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Review

Study on Cellular Mechanism of Improving Inflammatory Effect of Gastrodin

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Neuroinflammation is a major pathological mechanism of neurodegenerative disease-triggered cognitive disorders. Currently, no preventative measures or therapies are available. Gastrodin (GAS), an effective monomer derived from Gastrodia, is considered to be an anti-inflammatory candidate to attenuate microglia-induced neuroinflammation and neurodegenerative diseases. The present study first modelled the inflammatory activation of BV2 cells, which was induced by lipopolysaccharide (LPS) at the molecular level. The optimal concentration of GAS was screened out to preliminarily investigate its role in improving the inflammatory activation of BV2 cells during cellular death. Then, the research further discussed how GAS ameliorated inflammation via regulating ferroptosis. According to the results of our study, GAS up-regulates downstream heme oxygenase-1 (HO-1) and NAD(P)H:quinone oxidoreductase 1 (NQO1) expression while lowers reactive oxygen species (ROS) expression by Nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear transposition. Experimental results showed that 100 μM is the optimal concentration for gastrodin in the inflammatory activation model. GAS can promote Nrf2 nuclear translocation and the expression of HO-1 and NQO1 while reduce ROS level. Therefore, GAS can regulate ferroptosis in LPS-induced BV2 cellular inflammation model, thus attenuating inflammatory occurrence. In conclusions, GAS is considered to be an anti-inflammatory candidate that acts in LPS-induced BV2 cellular inflammation model by regulating ferroptosis.

Keywords: ferroptosis; gastrodin; neuroinflammation; nuclear factor erythroid 2-related factor 2; reactive oxygen species
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Introduction

Cognitive disorders have become a public concern, especially for the elderly, as the aging population is deteriorating. On the one hand, cognitive disorders are resulted from neural disease including Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD) etc. On the other hand, cognitive disorders are closely correlated with risk factors such as anesthesia and surgery. A typical attribute of cognitive disorders is neuronal injury that may lead to dementia, cognitive impairment, motor dysfunction and even death (Tan et al. 2021). Despite ignorance of the mechanism, the onset of cognitive disorders could be partially explained by several theories, including neuroinflammation, mitochondrial injury, Amyloid β-protein (Aβ) deposition (Huang et al. 2018; Song et al. 2021; Apatiga-Perez et al. 2022; Rump and Adamzik 2022). Currently, scholars favor the view that neuroinflammation is a major trigger of cognitive disorders (Li et al. 2022b; Poh et al. 2022). When microglia in the central nervous

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system (CNS) is activated, neuroinflammation is triggered by chemokines, pro-inflammatory cytokines and ROS. All the factors act together on neurons, so neurons are injured and neurological dysfunction arises. Regardless of tremendous achievements in pathophysiology of neuroinflammation, no effective preventative measures and therapies are at hand to ameliorate cognitive disorders. Thus, it is vital to further investigate into the mechanism of medicines that are targeted for neuroinflammation-induced cognitive disorders.

Gastrodia, a species of orchid family, has dry tubers that are rich in GAS, p-hydroxybenzyl alcohol, parahydroxybenzaldehyde, and barrisonsides (Liu and Huang 2017). GAS is a phenolic glycoside compound derived from the gastrodia rhizome, and its chemical name is p-hydroxymethylbenzene-β-D glucopyranoside. GAS is included in the Pharmacopoeia of the People's Republic of China as an indicator to assess Gastrodia quality. GAS now is effective in anti-inflammation, anti-oxidation, attenuating pyroptosis, retaining glial homeostasis, promoting neural growth and reducing ischemic cerebral injury (Li et al. 2023; Ma et al. 2024). GAS is confirmed to inhibit activated microglia, and lower pro-inflammatory mediators and pro-inflammatory cytokines of neurotoxicity. Hence, GAS is an effective candidate for microglia-induced neuroinflammation and neurodegenerative diseases (Dai et al. 2011; Li et al. 2018; Yao et al. 2020; Guo et al. 2021). Although researchers investigate GAS by modelling different kinds of cells and animals, studies on the effect of GAS on activated microglia remain rare. Therefore, it is an urgent need to explore new signaling pathways and mechanism of how GAS regulates microglia activation for anti-inflammation. Cellular death is irreversible. When the body is seriously injured, metabolism terminates, tissue structures are destroyed, and dysfunctions occur. Such a state is significant for biological process, including maintaining homeostasis and suppressing rapid proliferation of tumors cells (Fuchs and Steller 2011). Meanwhile, abnormal regulation of cellular death is closely related to neurodegenerative diseases. Previous studies have demonstrated that GAS can improve cognitive impairment in neuroinflammatory rats (Zheng et al. 2022). To this end, this research will further investigate the role of cell death in the inflammatory activation of BV2 cells at the cellular level to confirm that GAS can ameliorate the inflammatory response via regulating ferroptosis.

Materials and Methods

Gastrodin (CAS:62499-27-8) was purchased from Solarbio in China, Lipopolysaccharides (#0000090043) was purchased from Sigma in USA. Antibodies for Iba-1 (ab283119) was purchased from Abcam in UK. Antibodies for Nrf2 (16396-1-AP), GPX4 (67763-1-IG), GAPDH (10494-1-AP) were purchased from Proteintech in USA. Antibodies for HO-1 (ET1604-45), NQO1 (ET1702-50) were purchased from Huabio in China. CheKine Reactive Oxygen Species Detection Fluorometric Assay Kit was purchased from Abbkine in USA.

