Ginsenoside Rg1 Delays the Aging of Mouse Hippocampus and NSCs Through the Keap1-Nrf2/ARE Pathway

Lan Wang[1], Qi Wu[1], Shunhe Wang[1], Xiao Cheng[1], Ziling Wang[1], Lu Wang[1], and Yaping Wang[1]

[1]Chongqing Medical University

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Abstract

Aging can be caused by oxidative stress. Keap1-Nrf2/ARE signaling pathway is an antioxidant pathway. Ginsenoside Rg1 is one of the saponins found in ginseng having biological activity. It possesses anti-aging, anti-oxidant, and immune-strengthening properties. However, the molecular mechanism through which Rg1 affects brain aging is unknown. The purpose of this study was to investigate the effect of ginsenoside Rg1 in aging mice models and to decipher the molecular mechanisms underlying hippocampal aging and NSCs. D-gal was used to construct a mouse brain and NSCs aging model. Rg1 treatment improved brain function; decreased the structural damage of D-gal to hippocampal tissue and cells; decreased Ach neurotransmitter degradation; down-regulated the expression of the aging-related protein P53; alleviated oxidative stress damage: increased SOD and CAT activities, decreased MDA, 4-HNE and 8-OHdG content; down-regulated Keap1 protein expression and promoted Nrf2 dissociation from Keap1, and up-regulated Nrf2 and HO-1 protein expression, thus activating the Keap1-Nrf2/ARE pathway; Up-regulated expression of Nrf2/ARE pathway-related protective target genes (NQO1, GCLM, GSTM-1, and GCLC), reducing the aging process caused by D-gal-induced oxidative stress damage. Conclusion: Ginsenoside Rg1 can protect the hippocampus and NSCs of mice from oxidative damage and delay aging via the Keap1-Nrf2/ARE pathway.It advances our understanding of antioxidant therapy, serves as a critical reference for the prevention and treatment of aging-related disorders of the nervous system, and gives new scientific connotations to the Chinese medicine.

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Lan Wang[1*],Qi Wu[1.3*],Shunhe Wang[1],Xiao Cheng[1],Ziling Wang[2],Lu Wang[2],Yaping Wang[2#]

[1]Department of Pathology,Chongqing Medical University,Chongqing,400016,China.

[2]Department of Stem Cells and Tissue Engineering, Chongqing Medical University,Chongqing,400016,China.

[3]Department of Traditional Chinese Medicine Hospital,Tong Liang, Chongqing,402560,China.

*These authors contributed equally to this work.

#Corresponding authors:Chongqing Medical University,1 Yi xue yuan Road,Yuzhong District,Chongqing,400016,China.

Author email address:1946585123@qqq.com.

Corresponding author mail address:Prof.Yaping Wang:ypwangcq@aliyun.com.

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Keywords:

Ginsenoside Rg1; Hippocampus; NSCs; Oxidative Stress Damage; Aging; Keap1-Nrf2/ARE Pathway

Introduction

Aging is one of the most basic natural laws in the biological world. The damage, degeneration, apoptosis, and neuron loss caused by oxidative stress are significant contributors to neurodegenerative diseases and brain aging. As the human body ages, stem cells inevitably drop in number and function (DE HG et al,2018). The senescence of stem cells is closely related to the occurrence and progression of a wide variety of diseases (REN R et al,2017). The ability of neural stem cells (NSCs) to regenerate declines with age, resulting in brain aging and dysfunction. It has been linked to the development of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) (Zhu J et al,2014; Glorioso C et al,2011). The brain is closely related to the occurrence and development of NSCs, cognitive impairment, and neurodegenerative diseases (Zhang Q et al,2016). Therefore, Therefore, it is critical to investigate techniques to prevent the aging of the hippocampus and NSCs.

