Adenosine A1 receptor agonist alleviates cerebral ischemia/reperfusion injury by inhibiting Nrf2/NLRP3 signaling-mediated pyroptosis

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Abstract. The present study aimed to investigate whether adenosine A1 receptor (A1R) agonists can alleviate cerebral ischemia/reperfusion (I/R) injury by inhibiting pyroptosis mediated through the nuclear factor erythroid 2-related factor 2 (Nrf2)/NLR family pyrin domain containing 3 (NLRP3) signaling pathway. A total of 36 Sprague-Dawley rats were randomly assigned to the following four groups: Sham (sham group), I/R (model group), adenosine A1 receptor agonist preconditioning [model + adenosine A1R agonist 2-chloro-N(6)-cyclopentyladenosine] group and ML385 (model + adenosine A1R agonist group + Nrf2 pathway inhibitor group). A middle cerebral artery occlusion model was induced using the thread occlusion method. Neurological function was assessed using the Longa scale, brain infarction volume was determined through 2,3,5-triphenyltetrazolium chloride staining, protein expression of Nrf2, NLRP3, caspase-1, GSDMD and IL-1β was assessed using western blotting, and the expression of Nrf2 and GSDMD was assessed using immunofluorescence. The findings revealed that adenosine A1R agonist improved neurological function and reduced infarct size. Mechanistically, this was found to be associated with the activation of the Nrf2/NLRP3 signaling pathway and the suppression of pyroptosis-associated proteins (gasdermin D, caspase-1 and IL-1β) expression. Notably, the Nrf2 inhibitor ML385 reversed the aforementioned effects induced by the adenosine A1R agonist. These results suggest that adenosine A1R agonist can alleviate cerebral I/R injury in rats, potentially by modulating the Nrf2/NLRP3 signaling pathway to inhibit pyroptosis.

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Introduction

Ischemic stroke is a one of the worldwide leading causes of death, with an incidence of ~7 million individuals annually, and a leading cause of disability, as expressed by >160 million disability-adjusted life-years lost, representing a significant socioeconomic burden. The estimated global cost is >US$890 billion annually (1). The primary treatment strategy for ischemic stroke is the rapid restoration of blood flow. However, reperfusion of the ischemic brain tissue can worsen the injury, which is caused a phenomenon known as cerebral ischemia/reperfusion (I/R) injury (2). This condition involves multiple pathological processes, including pyroptosis, apoptosis, oxidative stress and the inflammatory response (3). Current treatment options for cerebral I/R injury are limited, as although substances such as antioxidants and anti-inflammatory agents have demonstrated neuroprotective effects in models of cerebral I/R injury, consistent results have not yet been achieved in further clinical trials (4). This highlights the urgent need for the development of novel therapeutic strategies.

Pyroptosis is a form of inflammatory programmed cell death that has been documented to serve a key role in cerebral I/R injury (5,6). It is characterized by activation of the NLR family pyrin domain containing 3 (NLRP3) inflammasome, which in turn activates caspase-1 to cleave gasdermin D (GSDMD) and promotes the release of various inflammatory cytokines, such as IL-1β and IL-18 (7). These cytokines, through binding to their respective receptors, initiate inflammatory signaling cascades that ultimately result in pyroptotic cell death (8).

Adenosine is an endogenous purine nucleoside and can mediate a variety of physiological functions through interactions with its receptors (9). There are four currently known subtypes of adenosine receptors (A1, A2a, A2b and A3), which are widely distributed throughout the body and are therefore implicated in a range of pathological conditions, including ischemic cerebrovascular diseases, immune disorders, cardiovascular diseases and inflammation, thereby underscoring the

critical importance of selecting adenosine receptors based on their subtypes (10). Amongst them, adenosine A1 receptors (A1Rs) exert neuroprotective effects in the central nervous system (11). Recent studies have shown that adenosine A1R agonist [2-chloro-N(6)-cyclopentyladenosine (CCPA)] can alleviate cerebral I/R injury in the MCAO model (12), although the exact mechanisms remain unclear.

The Nrf2/NLRP3 pathway is a key pathway in regulating pyroptosis (13). Therefore, the present study aimed to investigate the effects of the adenosine A1R agonist on the Nrf2/NLRP3 signaling pathway and pyroptosis in rats, to elucidate the possible mechanism of adenosine A1R agonist in cerebral I/R injury.

Materials and methods

Experimental animals. A total of 36 healthy, male Sprague-Dawley rats (age, 6-7 weeks; weight, 230-250 g) were provided by Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. [license no. SCXK (Beijing) 2021-0011]. The temperature in the animal room was controlled at 25±1˚C and the air humidity was 60-65%. Animals were housed under a 12-h light/dark cycle and had free access to food and water *ad libitum*. The present study was approved by the Animal Experiment Ethics Committee of Xinxiang Medical University (approval no. K2023-64-01; Xinxiang, China).

