
Normal	14	9.72±1.59	24.85±8.97▲	9.58±2.01
Model	13	11.25±1.32	16.63±4.39	9.74±0.56
Treatment 1	14	9.83±1.69	25.02±7.11▲	9.05±1.31
Treatment 2	15	9.32±2.79▲	22.39±5.67	9.15±1.43

Note: ANOVA, β-secretase: $F=1.581$, $P=0.211$; compared to model group, ▲$P<0.05$. α-secretase: $F=1.757$, $P=0.177$; compared to model group, ▲$P<0.05$. γ-secretase: $F=0.462$, $P=0.711$; no significant difference among groups.

The β-secretase activity was elevated in the model group and reduced in the treatment groups, with a more significant reduction in treatment group 2 ($P<0.05$). The α-secretase activity was

increased in the normal group and treatment groups compared to the model group (P<0.05). No significant difference was observed in γ-secretase activity among the groups (Table 1).

3.2. Serum Biochemical Indicators

No significant difference was observed in serum TG levels among the groups. Serum TC levels were significantly elevated in all groups except the normal group (P<0.05). Serum BUN levels were significantly lower in treatment group 1 compared to the model group (P<0.05). Serum TP levels were significantly different among the groups, with the model and treatment groups showing higher levels than the normal group, and treatment group 2 exhibiting the lowest levels (Table 2).

Table 2 Comparison of Serum Biochemical Indicators (mean ± SD)

Group	N	TC (mmol/L)	TG (mmol/L)	BUN (mmol/L)
Normal	14	1.94±0.59	2.02±0.94	25.73±5.59▲▲
Model	14	2.11±0.65●	1.79±0.28	22.33±10.45▲▲
Treatment 1	15	2.62±0.68▲▲	2.09±0.61	33.85±7.86
Treatment 2	12	2.46±0.45▲	1.74±0.33	27.02±4.48▲

Note: ANOVA, TC: F=3.437, P=0.024; compared to normal group, ▲P<0.05, ▲▲P<0.01; compared to treatment group 1, ●P<0.05. TG: F=1.093, P=0.360; no significant difference. BUN: F=6.139, P=0.001; compared to treatment group 1, ▲P<0.05, ▲▲P<0.01.

3.3. Serum Total Protein (TP) and Brain Acetylcholinesterase (AchE) Levels

Table 3 Comparison of Serum Total Protein (TP) and Brain Acetylcholinesterase (AchE) Levels (Mean ± SD)

Group	N	TP (mmol)	Brain AchE (U/mg.pro)
Normal	15	64.05±5.56●	0.08±0.04▲▲
Model	12	68.98±4.40▲▲	0.13±0.05
Treatment 1	14	67.07±3.48▲	0.09±0.04▲
Treatment 2	14	62.16±5.68	0.08±0.04▲▲

The results demonstrated statistically significant differences in serum total protein (TP) levels among the groups. Compared to treatment group 2, the model group and treatment group 1 exhibited significantly higher serum TP levels (P < 0.05). Conversely, serum TP levels in the normal group were significantly lower than those in the model group (P < 0.05). Brain AchE levels in the treatment groups were notably reduced compared to the model group, with treatment group 2 showing values closer to the normal group (Table 3).

Statistical Analysis

TP: F = 5.102, P = 0.004; ▲P < 0.05, ▲▲P < 0.01 vs. treatment group 2; ●P < 0.05 vs. model group.

Brain AchE: F = 3.811, P = 0.015; ▲▲P < 0.01 vs. model group.

4. Discussion

4.1. Aluminum Neurotoxicity and APP Processing Pathways

Aluminum, a neurotoxic substance, poses significant risks to human health, particularly in inducing neurodegenerative disorders such as Alzheimer's disease (AD). Aluminum can traverse the blood-brain barrier, accumulate in neural tissues, and severely impair cognitive functions, including

learning and memory[3] . Amyloid precursor protein (APP), a transmembrane glycoprotein widely distributed in humans, plays a pivotal role in AD pathogenesis. APP undergoes two distinct cleavage pathways: α-secretase-mediated non-amyloidogenic processing and β-/γ-secretase-mediated amyloidogenic processing.

