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Resveratrol Treatment Attenuates Focal Cerebral Ischaemic Injury in Rats by Activating the Sirt1/Gpx4 Pathway to Inhibit Ferroptosis

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Abstract:
Objective: *To investigate the neuroprotective effects of resveratrol (RSV) against ferroptosis in a rat model of cerebral ischaemia.*
Methods: *Photochemical embolisation was used to generate focal cerebral ischemic injury in Sprague-Dawley rats. The modified neurological severity score system and adhesive removal experiment were used to evaluate neurological deficits) in model rats. Cerebral infarction volume was determined using TTC staining. Commercially available glutathione (GSH), iron ion, reactive oxygen species (ROS), and malondialdehyde (MDA) kits were used to detect ferroptosis. Western blot was used to detect the expression of the ferroptosis-related proteins Sirt1 and Gpx4.*
Results: *We found in a rat model of focal cerebral ischaemia that RSV treatment could significantly alleviate the neurological deficit score, reduce the adhesive removal time, reduce cerebral infarct area, reduce brain water content, and alleviate the neurological damage caused by cerebral ischaemia. Meanwhile, RSV treatment can significantly restore GSH and iron ion levels, reduce ROS and MDA levels, and activate the expression of the ferroptosis-related proteins Sirt1 and Gpx4.*
Conclusion: *RSV improved neurological deficits, reduced the area of cerebral infarction, and alleviated neuronal damage. This protective effect may be achieved by upregulation of Sirt1 and Gpx4 protein expression to alleviate damage caused by ferroptosis.*

Keywords: *resveratrol; ferroptosis; Sirt1; Gpx4.*

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1. Introduction

Ischaemic stroke precipitates focal brain tissue necrosis through the disruption of cerebral perfusion and remains a leading cause of global mortality and disability (DeLong et al., 2022). Pathologically, this condition is defined by acute vascular occlusion—typically stemming from thrombosis or embolism—which triggers a deleterious ischaemic cascade, demarcating an irreversible necrotic core from the surrounding, hypoperfused penumbra (GBD 2019 Stroke Collaborators, 2021). Epidemiologically, ischaemic stroke constitutes approximately 62.4% of all incident strokes worldwide, with the highest burden observed in the elderly and populations with metabolic comorbidities such as hypertension and diabetes (Campbell et al., 2019). The aetiology is predominantly driven by large-artery atherosclerosis and cardioembolism, underscoring the heterogeneity of its pathogenesis (Martin et al., 2025). Amidst the complex molecular sequelae of occlusion, lipid peroxidation induced by cerebral ischemia emerges as a critical mechanism of injury (Adibhatla & Hatcher, 2008). Recent research has further elucidated that a novel form of regulated cell death driven by lipid peroxidation, known as ferroptosis, plays a pivotal role in cerebral ischaemic pathology (Xu, Li, & Wang, 2023).

Ferroptosis is a newly discovered form of regulatory cell death characterized by iron-dependent, excessive accumulation of lipid peroxides (Chen et al., 2021). In a recent study, ferroptosis was shown to be involved in multiple diseases, such as cancer, renal degeneration, intestinal ischemia and reperfusion (I/R) injury, and several neurodegenerative diseases (Li et al., 2020). Iron accumulation was found to exacerbate neuronal damage during cerebral ischemia in clinical pathology and laboratory studies (Campbell et al., 2019). Previous studies have demonstrated that mouse models of transient middle cerebral artery occlusion (tMCAO) exhibited increased lipid peroxidation and reduced glutathione (GSH) levels, whereas ferroptosis inhibitors ameliorated I/R injury in mice (Li et al., 2022). Based on these data, exploring the regulatory mechanism of ferroptosis may be a potential breakthrough in the treatment of ischaemic stroke.

RSV is a natural polyphenolic compound derived from plants, with antioxidant, anti-inflammatory, anti-cardiovascular disease, anticancer, and anti-aging effects (Zhou et al., 2021). Studies have shown that RSV treatment is neuroprotective against Alzheimer's disease (Sawda, Moussa, & Turner, 2017). It was found that RSV pretreatment significantly relieved cerebral ischaemic injury in a rat model of cerebral ischemia (Girbovan & Plamondon, 2015). However, the neuroprotective mechanism of RSV in cerebral ischemia has not been clarified. Ferroptosis is an important injury factor in newly diagnosed cerebral ischemia; however, the relationship between RSV and ferroptosis is unclear, and is a phenomenon worthy of exploration.

