

Ginsenoside Rb1 Protects Against Age-Related Cognitive Decline by Regulating Oxidative Stress and the SIRT1/eNOS/NO Pathway

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Received: 15 July 2023; Revised: 03 November 2023; Accepted: 06 November 2023

ABSTRACT

In traditional Chinese medicine, ginsenoside Rb1 (Rb1) is a major active ingredient from ginseng known for its capacity to alleviate oxidative stress, regulate autophagy, and suppress apoptosis. However, its potential influence on the natural aging process has not been fully explored. To examine the effects of Rb1 on natural aging and to uncover the possible underlying molecular mechanisms. Middle-aged (12-month-old) and aged (20-month-old) mice were treated with Rb1 at low (10 mg/kg·d) or high (20 mg/kg·d) doses for eight consecutive weeks. Changes in body weight and spatial learning ability were monitored in relation to aging manifestations. Oxidative stress indicators and nitric oxide (NO) concentrations were measured in hippocampal tissue following treatment, along with evaluation of aging-related biomarkers and associated pathways. Rb1 administration markedly mitigated several physiological signs of aging, such as slowing body weight decline, preventing the deterioration of visuospatial learning and memory, and reducing senescence in brain tissues. Mice receiving Rb1 showed lower serum malondialdehyde (MDA) levels, increased superoxide dismutase (SOD) activity, and decreased production of pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). Moreover, Rb1 elevated serum NO levels and enhanced Sirtuin 1 (SIRT1) protein expression, indicating that its anti-aging actions are closely linked to modulation of the SIRT1/eNOS/NO signaling axis. Rb1 delays aging progression in mice by regulating the SIRT1/eNOS/NO pathway, which helps reduce oxidative stress and inflammatory responses.

Keywords: Ginsenoside Rb1, Aged mice, Cognitive impairment, Oxidative stress, SIRT1/eNOS/NO axis

How to Cite This Article: Martínez S, Gómez C, Navarro L. Ginsenoside Rb1 Protects Against Age-Related Cognitive Decline by Regulating Oxidative Stress and the SIRT1/eNOS/NO Pathway. Spec J Pharmacogn Phytochem Biotechnol. 2023;3(2):100-11. <https://doi.org/10.51847/M7q10CZtyf>

Introduction

Aging represents a gradual, irreversible process involving disruption of homeostasis and progressive decline in the structure and function of tissues, organs, and cells throughout the body. This process heightens the risk of numerous age-associated disorders involving the neurological, cardiovascular, metabolic, musculoskeletal, and immune systems [1]. In the next thirty years, the global elderly population is anticipated to reach 1.6 billion, representing roughly one-sixth of the world's total inhabitants. Such demographic changes will create substantial economic, healthcare, and social burdens. Consequently, gaining deeper insight into aging mechanisms and developing strategies to postpone them are vital priorities in medical research.

Cognitive deterioration in the elderly continues to lack effective therapeutic solutions. Wang *et al.* demonstrated that age-related reductions in brain white matter volume and impaired myelin formation likely play a key role in the loss of spatial learning and memory in mice [2]. The diminished regenerative capacity in older mice impairs resolution of inflammation resulting from myelin damage and hinders reformation of damaged myelin tissue, ultimately leading to myelin loss [3].

In preclinical aging studies, laboratory mice and rats are the most commonly employed animal models. Mice, in particular, share 99% of human genes and display up to 78.5% genetic homology [4]. A variety of mouse models have been established for aging research, among which the natural aging mouse model is extensively utilized for screening anti-aging compounds and exploring their pharmacological mechanisms.

Rb1 is a principal bioactive constituent extracted from ginseng that exerts beneficial effects in the treatment of diabetes, neurological disorders, and cardiovascular diseases [5-7]. Its actions involve regulation of mitochondrial energy metabolism, mitochondrial fission and fusion balance, oxidative stress and reactive oxygen species generation, mitophagy, and mitochondrial membrane potential [6]. Earlier investigations have shown that Rb1 can protect neurons by promoting neurogenesis, inhibiting apoptotic pathways, and attenuating inflammation [8, 9]. Recent evidence further indicates that Rb1 boosts hippocampal neurogenesis, thereby enhancing learning and spatial memory performance in rats [10]. Zhang *et al.* reported that ginsenosides can alleviate spatial memory deficits induced by D-galactose combined with aluminum chloride [11]. Despite these findings, relatively few studies have investigated whether Rb1 can improve aging-associated cognitive deficits through its anti-inflammatory and antioxidant properties.

In addition, previous research has revealed that Rb1 provides protection to endothelial cells and cardiac tissue by suppressing fibrosis and inflammation [12] and by counteracting age-related endothelial senescence via modulation of the SIRT1/eNOS signaling pathway [13-15]. However, there remains a shortage of studies specifically addressing Rb1's role and detailed mechanisms concerning cognitive functions in the context of natural aging in mouse models.

To elucidate the protective role and potential molecular pathways of Rb1 in aging, we utilized a natural aging mouse model that closely recapitulates human physiological aging. We investigated Rb1's effects on visuospatial learning and memory capabilities in aged mice and assessed aging-related biomarkers in brain tissue, including SIRT1 levels, oxidative stress parameters, and inflammatory markers at various aging stages. Furthermore, metabolomics profiling was conducted to analyze alterations in aging-associated metabolites in plasma after Rb1 treatment.