Cellular, inflammatory activation model and grouping

BV2 microglia were purchased from Procell (Wuhan, China) (CL-0439). Based on Yao et al. (2019), the study stimulated BV2 cells with LPS (1 μg/mL) to establish an inflammatory activation model. The experiment was conducted in the translational neurology laboratory, affiliated hospital of Zunyi Medical University and lasted for six months. The methods of the experiment were as follows:

Experiment 1: Screen the optimal concentration of GAS for the model of inflammatory activation of BV2 cells. This experiment consists of 5 groups, namely the control (CON) group, LPS group, LPS+50GAS group, LPS+100GAS group and LPS+200GAS group. LPS (1 μg/mL) was used to treat the model. Then, BV2 cells were administered with GAS (50 μM, 100 μM, and 200 μM respectively) for 24 hours (h).

Experiment 2: GAS inhibits ferroptosis routes to improve inflammatory activation of BV2 cells. The experiment consists of 3 groups, namely the CON group, LPS group and LPS+GAS group. LPS (1 μg/mL) was used to treat BV2 cells for 24 h. Afterwards, the cells were administered with GAS (100 μM) for 24 h.

Experiment 3: The role of ferroptosis in improving inflammatory activation of BV2 cells via GAS. The experiment consists of 4 groups, namely the CON group, LPS group, LPS+GAS group, LPS+GAS+RSL3 group. LPS (1 μg/mL) was used to treat BV2 cells for 24 h. Next, the cells were administered with RSL3 (10 μM) + GAS (100 μM) for 24 h.

Experiment 4: The role of Nrf2 nuclear transposition in improving inflammatory activation of BV2 cells via GAS. The experiment consists of 4 groups, namely the CON group, LPS group, LPS+GAS group and LPS+GAS+ML385 group. LPS (1 μg/mL) was used to treat BV2 cells for 24 h. Then, the cells were administered with ML385 (10 μM) + GAS (100 μM) for 24 h.

CCK-8

Change in cell viability was determined by using the Cell Counting Kit-8 (CCK-8) assay. After washing, digestion, centrifugal and resuspension, cell suspension was obtained from each group. The cells were seeded into 96-well plates at a density of 8,000 cells/well in 100 μl of the complete medium and cultured at 37°C for 24 h. After specific medicine intervention for each group, the cells underwent incubation at 37°C for another 24 h. The morphology of BV2 cells was observed using microscopes (Mshot, Guangzhou, China). At the end of each experiment, 10 μl CCK-8 reagent was added into each well and the cells were further cultured for 3 h at 37°C and then the optical density value (OD 450) was measured by using a microplate reader (Huisong, Shandong, China). Cell viability = (experiment groups -blank groups) / (Con groups -

blank groups) ×100%.

Western Blot

Cell suspension was seeded into 6-well plates (1×10^6 cells/mL per well), and incubated in incubators, which were filled with CO_2, for 24 h. After specific medicine intervention for each group, the cells underwent incubation in CO_2 for another 24 h. After cleaning, total cellular protein and nucleoprotein were extracted from the cells using RIPA and PMSF. The samples were centrifuged at 12,000 rpm at 4°C for 20 min to collect the supernatants, and the protein concentration was measured using the bicinchoninic acid (BCA) protein assay kit. After that, the protein samples (10 μL each loading) were separated in 8~12% sodium dodecyl sulfate-polyacryla mide gel electrophoresis (SDS-PAGE) gels and then transferred onto the polyvinylidene fluoride (PVDF) membranes (Biorad, USA) in an ice bath. Next, the mixture was concealed using 5% skimmed milk powder and mixed with corresponding primary antibodies (GPX4, Nrf2, HO-1, NQO1) at 4°C overnight. Afterwards, incubation in secondary antibodies (goat antirabbit IgG) was performed. After that, the protein bands were detected with an enhanced chemiluminescence reagent using the FluorChem M System (Biorad, USA) and quantified using ImageJ software after being normalized to the GAPDH and HistoneH3 level.

Immunofluorescence

Cell suspension was seeded on coverslips (1×10^6 cells/mL per well), and incubated in incubators, which were filled with CO_2, for 24 h. After specific medicine intervention for each group, the cells underwent incubation in CO_2 for another 24 h. After treatment, the cells were fixed with paraformaldehyde for 20 min, permeabilized with 0.5% Triton X-100 for 15 min, and blocked with 5% BSA for 30 min. The coverslips were placed in a wet box for Iba1 antibody incubation at 4°C overnight. After washing the coverslips with PBS, fluorescent secondary antibodies were added and incubated for 1 h. Nucleus were stained using DAPI without exposure to light. Five min later, the slices were covered with glass slides. Samples were observed using confocal microscops (Zeiss, Germany) and analyzed using ipWin32.

ELISA

Cell suspension, which was collected from each group, was frozen and thawed for 3-4 times. After centrifugal (2,000 rpm) at 4°C for 20 min, the supernatant was obtained. The standard wells and the sample wells to be tested were loaded respectively, which was followed by addition of enzymes, incubation, washing, coloration. After the reaction terminated, optical density value (OD 450) were measured.

Fluorescent probes

After preparing ROS probes, the slides were fully covered and incubated in incubators, which were filled with CO_2, for 30 min without exposure to light. Samples were observed using confocal microscops and analyzed using ipWin32.

Statistical assessment

All data are expressed as the mean ± standard ($\overline{x} \pm s$). Statistical data were processed using IBM SPSS Statistics for Windows, version 29.0 and GraphPad Prism 8.0 software. One-way analysis of variance was used to determine the least significant difference in each group (homogeneity of variance and conformation to normal distribution). Otherwise, Dunnett's test was used to determine the significant differences between groups. Differences were considered statistically significant at $P < 0.05$.

