Neuroprotective effects of salidroside administration in a mouse model of Alzheimer's disease

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Abstract. Salidroside administration improves memory in different models of learning. However, its influence on models of Alzheimer's disease (AD) has not been widely studied. In the present study, the therapeutic effect of salidroside was investigated in an animal model of AD. APPswe/PS1ΔE9 mouse (n=20) were randomly divided into either the AD model group or the salidroside + AD model group (n=10 in each group), and C57BL/6J mouse (n=20) of identical age and genetic background were randomly divided into either the normal control (NC) group or the salidroside + NC group (n=10 in each group). The Morris water maze behavioral test was applied to all mice in order to investigate the effects of salidroside administration on learning and memory functions. The concentrations of malondialdehyde (MDA), glutathione (GSH) and nitrate in the hippocampus of the mice were determined, and hippocampal superoxide dismutase (SOD) activity was also determined. In addition, terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling was used to investigate the rate of neuronal apoptosis in the hippocampus. Furthermore, the concentrations of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were tested for in the brain tissues of AD mice. Learning and memory functions in AD mice were revealed to decrease the apoptotic rate of hippocampal neurons, and increase the activity of SOD and the concentration of GSH in hippocampal tissue. In addition, it was demonstrated that salidroside administration suppressed the expression levels of IL-6 and TNF-α. In conclusion, this study revealed that the administration of salidroside could attenuate the effects of AD-associated memory and learning impairment in mice. Furthermore, it was demonstrated that the effects of salidroside administration on AD mice were, at least partially, via inhibition of brain oxidative/nitrosative damage, suppression of both IL-6 and TNF-α expression levels, and suppression of the hippocampal neuronal apoptotic rate.

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which is characterized by severe memory loss and behavioral disturbances (1-3). AD is a multifactorial disease and is affected by genetic risk factors, aging and oxidative stresses (4,5). The impairment of memory and cognition in patients with AD is caused by synaptic loss, enhanced inflammatory signaling, progressive deposition of senile plaques, neurofibrillary tangles and neurodegeneration (6-8). Additionally, amyloid-β (Aβ) peptides, as well as the tau protein, in the form of neurofibrillary tangles, are implicated in the pathogenesis of AD (9). In addition to the tau-amyloid signature, oxidative damage, neuroinflammation, widespread synaptic loss and neuronal death are considered hallmark features of AD (9).

With an aging population, AD is on the increase, and there are currently no effective treatments or cures. Therefore, the pursuit of novel disease-modifying therapeutics for AD is the subject of intense investigation.

Salidroside is a type of traditional Chinese medicine, which is extracted from rhodiola medical plants, named *Rhodiolasa chinensis* A Bor. Salidroside has numerous pharmacological activities, such as anti-anoxia (10), anticancer (11,12), anti-fatigue (13), and anti-toxin effects (14). Furthermore, numerous studies have suggested that salidroside may also improve cognitive functioning in disease models (15,16); however, the mechanisms were not determined. In this study, the potential therapeutic effect of salidroside in APPswe/PS1ΔE9 mice and its underlying mechanisms were investigated.

**Key words:** APPswe/PS1ΔE9 mouse, salidroside, SOD, MDA, GSH, nitrate, TNF-α, IL-10

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Abbreviations: AD, Alzheimer's disease; MWM, Morris water maze; SOD, superoxide dismutase; GSH, glutathione; MDA, malondialdehyde; TNF-α, tumor necrosis factor-α; IL-10, interleukin-10

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Materials and methods

Experimental animals. All the experiments were performed with the approval of the Ethics Committee of the Tianjin Medical University (Tianjin, China). APPswe/PS1ΔE9 mice (n=20; 3 months, male, 26-30 g) and C57BL/6J mice (n=20; 3 months, male, 23-26 g) in clean grade were obtained from Beijing Huafukang Biological Technology company (Beijing, China) and raised in the temperature of 25±2°C, relative humidity 70% and in a 12-h light/dark room at with free access to food and water. Following 1 week of adaption, all APPswe/PS1ΔE9 mice were randomly divided into either the AD model group or the salidroside + AD model group (n=10 in each group). C57BL/6J mice were also randomly divided into either the normal control (NC) group or the salidroside + NC group (n=10 in each group). The mice in the salidroside + NC and salidroside + AD model groups were administered salidroside (30 mg/kg) orally once daily for 3 consecutive months. Following treatment, behavioral tests and biochemical experiments were performed.