Materials and Methods

Animals and management

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of Sun Yat-Sen University. Female C57BL/6 mice were obtained from the Center of Experimental Animals at Sun Yat-Sen University. Animals were housed under specific pathogen-free (SPF) conditions with a standard diet, controlled temperature (24 ± 2 °C), humidity (50 ± 5 %), and a 12-hour light/dark cycle. Rb1 (purity > 98 %) was purchased from Victory (Sichuan, China).

Young control (YC) mice (4 months old, $n = 12$) served as the reference group. Thirty-six 12-month-old female C57BL/6 mice were randomly divided into three groups ($n = 12$ each): middle-aged control (MC), middle-aged + 10 mg/kg·d Rb1 (MRb1-10), and middle-aged + 20 mg/kg·d Rb1 (MRb1-20). Similarly, thirty-six 20-month-old mice were assigned to three groups ($n = 12$ each): aged control (AC), aged + 10 mg/kg·d Rb1 (ARb1-10), and aged + 20 mg/kg·d Rb1 (ARb1-20). Mice received daily intraperitoneal injections of either sterile saline (vehicle) or Rb1 solution for eight weeks. Body weight and food intake were recorded monthly.

Morris water maze

In the ninth week, spatial learning and memory were evaluated using the Morris water maze (MWM) test. The apparatus consisted of a circular pool (120 cm diameter, 50 cm height) filled with water maintained at 24.0 ± 1 °C. A hidden platform was submerged 1 cm below the water surface in one quadrant. The test included a hidden platform training phase (acquisition) and a probe trial (memory retention). This experiment was performed at the Medical Experimental Center of the North Campus of Sun Yat-sen University. Mouse swimming trajectories were recorded and analyzed with a video tracking system and SMART 2.0 software.

Collection of animal specimens

Serum collection

Following completion of the behavioral tests, mice were anesthetized and blood was collected. Samples were allowed to clot for 2 hours at room temperature, then centrifuged at 4000 rpm for 10 min. The supernatant was collected and stored at -80°C .

Isolation and preservation of mouse brain tissue

After blood collection, mice were perfused transcardially with 4% paraformaldehyde. Brains were carefully removed, post-fixed overnight in 4% paraformaldehyde, and then transferred to 15% and 30% sucrose solutions for cryoprotection.

SA- β -gal staining

Senescence-associated β -galactosidase (SA- β -gal) staining was performed according to the standard protocol [16]. Brain sections were fixed briefly and incubated in staining solution containing X-gal at 37°C for 18 hours in the dark. Blue-stained cells were counted as senescent, and the percentage of SA- β -gal-positive cells was calculated from 400 neighboring cells.

Western Blotting

Hippocampal tissue was lysed in RIPA buffer supplemented with protease inhibitors. Protein concentrations were determined using the BCA assay kit. Membranes were blocked with 5% skim milk, incubated with primary antibodies overnight at 4°C , followed by HRP-conjugated secondary antibodies for 1 hour. Signals were visualized by chemiluminescence and quantified with Quantity One software.

Co-immunoprecipitation

After cell transfection, lysates were prepared in RIPA buffer and centrifuged. Antibodies against eNOS and Caveolin-1 were added and incubated overnight. Protein complexes were captured with agarose beads, washed, and analyzed by SDS-PAGE followed by Western blotting procedures.

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of IL-6 and TNF- α were quantified using commercial ELISA kits according to the manufacturer's instructions.

NO, MDA, GSH content and SOD activity assaying

Levels of malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD) activity, and nitric oxide (NO) were measured in serum or tissue using commercial assay kits (Nanjing Jiancheng Bioengineering Institute, China) following the provided protocols.

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). Statistical comparisons were performed using one-way ANOVA followed by LSD post-hoc tests with SPSS 13.0 software. Differences with $p < 0.05$ were considered statistically significant.

Results and Discussion

Effect of ginsenoside Rb1 on mouse body weight

Prior to Rb1 intervention, no significant differences in body weight were observed across groups. After eight weeks of treatment, body weight in the young control and middle-aged groups remained relatively stable. In contrast, aged mice exhibited varying degrees of weight loss, with the most pronounced decline in the aged control group, followed by the low-dose Rb1 group, while the high-dose Rb1 group showed the smallest reduction (**Figure 1**).