In the non-amyloidogenic pathway, α-secretase cleaves APP within the Aβ domain, generating soluble α-APPs fragments that exhibit neuroprotective effects by inhibiting excitatory amino acid toxicity. In contrast, β- and γ-secretases sequentially cleave APP to produce Aβ peptides (e.g., Aβ40 and Aβ42). Aβ42, an aggregation-prone isoform, is the primary component of senile plaques, a hallmark of AD neuropathology .The transition of Aβ from soluble monomers to insoluble aggregates constitutes a critical event in the pathogenesis of Alzheimer's disease[2]. This conformational transformation is primarily mediated through β-secretase-mediated cleavage of the N-terminal peptide bond and subsequent γ-secretase-driven proteolysis at variable C-terminal sites, generating full-length Aβ peptides with heterogeneous molecular lengths[4].

Our findings revealed that aluminum-exposed model mice exhibited elevated β-secretase activity and reduced α-secretase activity, favoring Aβ generation and disease progression. Conversely, Suanzaoren treatment reversed these effects, suppressing β-secretase activity and enhancing α-secretase activity, thereby promoting non-amyloidogenic APP processing. The lack of significant differences between treatment groups may stem from insufficient dosage differentiation.

4.2. Acetylcholinesterase (AchE) and Aβ Pathology

AchE plays a critical role in synaptic neurotransmission by hydrolyzing acetylcholine (Ach) to terminate neuronal signaling. Notably, AchE colocalizes with Aβ plaques and exacerbates their cytotoxicity by forming stable complexes with Aβ peptides. This interaction accelerates cognitive decline in AD [6].

In our study, AchE activity was significantly elevated in the model group, correlating with pathological cortical alterations. Treatment with Suanzaoren, particularly at lower doses (treatment group 2), normalized AchE levels and ameliorated cortical pathology, suggesting its therapeutic efficacy against aluminum-induced AD-like pathology[5].

4.3. Serum Biochemical Indicators

Serum triglycerides (TG), total cholesterol (TC), total protein (TP), and blood urea nitrogen (BUN) reflect hepatic and renal function. While TG and TC levels showed no significant intergroup differences, BUN levels were elevated in treatment groups, indicating potential renal impairment unrelated to AD pathology. TP levels in treatment groups were reduced to near-normal values, with treatment group 2 (low-dose Suanzaoren) showing superior efficacy, implying dose-dependent benefits. Further studies are warranted to clarify the dose-response relationship of Suanzaoren on lipid and nitrogen metabolism. [5][6]

4.4. Serum Levels of TG, TP, TC, and BUN as Indicators of Liver and Kidney Damage

The content of TG, TP, TC and BUN in serum is an observation indicator reflecting the degree of liver and kidney damage. There was no significant difference between the TG group and the TC group, suggesting that this experiment had no significant effect on serum triglycerides and total cholesterol in patients with Alzheimer's disease. The BUN in groups 1 and 2 of the treatment was higher than that in the normal group, suggesting impaired renal function in mice. It was not stated that this experiment had a therapeutic effect on the regulation of serum urea nitrogen in patients with Alzheimer's disease. Both TP treatment groups 1 and 2 were lower than the model group and

close to the normal group, indicating that this experiment has a therapeutic effect on the regulation of total protein in the serum of patients with Alzheimer's disease, and the content of TP treatment in groups 2 was lower than that in treatment group 1. It is suggested that low-dose traditional Chinese medicine of jujube seed has a good therapeutic effect on mice with Alzheimer's disease model caused by aluminum poisoning. The therapeutic effect of TG, TC and BUN in the serum of patients with Alzheimer's disease by different sizes of jujube kernels still needs to be further studied and explored.