D-galactose (D-gal) is a well-established aging agent, and the aging model it induces is strikingly comparable to natural aging (Peng L 2011; Bei Y,2018; Chen LB,2018; Li G,2018; Zhao JC,2018). At present, conventional wisdom is that cell aging is typically induced by oxidative damage (WU Q et al,2020). The theory suggests that when the number of reactive oxygen species (ROS) produced in cells surpass the antioxidant defense mechanism and accumulates, oxidative stress occurs, promoting organisms and cells aging (Jiang LD et al,2017; Yan HL et al,2011).

Ginseng is an important drug in Chinese medicine for invigorating Qi. Ginsenoside Rg1 is the active ingredient of ginseng. Ginsenoside Rg1 has been shown to have considerable anti-aging properties(Han SH et al,2018; Leung KW et al,2007; Cheng Y et al,2005; Attele AS et al,2000). This study established that ginsenoside Rg1 can antagonize D-galactose-induced brain aging in mice, improve their behavior and enhance the neurogenesis of the entire NSCs (Xiang Y et al,2017). However, the molecular mechanism by which Rg1 delays hippocampal and NSC senescence is unknown.

The Keap1-Nrf2/ARE signal transduction pathway is a critical antioxidant pathway(Tu WJ et al,2019;Kundu JK et al,2010) . When stimulated by appropriate oxidants,nuclear factor E2-related factor 2 (Nrf2) and Kelch-like ECH-related protein 1 (Keap1) decompose and combine with antioxidants in the response element (ARE) of the nucleus to initiate transcription of cytoprotective genes, thereby protecting cells from Oxidative damage and aging (Hybertson BM et al,2011). Nrf2 has been found to be critical in preventing oxidative damage in studies (Ishii T et al,2000). Therefore, we hypothesized that ginsenoside Rg1 delays the aging of the hippocampus and NSCs via anti-oxidative stress damage and is associated with the activation of the Keap1-Nrf2/ARE signaling pathway.

This experiment combines traditional Chinese medicine, stem cell research, and the aging regulation theory. Using D-gal as a model of aging, we investigated whether ginsenoside Rg1 may delay the aging of the hippocampus and NSCs by reducing oxidative stress damage, and is mediated by the Keap1-Nrf2/ARE signaling pathway. By elucidating the mechanism by which ginsenoside Rg1 delays the aging of the hippocampus and NSCs, this study aims to provide a new direction for the prevention and treatment of neurodegenerative diseases.

Materials and methods

Reagents

Ginsenoside Rg1 (RSZD-121106, Purity = 98.6%) was purchased from Jilin Hongjiu Biological Technology Co, Ltd (Jilin, China), dissolved in PBS at a concentration of 20mg/mL, and sterilized by ultrafiltration. OriCell/C57BL/6J mice NSCs complete medium (MUBNF-90011) was obtained from Cyagen Biosciences Inc (Guangzhou, China). D-galactose (SG8010, Purity[?]98%) was obtained from Solarbio Science&Technology Co, Ltd (Beijing, China). SOD (A001-3-2), MDA (A003-1-2), CAT (A007-1-1), 4-HNE (H268) ,8-OHdG (H165) and ChAT(A079-1-1) kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China). AChE kit was purchased from Solarbio LIFE SCIENCES. BCA (P0012S), Mouse monoclonal anti-Nestin antibodies (AN203), Trypan blue staining (C0011), and SA-β-gal staining (C0602) kits were purchased from Beyotime Institute of Biotechnology (Nanjing, China).The antibody against P53(#ab26) was purchased from Abcam (United Kingdom), Nrf2 (#12721), Keap1 (#7705), and HO-1 (#86806) were purchased from Cell Signaling Technology Inc (Boston, USA). Trizol reagent (B511311) was purchased from Sangon Biotech (Shanghai, China). cDNA Reverse Transcription Kit (RR047A) and TBGreen® Premix Ex Taq II (RR820A) were obtained from Takara Biotechnology (Dalian, China).