Gpx4 is a confirmed key regulator of ferroptosis (Liu et al., 2023). The catalytic activity of Gpx4 weakens lipid peroxide toxicity and maintains homeostasis of the membrane lipid bilayer (Forcina & Dixon, 2019). Inhibition of Gpx4 function was found to cause the accumulation of intracellular peroxides, triggering ferroptosis (Seibt, Proneth, & Conrad, 2019). Overexpression of Gpx4 attenuates ferroptosis and ameliorates cognitive dysfunction in the rat TBI model (Fang et al., 2023). Silent information regulator 2-related enzyme 1 (Sirt1) is a nicotinamide adenine dinucleotide-dependent histone deacetylase that regulates the cellular response to damage (e.g., antioxidant defense and apoptosis) (Chen et al., 2020). Recent studies have found that Sirt 1 suppresses ferroptosis by upregulating Gpx4 protein expression in a rat model of contrast-induced nephropathy (Fang et al., 2021). In another study, Gpx4 protein expression was decreased after Sirt1 inhibition (Sun et al., 2023).

A recent study has established that RSV is an activator of Sirt1 (Parsamanesh et al., 2021). As such, we hypothesized that RSV treatment could reduce cerebral ischaemic injury by inhibiting ferroptosis by activating the Sirt1/Gpx4 signaling pathway.

2. Materials and Method

2.1. Animals

Adult male Sprague–Dawley (SD) rats (n = 40) weighing between 0.25 and 0.3 kg were obtained from the Experimental Animal Center. The animals were randomly assigned into groups as follows: Sham (n = 10); photochemical cerebral ischemia (PM; n = 15 [2 died]); and PM + RSV (n = 15 [2 died]). All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and approved by the Institutional Animal Care and Use Committee.

2.2. Reagents and Antibodies

RSV, dimethyl sulphoxide (DMSO), rose bengal, and 2,3,5-triphenyltetrazolium chloride (TTC) were obtained from Sigma Aldrich (St. Louis, MO, USA). The primary antibodies used were as follows: anti-Sirt1 (ab110304, Abcam, Cambridge, United Kingdom), and anti-β-actin (AC004, ABclonal, Wuhan, China). Secondary antibodies included horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (H+L) antibody (AS014, ABclonal) and HRP-conjugated anti-mouse IgG (H+L) (AS003, ABclonal).

2.3. Establishing a PM Model and Drug Treatment

Selected SPF adult male SD rats were anaesthetized and fixed to a brain stereotaxic instrument. The coronal plane of the skull and the vector midline were exposed at the anterior sacral point. A 561 nm wavelength laser with a diameter of 4 mm was stereotactically positioned at the skull; AP, 0.5 mm, ML, 3.5 mm surface. The skull was exposed for 20 min. Two minutes before irradiation, the animal was slowly injected through the femoral vein reserve catheter (0.133 ml/kg body weight, 10 mg/ml rose bengal). After the injection, the catheter was removed, the skin was sutured, and the animal was placed in an incubator. The same method was performed on the Sham group, but no laser irradiation was performed. Core body temperature of the animals was maintained at 37°C and monitored using a rectal thermometer.

RSV injection was started 5 days before establishing the PM model, with a daily dose of 100mg/kg (intraperitoneal). The management plan for RSV is based on previous reports.

2.4 Evaluation of Neurological Damage

The modified neurological severity score (mNSS) was used to assess neurological function. After 24 h of establishing the PM model, the rats were scored for motor, sensory, reflex, and balance by an independent observer blinded to the experimental design. The observer graded neurological function using scores of 0–18, with score directly proportional to the severity of injury.

Adhesive Removal Test

Adhesive removal tests were used to assess sensorimotor impairment in animals following ischaemic stroke. After stroke, the forearm somatosensory cortex is affected, and the forepaw sensation in this focal ischaemic model is also affected. Briefly, a sticker (Tough-Spots 3/8″ diameter, Diversified BioTech, Dedham, MA, USA) was cut into quarters and placed on the animal's left paw on the 5th day after modelling. The time elapsed (in seconds) for the animal to remove the sticky label was recorded.

