Ginsenoside Rg1 protects against H_2O_2-induced neuronal damage due to inhibition of the NLRP1 inflammasome signalling pathway in hippocampal neurons *in vitro*

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Abstract. Oxidative stress and neuroinflammation are important in the pathogenesis of ageing and age-related neurodegenerative diseases, including Alzheimer's disease. NADPH oxidase 2 (NOX2) is a major source of reactive oxygen species (ROS) in the brain. The nucleotide-binding oligomerisation domain (NOD)-like receptor protein 1 (NLRP1) inflammasome is responsible for the formation of pro-inflammatory molecules in neurons. Whether the NOX2-NLRP1 inflammasome signalling pathway is involved in neuronal ageing and age-related damage remains to be elucidated. Ginsenoside Rg1 (Rg1) is a steroidal saponin found in ginseng. In the present study, the primary hippocampal neurons were treated with H_2O_2 (200 μM) and Rg1 (1, 5 and 10 μM) for 24 h to investigate the protective effects and mechanisms of Rg1 on H_2O_2-induced hippocampal neuron damage, which mimics age-related damage. The results showed that H_2O_2 treatment significantly increased ROS production and upregulated the expression of NOX2 and the NLRP1 inflammasome, and led to neuronal senescence and damage to hippocampal neurons. Rg1 decreased ROS production, reducing the expression of NOX2 and the NLRP1 inflammasome in H_2O_2-treated hippocampal neurons. Furthermore, Rg1 and tempol treatment significantly decreased neuronal apoptosis and the expression of β-galactosidase, and alleviated the neuronal senescence and damage induced by H_2O_2. The present study indicates that Rg1 may reduce NOX2-mediated ROS generation, inhibit NLRP1 inflammasome activation, and inhibit neuronal senescence and damage.

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Introduction

Brain ageing is a progressive and gradual accumulation of harmful changes in the brain tissue with age, including oxidative stress injury and neuronal inflammation, which increase the risk of age-related learning and memory impairment (1,2). At present, the oxidative stress theory is recognised as the most feasible hypothesis to explain the ageing process, particularly age-related neuronal degeneration (3-5). Increasing evidence has suggested that reactive oxygen species (ROS) may cause neuronal oxidative stress, inflammation, and neurodegeneration with age (3,6-7). There are several enzymes that can generate intracellular ROS. NADPH oxidase (NOX) is a major source of ROS generation (4,5). It has been found that NOX2 (gp91phox) is constitutively expressed in neurons and significantly increased in the brain of ageing mice (4). The expression of NOX2 subunits p47phox and p67phox are also increased in the cortex of patients with mild cognitive disorders (5). Inflammasomes are NOD-like receptor (NLR) family multiprotein complexes that are responsible for the formation of pro-inflammatory molecules. Neuroinflammation mediated by inflammasomes is important in several age-related neurodegenerative diseases (8,9). NOD-like receptor protein 1 (NLRP1) was the first reported inflammasome (10). The NLRP1 inflammasome is widely expressed in the body and is the main inflammasome in neurons. An increasing number of studies have shown that the NLRP1 inflammasome is associated with age-related neuronal damage and cognitive impairment (8,11). NADPH oxidase-derived ROS accumulation is one of the main pathways for NLRP3 inflammasome activation (12,13). However, whether NOX2-derived ROS can activate the NLRP1 inflammasome, and whether it is involved in neuronal senescence and damage, remain to be elucidated.

Ginsenoside Rg1 (Rg1) is one of the active ingredients of ginseng, which has been used to improve health conditions and delay senescence in China (14,15). It has been reported that Rg1 has significant antioxidant, anti-ageing and neuroprotective

effects (16), and Rg1 can improve learning and memory impairment in ageing mice (17,18). Our previous studies showed that Rg1 significantly decreased ROS generation and attenuated the neuronal oxidative stress damage induced by chronic restrain stress in mice (19), and Rg1 protected against neuroinflammation and neuronal injury induced by chronic glucocorticoid exposure (20). These data suggest that Rg1 has a protective effect on neuronal damage due to decreasing ROS generation. Therefore, it was hypothesised that Rg1 may downregulate NOX2 and reduce the production of ROS, thereby inhibiting activation of the NLRP1 inflammasome in hippocampal neurons and protecting against age-related neuronal damage.

In the present study, primary cultured hippocampal neurons were treated with H_2O_2 (200 μM) for 24 h to mimic age-related neuronal damage. The study evaluated the protective effects of ROS scavenger tempol and Rg1 on H_2O_2-induced neuronal damage and assessed whether Rg1 regulated the activation of the NLRP1 inflammasome via the inhibition of NOX2 in hippocampal neurons.

Materials and methods

Culture and treatment of hippocampal neurons. Primary hippocampal neurons were prepared from postnatal Sprague-Dawley rats (within 24 h; 6-8 neonates/experiment) obtained from the Center of Laboratory Animals of Anhui Medical University (Hefei, China) and placed in plates coated with poly-L-lysine (10 μg/ml). Neurobasal medium with B-27 supplement (Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to culture the neurons at 37˚C with 5% CO_2 as described previously (21). The neurons were cultured for 7 days and divided into six groups: Control group, H_2O_2 (200 μM) group, H_2O_2 (200 μM) + tempol (100 μM) group, and H_2O_2 (200 μM) + Rg1 (1, 5 and 10 μM) groups. With the exception of the control group, the neurons in the groups were treated with H_2O_2 (200 μM) and tempol (100 μM) or Rg1 (1, 5 or 10 μM) for 24 h. Tempol (Santa Cruz Technology, Inc., Dallas, TX, USA) and Rg1 (content of Rg1 >98%; Chengdu Desite Biotechnology Co., Chengdu, China) were dissolved in distilled water and stored at -80˚C. All experimental procedures were performed in accordance with the approved protocol of the Ethics Committee of Anhui Medical University (Anhui, China).