Morris water maze (MWM). Following the 3 months of salidroside treatment, the cognitive behavior of all of the mice was investigated using MWM (17), which measures spatial learning and memory ability. The animals were subjected to a daily session of four training trials for 4 consecutive days. In each training session, mice were placed into the pool at four different starting points (different quadrants). The mice were then permitted to find the platform within a maximum time period of 120 sec and following that the mice were allowed to remain on the platform for a further 30 sec. If a mouse was unable to find the platform within the 120 sec time period, the mouse would then be guided to the platform by the experimenter, and the escape latency was recorded as 120 sec. At day 5 of spatial testing, the platform was removed and mice were placed into water at any starting point. The time period that the mouse remained in the quadrant where the platform had previously been placed was then recorded.

Terminal deoxynucleotidyl-transferase-mediated dUTP nick end labeling (TUNEL) assay. Detection of hippocampal neuronal apoptosis was carried out using the TUNEL assay. The hippocampal tissue was fixed in 4% formalin at 4°C for 48 h and then embedded in paraffin wax. Apoptosis rates were detected using the In Situ Cell Death Detection kit (Roche Applied Science, Rotkreuz, Switzerland) according to the manufacturer's instructions. Apoptotic rate changes were measured via a light microscope (Olympus Corporation, Tokyo, Japan). Hippocampal tissue was fixed in 4% paraformaldehyde for 48 h at 4°C and embedded into paraffin wax. In Situ Cell Death Detection kit was used to detect the apoptosis according to the manufacturer's instructions. The images were taken using a light microscope (Olympus Corporation). Apoptotic rates were then determined according to the method previously detailed by Soslow et al. (18). A positive expression rate of 0-1% was defined as score 0 (negative), 1-10% as score 1 (weakly positive), 10-50% as score 2 (positive), 50-80% as score 3 (medium positive) and 80-100% as score 4 (strongly positive). Three sections were used for each group, and five fields were randomly selected from each section (magnification, x400).

Statistical analysis. Statistical analysis was performed using SPSS 17.0 (SPSS, Inc., Chicago, IL, USA). All data were expressed as the mean ± standard deviation of the mean. One-way analysis of variance was used to compare differences among three or more groups, and this was followed by Student Newman-Keuls-test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Administration of salidroside improves the spatial learning and memory abilities of AD mice. MWM was used to investigate the effects of salidroside administration on the learning and memory abilities of mice. As revealed in Fig. 1A, the escape latency time of the AD group was longer than...
that of the normal group, and there was no significant difference between the normal and the salidroside + NC groups. Furthermore, the escape latency of the mice in the salidroside + AD model group was significantly reduced compared with the escape latency of the AD model group. As demonstrated in Fig. 1B, the time period that the AD model group spent in the target quadrant was significantly less than that spent by the normal group. Additionally, there was no significant difference in the time period spent in the target quadrant between the NC and the salidroside + NC groups. Furthermore, the time period that the mice of the salidroside + AD model group spent in the target quadrant was significantly higher than the time spent by the AD group, but significantly less than the time spent by the NC group. Results in the salidroside + AD group, the escape latency was significantly shortened, the time of swim in the platform quadrant significantly increased, suggesting that mice significantly improved learning and memory to escape the incubation period (P<0.05; Fig. 1).

**Salidroside administration mitigates the rate of neuronal apoptosis in the CA1 hippocampal region in AD mice.** As revealed by Fig. 2, the number of neurons undergoing apoptosis in the CA1 hippocampal region was significantly increased in the AD group compared with the other groups. However, the administration of salidroside reduced this effect, as there was a significant reduction in neuronal apoptotic rates between the salidroside + AD group and the AD group.