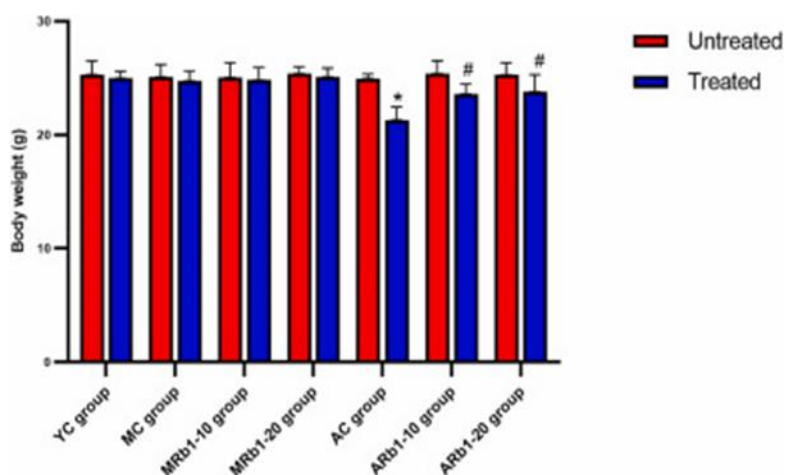


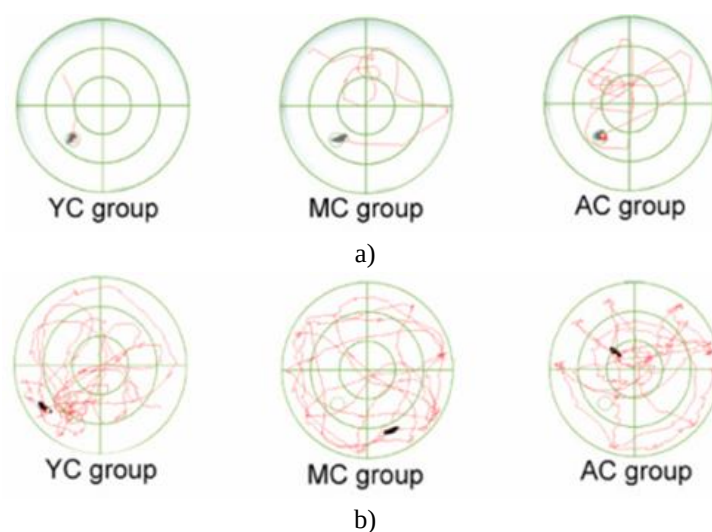
Figure 1. Impact of Rb1 treatment on body weight in mice.

The MRb1-20 and ARb1-20 groups received daily intraperitoneal injections of 20 mg Rb1/kg, while the MRb1-10 and ARb1-10 groups were administered 10 mg Rb1/kg per day. The YC, MC, and AC groups received equivalent volumes of sterile saline. All injections were given for eight weeks. Data are presented as mean $\pm$ SD, * $P < 0.05$ versus YC group, $n = 12$; # $P < 0.05$ versus AC group, $n = 12$.

Influence of Rb1 on learning, memory, and behavioral performance in mice

The Morris water maze test serves as a standard method for assessing spatial learning and memory in animals. During the hidden platform acquisition phase, both aged and middle-aged mice exhibited markedly increased swimming path lengths to locate the platform relative to young controls (**Figure 2a**), reflecting impaired visuospatial learning. Many older animals failed to locate the platform even after repeated trials. In the probe trial, aged and middle-aged mice displayed reduced capacity to recall and explore the spatial location, spending significantly less time in the target quadrant than young mice (**Figure 2c**). Their search patterns were erratic, often characterized by thigmotaxis (wall-hugging) and random swimming, especially in the aged cohort (**Figure 2b**).

Following Rb1 administration, middle-aged and aged mice showed substantially reduced escape latencies and path lengths to the hidden platform (**Figures 3a, 3b, 3i, 3j**). Probe trial performance also improved, with more directed swimming trajectories toward the former platform location (**Figures 3c and 3d**), higher platform crossings (**Figures 3e and 3f**), and greater time spent in the target quadrant (**Figures 3g and 3h**). These cognitive enhancements were dose-dependent, with stronger effects observed in the high-dose Rb1 groups. Notably, swimming speeds remained comparable across all groups in the water maze task.