2.5 Measurement of Infarct Volume

After 24 h of establishing the PM model, the rats were anaesthetized and euthanized by decapitation. Brains were quickly isolated and sectioned into coronal sections (2 mm) in an adult rat brain matrix (RWD, Shenzhen, China). The samples were treated with 2,3,5-triphenyltetrazolium chloride (2%) (TTC, Sigma, USA) for 20 min at 37°C in a dark room. Sections were rinsed in saline solution, and cross-linked in 4% paraformaldehyde for 24 h at 4°C. Healthy brain tissue stained red following this procedure, whereas infarct tissue was negative for staining. The sections were photographed using a camera (D5100, Nikon, Tokyo, Japan) and analyzed using Image J (version 1.37c, National Institutes of Health [NIH]). Infarct volume was quantified using the following equation:

Percentage hemisphere lesion volume (%HLV) = (Left hemisphere volume - Right hemisphere non-infarct volume) / (Left hemisphere volume × 100)

2.6. Assessment of Brain Water Content

After 24 h of establishing the PM model, the brains were rapidly removed and immediately weighed to obtain the wet weight. Brain tissues were then desiccated in a drying oven at 120°C for 24 h until the weight no longer changed and subsequently weighed again to obtain the dry weight. Brain water content was calculated as follows: (wet weight − dried weight) / wet weight × 100%.

2.7. Determination of GSH Levels

Quantitative analysis of GSH levels was performed using a commercially available reduced GSH assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) in accordance with manufacturer's instructions. Protein content was assessed using a commercially available BCA protein assay kit (Pierce Biotechnology, Waltham, MA, USA). Absorbance was determined using a microplate reader (ThermoScientific, Waltham, MA, USA) at a wavelength of 405 nm. GSH level was corrected based on protein content.

2.8. Iron Assay

Iron concentration in the brain tissue was determined using a commercially available kit. After homogenizing the brain tissue, the supernatant was processed in accordance with kit instructions (Sigma Aldrich, WI, USA). Finally, absorbance was measured at 593 nm using an enzyme marker, and iron concentration was calculated using a standard curve.

2.9. Western Blotting

The brains of the animals were washed by direct perfusion of saline into the tissue, and then isolated. The brains were then washed in 0.9% saline at 4°C. The ischemic penumbra cortical tissue was then homogenized in RIPA buffer. The resulting lysate was centrifuged at 12,000×g for 20 minutes at 4°C. The supernatant was then isolated, and protein concentration was quantified using an Enhanced BCA Protein Assay Kit (Beyotime, Jiangsu, China). Equivalent quantities of sample were separated by sodium-dodecyl polyacrylamide gel electrophoresis. The separated proteins were then transferred onto nitro-cellulose membranes (Millipore, Burlington, MA, USA), and blocked using 5% milk in TBS-T. The blots were incubated overnight at 4°C with primary antibodies. The antibodies used in this study included: Sirt1 (13161-1-AP, Proteintech, Rosemont, IL, USA), Gpx4 (67763-1-Ig, Proteintech) and β-actin (66009-1-Ig, Proteintech). Membranes were incubated for 1 h at room temperature with HRP-conjugated secondary antibodies (diluted 1:5000). Protein bands were visualized using electrochemiluminescence techniques. To quantify protein levels, the density of the separated protein bands was measured using Image J (NIH), and values were normalized to β-actin levels as a loading control.

2.10. Statistical Analysis

Data are expressed as mean ± standard error of the mean (SEM). Statistical variances between groups were assessed using Student's t-test (unpaired, two-tailed). Multiple comparisons (without rating scale data) were performed using one-way analysis of variance (ANOVA) followed by Tukey's range test. Neurological deficit scores were compared using the Mann–Whitney U test. Differences with $p < 0.05$ were considered to be statistically significant. Statistical analyses were performed using Prism (GraphPad Inc., San Diego, CA, USA).