Main drugs, reagents and instruments. Adenosine A1R agonist CCPA (cat. no. 119136) was purchased from MilliporeSigma. The NLRP3 (cat. no. 38679) and IL-1β antibodies (cat. no. 41059) were obtained from Signalway Antibody LLC. The GSDMD (cat. no. AF4012), caspase-1 (cat. no. AF5418), Nrf2 (cat. no. AF0639) and GAPDH antibodies (cat. no. AF7021) were obtained from Affinity Biosciences. The Nrf2 inhibitor ML385 (cat. no. T4360) was purchased from TargetMol Chemicals, Inc. Western blotting electrophoresis equipment was obtained from Bio-Rad Laboratories, Inc. A cryogenic grinder was purchased from Shanghai Jingxin Industrial Development Co., Ltd.

Animal grouping and model establishment. A total of 36 Sprague-Dawley rats were randomly divided into the following four groups: Sham, I/R, adenosine A1 receptor agonist preconditioning (AP) and ML385, with 9 rats in each group. Amongst these, 3 rats were used for 2,3,5-triphenyltetrazolium chloride (TTC) staining, 3 for western blot analysis and 3 rats for immunofluorescence. The sham and I/R groups were given intraperitoneal injections with an equal amount of physiological saline (0.9% NaCl, 2 ml/kg) (14) 3 days before surgery, once per day. The AP group was administered the adenosine A1R agonist (200 μg/kg dissolved in physiological saline) by intraperitoneal injection 3 days before surgery, once per day (15). The ML385 group received intraperitoneal injections of ML385 + adenosine A1R agonist (30 mg/kg + 200 μg/kg, respectively) 3 days before surgery, once per day. The dosages used were based on previous studies (16,17).

The middle cerebral artery occlusion (MCAO) model was prepared using the Longa method (18). After an intraperitoneal injection of 40 mg/kg pentobarbital sodium for anesthesia, the rats were fixed in a supine position and a midline longitudinal incision was made in the neck to separate the left common carotid artery (CCA), external carotid artery (ECA) and the internal carotid artery (ICA). A V-shaped oblique incision was made at the bifurcation of the ECA and ICA using vascular scissors. The arterial clamp was then reopened and the embolization line was inserted through the residual end of the ECA into the ICA until slight resistance was felt, after which the insertion was stopped. The upper end of the CCA was then ligated to prevent bleeding and movement. Blood flow was blocked for 2 h, the filament was withdrawn, and after reperfusion for 24 h, neurological function scoring was conducted. The sham group did not undergo middle cerebral artery embolization.

The following criteria were used to determine when animals should be immediately euthanized during the 24 h of reperfusion: i) Lack of movement or unresponsiveness to gentle stimuli; ii) respiratory distress (typical symptoms include drooling from the mouth or nose and/or cyanosis); iii) diarrhea or urinary incontinence; iv) weight loss of >20% compared with the pre-experiment body weight; v) inability to eat or drink; vi) persistent seizures or stereotyped behavior; and vii) skin lesions covering >30% of the body or signs of purulent infection. The surgical procedure was smooth, with no rat fatalities.

All rats were euthanized with an intraperitoneal injection of sodium pentobarbital (150 mg/kg) 24 h after MCAO. Death was confirmed based on the absence of pain responses, complete cessation of cardiac and respiratory activity and pupil dilation.

Neurobehavioral function score. Neurobehavioral function score was assessed at 24 h after reperfusion using the Longa method (19). Scores of 1-4 were considered indicative of a successful model. The scoring scale used was as follows: 0, no neurological deficits; 1, failure to fully extend the right forepaw (mild deficits); 2, turning in circles to the right while crawling (moderate deficits); 3, falling to the right (moderate deficits); 4, not able to walk independently and exhibiting a depressed level of consciousness (severe deficits).

TTC staining. After rat euthanasia, the brain was quickly removed, placed in a -20˚C freezer for 20 min. The brain was cut into five 2-mm coronal sections. The slices were soaked in a 2% TTC solution and stained in the dark at 37˚C, then the brain slices were fixed with 4% paraformaldehyde at 4˚C for 24 h. The non-ischemic area appeared red, whilst the infarcted area was white. ImageJ software (version 1.6.0; National Institutes of Health) was used for image analysis, where the infarct volume ratio (%) was calculated using the following formula: Infarct volume ratio=(infarct area/total brain volume) x100% = ($\sum$ infarct area x slice thickness)/($\sum$ total brain area x slice thickness) x100%.