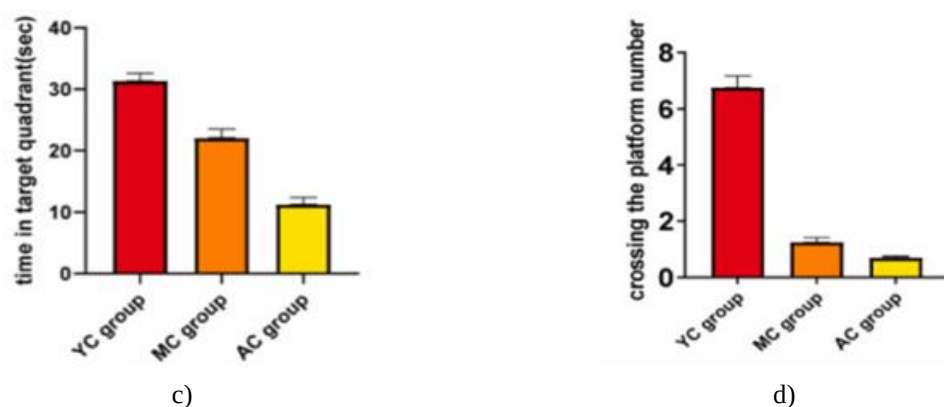
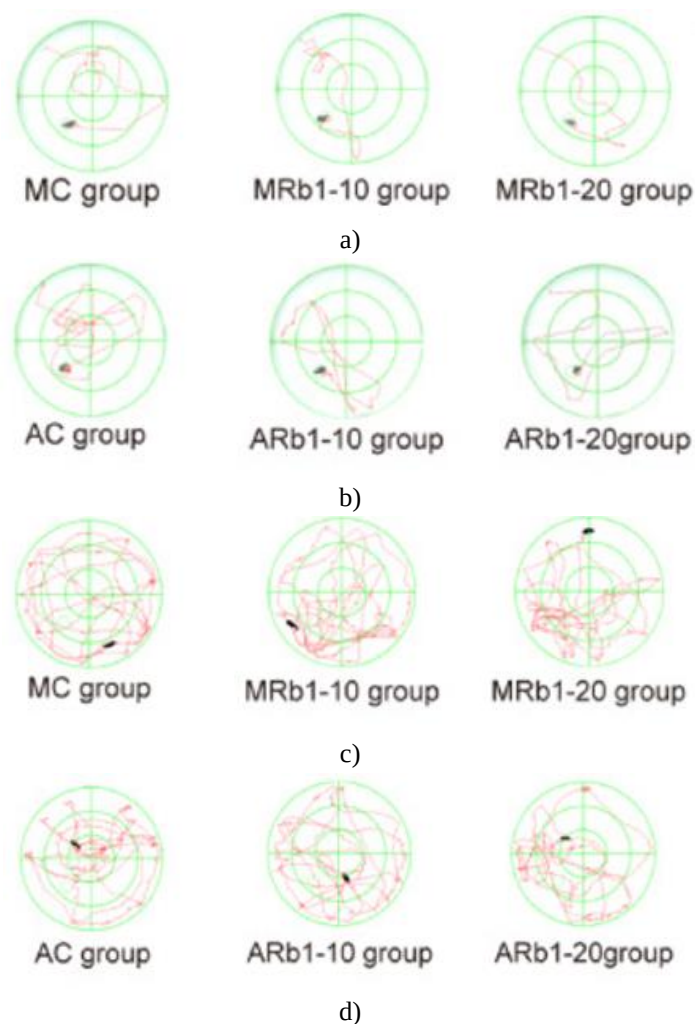


Figure 2. Age-related changes in mice spatial memory. a) Search paths illustrating how young, middle-aged, and aged mice navigated toward the hidden platform on training day 5. b) Movement patterns of young, middle-aged, and old mice recorded in the memory retention probe phase; c) Average time spent by young, middle-aged, and aged mice in the correct quadrant during probe trials; d) Mean platform crossing counts achieved by young, middle-aged, and aged mice in the spatial probe assessments.



