Neuroprotective effects of galectin-1 on cerebral ischemia/reperfusion injury by regulating oxidative stress

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Abstract. Oxidative stress contributes to the pathology of cerebral ischemia/reperfusion (I/R) injury. Galectin-1 has shown an anti-oxidative stress effect. The present study investigated whether this anti-oxidative stress effect can account for the neuroprotective actions of galectin-1 induced by cerebral I/R injury. A cerebral I/R injury model was created in C57Bl/6 mice by transient occlusion of the middle cerebral artery, after which the mice were treated with galectin-1 for 3 days. Infarct volumes were measured. A rotarod test and neurological deficit score assessment was performed to evaluate the neurological deficits. Oxidative stress was evaluated by measuring the levels of reactive oxygen species (ROS) and lipid peroxidation malondialdehyde (MDA), while the anti-oxidative stress status was assessed by measuring molecules such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidation enzyme (GSH-Px) in the ischemic cerebral hemisphere of mice. The inflammatory cytokines, including Interleukin 1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α) were measured, and the expression of microglia was evaluated by immunohistochemistry in the ischemic cerebral hemisphere of mice. Galectin-1 treatment ameliorated neurological deficits and reduced infarct volumes in the mice model with cerebral I/R injury. Moreover, it was demonstrated that galectin-1 can significantly alleviate cerebral I/R injury in the ischemic cerebral hemisphere by decreasing the production of ROS and MDA, but increasing the production of CAT, SOD and GSH-Px. Galectin-1 treatment decreased microglia expression, and IL-1, IL-6 and TNF-α levels in the ischemic cerebral hemisphere of mice. Galectin-1 could improve the outcome of cerebral I/R injury by alleviating oxidative stress. Moreover, the neuroprotective effect of galectin-1 in cerebral ischemia could be related to its anti-oxidative stress effect.

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Introduction

Stroke is the second leading cause of death and a significant cause of disability globally. In 2019, the worldwide prevalence of stroke was 101.5 million individuals, with ischemic stroke accounting for 77.2 million of these cases (1). Severe and/or prolonged reductions in cerebral blood flow result in a lack of oxygen and energy supply to brain tissues, as well as an accumulation of potentially harmful substances. The substantial damage to brain tissues can be attributed to energy failure, loss of cellular homeostasis, acidosis, increased intracellular calcium, excitotoxicity and toxicity mediated by free radicals.

Currently, the primary therapeutic strategy against cerebral ischemia is thrombolysis, which involves dissolving blood clots to restore blood flow before damage occurs. However, thrombolysis often leads to reperfusion of the infarcted brain tissue, thereby causing reperfusion injury (2). Furthermore, numerous studies have established a time window of 4.5 h for systemic thrombolysis and early recanalization and reperfusion in ischemic stroke (3). Beyond this window, the risk of ischemia/reperfusion (I/R) injury significantly escalates and could lead to catastrophic outcomes, such as fatal edema. Indeed, oxidative stress is a crucial mechanism involved in the pathogenesis and disease progression of both cerebral ischemia and I/R injury. In the pathology of cerebral ischemia and I/R injury, oxygen and energy deprivation, along with post-translational modification of oxidative phosphorylation proteins, could elevate the mitochondrial membrane potential. This, in turn, results in the excessive generation of reactive oxygen species (ROS) (4). In a clinical study involving stroke patients, Domínguez *et al* (5) found that oxidative stress markers, including malondialdehyde (MDA) and myeloperoxidase, increased when a stroke occurred. In rat brains with cerebral I/R, an enhanced ROS production that overwhelmed antioxidant capacity was detected (6). ROS directly damages all cellular components, including proteins, DNA, RNA and lipids, subsequently inducing apoptosis of neuronal cells (7). Therefore, anti-oxidative stress during cerebral ischemia may be a strategy to rescue the neurons of the penumbra and ensure their survival. In previous studies, anti-oxidative

stress therapy was shown to improve the prognosis of cerebral ischemia (8-10).

Galectin-1 is a carbohydrate-binding protein that belongs to the galectin family. Galectin-1 (Uniprot ID: P09382) is a small protein consisting of ~135 amino acids. The expression of galectin-1 is widespread across different tissues and cell types, including immune cells, endothelial cells, epithelial cells and neurons. It contains a conserved carbohydrate recognition domain that allows it to bind specifically to beta-galactoside-containing glycoconjugates (11). It plays a crucial role in a wide range of biological processes, including cell adhesion, immune modulation, angiogenesis and tumor progression. Galectin-1 has also been implicated in neural development, tissue remodeling and various disease states. Galectin-1 influences neural development, axon guidance, and synapse formation, making it relevant in the context of neuro-regeneration and repair. Therefore, galectin-1 may contribute to the survival of neurons in the pathology of cerebral ischemia. In the present study, the anti-oxidative stress effect of galectin-1 was investigated, and it was identified that galectin-1 decreased the ischemic area by an antioxidant action in a cerebral I/R injury mouse model.

Materials and methods

Animals and model of middle cerebral artery occlusion-reperfusion (MCAO/R). All the procedures were strictly in line with the regulations of the National Institutes for Animal Research. All the animal-related experiments were approved (approval. no. 2022023) by the Animal Care and Management Committee of Beijing Geriatric Hospital (Beijing, China). A total of 90 male C57BL/6 mice (25-30 g, 10 weeks-old) were obtained from the Beijing Vital River Laboratory Animal Technology Co., Ltd. The experimental animals were housed in conditions with 12/12-h light/dark cycles, a temperature of 22±3˚C and 60±5% humidity. All the mice had access to a standard rodent diet and tap water.