Western blotting. The separated cerebral cortex was stored at -80˚C for subsequent protein extraction. The tissue samples (50 mg each) were lysed with RIPA lysis buffer (Beyotime Institute of Biotechnology) at 4˚C, before the tissue homogenate was centrifuged at 4˚C and at 12,000 x g for 30 min to extract proteins. The protein concentration was determined using the BCA method (Bio-Rad Laboratories, Inc.). Proteins samples

(50 μg/lane) were separated on a 10% gel by SDS-PAGE and transferred onto a PVDF membrane. After blocking the membrane with 5% milk at room temperature for 2 h, diluted primary antibodies (1:1,000; diluted in blocking solution) against GAPDH, Nrf2, NLRP3, caspase-1, GSDMD and IL-1β were added and incubated overnight at 4˚C. Subsequently, anti-rabbit IgG (H+L) secondary antibody (cat. no. 14708; 1:1,000; Cell Signaling Technology, Inc.) was added and the membrane was incubated in the dark at room temperature for 1 h. The membrane was visualized using an enhanced chemiluminescence detection kit (cat. no. WBULS0100; Merck KGaA). ImageJ software (version 1.6.0; National Institutes of Health) was used to analyze the target bands and calculate the band density, before protein expression was normalized to GAPDH levels.

Immunofluorescence. The brain tissue was fixed in a 4% paraformaldehyde solution at 4˚C for 24 h, before 20-μm-thick slices were prepared in coronal position using a cryostat at -20˚C. Slices containing the hippocampal dentate gyrus region were selected for immunofluorescence analysis. The slices were permeabilized with 0.2% Triton X-100 and blocked with 10% normal goat serum (cat. no. 16210064; Thermo Fisher Scientific, Inc.) at room temperature for 45 min. Subsequently, 35 μl diluted (1:200) Nrf2 and GSDMD primary antibodies were added to the sections and incubated overnight at 4˚C. After washing three times with PBS, the slices were incubated with 35 μl goat anti-rabbit IgG (H+L) Fluor488-conjugated secondary antibody (cat. no. S0018; 1:1,000; Affinity Biosciences) at room temperature in the dark for 2 h. The tissue was stained with DAPI (cat. no. C1005; 1:1; Shanghai Biotium Biological Co., Ltd.) at 25˚C for 5 min, followed by mounting with glycerol. Images were obtained using a Leica microscope (Leica DM 2000, Leica Microsystems, Inc.) and Leica microscope software (LAS V3.7). The number of positive stained cells was counted using ImageJ software (version 1.6.0; National Institutes of Health).

Statistical analysis. Statistical analysis was performed using GraphPad Prism 9 software (Dotmatics). The neurobehavioral function score is presented as the median (interquartile range), whilst all other data are presented as the mean ± standard deviation. The normality of the data was assessed using the Shapiro-Wilk test, whereas the homogeneity of variance was examined using the Brown-Forsythe test. One-way ANOVA followed by Tukey's post hoc test was used for comparisons among multiple groups, whilst the neurobehavioral function score was evaluated using Kruskal-Wallis followed by Dunn's post hoc test. P<0.05 was considered to indicate a statistically significant difference.

Results

Neurobehavioral function score and cerebral infarction volume of rats. Compared with that in the sham group, the neurobehavioral function score in the I/R group was found to be significantly increased (P<0.05). Compared with that in the I/R group, the AP group exhibited a significant decrease in the neurobehavioral function score (P<0.05). By contrast, compared with that in the AP group, the neurobehavioral function score of the ML385 group was significantly higher (P<0.05; Table I).

Table I. Neurobehavioral function score in each group.

Group	Neurobehavioral function score
Sham	0 (0,0)
Ischemia/reperfusion	4 (3,4)[a]
AP	2 (1,2)[b]
ML385	3 (3,3.5)[c]
Overall P-value	<0.01

Data are presented as the median (25th percentile, 75th percentile) with n=9 per group. [a]P<0.05 vs. sham; [b]P<0.05 vs. I/R; [c]P<0.05 vs. AP. AP, adenosine A1 receptor agonist preconditioning.

TTC staining of the brain tissue showed minimal infarcted area in the sham group. However, compared with that in the sham group, the I/R group showed a significantly larger area of ischemic infarction (P<0.05). Compared with that in the I/R group, the cerebral infarction volume of the rats in the AP group was significantly decreased (P<0.05). Compared with that in the AP group, the ML385 group exhibited a significant increase in the cerebral infarction volume (P<0.05; Fig. 1 and Table II).