Separation, purification, and grouping of NSCs

Whole-brain tissue was obtained from C57BL/6J fetal mice, separated, purified, and culture cells, culture them in serum-free medium to the third generation, NSCs were identified using Nestin immunofluorescence, and randomly divided into four groups. In the Rg1 group, NSCs were treated with PBS and incubated for 24 hours before being treated with Rg1 (20 μg/mL) and incubated for another 24 hours. NSCs were treated with D-gal (10 mg/mL) and cultured for 48 hours in the D-gal group. The NSCs in the D-gal + Rg1 group were treated with D-gal (10 mg/mL) and incubated for 24 hours, and then treated with Rg1 (20 μg/mL) and incubated for 24 hours. The Control group was incubated for 48 hours with the same amount of PBS. Relevant indicators were detected on the second day after Modeling.

Immunofluorescence identification of Nestin protein expressed by NSCs

Take the 3rd generation neurosphere cultured NSCs, and fix for 4% paraformaldehyde for 20 min, break the membrane with 0.3% TritonX-100 for 15 min, block with 10% goat serum for 60 min at room temperature, add Nestin antibody (1:100), and incubate at 4 Overnight, add fluorescent secondary antibody, incubate at 37°C for 1h in the dark, add DAPI working solution dropwise, drop anti-fluorescence quenching solution 5 minutes later, mount the slide with neutral gum, and observe the Nestin expression of NSCs under a fluorescence microscope.

CCK-8 assay

Cell viability was determined using the cell counting kit (CCK) 8-test. Neural stem cells (1×104 cells per well) were incubated in a culture medium. CCK-8 solution (10 μL) was added to the cell culture medium and incubated at 37°C for 4h. The absorbance was measured at 450 nm using a 96-well multi-scanner automatic reader.

Trypan blue staining

NSCs were collected in centrifuge tubes and stained with Trypan Blue for 3 min. Counting 500 NSCs with an optical microscope. Cell survival rate=(the total number of cells-the number of trypan-blue-stained cells)/the total number of cells*100%.

Animal treatment

Two months old C57BL/6J male mice(20-25g) were purchased from Chongqing Medical University's Laboratory Animal Center. All animal procedures and experiments complied with the approved guidelines and were approved by the Laboratory Animal Ethics Committee of Chongqing Medical University (Animal Certificate of Conformity: SYXK (Chong Qing) 2018-0003).The laboratory animals'rooms were ventilated regularly.The animals were housed in a temperature, humidity, and light-controlled room with free access to water and standard feed at the same time every day. 48 male mice were randomly divided into four groups, 12 mice/group. D-gal (120mg/kg/day) was administered subcutaneously into mice daily for 42 days in the D-gal group. The D-gal+ Rg1 group received the same treatment as the D-gal group, except for a 15th-day intraperitoneal injection of Rg1 (40mg/kg/dayx28d) (Dong ZY et al.2017). In the Rg1 group, the same amount of normal saline was administered subcutaneously for 14 days, followed by an intraperitoneal injection of Rg1 (40mg/kg/dayx28d) on the 15th day. In the Control group, an equal volume of normal saline was injected subcutaneously at the same time.

Morris water maze

Following the 42-day treatment, we used the Morris water maze test to assess the spatial learning and memory ability of mice(*Yang*XY et al,2021;Wang P et al,2017). Place navigation test was performed once a day for 5 days. The mice faced the pool wall and took turns to enter the water from 4 water entry points, and the time they took to locate the platform within the 60s was recorded. If they did not find the platform for more than 60s, the latency was recorded as 60s, and the mouse was guided on the platform and allowed to stay for 10s. The spatial probe test was performed on the 6th day, where the invisible platform was removed, the mouse launched from a certain entry point, and the swimming track of the mouse within the 60s after entering the water was recorded. The latency period of the mouse's escape, the number of times that the mouse crossed the position of the original platform and the original platform were calculated as a percentage of residence time in the quadrant.