A MCAO/R mouse model was created as previously described (12,13). The mice were anesthetized with isoflurane (induction dosage 3%; maintenance dosage 1.5%). Through a midline incision on the ventral side of the neck, the common carotid arteries were exposed, the external carotid artery (ECA) and the internal carotid artery (ICA) were carefully isolated. Subsequently, a 6-0 silicone suture was introduced through the ECA and advanced into the ICA to occlude the origin of the middle cerebral artery, ~10-11 mm from the common carotid artery bifurcation. A laser Doppler flow probe was used to confirm the development of MCAO. After 60 min of ischemia, the suture was withdrawn to allow reperfusion. Concurrently, a sham operation was conducted using the same procedure, except for MCA occlusion. After 72 h of the experiment, the mice were euthanized via CO_2 inhalation (50% of the chamber volume per minute), and their brain tissues were collected.

The protocol of the present study, which involves treating with galectin-1 for 3 days, was developed based on previous studies and experimental data of the present study. According to previous data by the authors on zebrafish (14), a 3-day treatment with galectin-1 significantly reduced the generation of oxidative stress *in vivo*. Additionally, the study by Vallecillo-Zúniga *et al* (15) observed the anti-ROS effect of galectin-1 in myogenic cells after 2-3 days of treatment (15). Cheng *et al* (16) found that a 3-day treatment with galectin-1 could promote vascular remodeling, while Arda-Pirincci and Aykol-Celik (17) demonstrated that galectin-1 can reduce the severity of dextran sulfate sodium-induced ulcerative colitis after at least 3 days of treatment. The preliminary data of the present study demonstrated the antioxidative stress effects of galectin-1 in cerebral I/R. Therefore, in the present study, a treatment duration of 3 days was selected.

The dose and administration route for the present study were selected based on previous studies and the preliminary experimental data of the present study. In addition, in the study by Carlos *et al* (18), rats were treated with recombinant human galectin-1 at a dose of 100 μg per animal, administered intravenously. Similarly, the study by Ye *et al* (19) involved injecting mice with recombinant human galectin-1 at doses ranging from 250-500 μg per animal, administered intraperitoneally. The preliminary data of the present study indicated that an injection of 500 μg of galectin-1 significantly reduced ROS levels in the mouse brain.

All the male C57BL/6 mice were randomly allocated to three groups (30/group): i) galectin-1 group, the mice received the MCAO/R operation and were treated with recombinant human galectin-1 (500 μg/mouse) (18,19) intraperitoneally starting 1 h after the operation, and this treatment continued for 3 days; ii) MCAO/R group, the mice received the MCAO/R operation and were treated with saline (same amount as galectin-1 intraperitoneally from 1 h after operation, and this treatment continued for 3 days; and iii) sham surgery group, the mice underwent the sham surgery and received saline (same amount as galectin-1) intraperitoneally from 1 h after operation, and this treatment continued for 3 days.

After the brain tissues were collected, 10 mice brain tissues from each group were subjected to examine cerebral infarct volume and microglial cell infiltration, 10 were used for immunohistochemistry (IHC) analysis of allograft inflammatory factor 1 (IBA-1) expression, and the brain tissues of the remaining 10 mice brain tissues from each group were homogenized and stored for further tests (ROS and cytokines levels). Galectin-1 (cat. no. P00388) was purchased from Solarbio Science & Technology Co., Ltd. and dissolved in saline for the experiments based on the previous published study (16).

Cerebral infarct volume measurement. After the mice were euthanized, the brain tissues were collected and then sliced to a thickness of 2 mm. The slices were stained with 2, 3, 5-triphenyl tetrazolium chloride (2%) for 20 min at 37˚C. Thereafter, the slices were fixed using 4% paraformaldehyde overnight at room temperature. Images of all the stained slices were captured and quantified using ImageJ software (version 1.54; National Institutes of Health). Infarct volumes were calculated using the following formula: Percentage hemisphere lesion volume (% HLV)=[total infarct volume-(volume of intact ipsilateral hemisphere-volume of intact contralateral hemisphere)]/contralateral hemisphere volume x100%.

Rotarod test. The neurological deficits of the mice were evaluated using the rotarod test. On 4 consecutive days (before surgery, and 24, 48 and 72 h after surgery), the mice were placed on rotating rods and practiced three times a day.

The rods accelerated in speed from 4 to 40 rpm over a 5-min period. The results were expressed as the latency to fall from the rod.

Neurological deficit scores. A neurological test was performed to investigate neurological deficit after 72 h of surgery. The degree of neurological impairment was evaluated based on the following features: i) absence of deficits; ii) body bending towards the opposite side; iii) involuntary contralateral circling; iv) tendency to fall to the opposite side; and v) lack of spontaneous movement or a state of unconsciousness (20).

In the present study, the data for the neurological deficit score were presented, exclusively at the 72-h mark, following the precedent set by several other studies in the field (21-25). These studies, focusing on cerebral ischemia in mouse models, typically report neurological deficit scores only at the experiment's endpoint, which is often at 72 h. Consequently, the reporting methodology of the present study was aligned with this standard practice.

ROS level measurement. ROS generation was estimated using the oxidation-sensitive 2',7'-dichlorodihydrofluorescein diacetate (CM-H2DCFDA) dye (cat. no. KGAF018, Nanjing Jiancheng Bioengineering Institute). CM-H2DCFDA becomes fluorescent when oxidized by ROS. In brief, the cerebral cortex tissues of the ischemic hemisphere were collected and gently homogenized in 500 μl of PBS. The concentration of proteins was determined by the BCA method. Thereafter, the brain homogenate was incubated with 25 μl CM-H2DCFDA (10 mM) for 30 min at 37˚C in dark conditions. Subsequently, 500 μl of each sample was loaded into the wells of a 96-well plate. The fluorescence intensities of ROS were measured using a fluorescent microplate reader (excitation: 485 nm, emission: 525 nm). The activity is expressed as U/mg protein (26).

