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Study on the Pathogenesis of Streptozocin (STZ)-Induced Type 2 Senile Diabetes in Mice and the Comparative Therapeutic Effects of Guangxi Sweet Tea and Shutangbao

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Abstract: This study investigated the pathogenesis and comparative therapeutic effects of Guangxi sweet tea versus Shutangbao in streptozotocin (STZ)-induced type 2 senile diabetic mice, and their effects on Alzheimer's disease. Mice were randomized into four groups: model group, treatment group 1, treatment group 2, and control group. The control group received intraperitoneal injection of 0.3 ml saline for 30 days, followed by 0.3 ml distilled water gavage for another 30 days. The other groups were modeled with STZ combined with aluminum maltolate: 0.3 ml/d intraperitoneal aluminum maltolate for 30 days, then 0.3 ml STZ daily for 5 days. From day 31, treatment groups received 0.3 ml/d Guangxi sweet tea or Shutangbao by gavage for 30 days. Blood glucose levels and learning-memory ability (Y-maze water labyrinth) were measured.Results showed: Model, treatment 1, and 2 groups had higher blood glucose than control group at day 5 post-modeling ($P < 0.01$). Brain β-secretase activity was higher in the model group ($P < 0.05$) but lower in treatment groups ($P < 0.01$, $P < 0.05$) compared to control group. Treatment groups needed fewer trials in the Y-maze test than the model group ($P < 0.01$)[1]. Successful modeling was confirmed by increased blood glucose and decreased α-secretase activity. Post-treatment, both groups showed reduced β-secretase activity, improved memory, and treatment 1 showed decreased blood glucose.

1. Preface

Alzheimer's disease (AD) is a neurodegenerative disorder predominantly characterized by spatial cognitive dysfunction, memory impairment, and behavioral abnormalities.[2]. Concomitant with population aging and rising obesity prevalence, the incidence of diabetes has increased annually. Epidemiological studies indicate that type 2 diabetes mellitus (T2DM) is associated with a 1.5–2.5-fold higher risk of Alzheimer's disease (AD)[3]. It represents a significant risk factor for the development of Alzheimer's disease (AD).Patients with type 2 diabetes mellitus (T2DM) exhibit persistent insulin resistance and elevated insulin-like growth factor levels, which contribute to the overexpression of Aβ and tau proteins and potently facilitate the formation of amyloid and neurofibrillary plaques associated with AD[4].Chronic hyperglycemia substantially increases the risk of multiple forms of cerebrovascular injury, including atherosclerosis in large and small vessels and cerebral amyloid angiopathy[5]. Given the bidirectional link between Alzheimer's disease (AD) and type 2 diabetes mellitus (T2DM), investigating their association to inform the development of preventive and therapeutic strategies holds substantial importance. This study investigates the underlying mechanisms by establishing a comorbid model of type 2 diabetes mellitus (T2DM) and Alzheimer's disease (AD) using streptozotocin combined with aluminum maltolate. Mice were treated with Guangxi sweet tea extract, while Shutangbao oral liquid served as a positive control. Relevant parameters, including blood glucose levels and brain β-secretase activity, were measured to explore the diabetes-aging association and evaluate the therapeutic effects of the interventions.

2. Materials and Methods

2.1. Experimental Animals

A total of 60 KM mice (supplied by Changsha Tianqin Biotechnology Co., Ltd., Changsha, China) were used, with an equal number of males and females.Mice weighed 30–35 g and were housed in a conventional environment with controlled temperature (22±2 ℃), 55±5% humidity, and a 12-h light/dark cycle.

2.2. Reagents and Drugs

Maltol, aluminum chloride, sodium chloride, 95% ethanol, 0.9% normal saline, o-toluidine, thiourea, boric acid, glacial acetic acid, and other small-molecule reagents were used. The h glucose test kit (oxidase method) and α-secretase/β-secretase/γ-secretase , following the instructions.

2.3. Methods

2.3.1. Animal Grouping and Aluminum Poisoning Model Establishment

Sixty KM mice were subjected to sex-separated housing and randomly assigned to four groups using a random number generator: the Control Group, Model Group, Treatment Group 1, and Treatment Group 2 (15 mice per group, with gender-separated cages within each group).The Control group received a daily intraperitoneal injection of 0.3 ml normal saline, while the other three model groups were administered a daily intraperitoneal injection of 0.3 ml aluminum maltol solution.Each aluminum poisoning model was continuously injected for 60 days. All mice had ad libitum access to food and water and were provided with standard rodent chow.