Brain organ index

The bodyweight of the mouse was weighed. The mouse was anesthetized using pentobarbital and the neck severed to death. The whole brain was removed, the surface PBS liquid absorbed using a filter paper, and the wet weight of the brain determined. The brain index was calculated by dividing the wet brain weight by the weight of the mouse.

H&E staining

Pentobarbital was used to anesthetize the mice, and the entire brain tissue was swiftly taken via decapitation. For 24 hours, the specimen was fixed in 4% paraformaldehyde, dehydrated in ethanol, made transparent with p-xylene, embedded in paraffin, coronal sectioned, and stained with hematoxylin and eosin (HE) (Yang L et al,2017). Under an optical microscope, the histology of the hippocampus CA1 region was examined.

Nissl staining

Nissl staining provided information about the pathology of hippocampal neurons.The brain tissue was fixed for 24h with 4% paraformaldehyde, ethanol dehydrated paraffin-embedded, coronal sectioned, deparaffinized by immersing it in 1% toluidine blue solution for 40min, washing three times with distilled water, ethanol dehydrated, transparent with xylene, and neutral resin sealed. An optical microscope was used to observe the Nissl body in the CA1 region of the hippocampus.

ΣA-β-γαλ σταινινγ

Because the buildup of endogenous lysosomal galactosidase is a hallmark of aging cells, we determined the senescence-related β-galactosidase (SA-β-gal), which is widely used in mammalian cells as a senescence biomarker (Zhang MS et al,2015). The Leica cryostat was used to prepare a frozen segment of the coronal hippocampus of brain tissue. The section and third-generation neurosphere were fixed for 30 minutes at room temperature with the kit fixative solution, washed 3 times with PBS, stained with the β-galactosidase

staining working solution, and incubated overnight at 37°C. Using an optical microscope, the hippocampal CA1 region and NSCs were observed. The blue-stained cells were senescent cells or positive cells.

Transmission electron microscopy of the hippocampal nerve cells ultrastructure

The mice were anesthetized using pentobarbital, and then perfused with normal saline and 4% paraformaldehyde, and the brain tissues were removed. The hippocampus was cut into a cube with a side length of about 1 mm, put in glutaraldehyde, and observed under an electron microscope.

Analysis of cholinergic function

Acetyl-CoA and choline were used as substrates in the determination of ChAT, following the manufacturer's instructions. ChAT and AChE activities were measured the absorbance at 324 nm and 412 nm(Ji Y et al,2019).

Measurement of oxidation-related biomarkers

Following treatment, the hippocampus and NSCs were lysed in RIPA Lysis Buffer (Beyotime, China), centrifuged and the supernatant (containing protein) was collected, and the protein concentration was measured with BCA kit(Beyotime Institute of Biotechnology ,China). SOD, CAT, and MDA(Zhou H et al,2022) activities in hippocampus homogenate and NSCs were determined by chemical colorimetric method following the manufacturer's instructions. 8-OHdG and 4-HNE activity in hippocampal homogenate and NSCs were determined by ELISA.

Western Blot

The same method was used to obtain the protein of the hippocampus and NSCs and measured the protein concentration. SDS-PAGE was used to separate the proteins, which were then transferred to a PVDF membrane and blocked for 1 hour with TBST and skimmed milk powder. The membranes were incubated using a primary antibody(1:1000) overnight at 4degC, then with a secondary antibody(1:10000) for 1 hour at room temperature. The ECL light-emitting system was used to develop colors, and quantitative analysis was performed using Image Lab software.

qRT- PCR

Total RNA from hippocampus and NSCs was extracted using Trizol reagent and following the manufacturer's instructions. The quality of RNA was determined by the ratio of A260/A280,and samples with A260/A280=1.8˜2.0 were used for further analysis.Followed the instructions of the cDNA Reverse Transcription Kit to reversed transcribe RNA into cDNA. Fluorescence quantification was performed using the TBGreen(r) Premix Ex Taq II (Takara, Japan), and then the amplifying conditions for cDNA were as follows: 95degC for 30s, 40 cycles of 95degC for 5s and 60 degC for 30s. Primer3 software (http://simgene.com/Primer3) was used to design specific primers. Primer sequences are listed in Table 1.