Results

The optimal concentration of GAS for inflammatory activation model

First, we observed the morphology of BV2 cells with a microscope (400 ×). Cells from the CON group were more inclined to adhere to cellular walls. Most of the cells were shaped in spindle. As for cells from the LPS group, adherence to cellular walls was apparently less. The cells deformed and extended pseudopodia. Spindle cells sharply reduced. In the LPS+50GAS group, adherence to cellular walls was more than that of the LPS group. Cellular deformation was improved. Spindle cells increased. Through a comparison among the LPS+100GAS group, the LPS+200GAS group and the LPS+50GAS group, adherence to cellular walls and spindle cells increased. Based on the observation, conclusions of the cellular viability results were obtained.The cellular activity were 1.00, 0.45 ± 0.09, 0.63 ± 0.21, 0.81 ± 0.14, 0.85 ± 0.18 respectively. Compared with the CON group, cellular viability of the LPS group significantly fell, so the differences were statistically significant. Compared with the LPS group, cellular viability of the LPS+100GAS and LPS+200GAS increased, so the differences were statistically significant. However, the differences between the LPS+100GAS group and the LPS+200GAS group were not statistically significant. Therefore, 100 μM is confirmed to be the optimal concentration of GAS for inflammatory activation model. This concentration (100 μM) was adopted in following experiments (Fig. 1).

The role of ferroptosis in the anti-inflammatory effects of GAS

Considering that ferroptosis is a new type of programmed cell death that has been discovered in recent years and is extensively involved in neuroinflammatory Neurodegeneration, we tested the expression of ferroptosis-related proteins regulated by GAS using Western Blot. Compared with the CON group, GPX4 expression of the LPS group decreased (0.58 ± 0.01 vs. 0.16 ± 0.01). Compared with the LPS group, GPX4 expression of the

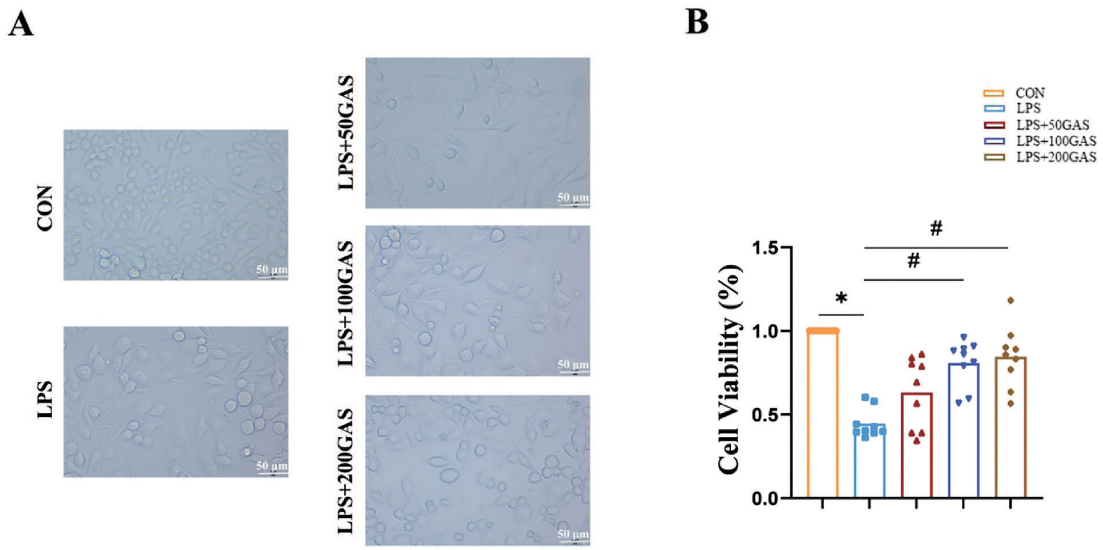

Fig. 1. Effects of GAS on inflammatory activation model at different concentrations.
(A) Cellular morphology. (B) Cellular viability. The results are presented as the mean ± S.D. *$P < 0.05$, #$P < 0.05$, compared between two groups that are shown by the lines.

LPS+GAS group increased (0.16 ± 0.01 vs. 0.30 ± 0.03), so the differences were statistically significant. According to the results of above experiments, GAS acted in inflammatory activation models and may be associated with the inhibition of ferroptosis. Hence, following experiments would center on the mechanism and effects of GAS on ferroptosis (Fig. 2).

Based on the above results, the effects of GAS on inflammatory activation models may be associated with ferroptosis. Therefore, RSL3 was adopted to explore the anti-inflammatory effects of GAS on inflammatory activation of BV2 cells.

The effect of RSL3 in the anti-inflammatory activation model via GAS

This research observed the morphology of BV2 cells with a microscope (400 ×). Cells from the CON group were more inclined to adhere to cellular walls. Most of the cells were shaped in spindle. As for cells from the LPS group, adherence to cellular walls was apparently less. The cells deformed and extended pseudopodia. Spindle cells sharply reduced. In the LPS+50GAS group, adherence to cellular walls was more than that of the LPS group. Cellular deformation was improved. Spindle cells increased. Compared with the LPS+GAS group, adherence to cellular walls and spindle cells of the LPS+GAS+RSL3 group decreased with pseudopodia being extended. Based on the observation, conclusions of the cellular viability results were obtained. The cellular activity were 1.00, 0.47 ± 0.18, 0.79 ± 0.15, 0.59 ± 0.10, 0.43 ± 0.09 respectively. Compared with the CON group, cellular viability of the LPS group significantly fell, so the differences were statistically significant. Compared with the LPS group, cellular viability of the LPS+GAS group increased, so the differences were statistically significant. Compared with the LPS+GAS group, cellular viability of the LPS+GAS+RSL3 groups decreased, so the differences were statistically significant. The above results suggested that inhibiting inflammatory activation of BV2 cells via GAS is closely related to regulation of ferroptosis (Fig. 3).