Expression of pyroptosis-associated proteins in the hippocampal tissue of rats. The protein expression levels of caspase-1, GSDMD and IL-1β in the hippocampus of rats were next detected through western blotting. The results showed compared with those in the sham group, the protein expression levels of caspase-1, GSDMD and IL-1β were all significantly increased in the I/R group (P<0.05). Compared with those in the I/R group, the expression levels of caspase-1, GSDMD and IL-1β were significantly reduced in the AP group (P<0.05). By contrast, compared with those in the AP group, the ML385 group exhibited significantly increased protein expression levels of caspase-1, GSDMD and IL-1β (P<0.05; Fig. 2 and Table II).

Immunofluorescence assay was then used to detect the expression of GSDMD in rat hippocampal dentate gyrus. The results showed that compared with that in the sham group, the I/R group showed significantly increased expression of GSDMD (P<0.05). Compared with that in the I/R group, the AP group showed a significant decrease in GSDMD expression (P<0.05). However, compared with that in the AP group, the ML385 group showed a significant increase in GSDMD expression (P<0.05; Fig. 3 and Table II).

Expression of Nrf2 and NLRP3 proteins in the hippocampal tissue of rats. The Nrf2/NLRP3 pathway is a key pathway in regulating pyroptosis (13). The protein expression levels of Nrf2 and NLRP3 were detected via western blotting. The results showed that compared with those in the sham group, the I/R group exhibited a significant decrease in the Nrf2 protein expression level (P<0.05) and a significant increase in the NLRP3 protein expression level (P<0.05). Compared with that in the I/R group, the expression level of Nrf2 was significantly increased (P<0.05) whilst the expression level

Table II. Cerebral infarction volume and protein expression levels of GSDMD, caspase-1 and IL-1β in each group.

Group	Cerebral infarct volume, %	Western blotting			Immunofluorescence
		GSDMD	Caspase-1	IL-1β	GSDMD
Sham	0.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	2.00±1.00
I/R	0.37±0.02[a]	1.43±0.03[a]	2.54±0.07[a]	2.99±0.16[a]	34.33±3.06[a]
AP	0.14±0.02[b]	1.14±0.02[b]	1.48±0.05[b]	1.67±0.08[b]	5.67±2.08[b]
ML385	0.30±0.01[c]	1.27±0.03[c]	2.39±0.06[c]	2.78±0.09[c]	27.00±2.65[c]
F-value	455.96	184.43	620.25	269.36	127.73
P-value	<0.01	<0.01	<0.01	<0.01	<0.01

Data are presented as the mean ± standard deviation (n=3 per group). Western blot results, protein expression levels are presented as relative protein expression, normalized to GAPDH as the internal control. For immunofluorescence quantification, the results are based on the counting of positive cells, with the number of positive cells quantified in each group. [a]P<0.05 vs. sham; [b]P<0.05 vs. I/R; [c]P<0.05 vs. AP. GSDMD, gasdermin D; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

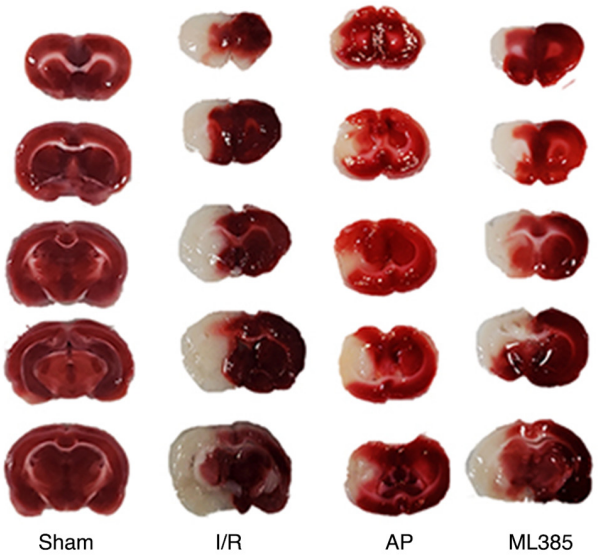

Figure 1. Cerebral infarct areas of rats in each group, assessed by 2,3,5-triphenyltetrazolium chloride staining (n=3 per group). The red brain tissue represents the normal area, and the white brain tissue represents the ischemic infarct area. Each rat's brain was sectioned into 5 coronal slices (2-mm thick). I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

Table III. Protein expression levels of Nrf2 and NLRP3 in each group.