Statistical analysis

All experimental data were presented as the mean+-standard deviation (SD), The statistical significance were analyzed using GraphPad Prism 7.0(GraphPad Software Inc., San Diego, CA, USA) statistical software by repeated-measure ANOVA and paired t test. $p < 0.05$ was considered statistically significant.

Results

Identification of the Nestin protein expressed by NSCs by immunofluorescence

Nestin staining could identify NSCs(Seonghee Jung et al.2020).Under a microscope, it was observed that the NSCs developed into neurospheres (Fig.1A). NSCs were identified using Nestin immunofluorescence staining(Fig.1B), with green fluorescence representing positive NSCs when observed under a fluorescence microscope (x100). The result showed that the real NSCS was extracted

Rg1 delayed the aging of NSCs

To explore whether Rg1 could delayed D-gal induced aging of NSCs,we used CCK-8 assay (Table.3) revealed a significant improvement in the ability of cells in the D-gal +Rg1 group to proliferate when compared to cells in the D-gal group,In comparison to cells in the Control group, the D-gal group had a strong inhibitory effect on cell proliferation.SA-β-gal (Fig.2 A, B) revealed that whereas the D-gal group had a higher percentage of senescence neurospheres than the Control group, the D-gal group had a significantly lower percentage of senescence neurospheres than the D-gal +Rg1 group(paired t test,n=3,p¡0.05). Trypan blue staining (Fig.2 C) results showed that the cell survival rate of the D-gal group was significantly lower than that of the Control group,However, as compared to the D-gal group, the D-gal +Rg1 group demonstrated a considerable increase in cell survival(paired t test,n=6,p¡0.05). The above results indicated that Rg1 could delayed the aging of NSCs induced by D-gal.

Rg1 reduced oxidative stress in NSCs by increasing antioxidant capacity

In order to study whether Rg1 could delayed the aging of NSCs by reducing oxidative damage, we measured the indicators related to oxidative stress.In comparison to the Control group, the D-gal group had lower SOD (Fig.3 A) and CAT (Fig.3 B) activities, but elevated MDA (Fig.3 C), 4-HNE (Fig.3 D), and 8-OHdG (Fig.3 E) contents. In comparison to the D-gal group, the D-gal +Rg1 group had higher SOD and CAT activity, but lower MDA, 4-HNE, and 8-OHdG contents(paired t test,n=4-6,p¡0.05). The results showed that Rg1 delayed the aging of NSCs by reducing oxidative damage.

The protective mechanism of Rg1 against oxidative stress damage was mediated by the Keap1-Nrf2/ARE pathway

To determine whether the protective mechanism of Rg1 against oxidative stress injury was mediated by the Keap1-Nrf2/ARE pathway, we determined the expression of proteins associated with the Keap1-Nrf2/ARE pathway (Fig.4 A-D),Nrf2 and HO-1 protein expression level were lower in the D-gal group than in the Control and D-gal +Rg1 group, while Keap1 protein expression level were greater in the D-gal group than in the Control and D-gal +Rg1 group (paired t test,n=3,p¡0.05). We also measured the expression of mRNA related to the Keap1-Nrf2/ARE pathway (Fig.4 E-H),in comparison to the Control group, the expression levels of NQO1,GCLM,GSTM-1 and GCLC mRNA was decreased in the D-gal group, but the expression levels of these mRNA increased in the D-gal +Rg1 group(paired t test, n=5, p¡0.05). The above results indicated that the protective mechanism of Rg1 against oxidative stress injury of NSCs was mediated by the Keap1-Nrf2/ARE pathway