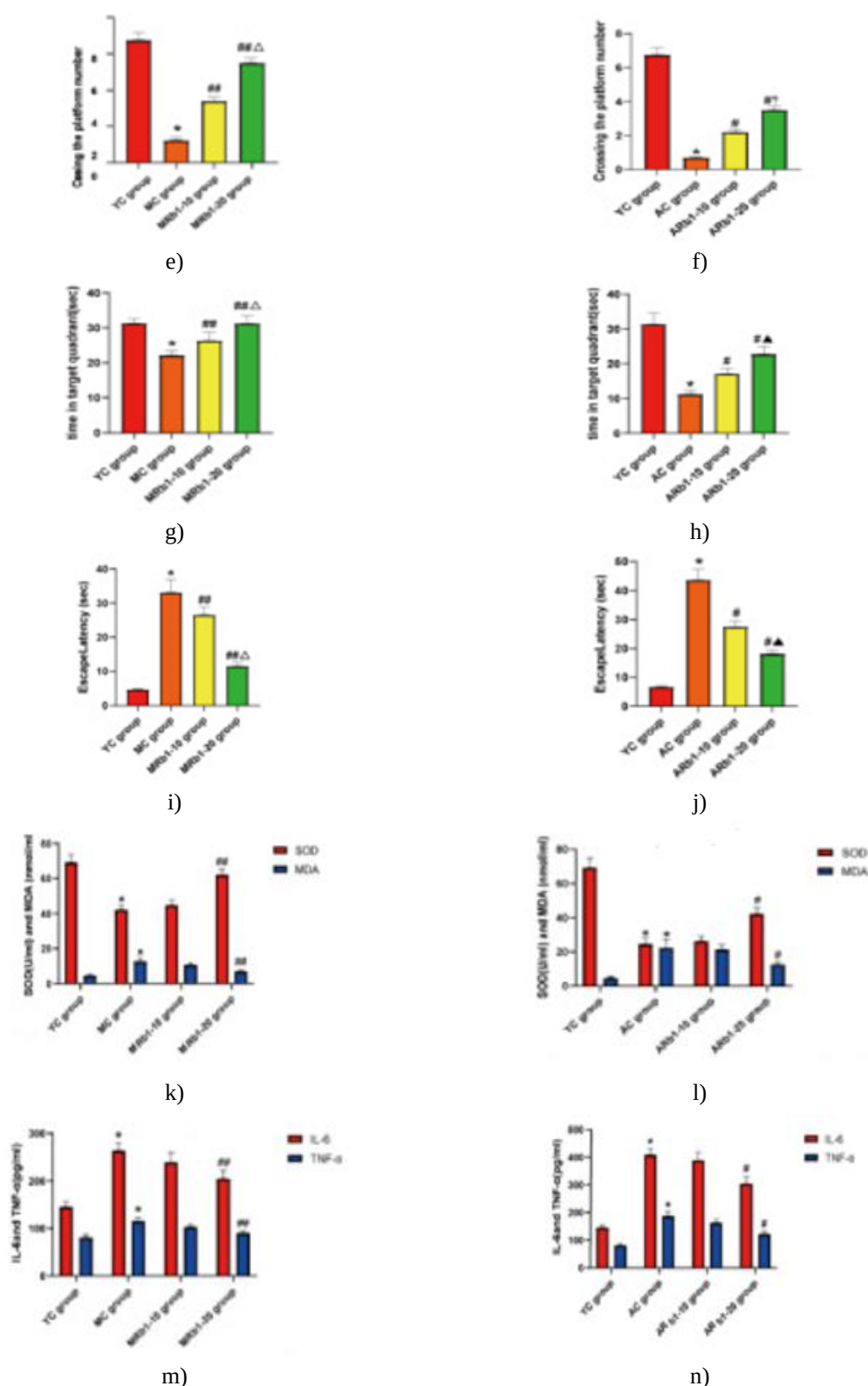


Figure 3. Rb1 enhances spatial memory in mice along with its influence on oxidative stress and inflammation markers in vivo. a,b) Representative swimming paths of middle-aged and aged mice in the hidden platform acquisition phase; c,d) Swimming paths of middle-aged and aged mice recorded during the spatial probe trial; e,f) Average number of platform crossings by middle-aged and aged mice in the probe test; g,h) Mean time spent in the target quadrant by middle-aged and aged mice during spatial probe trials; i,j) Average latency to locate the hidden platform in middle-aged and aged mice; k,l) Changes in serum SOD activity and MDA content in middle-aged and aged mice following Rb1 treatment; m,n) Alterations in serum IL-6 and TNF- α concentrations in middle-aged and aged mice after Rb1 administration. Results are shown as

mean $\pm$ SD, *P < 0.05 vs. YC group, n = 12; #P < 0.05 vs. AC group, n = 12; ▲P < 0.05 vs. ARb1-10 group, n = 12; ##P < 0.05 vs. MC group, n = 12; ΔP < 0.05 vs. MRb1-10, n = 12.

Rb1 treatment and its impact on oxidative stress in mice

Serum MDA levels and SOD activity were evaluated to determine the degree of oxidative injury and the potential protective role of Rb1. Compared with young controls, both aged and middle-aged mice displayed significantly reduced SOD activity. In contrast, high-dose Rb1 treatment substantially increased SOD activity and markedly lowered MDA concentrations in these animals (**Figures 3k and 3l**).

Rb1 treatment and its impact on inflammatory responses in mice

Aging is commonly associated with heightened inflammation. Previous in vitro observations showed that Rb1 lowered IL-6 and TNF- α production in senescent HUVECs. To determine whether similar anti-inflammatory effects occur in naturally aged animals, inflammatory cytokines were measured in mouse serum. Both elderly and middle-aged groups exhibited significantly higher serum IL-6 and TNF- α levels than young controls. High-dose Rb1 administration effectively decreased these elevated cytokine levels (**Figures 3m and 3n**).

Rb1 treatment and its influence on SA- β -gal activity in the mouse hippocampus

SA- β -gal staining is a well-established indicator of cellular senescence. To evaluate brain aging in naturally aged mice, SA- β -gal activity was assessed in hippocampal tissue. Young control mice showed very few positively stained cells, whereas middle-aged and aged control mice displayed a greater number of positive cells, some with strong staining intensity. In contrast, Rb1-treated animals demonstrated a notable reduction in the number of SA- β -gal-positive cells (**Figures 4a and 4b**).