4.5. Research Findings

The above research results show that the content of brain β-secretase in the treated 1 and 2nd groups of mice was significantly lower than that of the model group, and the content of brain α-secretory enzymes in the treated 1 and 2nd groups of mice was significantly higher than that of the model group, indicating that sour jujube kernel traditional Chinese medicine preparations have the therapeutic effect of changing the activity of brain β-secreting enzyme and α secretory enzymes in senile dementia patients. , because the content of brain beta-secretase in the treated 2 groups of mice was lower than that of the treated 1 group, it seems that the therapeutic effect of small doses of sour jujube kernel Chinese medicine is better than that of large doses. There is no quantitative-effect relationship between the size of the dose, and the difference between the doses may be relatively close. There is no significant difference in the activity of γ-secreting enzyme in the brain of each group of mice, and further experiments are needed to be explored. According to the theory of β-amyloid [2], the mechanism of action of traditional Chinese medicine preparations of sour jujube kernels is not necessarily caused by promoting the discharge of aluminum in mice, but may be a direct inhibitory effect on β-secretase to generate more α-APPs to protect the brain nerve unit; by reducing β-secretase and γ-secretase, the activity inhibits the internal swallowing of precursor proteins, reduces the generation of Aβ42, makes it difficult to deposit, delays aging with brain tissue, and achieves the effect of treating Alzheimer's disease.

5. Conclusion

Suanzaoren extract effectively modulates key enzymes in Aβ metabolism (α-, β-,γ-secretases, and AchE) in aluminum-induced AD model mice. The low-dose formulation exhibited superior therapeutic outcomes, highlighting its potential as a novel strategy for AD prevention and treatment. Further research is needed to elucidate the dose-dependent mechanisms and validate these findings in clinical settings.

Acknowledgement

1) 2023 Guangxi University Young and Middle-aged Teachers' Basic Scientific Research Ability Improvement Project (Natural Sciences) (Project No.: 2023KY0578).

2) This work was supported by the National College Student Innovation and Entrepreneurship Training Program (Project No. 202410599012).

References

[1] Sun Zhonglei, Chen Yao, Bai Jing. Research progress on aluminum poisoning. Medical Review. 2013, 19(15): 2741-2743.

[2] Xu Qingying. Recent advance1s in etiological research of Alzheimer's disease. Medical Theory and Practice. 2020, 33(14): 2262-2263+2266.

[3] Li Chenyu, Jia Yunjing, Gan Juefang, Lian Chunrong, Li Shasha, Ling Yanwu. Effect of chronic aluminum

poisoning on memory function in rats. Journal of Nanchang University (Medical Sciences). 2024, 64(01): 1-5+31.
[4] Ma Xiaofei. Amyloid-beta protein and Alzheimer's disease. China Medical Herald. 2011, 8(15): 13-15.
[5] Li Tao, Chen Jufang, Yu Ying, et al. Experimental study on therapeutic effects of Heifu on aluminum-induced Alzheimer's disease in mice. Industrial Hygiene and Occupational Diseases. 2012, 38(04): 224-227. DOI:10.13692/j.cnki.gywsyzyb.2012.04.015.
[6] Fu Jun. Effect of recombinant Ganoderma lucidum immune regulatory protein on Alzheimer's disease model rats and its mechanism. Dissertation, Jilin University. 2014.
[7] Long Qijun, Yang Meichun, Li Guangwei, et al. Establishment of Alzheimer's disease mouse model and efficacy of Coix seed in aluminum detoxification. Chinese Journal of Gerontology. 2021, 41(21): 4812-4815.
[8] Lin Kailing, Xue Yan, Yu Huizhen. Research progress on NLRP3 inflammasome and chronic inflammatory diseases. Chinese Journal of Clinical New Medicine. 2020, 13(07): 733-737.
[9] Guo Jianxiong, Cheng Houzhi, Zhang Lifeng, et al. Effect of schisandrin B on hippocampal neuronal apoptosis-related genes in rats with chronic aluminum poisoning. Journal of Youjiang Medical University. 2023, 45(03): 379-383.
[10] Luo Gang, Hu Chunshen, Chen Hui. Effect of Congming formula on behavioral ability and alpha-beta-secretase in Alzheimer's disease model rats. World's Newest Medical Information Digest. 2019, 19(10): 1-3+5. DOI:10.19613/j.cnki.1671-3141.2019.10.001.