2.3.2. Treatment

Treatment was carried out 30 days after the start of modeling. The control group and model group received a daily intragastric administration of 0.3 ml normal saline, while treatment group 1 and treatment group 2 were given sweet tea and Haierfu via intragastric gavage, respectively, 0.3 ml every day for 30 consecutive days. Sweet tea and Haierfu were diluted with the original solution and distilled water at a ratio of 1:2.

2.4. Determination Methods

2.4.1. Assessment of Learning and Memory Abilities in Mice Using the Y-Maze Test

He Y-maze test protocol and experimental procedures were adapted from previously published methods. This experiment aimed to evaluate spatial memory function in mice using the Y-maze test. Ten days prior to aluminum poisoning model establishment, mice underwent pre-training in the Morris water maze task. Each mouse received 3 trials per day for 3 consecutive days, and the latency to reach the submerged platform was recorded. Behavioral tests were conducted before, during, and after model establishment, with each phase consisting of 3 consecutive days of testing (3 trials per mouse per day). Spatial memory data, including latency and path length, were recorded and analyzed.

2.4.2. Blood glucose determination (o-toluidine micro-method)

Twenty microliters of tail blood was collected into an EP tube containing 0.2 mL of saturated boric acid solution (prepared at room temperature).Thirty percent trichloroacetic acid (TCA) was added, and the mixture was vortexed thoroughly before centrifugation at 5000 × g for 5 minutes. The supernatant was collected for glucose quantification. The procedure was performed according to the manufacturer's instructions for the o-toluidine glucose assay kit.

2.4.3. Preparation of mouse 10% brain homogenate and secretase assay

Mice were euthanized by cervical dislocation, and brain tissues were harvested, rinsed with 0.01 M phosphate buffer (pH 7.4) to remove surface blood, gently blotted dry with filter paper, and weighed. Brain tissues were transferred to a glass homogenizer and mixed with 1 mL of 0.01 M phosphate buffer (pH 7.4), then homogenized on ice for 10 minutes to prepare a 10% (w/v) brain homogenate. The activities of β-secretase, α-secretase, and γ-secretase in brain homogenates were measured using a double-antibody sandwich ELISA kit, with all procedures performed according to the manufacturer's protocol.

2.5. Statistical Processing

SPSS13 software was used for analysis of variance (ANOVA) on the detected data, with results expressed as mean ± standard deviation ($\bar{x}$ ±s). Data were compared between groups, and statistical significance was defined as $P < 0.05$ or $P < 0.01$. The results were processed and analyzed using SPSS13.0 statistical software, presented as ($\bar{x}$ ±S), and subjected to ANOVA and Q-test.

3. Results

3.1. Group composition and mouse numbers

The control group comprised 15 mice, the model group 14 mice, treatment group 1 thirteen mice, and treatment group 2 fifteen mice.

3.2. Activities of β-secretase, α-secretase, and γ-secretase (U/L) in mouse brain tissues

Compared with the model group, β-secretase 1 (BACE1) activity was significantly lower in treatment group 1 ($P < 0.05$) and treatment group 2 ($P < 0.01$; Table 1).α-Secretase activity was significantly higher in the control group than in the model group and treatment group 1 ($P < 0.05$ for both comparisons).γ-Secretase activity was significantly higher in the control group than in all other groups ($P < 0.01$).Data are presented in Table 1.

Table 1 β-Secretase, α-Secretase, and γ-Secretase Activities (U/L) in Mouse Brains across Experimental Groups ($\bar{x}$ ±S)

Group	Brain β-secretase 1	Brain α-secretase	Brain γ-secretase
Control group	12.87±1.77★	12.32±0.19,	16.95±1.47
Model group	13.19±1.07	10.34±1.61▲★	15.04±0.96▲▲
Treatment group 1	11.64±1.09▲▲	9.59±2.32★	15.08±0.81▲▲
Treatment group 2	11.97±0.96▲	11.90±0.58▲	15.10±1.23▲▲

Analysis of Variance and Intergroup Comparisons.Brain β-secretase 1:In comparison with the model group, significant statistical differences were observed, with ▲$P < 0.05$ and ▲▲$P < 0.01$. When contrasted against Treatment Group 1, a statistically significant difference was noted (★$P < 0.05$).Brain α-secretase:A significant statistical difference (★$P < 0.05$) was identified when compared to the control group.Brain γ-secretase: Compared with the control group, a highly significant statistical difference (▲▲$P < 0.01$) was detected.

3.3. Morris Water Maze Latency (s) in Mice across Experimental Phases

During the post-modeling phase, latency in all poisoned groups was significantly longer than in the control group ($P < 0.05$), indicating impaired spatial memory in poisoned mice.