The effect of RSL3 on activation of microglia

This study observed microglial activation using immunofluorescence. The confocal microscope (200 ×) found that Ibal and DAPI, markers for microglia, exhibited red and blue fluorescence. Compared with the CON group, mean integrated optical density (MIOD) of Ibal in the LPS group increased. Compared with the LPS group, MIOD of Ibal in the LPS+GAS group fell. Compared with the LPS+GAS group, MIOD of Ibal in the LPS+GAS+RSL3 group significantly increased. The results showed that microglial activation in the model is related to regulation of ferroptosis (Fig. 4).

The effect of RSL3 on inflammatory factors

GAS could inhibit microglial activation in the model, but microglial activation may trigger the release of inflammatory factors including IL-1β, IL-6 and TNF-α. Hence, the expression of inflammatory factors was tested using ELISA. The results of IL-1β (15.35 ± 2.23, 36.58 ± 3.71, 29.00 ± 3.43, 43.43 ± 6.07, 47.43 ± 4.19), IL-6 (104.14 ± 10.86, 136.60 ± 9.50, 112.40 ± 9.46, 152.52 ± 18.32, 160.44 ± 12.38), TNF-α (47.71 ± 11.85, 132.78 ± 14.35, 102.59 ± 22.12, 179.39 ± 16.68, 165.22 ± 23.82) were shown in Fig. 5. Compared with the CON group, the expression in the LPS group increased, so the differences were statistically significant. After GAS intervention, the expression in the LPS+GAS group reduced compared with the LPS group, so the differences were statistically significant. Compared with the LPS+GAS group, the expression

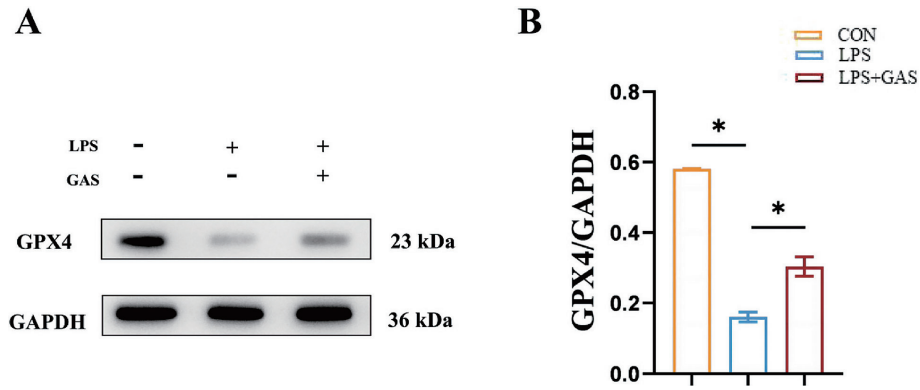

Fig. 2. Protein expression related to cellular death in each group.
(A) Western Blot assay of GPX4 of cells. (B) Related to (A), quantiative analysis of GPX4 protein expression level. The results are presented as mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

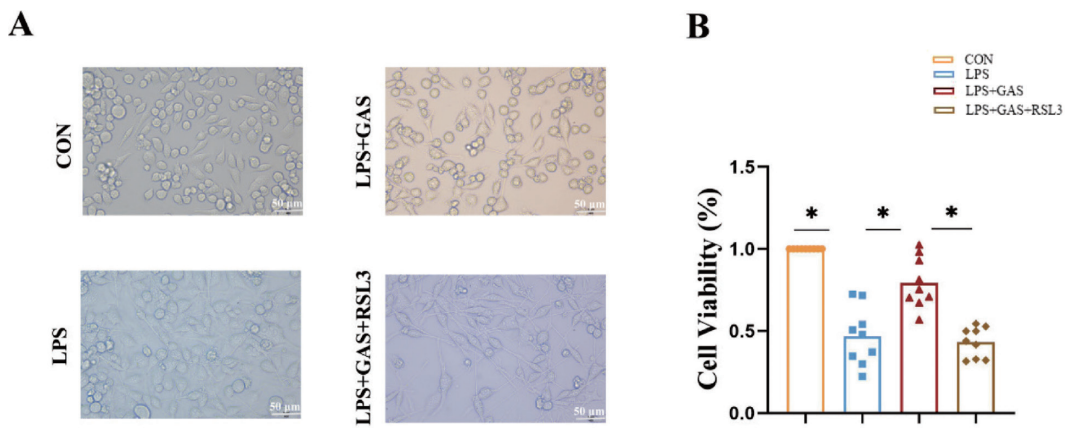

Fig. 3. Cellular morphology and viability in each group.
(A) Cellular morphology. (B) Cellular viability. The results are resented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

in the LPS+GAS+RSL3 group increased, so the differences were statistically significant. The results demonstrated that the generation of inflammatory factors is correlated with regulation of ferroptosis in the model (Fig. 5).