Neuronal apoptosis assay (Hoechst 33258 staining). Hoechst 33258 can stain the nucleus and is often used to assess the apoptotic rate of neurons (22). First, the neurons were fixed with 4% paraformaldehyde and incubated with Hoechst 33258 (5 μg/ml, Zhongshan Golden Bridge Biotechnology Co., Beijing, China) for 15 min. Following washing with PBS, the neurons were sealed onto slides with anti-fade mounting medium. Fluorescence microscopy was then used to examine the apoptosis of neurons (Olympus IX71; Olympus Corporation, Tokyo, Japan). Morphologically, the apoptotic neurons appeared smaller and bright blue, the nucleus was condensed and deeply stained (23). The apoptotic neurons were counted and the relative neuronal apoptotic rate was examined in each culture and compared with the control.

Examination of ROS production with dihydroethidium (DHE) staining. DHE is the most commonly used ROS fluorescent probe and is often used for labelling living cells to detect the production of ROS (24). For the ROS examination, DHE (10 μM) was added to the medium and incubated for 30 min at 37˚C. The production of ROS was then detected using a fluorescence microscope (Olympus IX71, Olympus Corporation). The red mean optical density was performed using Image-Pro Plus 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA), to indicate the ROS production in the hippocampal neurons.

Immunofluorescence. Caspase-3 is the common pathway and the executor of apoptosis (25). To confirm the effect of Rg1 on neuronal apoptosis in H_2O_2-damaged hippocampal neurons, the expression of caspase-3 was detected by immunofluorescence. First, the hippocampal neurons were fixed with 4% paraformaldehyde for 30 min and washed thoroughly with PBS. Following permeabilisation with Triton X-100 (0.25%) for 30 min, and blocking with 1% BSA (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) in PBS for 1 h, the neurons were incubated with caspase-3 primary antibody (cat. no. BS7003; lot no. CJ32131; 1:200; Bioworld Technology, Inc., St. Louis Park, MN, USA) overnight at 4˚C. Subsequently, the neurons were incubated in secondary antibody conjugated to FITC (cat. no. ZF-0311; lot no. 127805; 1:200; ZSGB-BIO; OriGene Technologies, Inc., Beijing, China) for 1 h at room temperature. The slides were then mounted and examined with a fluorescence microscope (Olympus IX71; Olympus Corporation). The relative fluorescent intensity was quantified from three random fields per slide using the Image-Pro Plus 6.0 analysis system to indicate the expression of caspase-3.

Immunoblotting assay. Radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China) were used to extract the total protein. The protein concentration was determined by BCA Protein Assay kit (Beyotime Institute of Biotechnology). Equal samples of protein (30 μg) were separated by 12% SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with 5% skim milk for 1 h at room temperature. The membrane was then incubated with primary antibodies targeting β-galactosidase (β-Gal), NOX2, p22phox, p47phox, NLRP1, apoptosis-associated speck-like protein containing a carboxy-terminal CARD (ASC), caspase-1 and β-actin (1:1,000) overnight at 4˚C. The primary antibodies for β-Gal (cat. no. ab9361; lot no. GR27325-12), NOX2 (cat. no. ab31092; lot no. GR91755-40), NLRP1 (cat. no. ab3683; lot no. GR281560-21) and caspase-1 (cat. no. ab1872; lot no. GR615230-38) were from Abcam (Cambridge, UK). The p22phox (cat. no. BS60290; lot no. CN21141) and p47phox (cat. no. BS4852; lot no. CN33161) antibodies were from Bioworld Technologies, Inc. ASC antibody (cat. no. SC-514414; lot no. G1916) was from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA) The dilutions of β-Gal, NOX2, NLRP1 and p47phox were 1:1,000. The dilutions of ASC, caspase-1 and p22phox were 1:500. The membranes were then washed with TBS-Tween 20 and incubated with anti-rabbit IgG antibody conjugated to HRP (cat. no. ZF-2301; lot no. 128615; 1:10,000; ZSGB-BIO; OriGene Technologies, Inc.) at room