Enzyme-linked immunosorbent assay (ELISA). The hemispheres containing the ischemic zones of mice were collected, and then gently homogenized. Tissue homogenates were diluted in the buffer provided by each kit at a concentration of 10 mg tissue/ml. The quantities of cytokines, including interleukin (IL)-6, IL-1 and tumor necrosis factor alpha (TNF-α), and the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidation enzyme (GSH-Px), were measured using ELISA kits (IL-1, cat. no. PI301; IL-6, cat. no. PI326; TNF-α, cat. no. PT513; SOD, cat. no. S0101S; CAT, cat. no. S0051; GSH-Px, cat. no. S0057S) from Beyotime Institute of Biotechnology. Briefly, 96-well plates were first coated with primary antibodies (provided within the kits; ready-to-use) and were filled with 100 μl of either the samples or standards. 100 μl of biotinylated detection antibodies (provided within the kits; ready-to-use) were then added to these wells and left to incubate at room temperature for 1 h. Following this, the wells were emptied, rinsed with PBS, and then filled with 100 μl of the streptavidin complex reagent, for another h of room temperature incubation. A total of 90 μl of 3,3',5,5'-tetramethylbenzidine substrate solution was next added to the wells and left to incubate for 20 min at room temperature. Then 50 μl of stop solution was added to the wells. Absorbance was evaluated using a multimode plate reader at 450 nm. Standard curves were produced using the optical density (OD) values of standard reagents, and these were used to ascertain the cytokine levels. The activity of SOD, CAT and GSH-Px were expressed as U/mg protein.

Assay of MDA levels. The lipid peroxidation product MDA is used as an indicator of oxidation. The tissue homogenate was analyzed using an MDA kit following the manufacturers' instructions via the thiobarbituric acid reaction (cat. no. A003-4-1; Nanjing Jiancheng Bioengineering Institute). The samples were measured by using a microplate reader at 532 nm and the values were expressed as nmol/mg of tissue protein.

IHC. Brain tissues were collected from euthanized mice. These tissues were first fixed with 10% formalin at room temperature for 24 h, then embedded in paraffin, sliced into 5-μm-thick sections, and arranged on glass slides. The tissue sections were subsequently deparaffinized with xylene at 55˚C and rehydrated in a descending alcohol series (ethanol; 100, 95, 75 and 50%; 3 min each). Thereafter, antigen retrieval was performed by boiling in citrate-EDTA buffer, followed by a 20-min cooling period at room temperature. After a blocking step with 5% goat serum (cat. no. C0265; Beyotime Institute of Biotechnology) at room temperature for 1 h, the sections were incubated with primary antibodies diluted against IBA-1 rabbit monoclonal (1:200; cat. no. MA5-36257; Thermo Fisher Scientific, Inc.) and left overnight at 4˚C. Following this, the slides were rinsed with TBS with 0.1% Tween-20, and treated with a horseradish peroxidase-labelled dextran polymer bound with an anti-rabbit antibody (1:1,000; cat. no. A0279; Beyotime Institute of Biotechnology) for 1 h at room temperature. Subsequently, the slides were incubated with DAB solution and inspected under a light microscope (Olympus Corporation). The expression of IBA-1 was assessed under a light microscope at a x40 magnification. The expression level was quantified using a standard IHC staining grade system by measuring positive expression in 5 random fields within the brain tissues, received from three separate non-adjacent sections per mouse.

In addition, a preliminary experiment was conducted to validate the protocol. This included optimizing blocking reagents and antigen retrieval, as well as determining the appropriate concentrations for primary and secondary antibodies. Additionally, the DAB staining step was carefully monitored under a light microscope to prevent overreaction. Furthermore, both positive and negative controls were employed in the staining process. The positive control tissue exhibited strong and specific staining for the target antigen, while no positive staining was observed in the negative control slides.

Statistical analysis. All the data were analyzed in SPSS 26 (IBM Corp.) for statistical computing and graphics. The quantitative data were expressed as the mean ± standard deviation (SD). Statistical comparisons were performed by one-way ANOVA followed by the Tukey's post hoc test or unpaired Student's t-test as described in figure legends. For data that were not normally distributed in the analyses of neurological scores, The Mann-Whitney U test was used for comparisons between 2 groups. Two-way mixed ANOVA followed by the Bonferroni's post hoc test was used to compare the difference