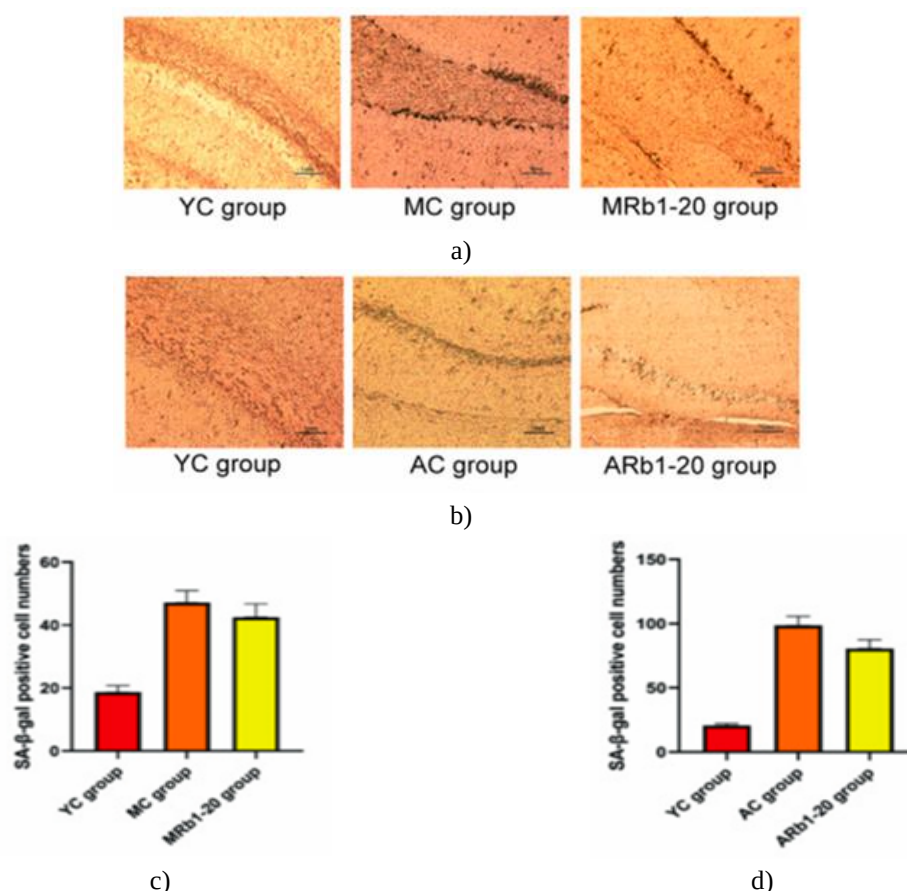


Figure 4. Rb1 treatment modulates SA- β -gal activity in the mouse hippocampus (objective 20 $\times$). a) Representative SA- β -gal staining images from the middle-aged groups; b) Representative SA- β -gal staining images from the aged groups; c) Quantification of SA- β -gal-positive cells in the middle-aged groups; d) Quantification of SA- β -gal-positive cells in the aged groups.

Rb1 treatment and its influence on SIRT1 expression in mouse hippocampal tissue

Both cell-based studies and naturally aged mouse models highlighted the important involvement of SIRT1 in the actions of Rb1. Given the clear benefits of Rb1 on cognitive performance (observed in the Morris water maze) and brain tissue aging (observed via SA- β -gal staining), we investigated whether these improvements were linked to changes in SIRT1. Accordingly, SIRT1 protein expression was analyzed in hippocampal tissue. As anticipated, SIRT1 levels were reduced in both middle-aged and aged mice, with a more substantial decline in the older group. Treatment with Rb1 led to a significant upregulation of SIRT1 protein, and this elevation was especially prominent in the high-dose Rb1 groups (**Figures 5a and 5b**).