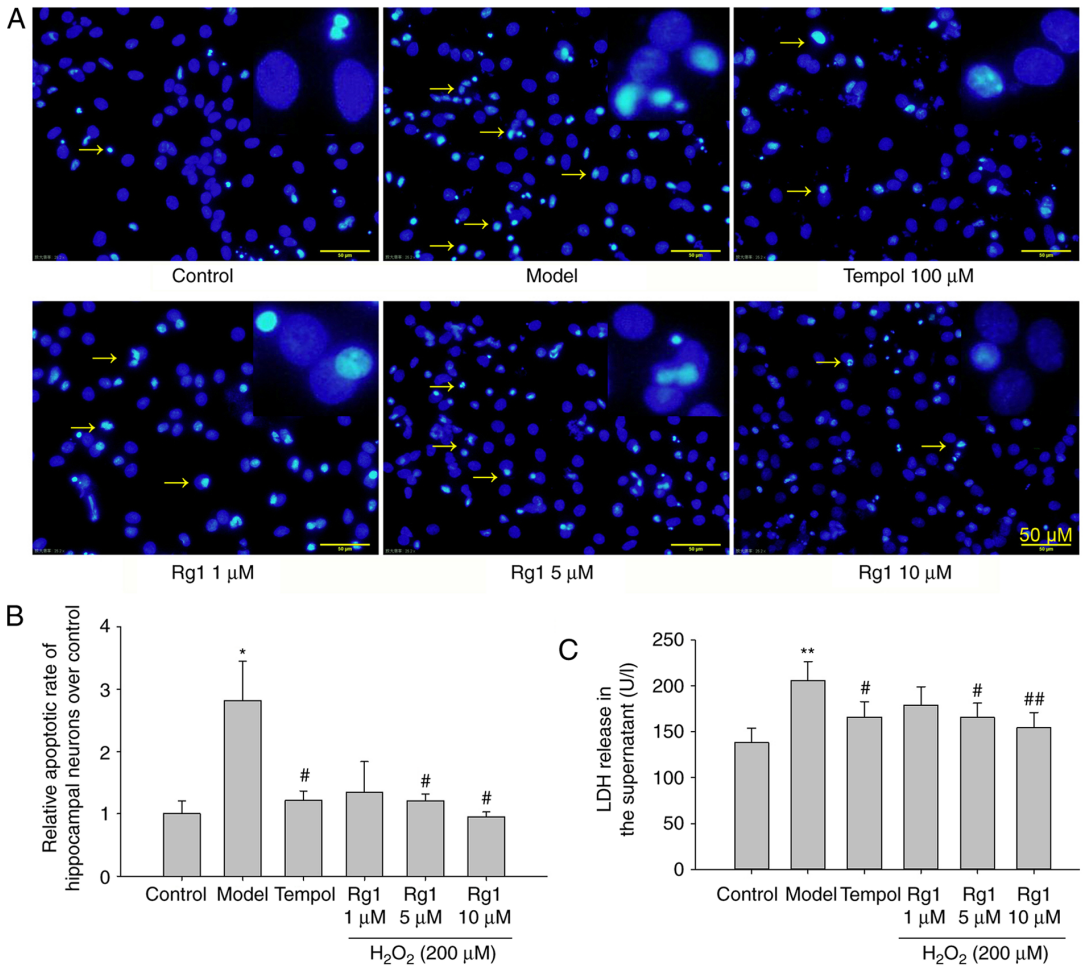

Figure 1. Effect of Rg1 on neuronal apoptosis in hippocampal neurons induced by H_2O_2 exposure (Hoechst 33258 staining, magnification, x400). (A) Representative images of neuronal apoptosis in hippocampal neurons. The apoptotic cells appeared smaller than normal and were more deeply stained (yellow arrow). (B) Quantitative analysis of the relative apoptotic rate of hippocampal neurons over the control. (C) The level of LDH released in the supernatant. Results are expressed as the mean ± standard deviation, n=3, *P<0.05 and **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the model group. Rg1, ginsenoside Rg1.

temperature for 1 h. The protein bands were visualised by using a Chemi Q4800 mini imaging system (Shanghai Bioshine Technology, Shanghai, China). The protein bands were measured with ImageJ 1.44 software (National Institutes of Health, Bethesda, MD, USA) and normalised to the corresponding β-actin bands. The relative density of each target protein over the control was used to represent the changes in expression of target proteins.

Enzyme-linked immunosorbent assay (ELISA). Following incubation with H_2O_2 and tempol or Rg1, the supernatants were collected from each group. ELISA kits were used to detect the levels of interleukin (IL)-1β and IL-18 (Cloud-Clone Corp., Houston, TX, USA). Briefly, the samples and the standards of IL-1β and IL-18 were placed into the assay plate and incubated for 1 h at 37˚C. Following incubation for 1 h, the HRP-conjugated reagent (100 μl) was added to the wells and incubated for 1 h at 37˚C. Subsequently, the chromogen solution (100 μl) was added to the wells. The plate was mixed and incubated for 15 min at 37˚C. Stop solution (50 μl) was then added to the wells and the absorbance at 450 nm was detected with a microplate reader (Thermo Fisher Scientific, Inc.) within 15 min.

Statistical analysis. Data are presented as the mean ± standard deviation. Statistical differences were analysed by one-way analysis of variance and the between-group comparisons were subjected to the Bonferroni post hoc test using SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA. P<0.05 was considered to indicate a statistically significant difference.

Results

Rg1 inhibits H_2O_2-induced neuronal apoptosis and the expression of caspase-3. The present study first examined the effects of Rg1 treatment on H_2O_2-induced neuronal apoptosis by staining with Hoechst 33258, which can bind to chromatin allowing the visualisation of normal and condensed chromosomes with a fluorescence microscope (22). The results showed that there were few apoptotic neurons in the control group. Compared with the control group, H_2O_2 exposure significantly increased neuronal apoptosis (Fig. 1A and B; P<0.05). Compared with the H_2O_2-treated group, Rg1 (5 and 10 μM) treatment significantly reduced neuronal apoptosis (Fig. 1A and B; P<0.05). Treatment with the ROS scavenger tempol (100 μM) significantly decreased the apop-

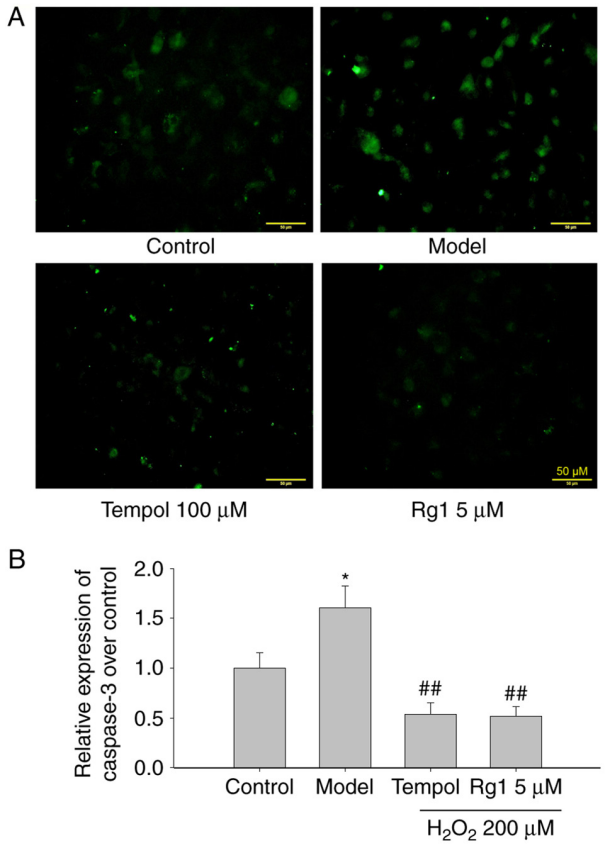

Figure 2. Effect of Rg1 on the expression of caspase-3 in H_2O_2-treated hippocampal neurons (immunofluorescence, magnification, x400). (A) Representative images of the expression of caspase-3 in hippocampal neurons. (B) Quantitative analysis of the relative expression of caspase-3 over the control. Results are expressed as the mean ± standard deviation, n=3, *P<0.05 compared with the control group; $^{\#\#}$P<0.01 compared with the model group. Rg1, ginsenoside Rg1.