Group	Western blotting		Immunofluorescence
	Nrf2	NLRP3	Nrf2
Sham	1.00±0.00	1.00±0.00	17.33±2.52
I/R	0.67±0.02[a]	2.34±0.16[a]	2.00±1.00[a]
AP	0.92±0.01[b]	1.46±0.08[b]	8.67±2.08[b]
ML385	0.81±0.01[c]	1.90±0.06[c]	2.67±1.53[c]
F-value	324.64	106.20	43.30
P-value	<0.01	<0.01	<0.01

Data are presented as the mean ± standard deviation (n=3 per group). Western blot results, protein expression levels are presented as relative protein expression, normalized to GAPDH as the internal control. For immunofluorescence quantification, the results are based on the counting of positive cells, with the number of positive cells quantified in each group. [a]P<0.05 vs. sham; [b]P<0.05 vs. I/R; [c]P<0.05 vs. AP. Nrf2, nuclear factor erythroid 2-related factor 2; NLRP3, NLR family pyrin domain containing 3; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

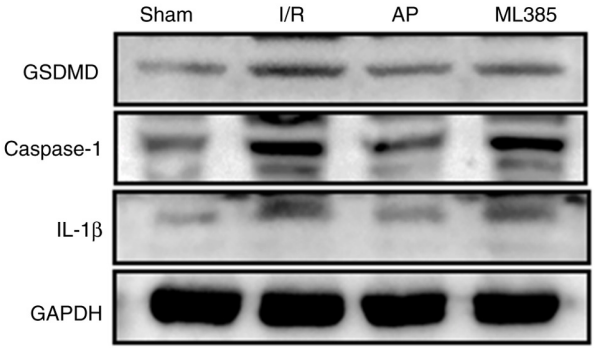

Figure 2. Protein expression levels of caspase-1, GSDMD and IL-1β in the hippocampal tissue of rats in each group as assessed by western blotting. GSDMD, gasdermin D; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

of NLRP3 was significantly decreased (P<0.05) in the AP group. Furthermore, compared with those in the AP group, the ML385 group showed a significant decrease in the Nrf2 protein expression level (P<0.05) and a significant increase in the NLRP3 protein expression level (P<0.05; Fig. 4 and Table III).

Immunofluorescence assay was then used to detect Nrf2 protein expression in rat hippocampal dentate gyrus. The results showed that compared with that in the sham group, the I/R group showed a significant decrease in Nrf2 protein expression (P<0.05). Compared with that in the I/R group, the expression of Nrf2 was significantly increased in the AP group (P<0.05). By contrast, compared with that in the AP group, the ML385 group exhibited a significant decrease in Nrf2 expression (P<0.05; Fig. 5 and Table III).