Inhibiting activation pathways of ferroptosis in the model via GAS

Activation of the ferroptosis pathway is a form of cellular death associated with ROS aggregation. After RSL3 intervention, the fluorescence expression of ROS were as follows 8.57 ± 2.59, 30.79 ± 8.43, 23.95 ± 4.30, 52.48 ± 5.69. Compared with the CON group, the expression of ROS increased in the LPS group. Compared with the LPS group, the expression decreased in the LPS+GAS group. Compared with the LPS+GAS group, the expression increased in the LPS+GAS+RSL3 group. The results demonstrated that inhibiting ferroptosis via GAS was closely related to generation of ROS (Fig. 6).

The role of Nrf2 nuclear transposition in the model

Previous studies have confirmed that GAS inhibits inflammation via regulating ferroptosis. ROS were also confirmed as the upstream molecules. Many studies suggest that Nrf2 signaling pathway could clear ROS-mediated oxidative defense system. Hence, ML385, an inhibitor for Nrf2, was applied in these studies. According to the results (11.76 ± 3.87, 28.73 ± 3.45, 20.20 ± 3.76, 68.61 ± 4.38), compared with the CON group, the expression of ROS increased in the LPS group. Compared with the LPS group, the expression decreased in the LPS+GAS group. Compared with the LPS+GAS group, the expression increased in the LPS+GAS+ML385 group (Fig. 7). Meanwhile, studies confirm that Nrf2 signal pathways are activated via Nrf2 nuclear translocation and up-regulating expression of antioxidant enzymes such as HO-1, NQO1, etc. Therefore, the research tested the expression of Nrf2, HO-1 and NQO1 using Western Blot. The results are shown in Fig. 8. Compared with the CON group, the expression of Nrf2 increased but the expression of HO-1 and NQO1 decreased in the LPS group. Compared with the LPS group, the expression of Nrf2, HO-1 and NQO1 increased in the LPS+GAS group. Compared with the LPS+GAS group, the expression Nrf2, HO-1 and NQO1 decreased in the LPS+GAS+ML385 group, so the differences were statistically significant (Fig. 8).

The above experimental results showed that GAS can

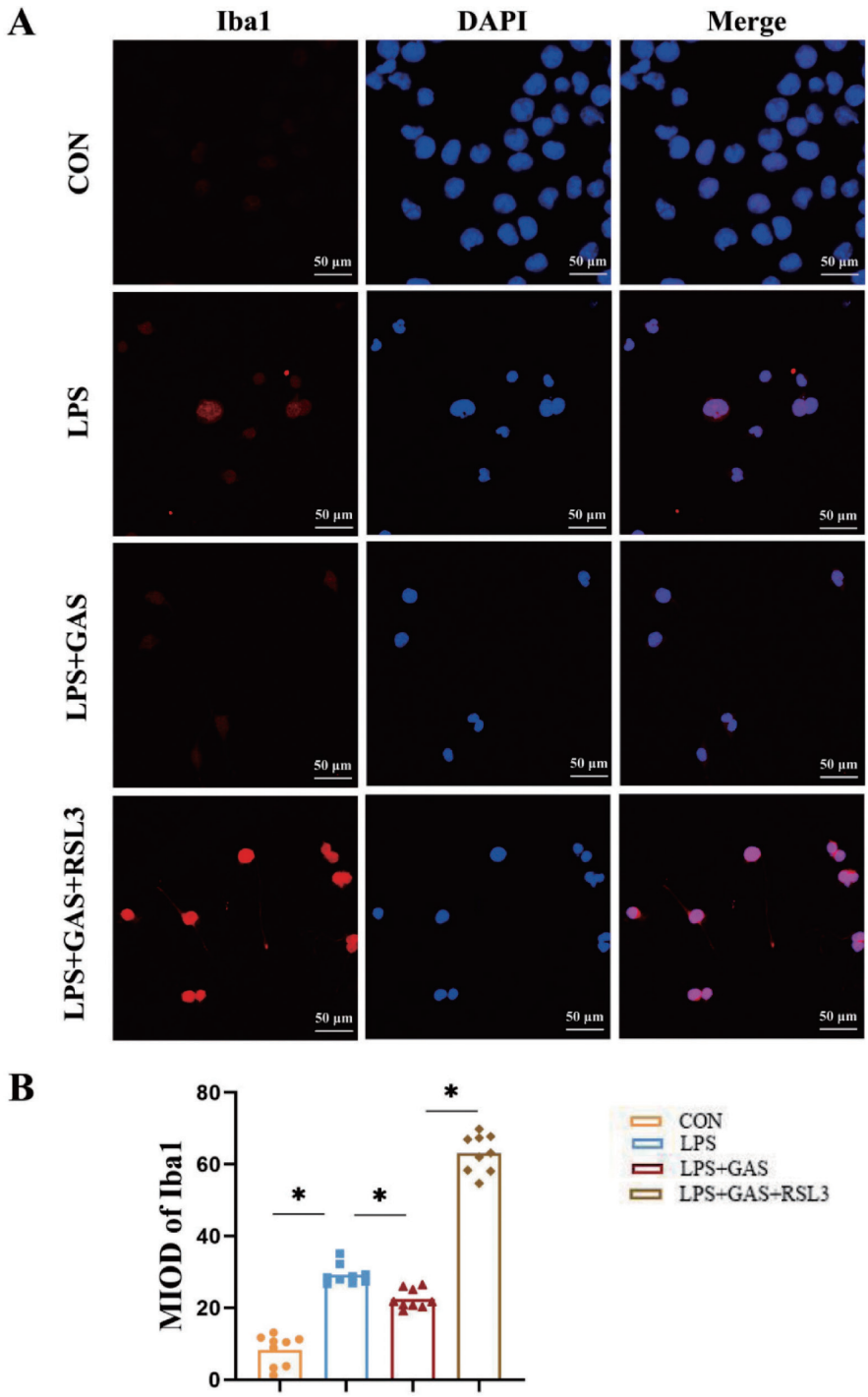

Fig. 4. Ibal expression of BV2 cells in each group.
(A) Immunofluorescence images. (B) MOID of Iba1. The results are presented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

promote Nrf2 nuclear translocation and the expression of HO-1 and NQO1 while reduce ROS level via regulating ferroptosis.