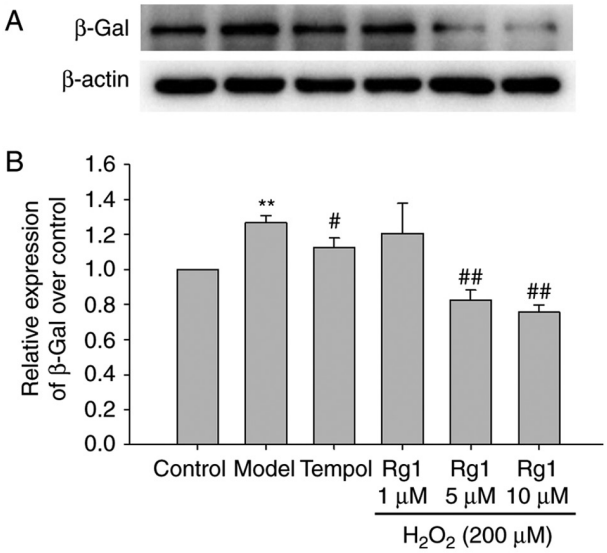

Figure 3. Effect of Rg1 treatment on the expression of senescence-associated β-Gal in H_2O_2-treated hippocampal neurons (immunoblot). (A) Representative blots of β-Gal in hippocampal neurons. (B) Analysis of the relative expression of β-Gal over the control. Results are expressed as the mean ± standard deviation, n=3, **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the model group. Rg1, ginsenoside Rg1; β-Gal, β-galactosidase.

tosis of hippocampal neurons induced by H_2O_2 treatment (Fig. 1A and B; P<0.05).

To confirm the anti-apoptotic effect of Rg1, the effects of Rg1 (5 μM) on the expression of caspase-3 in H_2O_2-damaged hippocampal neurons was further examined by immunofluorescence. The results showed that, compared with the control group, H_2O_2 treatment significantly increased the expression of caspase-3. Compared with the H_2O_2-treated model group, tempol and Rg1 treatments significantly decreased the expression of caspase-3 in H_2O_2-treated hippocampal neurons (Fig. 2A and B; P<0.05).

Rg1 decreases the expression of senescence-associated β-gal in H_2O_2-treated hippocampal neurons. Senescent cells exhibit increased senescence-associated β-Gal activity (26). In order to observe the effect of Rg1 on H_2O_2-induced senescence in hippocampal neurons, the expression of β-Gal was detected by western blot analysis. The results showed that H_2O_2 (200 μM) treatment significantly increased the expression of β-Gal in the hippocampal neurons compared with the control group (Fig. 3A and B; P<0.01). Compared with the H_2O_2-treated group, tempol (100 μM) and Rg1 (5 and 10 μM) treatments significantly decreased the expression of β-Gal in the hippocampal neurons (Fig. 3A and B; P<0.05 and P<0.01, respectively).

Rg1 reduces ROS generation in H_2O_2-treated hippocampal neurons. H_2O_2 exposure induces cell redox balance disorder, which may cause neuronal senescence and damage. Therefore, ROS production was detected using DHE staining. Compared with the control group, H_2O_2 treatment significantly increased the production of ROS in the hippocampal neurons (Fig. 4A and B; P<0.01). Compared with the H_2O_2-treated group, tempol (100 μM) and ginsenoside Rg1 (1, 5 and 10 μM) treatments significantly reduced the production of ROS in hippocampal neurons (Fig. 4A and B; P<0.05).

Effects of Rg1 on the expression of NOX2, p22phox and p47phox in H_2O_2-treated hippocampal neurons. To confirm whether NADPH oxidase is involved in H_2O_2-induced neuronal senescence and damage in primary hippocampal neurons, the expression levels of NOX2, p22phox and p47phox in hippocampal neurons were measured by immunoblotting. The results showed that, compared with the control group, the expression levels of NOX2, p22phox and p47phox were significantly increased in the H_2O_2-treated group (Fig. 5A-C; P<0.05). Compared with the model group, Rg1 (5 and 10 μM) treatment significantly reduced the expression levels of NOX2 and p47phox in the H_2O_2-treated hippocampal neurons, whereas tempol (100 μM) treatment had no significant influence on their expression. The results showed that Rg1 had no significant influence on the expression of p22phox, whereas tempol treatment increased the expression of p22phox in the H_2O_2-treated hippocampal neurons (Fig. 5B; P<0.05).

Effect of Rg1 on the expression levels of NLRP-1, ASC and caspase-1 in H_2O_2-treated hippocampal neurons. To confirm whether Rg1 regulated NLRP1 inflammasome activation, the expression levels of NLRP1, ASC and caspase-1 in primary