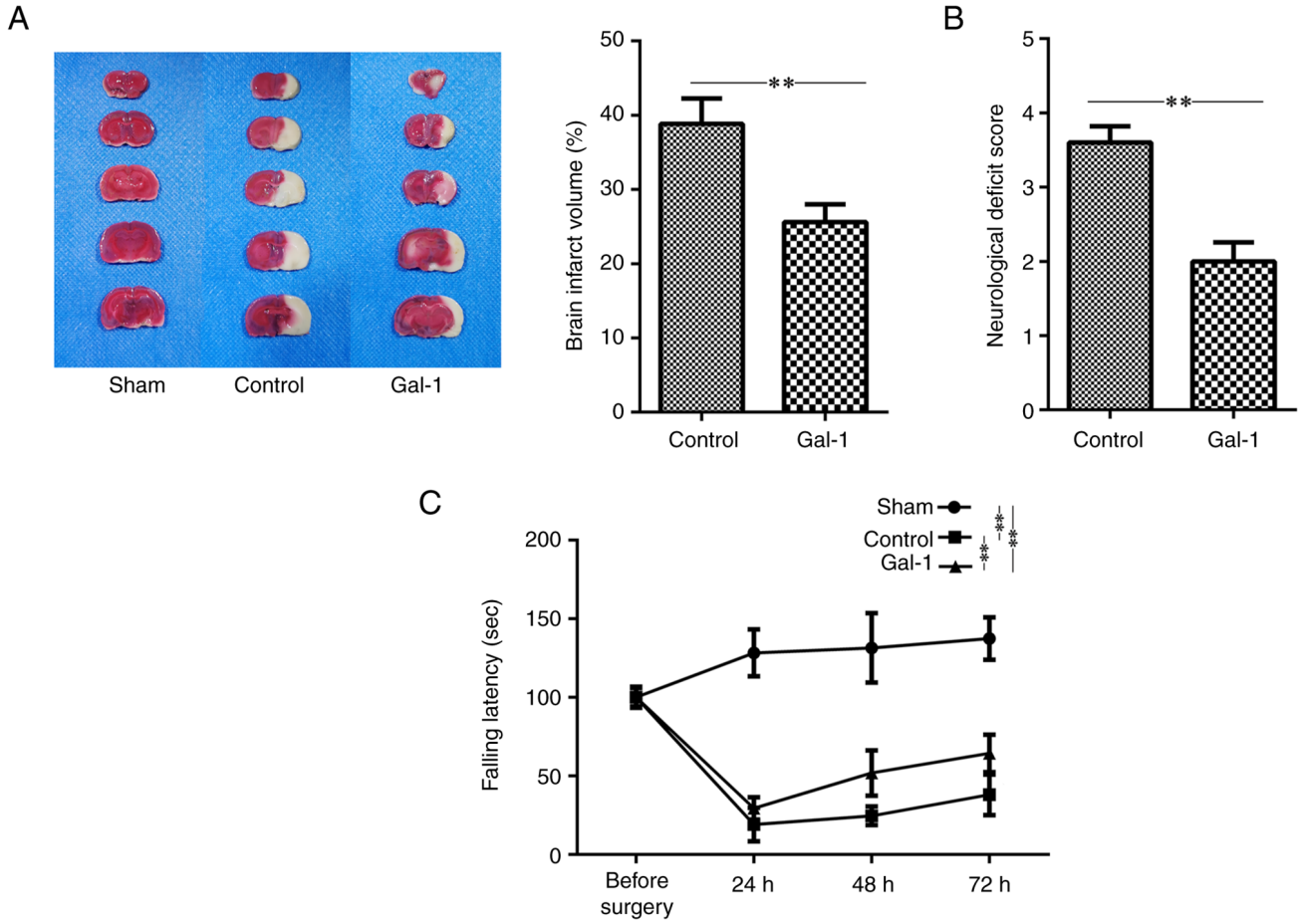

Figure 1. Galectin-1 ameliorates neurological deficits and reduces infarct volumes in MCAO/R mice. The mice received MCAO/R surgery, MCAO/R surgery + galectin-1 treatment, or sham surgery (n=10 per group). (A) Representative figures of brain sections and statistical results. The data revealed that the infarct volumes were significantly reduced in the MCAO/R + galectin-1 group compared with the non-galectin-1 MCAO/R group. (B and C) Neurological deficit scores were assessed at 72 h after surgery in MCAO/R mice, while rotarod tests were performed on 4 consecutive days (before surgery, and 24, 48 and 72 h after surgery). Galectin-1 treatment improved motor ability. The tests showed that mice which received galectin-1 treatment exhibited improved motor function, with (B) lower neurological deficit scores and (C) longer duration on the rotarod. Analyses were performed to compare the differences in the latency to fall from the rod among various groups at 24, 48 and 72 h after surgery. These groups included those undergoing MCAO/R surgery, MCAO/R surgery with galectin-1 treatment, and sham surgery. Compared with the sham surgery group at 24, 48 and 72 h after surgery, respectively, the latencies to fall from the rod were significantly decreased in both the MCAO/R mice with and without Gal-1 treatment. However, the latency to fall from the rod in the galectin-1 + MCAO/R group was significantly higher than that in the non-galectin-1 MCAO/R group at 24, 48 and 72 h after surgery, respectively (Sham vs. control at 24, 48 and 72 h after surgery, $P<0.01$; Sham vs. Gal-1 at 24, 48 and 72 h after surgery, $P<0.01$; Gal-1 vs. control at 24, 48 and 72 h after surgery, $P<0.01$). The data are presented as the mean ± SD. $^{**}P<0.01$ by Student's t-test or two-way mixed ANOVA followed by Bonferroni's post hoc test. MCAO/R, middle cerebral artery occlusion-reperfusion; Gal-1, galectin-1 treatment.

in rotarod test among different groups at 24, 48 and 72 h after surgery. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Galectin-1 ameliorates the neurological deficit and infarct size in MCAO/R mice. In the present study, the results revealed that cerebral ischemia developed in mice after MCAO/R surgery. The neurological outcome was investigated after 3 days of galectin-1 treatment in MCAO/R mice. Compared with the control group that underwent sham surgery, both the neurological deficit and the cerebral infarct area were observed in the MCAO/R mice group (Fig. 1A-D). Moreover, the results demonstrated that treatment with galectin-1 significantly improved the outcome of cerebral ischemia, as evidenced by longer latency on the rotarod, lower neurological deficit scores, and smaller infarct volumes. Data on the neurological deficit score for mice that underwent sham surgery were not included, as no deficits were detected in these mice.