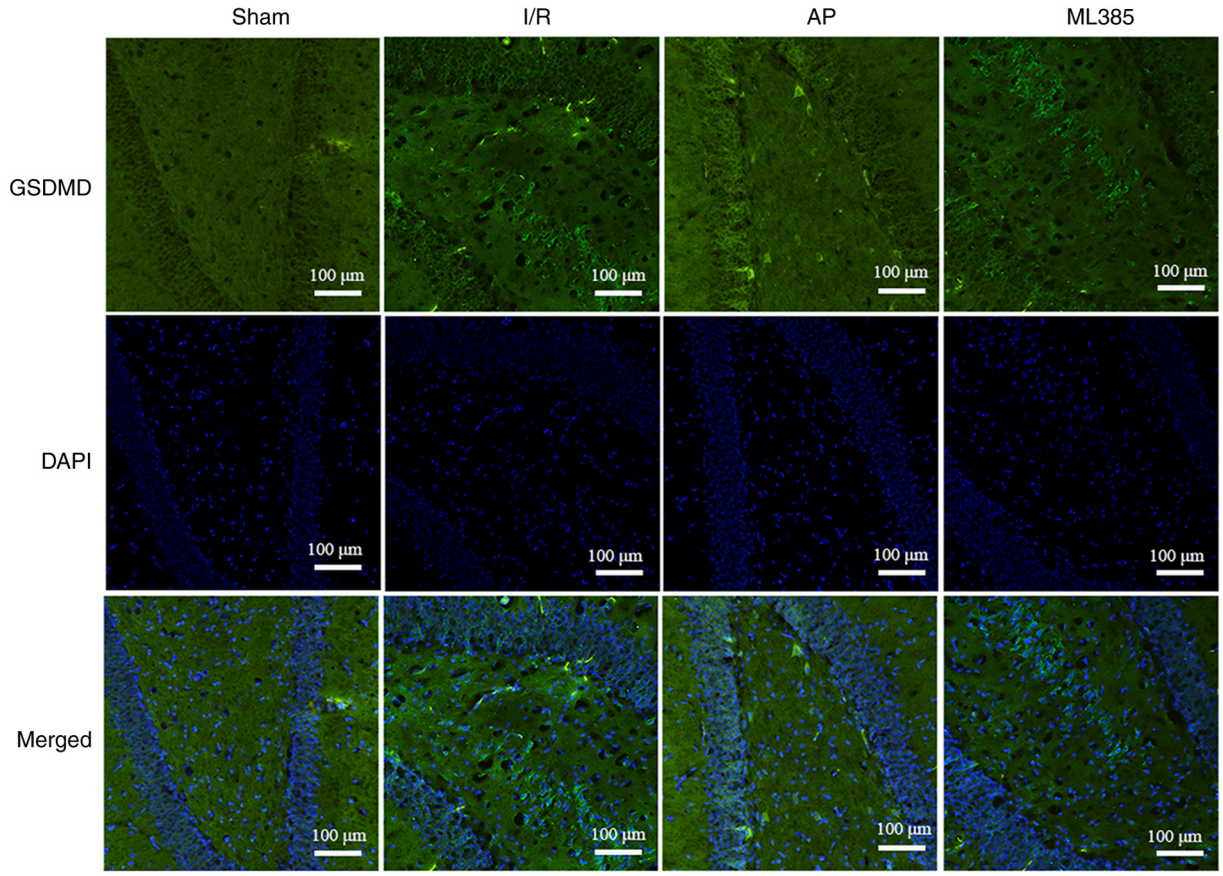

Figure 3. Expression of GSDMD in the hippocampal dentate gyrus region of rats in each group as assessed by immunofluorescence staining (scale bars, 100 μm). GSDMD, gasdermin D; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

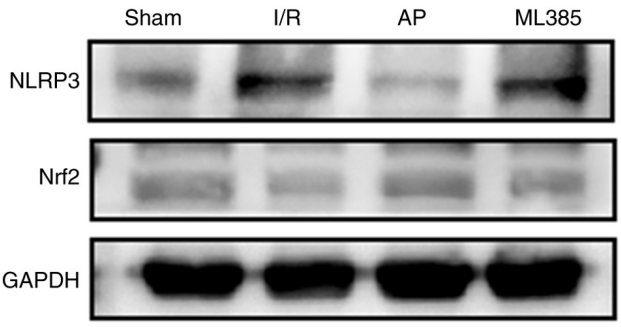

Figure 4. Protein expression levels of Nrf2 and NLRP3 in the hippocampal tissue of rats in each group as assessed by western blotting. Nrf2, nuclear factor erythroid 2-related factor 2; NLRP3, NLR family pyrin domain containing 3; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

Discussion

Under ischemic, hypoxic, inflammatory and/or stressful conditions, CD39 and CD73 catalyze the conversion of extracellular ATP into adenosine, resulting in a notable increase in adenosine concentrations (20). It has been previously demonstrated that the elevation of extracellular adenosine levels is associated with an endogenous neuroprotective response against neuronal damage caused by ischemic or inflammatory stress (21,22). In particular, adenosine A1R serves an important regulatory role in the central nervous system, participating in various physiological and pathological processes, such as the regulation of neurotransmitter release, modulation of neuronal excitability, neuroprotection in ischemic injury and the inhibition of inflammation (23). A previous study has shown that intraperitoneal injection of CCPA could reduce the infarct size in an MCAO rat model (24). Another previous study has also suggested that activation of adenosine A1R is beneficial for individuals with acute ischemic hypoxic injury, particularly in the brain (25). Consistent with the aforementioned previous studies, the present study demonstrated neuroprotective effects of adenosine A1R agonist in alleviating cerebral I/R injury. Unlike a previous study (12), the present study revealed that the adenosine A1R agonist not only significantly reduced the cerebral infarct volume and ameliorated neurological deficits in rats, but also inhibited pyroptosis downstream of the Nrf2/NLRP3 signaling pathway (Fig. 6). To the best of our knowledge, the present study was the first to reveal that adenosine A1R agonist can alleviate cerebral I/R injury, potentially by activating the Nrf2/NLRP3 pathway to inhibit pyroptosis, thereby providing novel insights into the mechanism by which adenosine A1R agonist can ameliorate cerebral I/R injury.