Discussion

As the aging population is deteriorating all over the world, cognitive disorder caused by neurodegenerative diseases have become a major issue that would downgrade the life quality of the elderly at present and in near future. Upon onset of cognitive disorder, the medical burden is greatly increased and the patient's life quality and ability to work is altered (Borges et al. 2017). The onset and development of the disease is closely related to inflammatory factor-mediated neuroinflammation (Wan et al. 2007), so the importance of neuroinflammation is highlighted day by day (Zheng et al. 2017; Safavynia and Goldstein 2018).

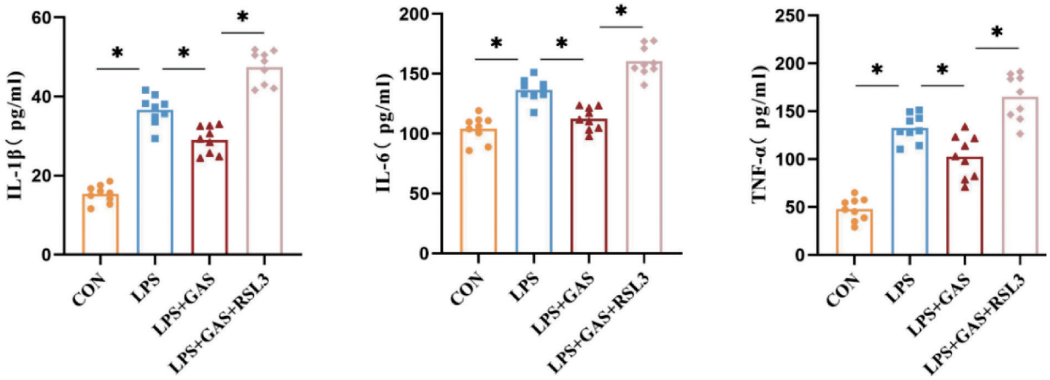

Fig. 5. Expression of inflammatory factors in each group.
The results are presented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

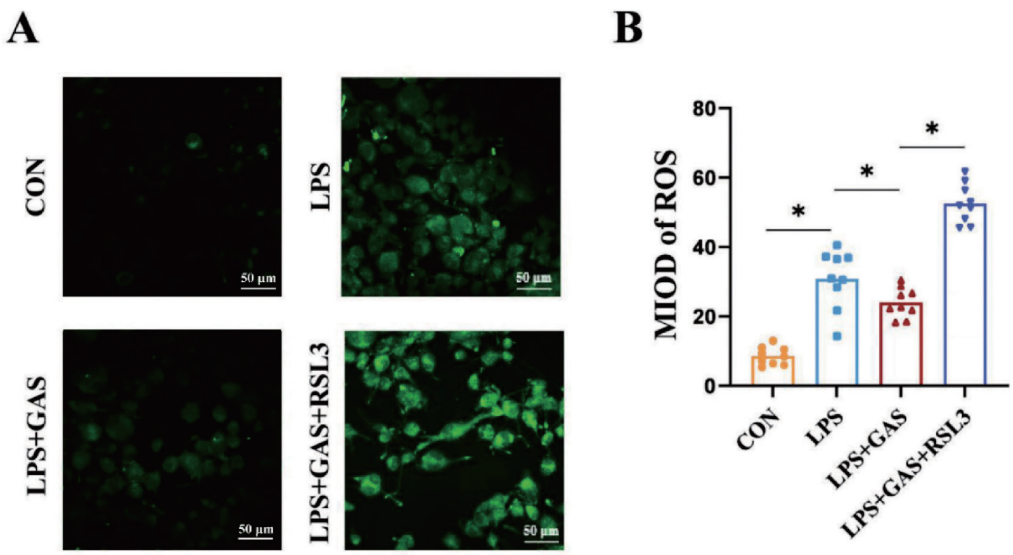

Fig. 6. Activation of ferroptosis pathways in each group.
(A) Immunofluorescence images of ROS. (B) MOID of ROS. The results are presented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