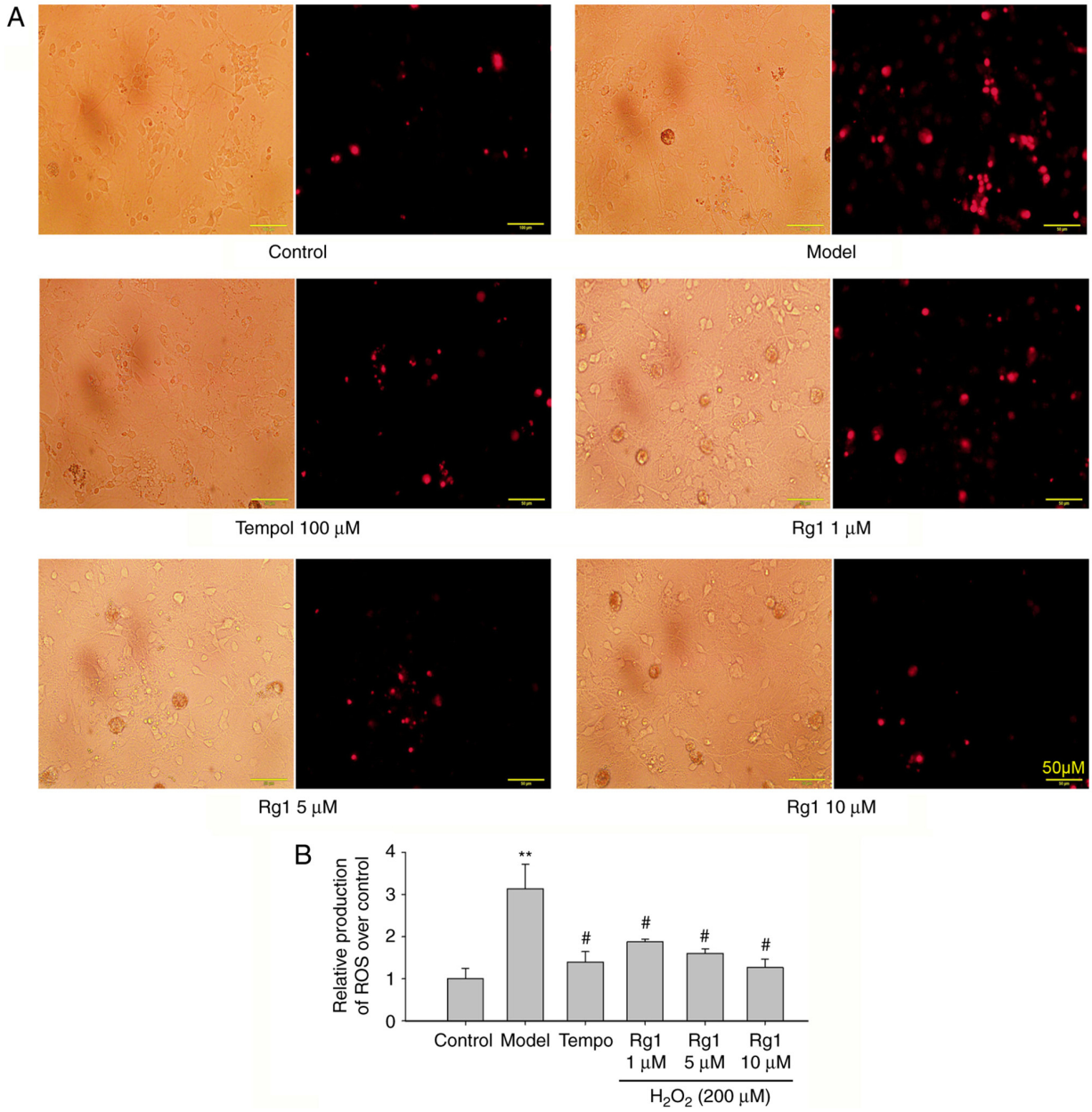

Figure 4. Effect of Rg1 treatment on ROS production in H_2O_2-treated hippocampal neurons (dihydroethidium staining). (A) ROS production was detected by fluorescence microscopy in hippocampal neurons (magnification, x400). Left-panel images are bright-field microscopy, representing cell morphology. Right-panel images are fluorescent microscopy, indicating the formation of ROS. (B) Relative production of ROS over the control group. Results are expressed as the mean ± standard deviation, n=3. **P<0.01 compared with the control group; $^{\#}$P<0.05 compared with the H_2O_2-treated model group. Rg1, ginsenoside Rg1; ROS, reactive oxygen species.

hippocampal neurons were detected by immunoblotting. The results showed that, compared with the control group, the expression levels of NLRP-1, ASC and caspase-1 were significantly increased in the H_2O_2-treated group. Compared with the model group, tempol (100 μM) and Rg1 (5 and 10 μM) treatments significantly decreased the expression levels of NLRP1, ASC and caspase-1 in the H_2O_2-treated hippocampal neurons (Fig. 6A-C; P<0.05).

Rg1 decreases the release of IL-1β and IL-18 in H_2O_2-treated hippocampal neurons. To confirm the effect of Rg1 on downregulation of the NLRP1 inflammasome, the levels of IL-1β and IL-18 released in supernatants were examined by ELISA. The results showed that, compared with the control group, the release of IL-1β and IL-18 was significantly increased in the H_2O_2-treated group (Fig. 7A and B; P<0.05). Compared with the model group, tempol (100 μM) and Rg1 (5 and 10 μM) treatments significantly reduced the release of IL-1β and IL-18 in supernatants from the H_2O_2-treated hippocampal neurons (Fig. 7A and B; P<0.05).

Discussion

It has been reported that oxidative stress and neuroinflammation are involved in neuronal senescence and neurodegenerative diseases, including Alzheimer's disease (27). The effect of Rg1 is largely associated with its antioxidant and other scavenging properties, for example, inhibiting the expression of inducible