Galectin-1 reduces ROS production and lipid peroxidation. ROS and MDA are both biomarkers for the status of oxidative stress. The data of the present study identified an increased level of both ROS (Fig. 2A) and MDA (Fig. 2B) in the ischemic hemisphere of MCAO/R mice, compared with that in the sham surgery. In addition, treatment with galectin-1 significantly decreased the levels of ROS and MDA in the ischemic hemisphere of MCAO/R mice, compared with that in MCAO/R mice without galectin-1 treatment.

Galectin-1 treatment improves antioxidant defenses. The data of the present study revealed a significantly decreased level of antioxidant defense molecules, including SOD, CAT

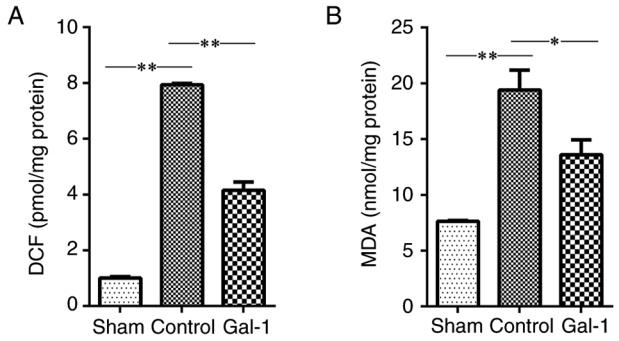

Figure 2. Galectin-1 attenuates oxidative stress levels in the ischemic hemisphere of MCAO/R mice. The mice received MCAO/R surgery, MCAO/R surgery + galectin-1 treatment, or sham surgery (n=10 per group). Subsequently, the levels of ROS and MDA in the ischemic hemisphere were measured. Compared with the sham surgery group, (A) ROS and (B) MDA production was significantly increased in the ischemic hemisphere of MCAO/R mice. However, galectin-1 treatment inhibited oxidative stress by decreasing the production of both ROS and MDA. Both ROS and MDA levels in the galectin-1 + MCAO/R group were significantly lower than those in the non-galectin-1 MCAO/R group. The data are presented as the mean ± SD. *P<0.05 and **P<0.01 by one-way ANOVA followed by Tukey's post hoc test. MCAO/R, middle cerebral artery occlusion-reperfusion; ROS, reactive oxygen species; MDA, malondialdehyde; Gal-1, galectin-1 treatment.

and GSH-Px, in the ischemic hemisphere of MCAO/R mice, compared with that in the sham surgery. However, compared with the non-galectin-1 treatment group, treatment with galectin-1 significantly increased the levels of SOD, CAT and GSH-Px in the ischemic hemisphere of MCAO/R mice (Fig. 3).

Administration of galectin-1 decreases microglial activation and inflammatory level in the brain of MCAO/R mice. IHC staining was performed to evaluate the activation of microglial cells in the ischemic hemisphere of mouse brain cortex and hippocampus. Galectin-1 treatment significantly decreased the number of IBA-1-positive microglial cells in the ischemic hemisphere of MCAO/R mice, compared with mice without galectin-1 treatment (Fig. 4A and B). In addition, cytokine levels in the ischemic hemisphere tissues of MCAO/R mice were assessed. The results revealed that mice treated with galectin-1 had lower inflammatory cytokines, including IL-6, IL-1 and TNF-α, compared with those in the brains of mice without galectin-1 treatment (Fig. 5A-C).

Discussion

In the present study, the neuroprotective effect of galectin-1 on MCAO/R mouse model was investigated and it was demonstrated that galectin-1 treatment improved cerebral I/R outcomes, as evidenced by the lower neurological deficit and lower infarct volumes.

Oxidative stress is closely associated with cerebral I/R injury. When an ischemic event occurs, oxygen and nutrient supply to brain tissues is halted, leading to energy depletion and cell death. However, paradoxically, restoration of blood flow, termed reperfusion, could exacerbate injury due to the sudden burst of ROS. ROS are a group of small molecules that include free radicals and peroxides. including stable oxidants, such as H_2O_2, and unstable free radicals, such as superoxide anion, nitric oxide, hydroxyl moiety and hypochlorite. Under normal condition, intracellular ROS can be effectively eliminated by the combined action of the antioxidant systems, including SOD, CAT, GSH-Px and other endogenous antioxidants, providing a repair mechanism for oxidized membrane components (27). However, excess ROS production can overwhelm the effect of antioxidant molecules, leading to protein denaturation, lipid peroxidation and DNA damage.

Moreover, ROS production induced by I/R may exacerbate the inflammatory response following transient focal ischemia. Nishi *et al* (28) demonstrated that reducing ROS production by enhancing the expression of SOD-1 in mice can decrease levels of pro-inflammatory cytokines, including TNF-α, IL-1, IL-6, monocyte chemoattractant protein-1 and macrophage inflammatory protein-1. Similarly, Bowler *et al* (29) found that the administration of the antioxidant AEOL 10150 attenuated pro-inflammatory genes and modulated the immune response after transient focal ischemia in mice. Furthermore, ROS are implicated in the activation of immune cells and endothelial cell functions through the activation of oxidative stress-sensitive nuclear transcriptional factors, such as NF-κB (30). Oxidative stress may also mobilize pools of preformed adhesion molecules in leukocytes and endothelial cells (31). The activation of microglia has been identified to parallel the induction of cellular apoptosis and correlates with the severity of the ischemic insult (32), suggesting a significant role for inflammation in the progression of cerebral ischemic injury. The second critical process involves the mobilization and infiltration of inflammatory cells, such as leukocytes, from the periphery and the activation of the endothelium. This leads to excessive ROS production, causing oxidative damage to endothelial cells and tissues (33-35).