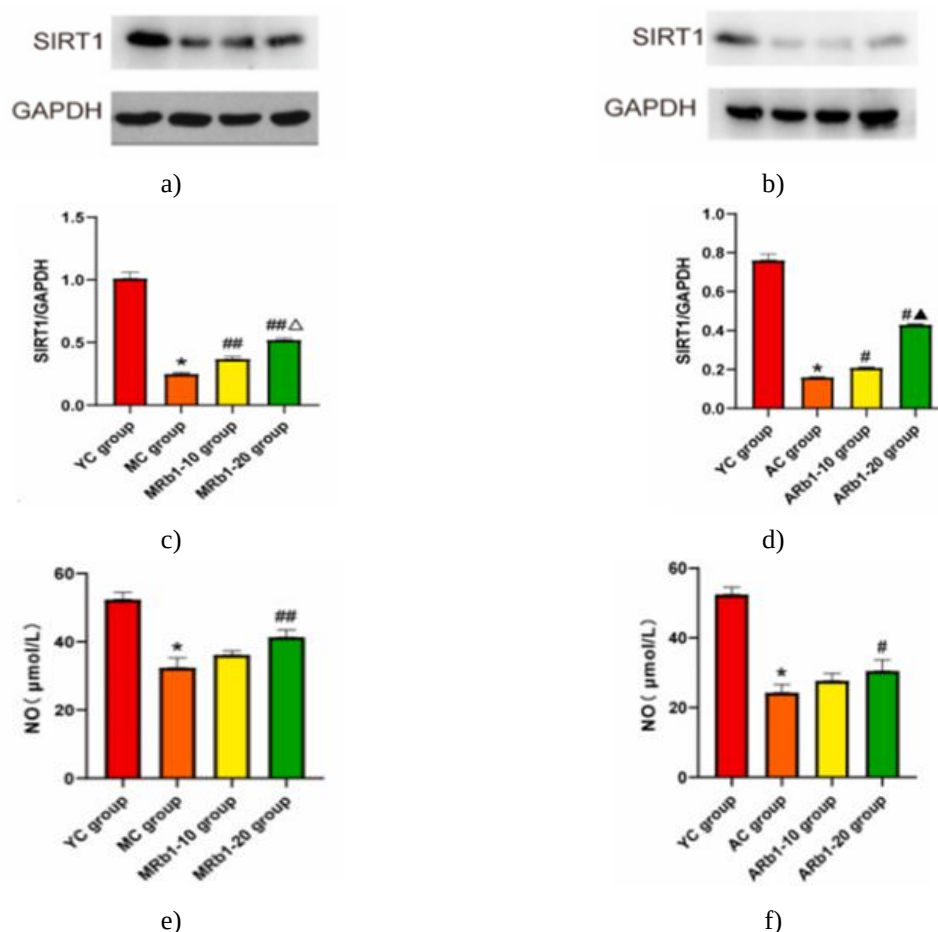


Figure 5. Rb1's influence on SIRT1 protein expression in the mouse hippocampus. a) SIRT1 protein expression in middle-aged mice; b) SIRT1 protein expression in aged mice; c) Quantitative analysis of SIRT1 expression in middle-aged mice; d) Quantitative analysis of SIRT1 expression in aged mice. e) Serum NO levels in middle-aged mouse groups; f) Serum NO levels in aged mouse groups. Results are expressed as mean $\pm$ SD, * $P < 0.05$ vs. YC group, $n = 12$; # $P < 0.05$ vs. old control group, $n = 12$; ▲ $P < 0.05$ vs. ARb1-10 group, $n = 12$; ## $P < 0.05$ vs. MC group, $n = 12$; Δ $P < 0.05$ vs. MRb1-10 group, $n = 12$.

Rb1 treatment and its impact on NO levels in mice

NO functions as a key signaling molecule and neurotransmitter. To explore Rb1's anti-senescence mechanisms, serum NO content was quantified. Results showed decreased NO levels in middle-aged mice, with a more substantial reduction observed in older mice. Rb1 administration effectively restored and elevated serum NO concentrations (**Figures 5e and 5f**).

Aging constitutes a major risk factor for cardiovascular disease [17]. Given the growing global elderly population, expanding aging research is vital to improve quality of life and identify agents that can delay senescence [18]. Rb1 is one of the most abundant and bioactive compounds in ginseng, exhibiting pro-cognitive, anti-inflammatory, antioxidant, anti-aging, and immune-enhancing properties [10, 17, 18]. Prior evidence indicates that Rb1 can alleviate aging-related vascular damage by inhibiting vascular calcification and fibrosis [19]. Our

previous investigations confirmed that Rb1 reduces senescence in mouse brain tissue and HUVECs through SIRT1 activation and involvement of the eNOS pathway [12, 20]. Nevertheless, the effects of Rb1 on age-associated cognitive and behavioral deficits, brain tissue senescence, inflammatory responses in mice, and the underlying molecular mechanisms were previously unknown. This study specifically utilized female mice, as postmenopausal women represent a population with high rates of cognitive decline. Estrogen variations and age-related oxidative stress in female animals more closely parallel human pathological conditions [21]. Furthermore, male mice frequently exhibit territorial aggression that may introduce experimental bias, whereas female mice display lower aggression and better adaptation to group housing [22]. Female mice also tend to have longer lifespans than males [17], and brain-specific SIRT1 overexpression extends lifespan more substantially in females than in males [23]. Therefore, female mice serve as an ideal model for investigating SIRT1–aging interactions. Through *in vivo* behavioral evaluations and SA- β -gal staining of brain tissues, we confirmed that Rb1 enhances cognitive function and attenuates brain tissue aging in middle-aged and elderly mice. We further examined how Rb1 exerts these benefits by assessing the SIRT1/NO system, oxidative stress, and inflammatory responses.

Rb1 improves memory function in mice

Earlier studies showed that Rb1 protects against memory impairments induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in rats, suggesting its potential as a treatment for memory deficits in Parkinson's disease [24]. Rb1 has also been found to enhance spatial learning and memory by modulating cell proliferation in hippocampal subregions [10]. Ginsenoside Rg1, another major ginseng component, significantly improves memory deficits in aged rats [25]. Both Rg1 and Rb1 ameliorate memory impairments in animal models of Alzheimer's disease (AD), with Rb1 demonstrating stronger efficacy in rescuing acquisition deficits and suppressing neuroinflammation [26]. However, the specific influence of Rb1 on age-related memory decline remained unclear. In this study, the Morris water maze test was employed to evaluate visuospatial learning and memory in mice. This paradigm is a classical method for assessing spatial learning and memory in rodents, relying on distal cues to locate a submerged escape platform [27]. Although the Morris water maze can provoke anxiety in aged rats, the primary active ginsenosides with anxiolytic properties are Rg1 and Rg2 [28, 29]. In contrast, Rb1's pharmacological effects mainly involve regulation of oxidative stress and neuroinflammation [30, 31]. The present experiment shows that ginsenoside Rb1 strongly activates the SIRT1/eNOS/NO axis and reduces pro-inflammatory cytokines (such as IL-6 and TNF- α). This pathway is known to provide cognitive protection by mitigating neuronal oxidative damage [32]. No reports link Rb1's mechanisms to anxiety-related molecular targets such as the 5-HT1A or GABAA receptors. Therefore, the cognitive enhancements observed here are distinct from anxiolytic effects. Results demonstrated that Rb1 significantly improved visuospatial learning and memory capacities in middle-aged and old mice. Subsequent analysis of hippocampal SA- β -gal activity revealed an age-dependent increase in senescent neuronal cells, consistent with the behavioral data. In contrast, mice receiving Rb1 intervention (20 mg/kg·d) exhibited a marked reduction in SA- β -gal-positive cells, indicating that prolonged Rb1 treatment can delay neuronal senescence and thereby enhance cognitive function during normal brain aging.