Rg1 protected the hippocampus against D-gal induced aging

To determine whether Rg1 could antagonize brain aging caused by D-gal, we used Rg1 and D-gal to intervene.The biological dynamics of aging in mice revealed that as the injection time and cumulative dose of D-gal increased, the hair of the mice in the aging model group became duller, their activity decreased, their eating decreased, their stool did not form, their weight gain was slow, and the typical signs of natural aging were observed,the other three groups exhibited no clear symptoms of aging.The navigation test of Morris water maze (MWM) revealed that the D-gal group exhibited learning deficits compared to Control group and D-gal+Rg1 groups (repeated-measure ANOVA; ($F[5, 25] = 2.3$, not statistically significant; Fig 5A),in the spatial exploration test (Fig.5 B, C), the D-gal group number of times crossing the original platform position and percentage of time spent in the original platform quadrant were significantly lower than those of the Control and D-gal+Rg1 groups,the swimming speed of D-gal group was slower than that of the Control group and D-gal+Rg1 group,and the swimming distance of D-gal group was longer(Fig.5 D,E) (paired t test, n=12, p¡0.05).The Brain Organ Index(Table.2) was lower in the D-gal group, but higher inthe D-gal + Rg1 group.HE staining (Fig.5 K,L) revealed that in comparison to the D-gal+Rg1 group, the D-gal group's cells were arranged in a disorderly manner, with varying sizes, uneven staining, and apparent neuronal necrosis, indicating enhanced cytoplasmic eosinophilia,the other three groups exhibited no clear symptoms of aging.Nissl staining (Fig.5 M,N) showed that nerve cells and the Nissl body were reduced in the D-gal group, but increased in the D-gal + Rg1 group.Transmission electron microscope (Fig.5 J) showed

that the intracellular organelles in the D-gal group were vacuoles, the RER was expanded and vesicles were present, mitochondria were swollen, the edges of heterochromatin were visible, and the perinuclear space increased,the other groups showed no obvious changes.SA-β-gal staining (Fig.5 O,P) showed that the number of blue-stained senescent cells increased in the D-gal group, whereas the number of blue-stained senescent cells reduced in the D-gal + Rg1 group.P53 protein (Fig. 5 F,G) expression increased in the D-gal group but decreased in the D-gal + Rg1 group. ChAT (Fig. 5 H) and AChE (Fig. 5 I) activity were determined, compared with D-gal group, ChAT activity in Control group and D-gal+Rg1 group increased, while AChE activity decreased(paired t test, n=3, p¡0.05). Taken together, D-gal can induce brain senescence in mice, while Rg1 can antagonize this brain senescence.

Rg1 protected the hippocampus against oxidative stress damage induced by D-gal

In order to determine whether the aging damage of hippocampus was caused by oxidative stress, the indexes related to oxidative stress were determined.The D-gal group SOD(Fig.6 A) and CAT(Fig.6 B) activity reduced in comparison to the Control group, while their MDA(Fig.6 C), 4-HNE(Fig.6 D), and 8-OHdG(Fig.6 E) concentrations increased. In comparison to the D-gal group, the D-gal + Rg1 group enhanced its SOD and CAT activities while decreasing its MDA, 4-HNE, and 8-OHdG content(paired t test, n=3, p¡0.05). In short, oxidative stress damage can cause aging.

The protective mechanism of Rg1 against oxidative stress damage was mediated by the Keap1-Nrf2/ARE pathway

Same as in vitro experiment, We measured the expression of proteins related to the Keap1-Nrf2/ARE pathway in the hippocampus of mice(Fig.7 A-D),we found that Nrf2 and HO-1 protein expression levels were significantly lower in the D-gal group than in the Control and D-gal + Rg1 group, Keap1 protein expression levels were significantly higher in the D-gal group than in the Control and D-gal + Rg1 group (paired t test,n=3,p¡0.05). We also measured the expression of mRNA related to the Keap1-Nrf2/ARE pathway (Fig.7 E-H), compared with D-gal group, the mRNA expression levels of NQO1, GCLM, GSTM-1 and GCLC decreased significantly, but the expression levels of D-gal+Rg1 group and Control group increased significantly (paired t-test, n=3, $p<0.05$). The above results indicated that the protective mechanism of Rg1 against oxidative stress injury in hippocampus was mediated by the Keap1-Nrf2/ARE pathway.