In the present study, ROS production in the brain tissues of MCAO/R mice was examined and the ROS induction by MCAO/R was observed. The excess ROS production could affect neurofunctional recovery from MCAO/R. Hsieh *et al* (36) demonstrated that urine 8-OHdG levels were negatively correlated with functional outcomes after a stroke and significantly decreased after rehabilitation. By contrast, antioxidant molecules play a critical role in cell defense against the toxic effects of oxygen radicals, reducing superoxide anions to hydrogen peroxide. A short-term increase in ROS could elevate the level of antioxidant molecules as an adaptation and defense response against ROS production. However, long-term excess production of ROS could exhaust the antioxidant system, leading to a decrease in antioxidant levels. This is especially true in the neuronal system, which is more sensitive to oxygen deprivation than other organs. Consistent with this, the present study observed a decrease in antioxidant molecules in ischemic brain tissues. Therefore, antioxidants, compounds that neutralize ROS, and strategies targeting the reduction of ROS generation have been investigated as potential therapies for I/R injury in recent years. In the present study, treatment with galectin-1 increased the levels of antioxidant molecules in the ischemic hemisphere. These molecules then scavenged ROS and other free radicals, including SOD and CAT, removing hydrogen and lipid peroxides. This process ultimately prevented the oxidation of biomolecules, thereby reducing oxidative stress

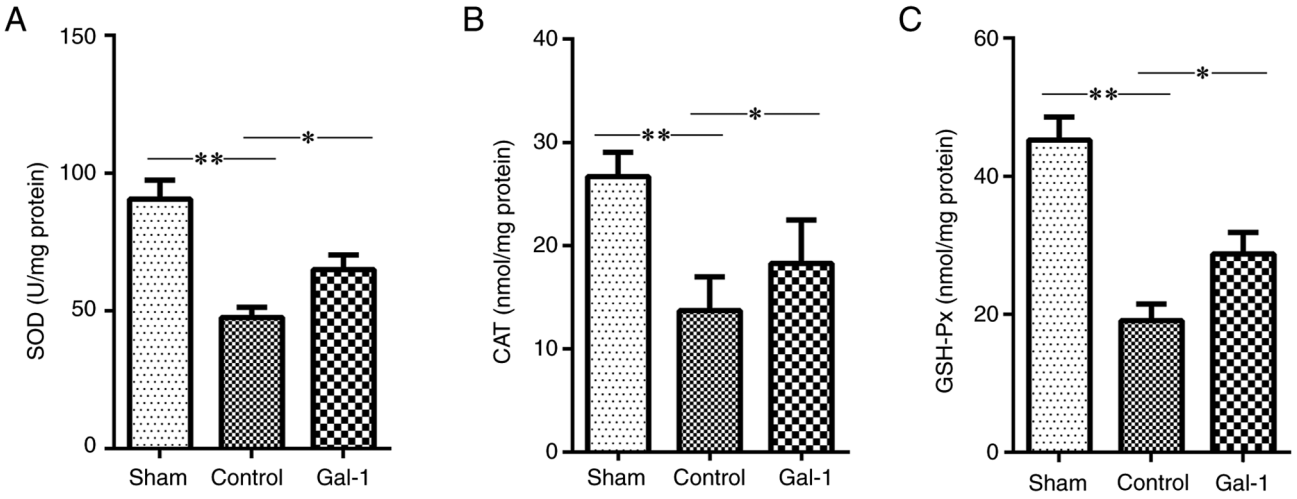

Figure 3. Levels of SOD, CAT and GSH-Px in the ischemic hemisphere of MCAO/R mice. The mice received MCAO/R surgery, MCAO/R surgery + galectin-1 treatment, or sham surgery (n=10 per group). Subsequently, the levels of (A) SOD, (B) CAT and (C) GSH-Px in the ischemic hemisphere were measured. Compared with the sham surgery group, the levels of SOD, CAT and GSH-Px were significantly decreased in the ischemic hemisphere of MCAO/R mice. However, galectin-1 treatment promoted the levels of anti-oxidative stress molecules, including SOD, CAT and GSH-Px. All of these were increased in the galectin-1 + MCAO/R group compared with the non-galectin-1 MCAO/R group. The data are presented as the mean ± SD. *P<0.05 and **P<0.01 by one-way ANOVA followed by Tukey's post hoc test. SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidation enzyme; MCAO/R, middle cerebral artery occlusion-reperfusion; Gal-1, galectin-1 treatment.