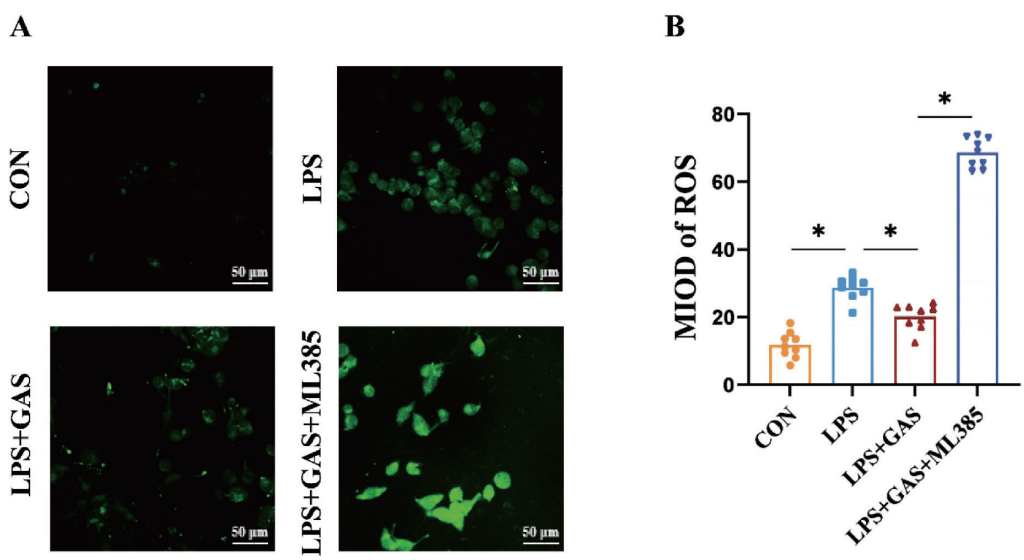

Fig. 7. Activation of ROS during ferroptosis in each group.
(A) Immunofluorescenct images of ROS. (B) MOID of ROS. The results are presented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

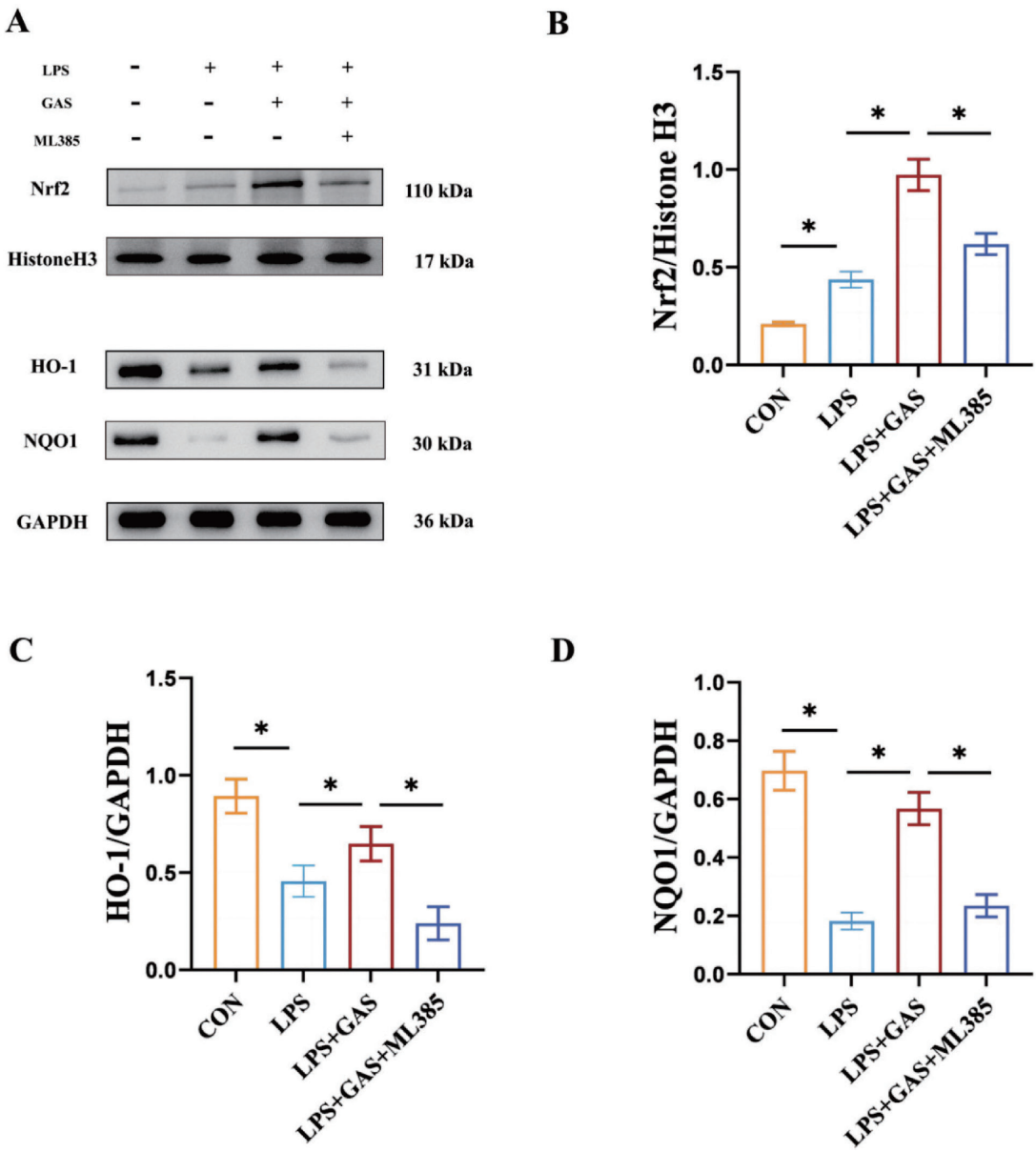

Fig.8. Expression of Nrf2, HO-1 and NQO1 in each group.
(A) Western Blot assay of Nrf2, HO-1, NQO1. (B) Related to (A), quantiative analysis of Nrf2 protein expression level. (C) Related to (A), quantiative analysis of HO-1 protein expression level. (D) Related to (A), quantiative analysis of NQO1 protein expression level. The results are presented as the mean ± S.D. *$P < 0.05$, compared between two groups that are shown by the lines.