Discussion

There is no doubt that the global population is aging. Neurodegenerative diseases have developed into a severe concern to the health of the world's elderly population. However, as humans age, NSCs age and degenerate, limiting the ability of the neurological system to repair and protect itself, leading to cognitive impairment (Xiang Y et al,2017). Damage to nerve cells caused by aging is a significant factor in the development of neurodegenerative disorders. There is currently no viable strategy for the prevention or treatment of neurodegenerative disorders. The hippocampus is composed of nerve cells, that perform memory and spatial positioning tasks. Therefore, postponing nerve cell aging damage is an efficient strategy for preventing and treating neurodegenerative diseases. Our previous research report stated that Ginsenoside Rg1 is the primary component of ginseng and has been shown to protect the brain and NSCs from the aging effects of D-gal (Xiang Y et al,2019). However, this process warrants additional investigation.

While a small amount of D-gal can be converted to glucose and participate in cell metabolism, a significant amount of D-gal will disrupt cell metabolism disorders and produce a large number of oxidation products, resulting in cell oxidative damage and senescence (Wang ZL et al,2016). As a result, in this study, we successfully extracted, cultured, purified, and identified NSCs using immunofluorescence. The cells were Nestin (green fluorescence) positive, and an in vitro model was constructed. CCK-8 assay results showed that D-gal inhibited cell proliferation, whereas Rg1 promoted cell proliferation. Trypan blue staining revealed that D-gal decreased cell survival rate, but Rg1 increased cell survival rate. The results of SA-β-gal staining showed that Rg1 can antagonized the aging effect of D-gal on NSCs and effectively reduced the senescence level of NSCs. The above experimental results demonstrated that D-gal can induced senescence in NSCs and that Rg1 can protected NSCs against D-gal induced senescence.To gain a better understanding of

what causes this aging, we measured indicators of oxidative stress which is SOD, CAT, MDA, 4-HNE , 8-OHdG,these findings suggested that aging was associated with oxidative stress damage, that D-gal can generated oxidative stress damage in NSCs, and that Rg1 can reversed the oxidative stress damage caused by D-gal, hence delaying NSCs aging. Is Rg1's resistance to oxidative stress damage related to oxidation-related pathways? As a result of our investigation into the molecular mechanisms behind oxidation, we discovered the Keap1-Nrf2/ARE pathway, an antioxidant route. Firstly, we explored the expression of pathway-related proteins. The Nrf2 and HO-1 proteins were expressed at a lower level in the D-gal group than in the Control or D-gal + Rg1 groups, although the Keap1 protein content was increased. The results indicate that Rg1 can down-regulate the expression of Keap1 protein, promote the dissociation of Nrf2 and Keap1, while simultaneously up-regulating the expression of Nrf2 and HO-1 proteins, activating this pathway and thereby reducing the aging caused by D-gal. Then, we examined the levels of expression of genes involved in this pathway. NQO1, GCLM, GSTM-1, and GCLC are all Nrf2/ARE pathway target genes. Compared with the D-gal group, the mRNA expression levels of each gene in the Control group and D-gal+Rg1 group were higher. The data indicated that Rg1 can activate this pathway, promoting the association of Nrf2 and ARE sequences and transcription of associated target genes, thereby alleviating D-gal-induced senescence. The above findings suggested that Rg1 protect against oxidative stress damage via the Keap1-Nrf2/ARE pathway, hence delaying NSCs aging.