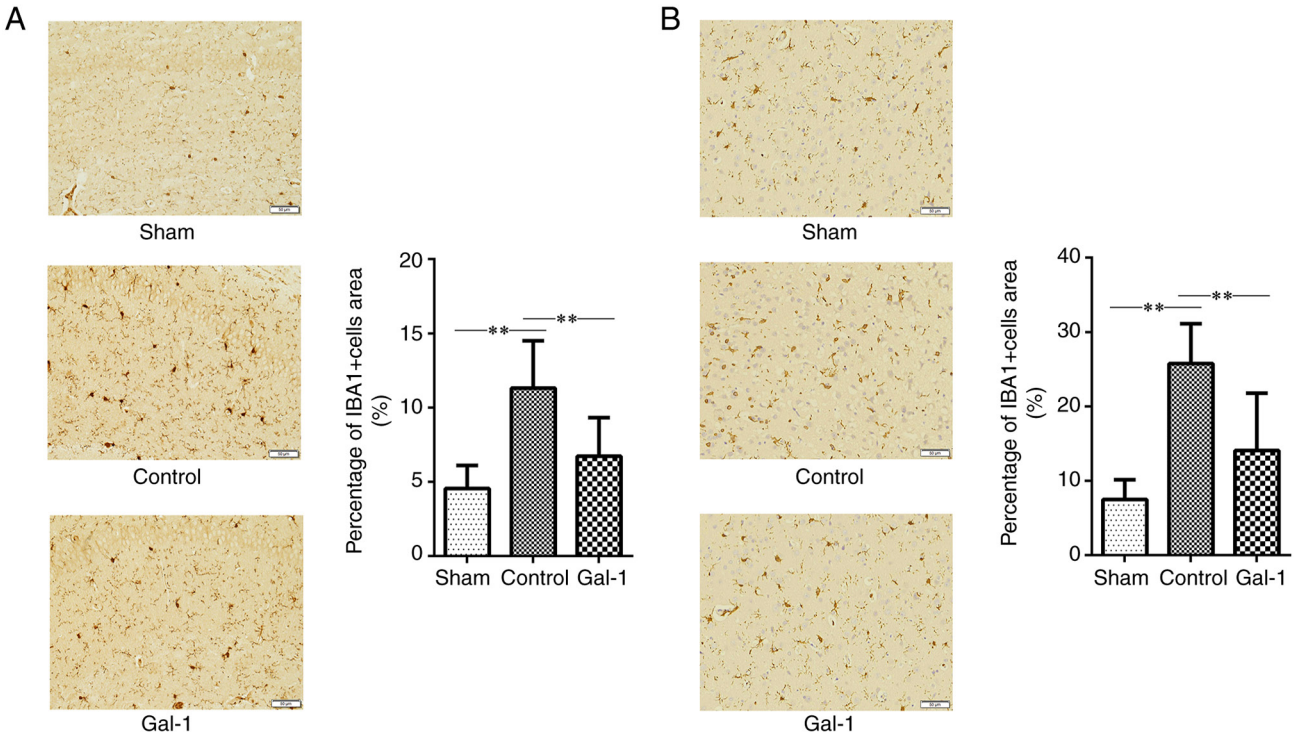

Figure 4. Microglia infiltration in the ischemic hemisphere of MCAO/R mice. The mice received MCAO/R surgery, MCAO/R surgery + galectin-1 treatment, or sham surgery (n=10 per group). Sections of the ischemic hemisphere tissues were stained with an antibody against the microglia biomarker IBA-1, and the levels of IBA-1 expression in the ischemic hemisphere were measured. Sham mice displayed a normal distribution of ramified microglia in the brain (A) hippocampus and (B) cortex, whereas strong activation of microglia was detected in the ischemic hemisphere of MCAO/R mice. Compared with non-galectin-1 treatment control mice, treatment with galectin-1 significantly decreased the quantities of activated microglia in both the (A) hippocampus and (B) cortex. Representative images and semi-quantitative data of IBA-1 are included. A total of five random areas within the brain hippocampus and cortex were analyzed from three independent non-adjacent sections per mouse. Data are expressed as the mean ± SD. **P<0.01 by one-way ANOVA followed by Tukey's post hoc test. Scale bars, 50 μm. MCAO/R, middle cerebral artery occlusion-reperfusion; IBA-1, allograft inflammatory factor 1; Gal-1, galectin-1 treatment.

in the brains of mice with reperfusion injury. Consequently, galectin-1 treatment improved the outcome of ischemia in MCAO/R mice.

Previous studies have investigated the anti-oxidative stress effect of galectin-1 in various other pathological conditions (37). Liu *et al* (14) reported that galectin-1