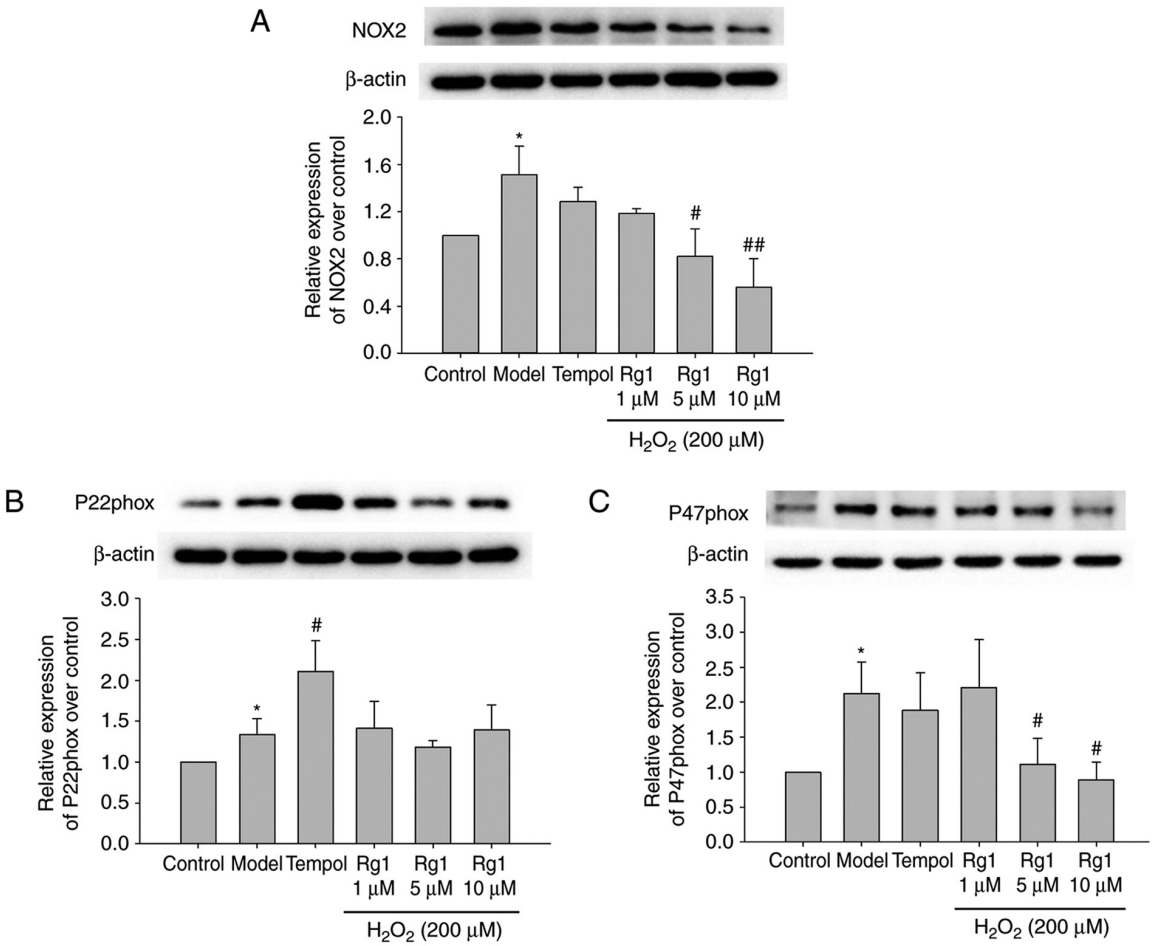

Figure 5. Effect of Rg1 treatment on the expression of NOX2, p22phox, and p47phox in H_2O_2-treated hippocampal neurons (immunoblot). (A) Relative expression of NOX2 over the control. (B) Relative expression of p22phox over the control. (C) Relative expression of p47phox over the control. Results are expressed as the mean ± standard deviation, n=3, *P<0.05 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the model group. Rg1, ginsenoside Rg1; NOX2, NADPH oxidase 2.

nitric oxide synthase and the overgeneration of nitric oxide. However, whether Rg1 can alleviate H_2O_2-induced senescence and neuronal damage, and its mechanism, warrants further investigation. In the present study, it was demonstrated that H_2O_2 treatment significantly induced oxidative stress damage and apoptosis in primary hippocampal neurons, and the NOX2-NLRP1 inflammasome pathway was important in H_2O_2-induced neuronal senescence and damage. In addition, Rg1 significantly reduced the production of ROS and the expression of NOX2 and the NLRP1 inflammasome, and inhibited neuronal senescence and damage in the H_2O_2-treated hippocampal neurons.

Oxidative stress is caused by the imbalance of redox in the body, resulting in excessive ROS generation. The accumulation of ROS can induce neuronal oxidative stress damage and is involved in the development of ageing-related neurodegenerative diseases (28). In the process of ageing, excessive ROS can lead to destruction of neuronal structure and impairment of learning and memory (29,30). H_2O_2 treatment is often used to simulate ROS accumulation-induced neuronal damage (31). In the present study, the results showed that H_2O_2 treatment significantly increased the generation of ROS, promoted neuronal apoptosis and increased the expression of senescence-associated β-Gal in hippocampal neurons.

Ginseng has been used as an anti-ageing drug for thousands of years in China. Numerous studies have shown that Rg1, a main active ingredient in ginseng, has antioxidant and anti-ageing effects, in addition to promoting cognitive function and improving immunity (32,33). Rg1 protects against neuronal damage induced by various mechanisms, including oxidative stress and inflammatory responses (20,34). Tempol is an effective antioxidant and can attenuate the damage caused by excessive accumulation of ROS (35). In the present study, the results showed that both Rg1 and tempol significantly decreased ROS generation and inhibited the expression of β-Gal in H_2O_2-treated hippocampal neurons. In addition, the Hoechst 33258 staining showed that Rg1 (5 and 10 μM) had a significant effect on H_2O_2-induced hippocampal neuron apoptosis, whereas Rg1 (1 μM) had no effect on the neuronal apoptosis. Caspase-3 is the most important terminal shear enzyme in the process of apoptosis (33). To confirm the anti-apoptotic effect of Rg1, the effects of Rg1 (5 μM) on the expression of caspase-3 were examined by immunofluorescence. The results showed that Rg1 (5 μM) significantly decreased the expression of caspase-3 in H_2O_2-induced hippocampal neurons. These results indicate that Rg1 may decrease ROS generation and inhibit neuronal senescence and apoptosis. However, the