3. Results

3.1. RSV Ameliorated Cerebral Ischaemic Injury in Rats

To explore the protective effects of RSV against cerebral ischemia injury, the effects of RSV treatment on cerebral infarct volume, neurological scores, and brain water content were examined. Results revealed that infarct volume in the PM + RSV group was significantly decreased compared with the PM group (Fig. 1A, B). Similarly, compared with the PM group, RSV significantly alleviated brain tissue oedema, improved neurological scores, and shortened adhesive removal time (Fig. 1C-E). Therefore, RSV treatment significantly reduced cerebral infarction volume and cerebral oedema in animals with cerebral ischemia, improved neurological deficits, and demonstrated pronounced neuroprotective effects.

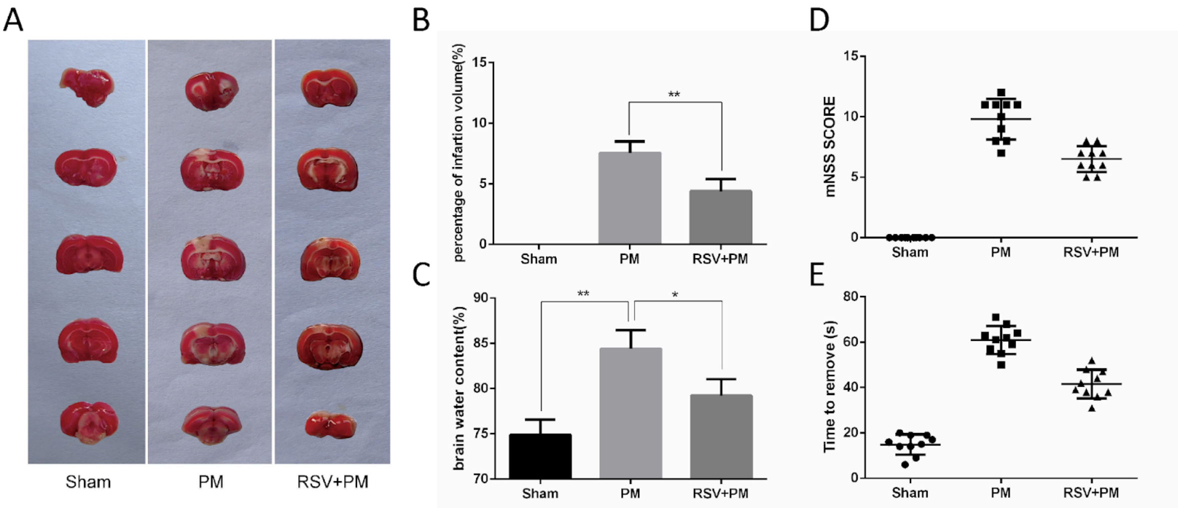

*Figure 1. Effects of RSV on cerebral infarct volume, brain water content, neurological scores, and adhesive removal time. (A, B) Cerebral infarct volume was greatly reduced after RSV treatment compared with the PM group. (C) Brain water content was significantly decreased after treatment with RSV compared with the PM group. (D) Neurological scores were clearly improved after RSV treatment compared with the PM group. (E) Adhesive removal time was significantly shorten after treatment with RSV compared with the PM group. Error bars represent mean ± SEM. (*P < 0.05, **P < 0.01 vs. PM). n = 10 in the Sham group, n = 10 in other groups.*

3.2. RSV Reduced Ferroptosis to Relieve Cerebral Ischaemic Injury in Rats

To explore the relationship between RSV and ferroptosis, the effects of RSV on GSH, iron ions, ROS and MDA content of the penumbra tissue in rat cerebral ischemia model animals were examined. Results revealed that GSH content and iron ions, ROS and MDA levels were significantly increased in the PM group compared with the Sham group (Fig. 2A, B, C, D). This indicates that the level of ferroptosis increased significantly after cerebral ischemia. Compared with the PM group, GSH content was significantly increased in the PM + RSV group, while iron ion, ROS and MDA levels were reduced (Fig. 2A, B, C, D), which indicated that the level of ferroptosis was significantly reduced after RSV treatment. Experimental results revealed that RSV could reduce ferroptosis and alleviate cerebral ischaemic injury. Furthermore, RSV treatment exhibited a strong correlation with ferroptosis, and that RSV treatment can reduce the level of ferroptosis, and reduce cerebral ischaemic injury.