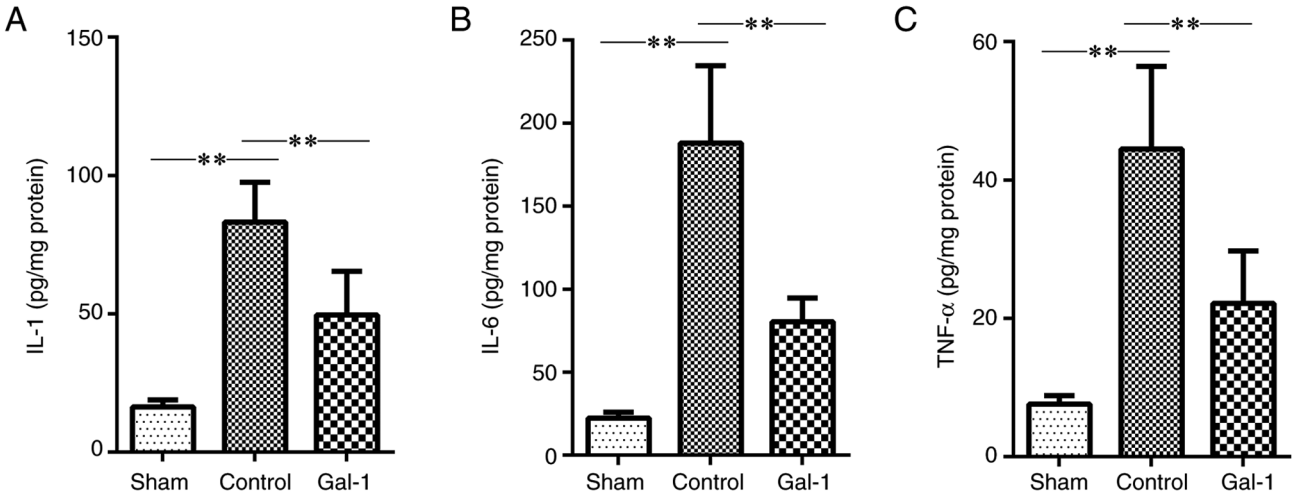

Figure 5. Inflammatory cytokine levels in the brains of MCAO/R mice. The mice received MCAO/R surgery, MCAO/R surgery + galectin-1 treatment, or sham surgery (n=10 per group). Thereafter, ischemic hemisphere tissues were collected. The levels of inflammatory cytokines (IL-1, IL-6 and TNF-α) were examined by ELISA. (A-C) Compared with the sham surgery group, the levels of IL-1, IL-6 and TNF-α were all significantly increased in the ischemic hemisphere of MCAO/R mice. However, treatment with galectin-1 decreased the levels of IL-1, IL-6 and TNF-α, compared with the non-galectin-1 MCAO/R group. Data are expressed as the mean ± SD. **P<0.01 by one-way ANOVA followed by Tukey's post hoc test. MCAO/R, middle cerebral artery occlusion-reperfusion; IL, interleukin; TNF-α, tumor necrosis factor alpha; Gal-1, galectin-1 treatment.

inhibited the production of ROS in neuronal cells induced by 1-methyl-4-phenyl pyridine ion. Rodrigues *et al* (38) found that galectin-1 could regulate the production of ROS in neutrophils. Moreover, Arda-Pirincci *et al* (39) reported that galectin-1 decreased the level of ROS formation in livers of mice. Ou *et al* (40) found that it protected the myocardial tissue after I/R, while Huang *et al* (41) suggested that galectin-1 ameliorated acute lung injury induced by lipopolysaccharide in mice. Moreover, galectin-1 has been revealed to protect the liver (19) and kidney (18) from I/R injury. Thus galectin-1 administration could attenuate the cell injury induced by oxidative stress. Researchers have identified various potential pathways involving galectin-1 in the regulation of oxidative stress. In a recent study by the authors, it was found that galectin-1 can promote Nrf-1 activity to increase the production of anti-ROS molecules (14), while Huang *et al* (41) demonstrated that galectin-1 can target the AMPK-Nrf2 pathway in mice to inhibit the level of oxidative stress. Li *et al* (42) discovered that galectin-1 had an effect on NF-κB activation through the regulation of the MAPK pathway.

Galectin-1 is expressed in immune cells. Aalinkeel *et al* (43) identified that galectin-1 can suppress the inflammation response of microglial cells. Castillo-González *et al* (44) found that deficient of galectin-1 resulted in increased infiltration of $CD8^+$ T cells and neutrophils in the skin of mice. Thus galectin-1 can attenuate the activation of immune cells, regulate the oxidative stress and inflammation, and eventually ameliorate the I/R injury. In the present study, it was observed that administration of galectin-1 alleviated the infiltration of microglial cells in the ischemic hemisphere of MCAO/R mice. Furthermore, it was found that galectin-1 can inhibit the generation of pro-inflammatory cytokines in pathology of I/R injury. Similarly, Ye *et al* (19) suggested that administration of galectin-1 significantly reduced proinflammatory cytokines including TNF-α, IL-6, IL-1β, IL-12, IFN-γ and IL-17 in mice with hepatic ischemia reperfusion injury. Meanwhile, Ou *et al* (40) suggested that administration of galectin-1 reduced the proinflammatory cytokines, such as IL-6 and IL-1β in myocardial tissue of rats with myocardial I/R injury. Moreover, galectin-1 can suppress the expression of pro-inflammatory cytokines, further contributing to its anti-oxidative stress effects through inhibition of the pathway of NF-κB (45). Besides, galectin-1 has been found to promote angiogenesis (46) and neurogenesis (47), which are crucial for the recovery of brain function after ischemic stroke. It also inhibits apoptosis, further contributing to its neuroprotective effects (45).

In addition, the data of the present study indicated that galectin-1 treatment resulted in limited improvement in the rotarod test; it was proposed that this may be due to the inherent limitations of the rotarod test itself. While the rotarod test is a widely accepted method in neuroscience research for assessing motor coordination and balance in mice, it has certain drawbacks. Specifically, the test can be stressful and fatiguing for the animals, potentially affecting their performance in ways that are not directly related to their motor coordination abilities. Furthermore, the rotarod test primarily evaluates gross motor coordination. As such, it may not be sufficiently sensitive to detect subtle changes in fine motor skills, which are also known to be affected in ischemic conditions. This limitation could account for the observed minimal improvements in mice treated with galectin-1, as the test may not fully capture the nuances of their motor skill enhancements.

Therefore, galectin-1 might represent a promising strategy in cerebral I/R injury, although to the best of the authors' knowledge, the data on galectin-1 treatment is still preliminary. Further research is needed to validate the outcomes of anti-oxidative stress and anti-inflammation treatments.

In conclusion, the present study demonstrated that galectin-1 can alleviate oxidative stress in the brains of the MCAO/R mouse model. Moreover, galectin-1 treatment

improved outcomes in cerebral I/R, suggesting that it might be a promising therapeutic strategy for stroke.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

JZ, XM and XL designed the study. JZ, RZ and FH acquired the data. JZ, MW and YW analyzed and interpreted the data. JZ, XM and XL wrote, reviewed and edited the manuscript. JZ and XL confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All the animal-related experiments were approved (approval no. 2022023) by the Animal Care and Management Committee of Beijing Geriatric Hospital (Beijing, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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