Neuroinflammation is initially a defensive response in the brain that removes or inhibits various pathogens. This response can produce beneficial effects by promoting tissue repair and removing cellular debris. However, persistent neuroinflammation produces harmful stimuli to the brain, leading to neuronal damage (Wyss-Coray and Mucke 2002; Kwon et al. 2020; Stuckey et al. 2021). Microglia, as major immune cells in the CNS, are key regulators of harmful stimuli of neuroinflammation. Meanwhile, a series of complex reactions depend on microglia (Mesquida-Veny et al. 2021). For one thing, when microglia are in the resting state, their cytosol is small and exhibits irregularly-branched synapses. For another thing, when they are activated after being invaded by pathogens or damaged cellular debris, the branching synapses shorten and the cytosol becomes larger, presenting an amoeba-like state. After activation, microglia could express multiple pro-inflammatory factors (such as IL-1β, IL-6 and TNF-α) and induce the tremendous generation of NO and ROS which produces harmful effects for neuroinflammation-induced neurodegenerative disease (Liddelow and Barres 2017; Friker et al. 2020). Thus, based on BV2 microglia, the study investigated the mechanism of GAS in preventing and treating neuroinflammation-induced cognitive disorder.

Cellular death, regulated by multiple factors, is a dynamic process that is tightly integrated into disease pro-

gression and plays a significant role in maintaining homeostasis and inhibiting the rapid proliferation of tumor cells and other biological processes (Fuchs and Steller 2011). With advances in studies on cellular death, the significance of GAS is increasingly recognized especially in terms of prevention and treatment of related diseases and the development of targeted medicine. Therefore, this part of the experiments sequentially screened the expression of GPX4 in ferroptosis based on the model and detected which cellular death pathway is regulated by GAS to improve inflammation, thus confirming that GAS may exert anti-inflammatory effects by inhibiting ferroptosis. Evidence shows that potential neuroprotective effects may be exhibited through inhibition of ferroptosis by intestinal flora (Liu et al. 2018). So GAS is expected to be an effective medicine for prevention and treatment of neuroinflammation.

Ferroptosis, a new type of programmed cellular death discovered recently, is a non-apoptotic form of death that depends on Fe^{3+} (Dixon et al. 2012). According to some studies, Erastin can specifically induce the death of RAS mutant cells, but there was no formation of apoptotic vesicles, activation of Caspase family and DNA breakage (Dolma et al. 2003; Nguyen et al. 2020). Hence, it is different from apoptosis and autophagy (Galluzzi et al. 2018). In the present, ferroptosis is another route for GAS to regulate cellular death. Ferroptosis, nonetheless, can be inhibited by iron chelators and be activated by RSL3. After intervention with RSL3, microglial activation was inhibited by LPS-mediated inflammatory activation model via GAS. Microglial activation was observed after using RSL3. On the other hand, the expression of IL-1β, IL-6 and TNF-α increased, indicating that RSL3 weakened the effects of GAS in inhibiting microglial activation and attenuating inflammatory factors. Ferroptosis is triggered by the aggregation of ROS due to abnormal proliferation. The present study also demonstrated that increase in ROS was closely correlated with the fact that GAS improves inflammatory activation of BV2 cells via inhibiting ferroptosis.

However, the role of ROS in the model remains unclear. According to some studies, clearing oxidative defense system of ROS is associated with activation of Nrf2 signaling pathway (Kaspar et al. 2009). Nrf2 is a basic leucine zipper transcription factor. It up-regulates NQO1 through interaction with the antioxidant response element (ARE). Meanwhile, heme oxygenase-1 (HO-1), an antioxidant enzyme upregulated by Nrf2, inhibits the activation of NOX2 and attenuates the tissue damage caused by oxidative stress (Kaspar et al. 2009; Wang et al. 2020). There is evidence that Nrf2 plays a critical defensive role in the brain for coordinating antioxidant responses (Villavicencio Tejo and Quintanilla 2021). Another important association of Nrf2 is with neuroinflammation in pathological changes such as Alzheimer's and Parkinson's disease (Castro-Sanchez et al. 2019; Sivandzade et al. 2019). Neuroinflammation is a process characterized by changes in the morphology of glial cells, such as microglia and astrocytes, and the release of inflammatory factors. Long-term and chronic inflammation in the CNS leads to increase in release of inflammatory mediators and oxidative stress, which sustains the neuroinflammatory cascade response and accelerates neuronal dysfunction (Scuderi et al. 2020). Nrf2 is activated in stressed microglia, so there is a correlation between damage to these cells and oxidative stress in neurodegenerative diseases (Sivandzade et al. 2019). Activation of the Nrf2 signalling pathway and clearing ROS in the oxidative defense system are closely linked to inflammation associated with neurodegenerative diseases. In a related study on the interaction between asparagine and Nrf2, Li et al. (2022a) found that GAS inhibited ferroptosis via the Nrf2/Keap-1-GPX4 pathway, thereby ameliorating cognitive dysfunction in rats with vascular dementia. In addition, Huang et al. (2022) demonstrated in a model of podocyte damage caused by diabetic nephropathy that GAS could effectively inhibit high glucose-induced inflammation, oxidative stress and apoptosis through the AMPK-Nrf2 pathway. Hence, GAS could take effect via Nrf2 pathways. In the present study, GAS could up-regulate the expression of downstream HO-1 and NQO1 via activating Nrf2 pathways by transferring Nrf2 protein to the nucleus.

In conclusion, the present study demonstrated an in-vitro analysis and posed an anti-inflammatory effect of GAS. It has shown that GAS can promote Nrf2 nuclear translocation and the expression of HO-1 and NQO1 while reducing ROS level. Therefore, GAS can regulate ferroptosis in LPS-induced BV2 cellular inflammation model, thus attenuating inflammatory occurrence.

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Conflict of Interest

The authors declare no conflict of interest.

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