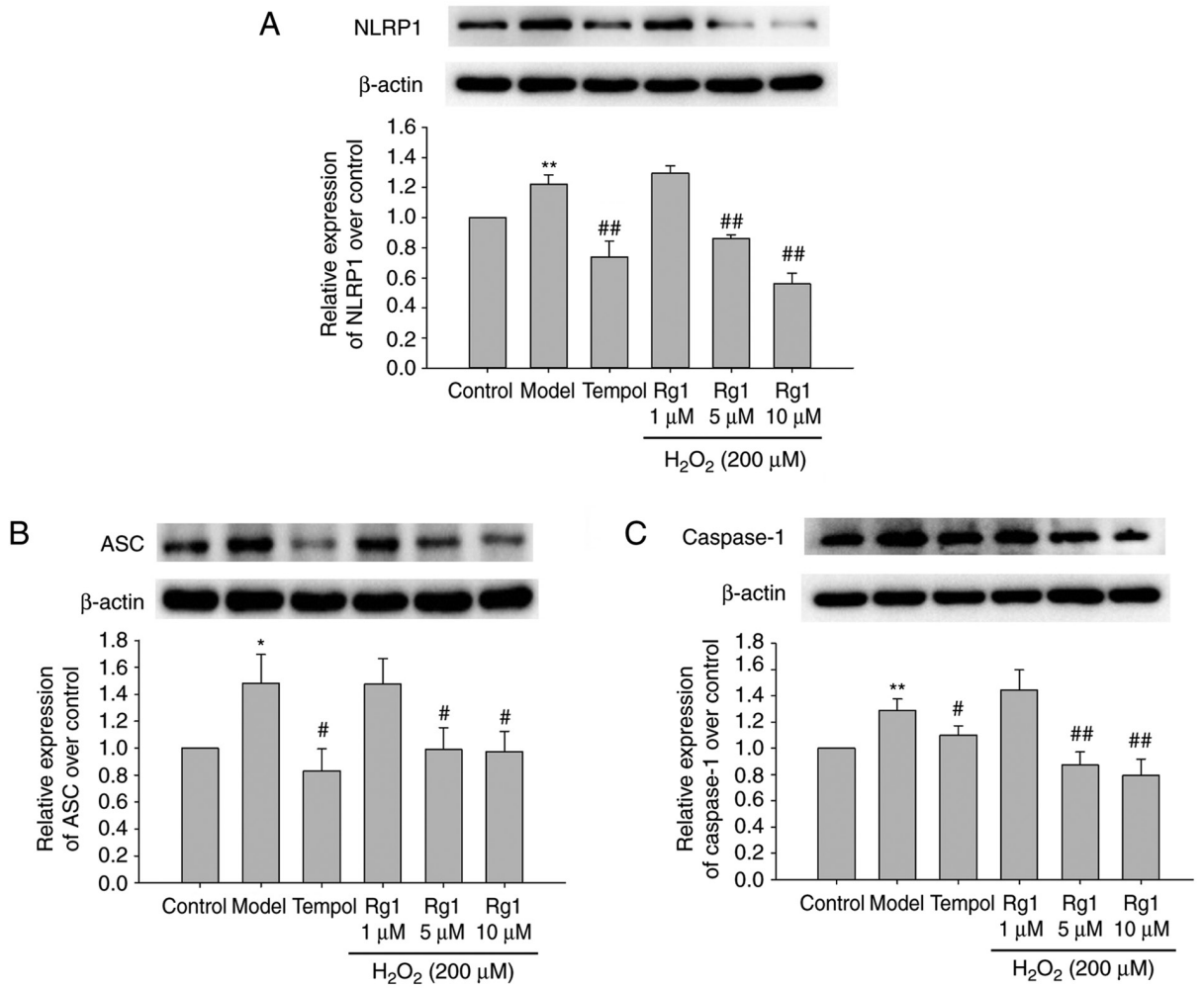

Figure 6. Effect of Rg1 treatment on the expression of NLRP-1, ASC and caspase-1 in H_2O_2-treated hippocampal neurons (immunoblot). (A) Relative expression of NLRP-1 over the control. (B) Relative expression of ASC over the control. (C) Relative expression of caspase-1 over the control. Results are expressed as the mean ± standard deviation, n=3, *P<0.05 and **P<0.01 compared with the control group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 compared with the model group. Rg1, ginsenoside Rg1; NLRP-1, nucleotide-binding oligomerisation domain-like receptor protein 1; ASC, apoptosis-associated speck-like protein containing a carboxy-terminal CARD.

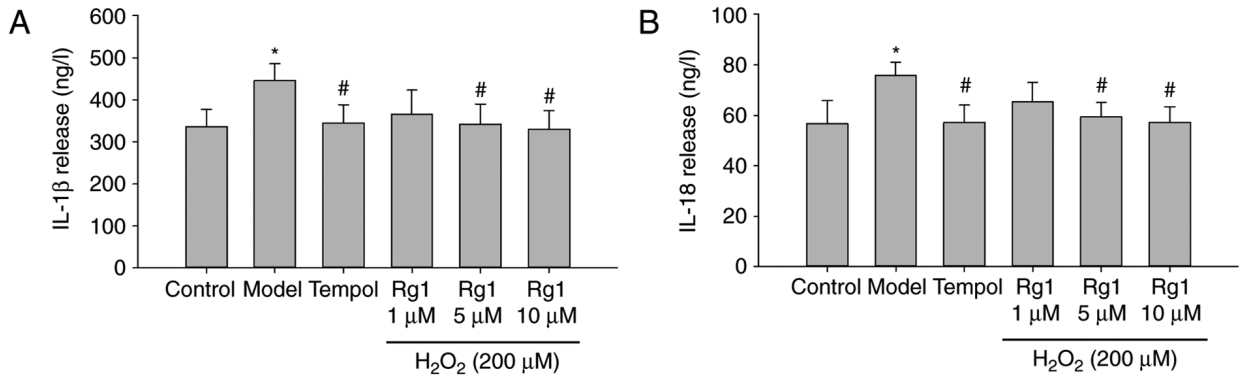

Figure 7. Effect of Rg1 on the levels of IL-1β and IL-18 released into supernatants in H_2O_2-treated hippocampal neurons (enzyme-linked immunosorbent assay). (A) Release of IL-1β into supernatants. (B) Release of IL-18 into supernatants. Results are expressed as the mean ± standard deviation, n=3, *P<0.05 compared with the control group; $^{\#}$P<0.05 compared with the model group. Rg1, ginsenoside Rg1; IL, interleukin.

mechanism underlying the effect of Rg1 on reducing the production of ROS remains to be fully elucidated.