Pyroptosis is a form of cell death that is typically associated with inflammatory responses. During pyroptosis, rupture of the cell membrane leads to the release of intracellular contents and proinflammatory factors into the extracellular environment. Pro-caspase-1 is cleaved into its active form,

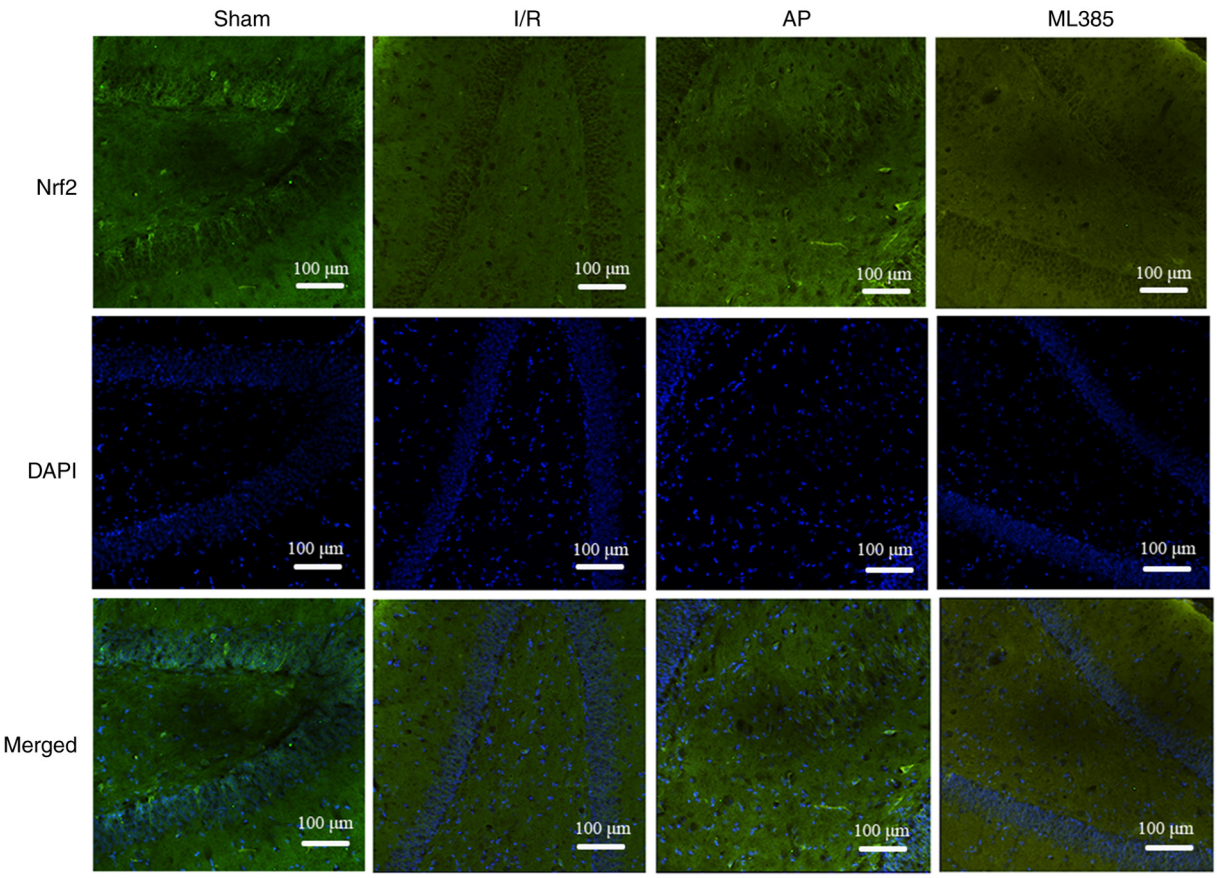

Figure 5. Expression of Nrf2 in the hippocampal dentate gyrus region of rats in each group as assessed by immunofluorescence staining (scale bars, 100 μm). Nrf2, nuclear factor erythroid 2-related factor 2; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning.