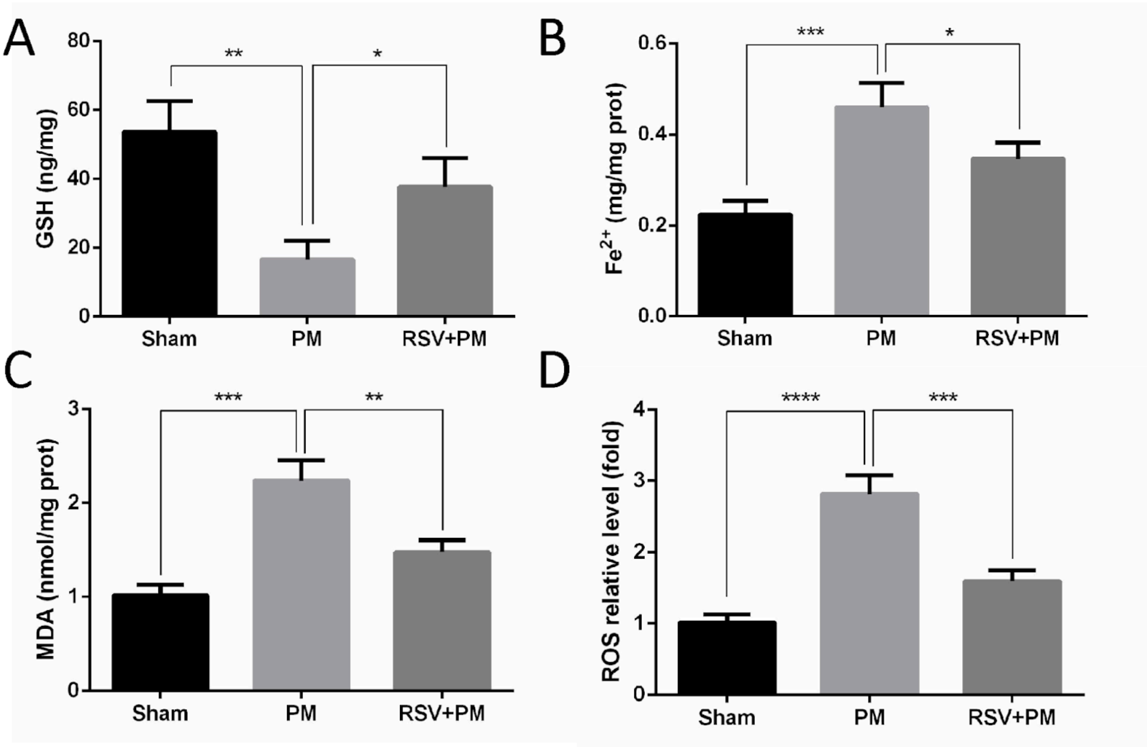

*Figure 2. Effects of RSV on indicators related to ferroptosis. (A) GSH content was significantly increased after RSV treatment compared with the PM group. (B) Iron ion content was reduced after RSV treatment compared with the PM group. (C) ROS levels were significantly reduced after RSV treatment compared with the PM group. (D) MDA levels were reduced after RSV treatment compared with the PM group. Error bars represent mean ± SEM. (*P < 0.05, **P < 0.01, ***P < 0.001 vs. PM). n = 6 in each group.*

3.3. RSV Alleviates Cerebral Ischaemia Injury by Activating the Sirt1/Gpx4 Signalling Pathway

To explore how RSV attenuates ferroptosis, the Sirt1/Gpx4 signalling pathway that affects ferroptosis was examined. Results demonstrated that the relative expression of both Sirt1 and Gpx4 proteins was significantly reduced in the PM group compared with the Sham group (Fig. 3A, B, C). The relative expression of Sirt1 and Gpx4 proteins was significantly higher in the PM + RSV group compared with the PM group (Fig. 3A, B, C).