**Salidroside administration increases the concentration of GSH and the activity of SOD in the hippocampus of AD mice.** The activity of SOD and the concentration of GSH in hippocampal tissue were investigated. As demonstrated in Fig. 3A, the relative SOD activity was significantly decreased in the AD group compared with the NC group; whereas SOD activity was upregulated in the salidroside + AD group compared with the AD group. Furthermore, there was no significant difference between the NC group and the salidroside + NC group (Fig. 3B).

**Salidroside administration reduces the concentrations of MDA and nitrate in the hippocampus of AD mice.** The hippocampal concentrations of MDA and GSH in mice were also investigated. As revealed in Fig. 4, in comparison with the NC group, MDA and nitrate concentrations were both increased in the AD group; whereas both MDA and nitrate concentrations were downregulated in the salidroside + AD group compared with the AD group. Furthermore, there was no significant difference between the MDA and nitrate concentrations of the NC group and the salidroside + NC group.

**Salidroside administration decreases the expression of TNF-α and IL-6 in the hippocampus of AD mice.** The effects of salidroside administration with regards to induced inflammation were investigated. The expressions of TNF-α and IL-6 inflammatory factors decreased significantly in the hippocampus of the salidroside + AD group compared with the AD group (Fig. 5). There was no significant difference between the expression levels of TNF-α and IL-6 of the NC group and the salidroside + NC group.
The number of people with AD worldwide is increasing as a result of the aging population. Previous research has revealed that 46.8 million people worldwide suffer from dementia, and further research has indicated that this number may increase 2-3-fold by 2030 (19). AD is a severe neurodegenerative disease of the central nervous system, which results in learning and memory impairment. The rising rate of senile dementia is of serious concern to global healthcare systems and causes serious economic burdens to affected families and wider society. AD is a neurologically complex disorder, and thus it is likely that a combinatorial therapeutic approach will be needed for effective treatment.

Chinese herbology has been heavily used globally and is regarded as an importance source of medicines. Salidroside is a component of *Rhodiolarosea*, and possesses medicinal properties, such as anti-inflammatory (20,21), antioxidative (14)
and neuroprotective effects (22). In the present study, it was demonstrated that salidroside administration attenuates the memory impairment exhibited in the AD rat model.

Previous studies have reported that one of the major brain regions affected by AD-induced neurogenesis is the hippocampus, which is a vital brain region for learning and memory (23). Hippocampal neuronal damage adversely affects neural plasticity and synaptic regulation, and thus is heavily implicated in the pathogenesis of AD (24). In the present study, it was revealed that the rate of hippocampal neuronal apoptosis was significantly increased in the rat AD model. Furthermore, it was demonstrated that the rate of apoptotic neurons in the CA1 hippocampal region was clearly increased in the AD group, and that administration of salidroside could attenuate this effect. In addition, the results of this study suggest that the administration of salidroside improves cognitive function in the AD model via suppression of the rate of apoptosis of hippocampal neurons.

It has previously been reported that salidroside, a potent antioxidant, ameliorates memory impairment via anti-oxidative damage activity. The decline of learning and memory function in rodents and humans has previously been linked to excessive oxidative stress, which may lead to neuronal death. There is a growing body of research suggesting that oxidative stress is a major cause of neuronal death in AD rat model with memory deficit, these adverse effects were additive, oxidative damage may also cause damage to components of the antioxidant system, such as MDA (28,29). The present study demonstrated that oxidative damage downregulated the activity of SOD and increased MDA concentration in rat hippocampal tissue. Furthermore, it was revealed that in the AD rat model with memory deficit, these adverse effects were greatly attenuated when salidroside was administered.

IL-6 is a cytokine that is secreted by T cells and macrophages, and acts as both a pro-inflammatory and as an anti-inflammatory in order to stimulate an immune response (30,31). TNF-α can promote T cells to produce a variety of inflammatory factors, and therefore trigger inflammatory reactions (32,33). In the present study, salidroside administration significantly decreased the expression of TNF-α and IL-6 cytokine inflammatory factors.

In conclusion, the present study demonstrated that salidroside administration may improve cognitive function in AD mice. The protective effects may be associated with alteration of levels of free radicals in the hippocampus.

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