To validate the aforementioned findings, we conducted relevant in vivo research. we established an animal model. MWM experiment was used to assess mice's brain function, the results indicated that D-gal has a clear detrimental effect on the cognitive function and spatial memory of mice and that Rg1 can reverse the cognitive and spatial memory impairment induced by D-gal. D-gal enhanced hippocampus neuron necrosis, which Rg1 could ameliorate. The results of Nissl staining showed that D-galactose could damage the physiological state of neurons, while Rg1 could restore the physiological state of neurons.SA-β-gal staining indicates that Rg1 can prevent hippocampus neurons from succumbing to D-gal-induced senescence.The results of transmission electron microscopy indicated that D-gal causes neuronal damage and that Rg1 can reverse this effect. P53 protein is a critical aging-related protein, the result showed that Rg1 down-regulated aging-related protein expression. The cholinergic test result Rg1 has been shown to inhibit the D-gal breakdown of the Ach neurotransmitter. The data above demonstrated that D-gal promoted hippocampal aging in mice and Rg1 delayed D-gal-induced aging.We continue to measured indicators related to oxidative stress, which are the same as the results of in vitro experiments ,the results indicated that the aging of hippocampals caused by D-gal was related to oxidative stress and that Rg1 was resistant to oxidative stress. Finally, we determined the indicators of proteins and genes involved in the Keap1-Nrf2/ARE pathway and obtained same results to those observed in vitro model. The results indicated that Rg1 can reduce the oxidative damage in hippocampals via the Keap1-Nrf2/ARE pathway to delay aging.

Ginseng is widely used in traditional Chinese medicine. Among the more than 30 varieties of ginsenosides, Rg1 is considered to be the main active ingredient, responsible for the attributes of multi-target pharmacological effects. Previous research has established that Ginsenoside Rg1 possesses anti-aging, and anti-oxidant properties, as well as the ability to promote hematopoiesis, improve immunity, protect the liver, and other functions. Ginsenoside Rg1 can enhance antioxidant activity by modulating the Wnt/β-catenin signaling pathway(Qi RJ et al,2020). Rg1 plays an anti-aging effect by regulating the p19-p53-p21 pathway (Cheng X et al,2019). However, Rg1 needs further research,for example, study the role of Rg1 in gene knockout, pathway agonists or inhibitors et al.

Conclusions

We successfully established an aging model for the hippocampus and NSCs using D-gal. The mechanism by which ginsenoside Rg1 delays the aging of mouse hippocampus and NSCs is related to the activation of the Keap1-Nrf2/ARE pathway, which increases the expression of related antioxidant enzymes and genes, thereby reducing the level of oxidative stress. Additional research is required to determine how Rg1 protects the hippocampus from damage.

Data Availability Statement

The data that support the findings of this study are available upon reasonable request from the respective authors.

Conflict of Interest

Lan Wang, Qi Wu, Xiao Cheng, Ziling Wang declare they have no conflict of interest.

Acknowledgments

L.W and Q.W performed the experiments and analyzed the data; X.C and L.W assisted in the design of the experiment; Z.W provided some chemical reagents; S.W and P.W guided the writing and revision of the articles. The final manuscript was read and approved by all authors.

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List of Abbreviations

AChE,Acetylcholinesterase; AD, Alzheimer disease; ARE, Antioxidants in the response element; CAT, Catalase; ChAT, Acetylcholine transferase; D-gal, D-glalctose; GCLC, Glutamate-cysteine ligase catalytic subunit; GCLM, Glutamate cysteine ligase; GSTM-1, Glutathione S-transferase M1; HO-1, Hemeoxygenase1; Keap1, Kelch Like ECH Associated Protein 1; MDA, alondialdehyde; MWM, Morris Water Maze; Nrf2, Nuclear Factor E2-related factor2; NQO1, NAD(P)H:Quinone Oxidoreductase1; PD, Parkinson's Disease; ROS, Reactive oxygen species; SA-β-Gal, Senescence-associatedβ-galactosidase; SOD,Superoxide dismutase;PAGE,Polyacrylamide gel electrophoresis;PBS,Phosphate-buffer sline;PVDF,Poly (vinylidene fluride);4-HNE,4-hydroxynonenal;

8-OhdG,8-hydroxy-2 deoxyguanosine.

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