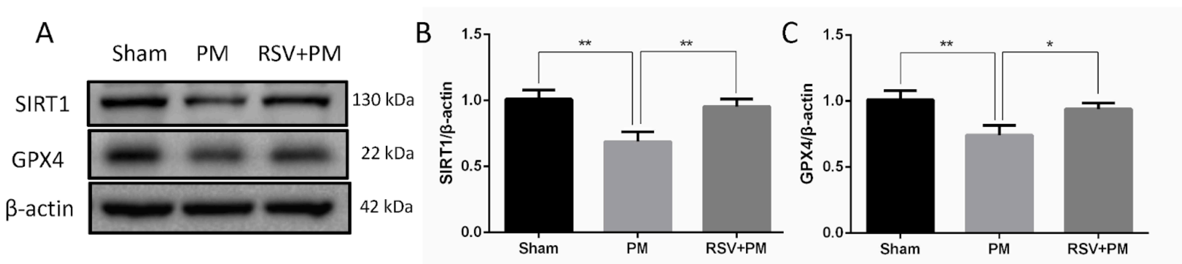

*Figure 3. RSV treatment activates the Sirt1 / Gpx4 signaling pathway associated with ferroptosis in cerebral ischemia injury in rats. (A) Representative western blots showing protein levels of Sirt1 and Gpx4. Protein levels were normalized to β-actin. Quantification of protein levels such as those in (A) is shown in (B, C). Error bars represent mean ± SEM. (*P < 0.05, **P < 0.01 vs. PM). n = 6 in each group.*

4. Discussion

Results of the present study demonstrated that RSV treatment was neuroprotective against focal cerebral ischaemic injury in PM model rats. We found that RSV significantly reduced infarct volume and brain water content and improved neurological scores. Moreover, RSV treatment reduced cellular iron ion, ROS and MDA content, increased GSH levels and, additionally, that RSV treatment activated the Sirt1/Gpx4 signalling pathway associated with ferroptosis. These experimental results indicate that RSV can reduce cerebral ischaemic injury by inhibiting ferroptosis.

Ferroptosis is a novel regulator of cell death caused by excess free radicals (Martin et al., 2025). The essence of ferroptosis is the accumulation of lipid peroxides and is accompanied by iron overload (Sun et al., 2022). Unique morphological features of ferroptosis include mitochondrial atrophy and increased mitochondrial membrane density (Gao et al., 2019). Accumulating evidence indicates that ferroptosis plays an important role in multiple neurological diseases (David et al., 2023; Li et al., 2022; Ou et al., 2022), and studies have found that inhibition of ferroptosis significantly attenuates nervous system damage (Cao et al., 2021; Ryan et al., 2023). However, the role of ferroptosis in ischaemic stroke remains to be extensively investigated. Some studies have shown that baicalein can relieve cerebral I/R injury by inhibiting ferroptosis and, in the rat MCAO/R model (Xu, Li, & Wang, 2023)., dihydromyricetin inhibited ferroptosis by inhibiting the SPHK 1/mTOR signalling pathway, thus reducing brain I/R injury (Xie et al., 2022). In this study, we found that after inhibiting ferroptosis, cerebral infarct area, brain water content, neurological deficit score, and the time elapsed for adhesive removal were reduced, and experimental results demonstrated that inhibiting ferroptosis could significantly alleviate neurological damage in focal cerebral ischemia model in rats.

The Gpx4-mediated anti-ferroptosis pathway may represent a basis for studying ferroptosis progression (Ursini & Maiorino, 2020). Gpx4 reduces the production of cytotoxic phospholipid hydrogen peroxide to the corresponding phospholipid alcohol by converting GSH to oxidized glutathione (GSSG) (Rochette et al., 2022). An increasing number of studies have found that Gpx4 inhibition leads to the accumulation of lipid peroxides and increases the level of ferroptosis (Wang et al., 2022), while overexpression of Gpx4 inhibits ferroptosis. Gpx4 has emerged as a marker of ferroptosis (Liu et al., 2023). Sirt1 is a class III histone deacetylase that regulates various cellular functions, including oxidative stress, inflammation, energy metabolism, DNA damage repair, and cell death (Jiao & Gong, 2020; You & Liang, 2023; Shen et al., 2021). Numerous studies have shown that Sirt1 is protective against neurological diseases (Tang et al., 2023). It has been shown that Sirt1 reduces cerebral ischemia-induced neuroinflammation and neuronal death by inhibiting p53 and NF-κB acetylation (Khan, Shah, & Kim, 2018). Sirt1 can also exert its neuroprotective effects by inhibiting the NLRP3 inflammasome and the mitogen-activated protein kinase (MAPK) pathway (Li et al., 2023). Investigation of the neuroprotective role of Sirt1 in ferroptosis and its underlying molecular mechanisms revealed that Sirt1 significantly reduced lipid peroxidation levels by promoting the expression of Gpx4, thereby significantly alleviating ferroptosis (Deng et al., 2023). The above evidence suggests that Sirt1 plays a neuroprotective role in ferroptosis by activating the function of the intracellular antioxidant system. A similar result regarding Sirt1 involvement in caloric restriction in contrast-induced nephropathy through upregulation of Gpx4 expression was also reported by Fang et al and Seibt, Proneth, and Conrad (2019). Thus, Sirt1 may represent a new therapeutic target for inhibiting ferroptosis after cerebral ischemia.