NOX is the most important enzyme system involved in the generation of ROS and has been shown to be important in various neurological diseases (28). NOX2 is a major source of ROS involved in the development of age-related cognitive dysfunction (28). The expression of NOX2 has been found to be increased in the brains of ageing mice (36). NOX2 consists

of a membrane-bound catalytic core, including NOX2 (gp91phox) and p22phox), and several cytosolic subunits, including p40phox, p47phox, p67phox and rac1 (37). NOX2 is activated when the cytosolic subunits translocate to the membrane and combine with gp91phox (37,38). There is a linear relationship between the activity of NOX2 and cognitive decline in aged mice (39). In addition, NOX contributes to oxidative stress and neuronal apoptosis. NOX-deficient mice exhibit reduced injury following stroke (40). It is not entirely clear whether Rg1 can reduce NOX2 and, thus, reduce the production of ROS in H_2O_2-treated hippocampal neurons. In the present study, the results showed that the expression levels of NOX2, p22phox and p47phox were significantly increased in H_2O_2-treated hippocampal neurons. Rg1 treatment significantly reduced the expression levels of NOX2 and p47phox in H_2O_2-treated hippocampal neurons, whereas tempol (100 μM) treatment had no significant influence on their expression. The results also showed that Rg1 had no significant influence on the expression of p22phox, whereas tempol treatment increased the expression of p22phox in H_2O_2-treated hippocampal neurons. Therefore, Rg1 may protect against neuronal ageing and damage by scavenging ROS and inhibiting the formation of ROS derived from NOX2.

Neuroinflammation can accelerate brain ageing, causing neuronal damage and cognitive deficits (41). The chronic, progressive pro-inflammatory response is an important feature of the ageing process and the leading cause of neuronal apoptosis (42). Inflammasomes are multimeric proteins in the cytoplasm of a wide variety of cells (43). Among them, the NLRP1 inflammasome was the first characterised and is expressed widely in the body, particularly in neurons (44,45). The NLRP1 inflammasome consists mainly of NLRP1, caspase-1 and ASC proteins. NLRP1 can activate the key regulatory agent caspase-1 through its own oligomerization. Activated caspase-1 can further cleave pro-IL-18 and pro-IL-1β into active IL-18 and IL-1β, which in turn promote inflammatory responses and apoptosis (46). It has been reported that the NLRP3 inflammasome can be activated by excessive ROS production (47). Our previous study showed that Rg1 protected against chronic dexamethasone-induced neuronal degeneration by inhibiting NLRP-1 inflammasomes in mice (19). It has also been reported that Rg1 can protect against H_2O_2-induced neuronal apoptosis (33). These studies suggest that Rg1 has an anti-oxidative effect and can inhibit the NLRP1 inflammasome in hippocampal neurons. However, whether Rg1 can downregulate the NOX2-mediated generation of ROS and thereby inhibit the NLRP1 inflammasome in H_2O_2-treated hippocampal neurons remained to be elucidated. The present study found that H_2O_2 treatment significantly increased the expression levels of NLRP-1, ASC and caspase-1 in hippocampal neurons and the levels of IL-1β and IL-18 released into the supernatant. These results suggest that the accumulation of ROS can activate the NLRP1 inflammasome, which may be involved in senescence and damage in hippocampal neurons. The results also showed that tempol and Rg1 treatments significantly decreased the expression levels of NLRP1, ASC and caspase-1, and reduced the levels of IL-1β and IL-18 released into the supernatant. A previous study showed that Rg1 attenuated H_2O_2-induced neuronal oxidative stress and apoptosis via the downregulation of caspase-3, Rho-associated kinase1 activation and myosin light chain (Ser-19) phosphorylation (33), whereas the present study suggested that Rg1 alleviated H_2O_2-induced neuronal oxidative stress and apoptosis via the inhibition of NOX2-derived ROS generation and downregulation of the NLRP1 inflammasome.

Overall, the present study demonstrated that Rg1 has antioxidant effects and can reduce the expression of NOX2 and the production of ROS, which in turn inhibits the activation of the NLRP1 inflammasome in H_2O_2-treated hippocampal neurons. These findings provide support for the hypothesis that Rg1 reduces NOX2-mediated ROS generation and inhibits NLRP1 inflammasome activation. However, whether the overexpression of NOX2 can activate the NLRP1 inflammasome and accelerate neuronal senescence remains to be fully elucidated. Other associated mechanisms of Rg1 on neuronal senescence associated with the NOX2-NLRP1 inflammasome signalling pathway warrant further investigation.

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Availability of data and materials

The datasets used and analysed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

TZX analysed data, performed the experiments, and was a major contributor in writing the manuscript. XYS, LLS and YLC collated the data, BQZ contributed to the immunoblot analysis and interpretation of the results. DKH was mainly responsible for the immunofluorescence detection. WZL designed the study, critically revised the manuscript for intellectually important content, supervised the study and wrote the manuscript. All authors read and approved the final submitted manuscript.

Ethics approval and consent to participate

All experiments involving animals were approved by the Ethics Committee of Laboratory Animals of Anhui Medical University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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