Possible mechanism of Rb1 to ameliorate brain aging in mice: oxidative stress

Oxidative stress is widely accepted as a primary contributor to brain aging and leads to cognitive and memory impairment by disrupting synaptic plasticity and neuronal function [21, 22]. Numerous compounds have been shown to improve cognition through modulation of oxidative stress, including lycopene, vitamin D, and sesamol [21, 23, 24]. However, the detailed mechanisms remain to be fully elucidated. Our prior research demonstrated that Rb1 effectively protects endothelial cells from H₂O₂-induced senescence by restoring redox homeostasis [33]. Rb1 also improves mitochondrial function in myofibroblasts and protects them from oxidative damage by inhibiting NF- κ B activation [34]. Consequently, we hypothesized that Rb1's ability to delay cellular senescence is linked to its antioxidant properties. In this study, serum SOD activity was significantly decreased and MDA levels substantially increased in both aged and middle-aged groups compared to young controls. High-dose Rb1 treatment markedly elevated SOD activity and reduced MDA content. These findings indicate that antioxidant capacity begins to decline from middle age onward. The results support our hypothesis that Rb1 attenuates oxidative damage in senescent cells and brain tissues of middle-aged and aged mice while enhancing antioxidant enzyme activity (such as SOD and GSH-Px), thereby slowing cellular and brain aging. These observations are

consistent with our group's previous *in vitro* data showing that Rb1 decreases MDA and ROS levels while increasing SOD activity in H₂O₂-induced premature HUVECs [33].

Possible mechanism of Rb1 to ameliorate brain aging in mice: anti-inflammatory response

Inflammaging refers to a state of chronic, low-grade inflammation that accompanies aging [35]. As age advances, the immune system undergoes complex alterations leading to sustained elevation of pro-inflammatory cytokines in blood and tissues, along with impaired inflammatory resolution. Pro-inflammatory cytokines such as TNF- α and IFN- γ can induce epithelial cell senescence via ROS production and downstream signaling [36]. Chronic systemic inflammation also promotes glial cell senescence in rats, resulting in learning and memory deficits observable in middle-aged animals [37]. A key indicator linking aging to excessive inflammation is the rise in circulating pro-inflammatory mediators, particularly IL-6 and TNF- α [38]. Elevated IL-6 during aging correlates with impaired vascular mitochondrial function, creating a vicious cycle that accelerates atherosclerosis and cardiovascular events [39]. Our results revealed significantly increased IL-6 and TNF- α levels in senescent groups compared to young controls. This suggests that inflammatory responses contribute to endothelial and mouse brain tissue senescence, beginning in middle age. Early intervention targeting inflammation is therefore critical for delaying senescence. High-dose Rb1 substantially lowered serum IL-6 and TNF- α levels in naturally aging middle-aged and old mice, demonstrating that Rb1 can slow cellular and brain tissue aging by suppressing age-related inflammatory mediators.

Possible mechanism of Rb1 to improve brain aging in mice: effect on SIRT1/NO system

Aging is regulated by numerous factors, with established theories including the free radical theory, DNA damage, telomere shortening, and inflammation. The discovery of the Sirtuins family has provided new insights into aging mechanisms. SIRT1, a histone deacetylase homologous to yeast Sir2, plays a central role in regulating various intracellular processes and has shown strong protective effects against cellular senescence and neurodegenerative diseases, becoming a major focus of current research. Our previous studies demonstrated that Rb1 inhibits H₂O₂-induced HUVEC senescence via SIRT1 pathway activation [40]. Recent findings indicate that SIRT1 exerts its effects on vascular endothelial function and the nervous system primarily through modulation of the eNOS/NO system. The SIRT1/eNOS axis is considered a promising target for treating age-related disorders and combating aging [41]. NO serves as an essential signaling molecule and neurotransmitter involved in cognition, vascular regulation, immune function, and aging. SIRT1 reverses endothelial senescence by deacetylating eNOS, thereby increasing eNOS activity and NO production. Our earlier *in vitro* work showed that Rb1 regulates eNOS expression and activity through SIRT1, leading to elevated NO levels and reduced endothelial cell senescence. In the current experiments, SIRT1 protein levels in hippocampal tissue were significantly decreased in middle-aged and aged mice (**Figure 4**). Rb1 intervention produced a substantial increase in SIRT1 expression, elevated NO content, and significantly alleviated cellular senescence, cognitive impairment, and brain tissue aging. These results highlight the critical role of the SIRT1/eNOS/NO axis in mediating Rb1's anti-aging effects.

This study, integrating *in vitro* cellular and *in vivo* animal experiments, confirms that Rb1 improves cognitive function and slows brain tissue aging in mice. The primary mechanisms involve regulation of the SIRT1/eNOS/NO system, antioxidant protection, and anti-inflammatory actions by ginsenoside Rb1. Rb1 achieves these benefits through multi-targeted, integrative effects that delay cellular and brain tissue senescence, thereby promoting overall health. The findings offer new theoretical and experimental support for the treatment of aging-related cardiovascular and neurodegenerative diseases. Further studies are warranted to clarify the precise mechanisms.

Conclusion

In conclusion, we have demonstrated that Rb1 postpones the aging process in mice. This effect is associated with the suppression of oxidative stress and inflammatory responses through regulation of the SIRT1/eNOS/NO pathway.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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