After treatment, latency in the model group remained significantly longer than in Treatment 1 and Treatment 2 groups ($P < 0.01$), suggesting treatment efficacy. Both treatment groups showed significantly reduced latency post-treatment ($P < 0.01$ vs. pre-treatment), indicating memory improvement following intervention. See Table 2.

Table 2 Morris Water Maze Latency (s) Across Experimental Phases ($\bar{x}$ ±S)

Group	Number of animals	Before poisoning	During poisoning	After poisoning
Control group	15	3.48±0.47	3.52±0.46	3.67±0.61▲▲
Model group	14	4.1±0.72	4.87±0.76▲	5.42±1.20
Treatment group 1	13	3.39±0.79[a]	5.27±0.97▲	3.68±0.81▲▲[a]
Treatment group 2	15	3.62±0.45[b]	4.50±0.53▲	3.71±0.62▲▲[b]

Analysis of Variance (ANOVA).When compared to the control group, a significant statistical difference was observed (▲$P < 0.01$). In contrast, the comparison with the model group revealed a highly significant difference (▲▲$P < 0.01$).Intragroup Comparisons (Before, During, and After

Poisoning).Treatment Group 1: A statistically significant reduction (aP < 0.01) was noted when comparing the post-treatment phase with the during-poisoning phase. Treatment Group 2: Similarly, a significant difference (bP < 0.01) was identified between the post-treatment and during-poisoning phases.

3.4. Results of blood glucose measurement before and after modeling

Compared with the control group, all experimental groups exhibited significantly higher blood glucose levels at the end of the 60-day aluminum injection period (P < 0.01), confirming successful establishment of the aluminum poisoning model. See Table 3.

Table 3 Blood Glucose Levels in Experimental Groups Before and After Aluminum Poisoning Model Establishment($\bar{x}$ ±S)(mmol/L)

Group	Number of animals	Before modeling	48h after modeling	72h after modeling	5d after modeling
Control group	15	7.71±3.73▲	7.37±2.89▲▲	4.58±1.40	7.43±1.93
Model group	14	7.61±3.52▲	7.71±2.31▲▲	13.02±4.36▲	12.85±4.14▲▲
Treatment group 1	13	5.66±1.99	7.14±2.37▲▲	11.78±3.08▲	11.78±4.61▲▲
Treatment group 2	15	5.16±2.82[c]	10.35±2.35	10.92±2.82▲	12.95±2.07▲▲

Analysis of Variance (ANOVA): In comparisons with the control group, both ▲P < 0.01 and ▲▲P < 0.01 indicated statistically significant differences.

3.5. Comparison of Blood Glucose Levels After Medication ($\bar{x}$ ±S)(mmol/L)

At 3 days post-treatment, blood glucose levels in Treatment 1 were significantly lower than in the model group (P < 0.05).By day 14 post-treatment, blood glucose levels in the model group and Treatment 2 were significantly lower than in the control group (P < 0.05).See Table 4.

Table 4 Blood Glucose Levels After Treatment in Experimental Groups ($\bar{x}$ ±S)(mmol/L)

Group	Number of animals	3 days after taking medicine	14 days after taking medicine
Control group	15	6.20±1.73	5.70±1.00
Model group	14	12.31±2.17	7.30±0.75▲▲
Treatment group 1	13	8.64±1.12▲	7.47±0.15▲▲
Treatment group 2	15	9.32±1.03	7.14±0.32▲▲

Analysis of Variance (ANOVA).When contrasted against the model group, a statistically significant difference was observed (▲P < 0.05). In comparison with the control group, a highly significant difference was noted (▲▲P < 0.01). Both comparisons exhibited significant statistical differences.($\bar{x}$ ±S).

3.6. Serum Total Cholesterol (TC) and Triglyceride (TG) Levels (mg/dL) in Mice Across Experimental Groups

Reatment 1 showed significantly lower serum total cholesterol (TC) and triglyceride (TG) levels than the control group (P < 0.01 for both). Additionally, TG levels in Treatment 1 were significantly lower than in the model group (P < 0.01).Treatment 2 had significantly lower serum TC levels than the model group (P < 0.01).See Table 5. Analysis of Variance (ANOVA).Serum Total Cholesterol (TC): A highly significant statistical difference (▲▲P < 0.01) was observed when comparing TC levels with those of the control group. Serum Triglyceride (TG): When contrasted with Treatment

Group 2, TG levels exhibited a significant difference (▲P < 0.01).In comparison with Treatment Group 1, a statistically significant difference (★P < 0.01) was also detected.