RSV is a natural antioxidant found in plants and exerts antioxidant effects mainly by scavenging free radicals, inhibiting lipid peroxidation, and regulating the activity of antioxidant-related enzymes (Tian & Liu, 2020). RSV has many biological and therapeutic properties, including hepatoprotective, anticancer, cardioprotection, antiatherogenic, endothelial protection, antithrombosis, antiangiogenic, lipid regulation, mitochondrial biogenesis, antidiabetic and antihypertensive effects (Breuss, Atanasov, & Uhrin, 2019; Ren et al., 2021; Pyo et al., 2020).

Results of a previous study suggest that RSV may reduce peroxylipid levels, increase superoxide dismutase activity, and decrease water content in the brain tissue of I/R rats to exert neuroprotective effects. RSV pretreatment also reduced the levels of cleaved caspase-3 and MDA (He, 2017). Furthermore, RSV exhibited strong cardioprotective and antiarrhythmic effects after myocardial I/R (Liu et al., 2023). RSV treatment attenuated lipid oxidation, normalized MDA levels, and alleviated renal I/R injury (Sener et al., 2006). Recent studies have revealed that RSV, as an activator of Sirt1, can exert a neuroprotective effect by activating Sirt1 (Hosoda et al., 2023). It has been found that RSV alleviates damage from oxidative stress and inflammatory damage in AD models by activating Sirt1 (Anekonda, 2006). It was also found that Sirt1 activation by RSV attenuated mitochondrial dysfunction in mice (Tabassum et al., 2023). In our experiments, we injected RSV intraperitoneally, and the results revealed that SD rats injected with RSV exhibited significantly reduced brain infarction area, brain water content, neurofunction score, and adhesive removal time. The results demonstrated that RSV could significantly alleviate cerebral ischaemic injury. Experimental results also demonstrated that the brain tissue of SD rats injected with RSV exhibited increased GSH content, decreased iron ion content, and increased expression of Sirt1 and Gpx4 proteins, which suggested that RSV may inhibit ferroptosis by activating the Sirt1/Gpx4 signalling pathway, thus alleviating cerebral ischaemic injury and playing a neuroprotective effect.

While the photothrombotic model in Sprague-Dawley rats served as a robust platform for elucidating ischaemic mechanisms, it is imperative to recognize the inherent translational gap between rodent models and human clinical reality. Fundamental disparities in neuroanatomical complexity—most notably the contrast between the lissencephalic (smooth) rodent cortex and the highly gyrencephalic (folded) human brain—may influence the pathophysiology of ischemia and the distribution of therapeutic agents. These structural and physiological differences, alongside variations in white matter composition, necessitate caution when extrapolating preclinical success to human patients, as they can significantly alter the clinical applicability of neuroprotective strategies. Nevertheless, within the context of these limitations, results of the present study provide new evidence that RSV may relieve cerebral ischaemic injury by inhibiting ferroptosis. The inhibition of ferroptosis by RSV through activation of the Sirt1/Gpx4 pathway may be a potential mechanism by which RSV exerts neuroprotection after cerebral ischaemic injury. These findings may provide new clues in the search for the treatment of cerebral ischaemic injury.

Conclusion

RSV treatment attenuated focal cerebral ischaemic injury in model rats by activating the Sirt1/Gpx4 pathway to inhibit ferroptosis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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