Table 5 Serum Total Cholesterol (TC) and Triglyceride (TG) Levels (mg/dL) in Mice across Experimental Groups ($\bar{x}$ ±S).

Group	Number of animals	TC(mmol/L)	TG(mmol/L)
Control group	15	5.83±3.14	2.86±1.02▲★
Model group	14	3.42±0.83	2.57±0.51▲★
Treatment group 1	13	2.92±0.70▲▲	1.25±0.55▲
Treatment group 2	15	2.24±0.41▲▲	4.60±1.54★

4. Discussion

Alzheimer's disease (AD), also referred to as senile dementia, is a neurodegenerative disorder characterized by clinical manifestations such as memory impairment, behavioral abnormalities, and changes in personality and behavior[6]. According to statistics, it is expected that by 2030, The global prevalence of diabetes is projected to rise from 5.9% in 2012 to 7.6%[7]. There are approximately 694 million diabetes patients and 88 million AD patients, and T2DM accounts for the vast majority of diabetes types[8]. The prevalence of type 2 diabetes (T2DM) in 65-year-old and older dementia patients (predominantly Alzheimer's disease [AD]) reaches 80%, indicating a high comorbidity rate between diabetes and AD.T2DM can increase the risk of cognitive impairment, and mild cognitive impairment (MCI) significantly increases the risk of AD[9].

Literature indicates that Aβ mainly originates from amyloid precursor protein (APP) in neurons. This protein is divided into a non-amyloid metabolic pathway and an amyloid metabolic pathway[10].In the non-amyloidogenic pathway, α-secretase primarily cleaves amyloid precursor protein (APP) to generate the soluble N-terminal fragment sAPPα, which is secreted into the extracellular space. The intracellular C-terminal fragment (αCTF) is then further processed by γ-secretase into soluble short peptides and non-toxic APP-derived cellular fragments. In the amyloidogenic pathway, β-secretase (BACE1) cleaves APP to produce sAPPβ, which is also secreted extracellularly. The resulting intracellular βCTF is subsequently cleaved by γ-secretase to generate numerous Aβ monomers containing 36–43 amino acid residues[10].

Insulin resistance and deficiency, the main characteristics of type 2 diabetes (T2DM), participate in the pathogenesis of Alzheimer's disease (AD) and are considered one of the mechanisms underlying the coexistence of these two diseases[9]. Peripheral insulin can enter the central nervous system of the brain through selectively distributed insulin receptor proteins. In the brain tissue of Alzheimer's disease (AD) patients, alterations in the sensitivity of brain insulin receptors lead to insulin resistance. Subsequently, these brain insulin receptors influence the enzymatic degradation during metabolism and expression of beta - amyloid protein and tau protein[8].

Brain α-secretase activity was significantly higher in the control group than in the model group and Treatment Group 1 (all P < 0.05).Following modeling, blood glucose levels were significantly higher in all mouse groups compared to the control group (all P < 0.01), which confirmed successful establishment of the type 2 diabetes mellitus-combined Alzheimer's disease (AD) model. Brain β-secretase 1 activity was significantly lower in Treatment Group 1 (P < 0.01) and Treatment Group 2 (P < 0.05) compared to the model group. Control group brain γ-secretase activity was significantly higher than in all other groups (P < 0.01).Post-treatment water maze test duration was significantly shorter in both Treatment Group 1 and Treatment Group 2 compared to pre-treatment levels. These findings suggest that sweet tea and Haierfu may reduce brain β-secretase 1 activity and enhance α-secretase activity in mice, potentially alleviating Alzheimer's disease symptoms.

Three days after treatment, blood glucose levels in Treatment Group 1 were significantly lower than those in the model group ($P < 0.05$), suggesting that the Guangxi sweet tea beverage reduced blood glucose in mice. Treatment Group 1 exhibited lower serum total cholesterol (TC) and triglyceride (TG) levels compared to the control group, and lower TG levels compared to the model group (all $P < 0.01$).Treatment Group 2 had significantly lower serum TC levels than the model group ($P < 0.01$).Collectively, these findings indicate that both Guangxi sweet tea and Haierfu beverages reduced serum TC and TG levels, potentially by regulating lipid metabolism, and contributed to blood glucose lowering in mice.

5. Conclusion

In summary, both sweet tea and Haierfu beverages regulated α-secretase and β-secretase activities in the Aβ pathway of AD model mice, thereby reducing Aβ generation, alleviating AD symptoms, and lowering serum total cholesterol (TC) and triglyceride (TG) levels. The Guangxi sweet tea beverage significantly reduced blood glucose levels in mice.

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