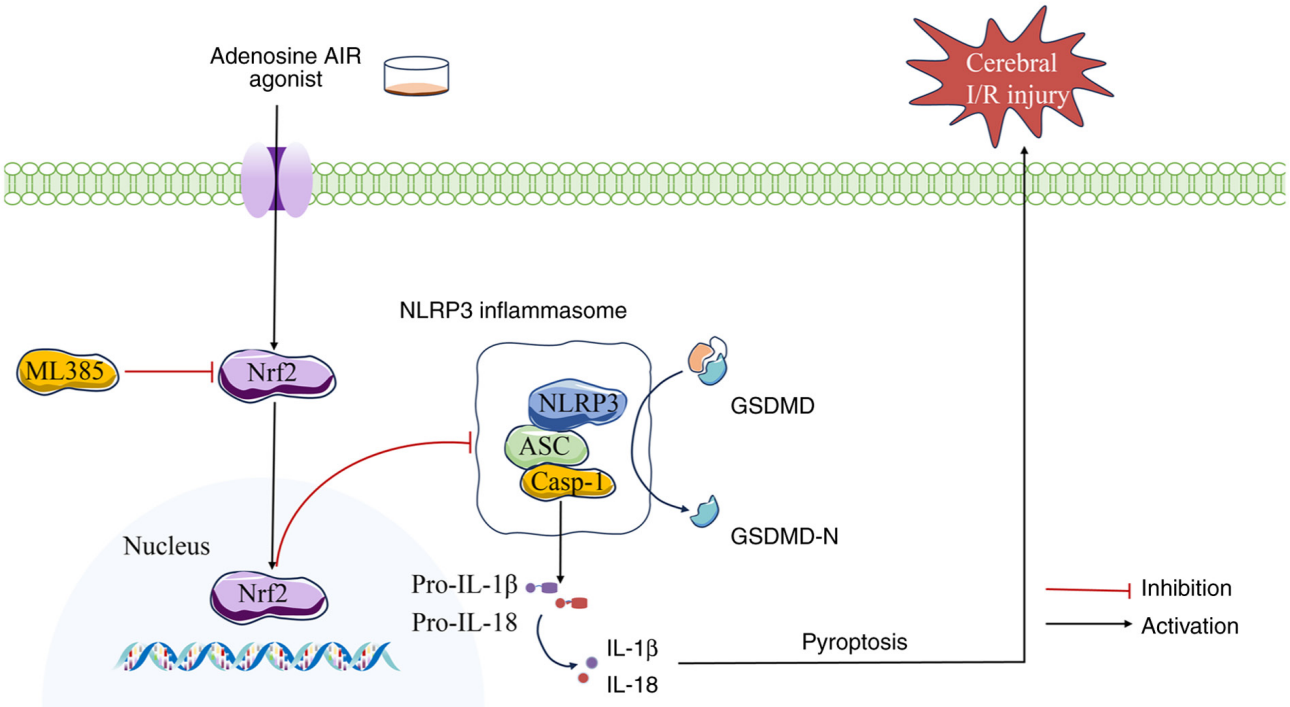

Figure 6. Adenosine A1R agonist inhibits pyroptosis mediated by the Nrf2/NLRP3 signaling pathway, thereby ameliorating cerebral I/R injury. The adenosine A1R agonist improves cerebral I/R injury by activating Nrf2, inhibiting the NLRP3 inflammasome and downregulating pyroptosis-related proteins such as caspase-1, GSDMD and IL-1β, thereby suppressing pyroptosis. The effects of the A1R agonist are reversed upon the use of the Nrf2 inhibitor ML385. A1R, A1 receptor; Nrf2, nuclear factor erythroid 2-related factor 2; NLRP3, NLR family pyrin domain containing 3; GSDMD, gasdermin D; I/R, ischemia/reperfusion; AP, adenosine A1 receptor agonist preconditioning. Casp-1, caspase-1; GSDMD-N, N-terminal domain of GSDMD; ASC, apoptosis-associated speck-like protein containing a caspase recruitment domain.

caspase-1, by inflammasomes, which then cleaves GSDMD into two N-terminal fragments and mediates the formation of membrane pores, allowing water to enter the cell, causing swelling, cell lysis and the release of IL-1β and IL-18 (26). It has been previously demonstrated that pyroptosis is associated with the increased expression of caspase-1, GSDMD, IL-1β and IL-18 (27). A previous study demonstrated that the expression of pyroptosis-associated proteins increases after cerebral I/R injury, thereby indicating that pyroptosis may be involved in this process (28). Consistent with the aforementioned studies, the present study observed an increase in the expression of caspase-1, GSDMD and IL-1β after cerebral I/R injury. Notably, administration of adenosine A1R agonist resulted in the decreased expression of these proteins, suggesting that the neuroprotective effect of the adenosine A1R agonist in cerebral I/R injury is closely associated with pyroptosis.

To investigate the specific mechanism by which adenosine A1R agonist alleviates pyroptosis after cerebral I/R injury, the signaling pathways involved were further examined. Nrf2 is an important regulatory factor in cerebral I/R injury. Upon cellular injury, Nrf2 is activated and transferred to the nucleus, where it binds to antioxidant response elements in the promoter regions of various target genes. These target genes include those involved in oxidative stress regulation, cellular repair and inflammation (29). Notably, activation of the Nrf2/NLRP3 pathway has been demonstrated to mitigate cerebral I/R injury in the rat MCAO model (30). The present study also supports this finding, revealing that the amelioration of cerebral I/R injury after the administration of adenosine A1R agonist was accompanied by the upregulation of Nrf2 and the downregulation of NLRP3. The Nrf2/NLRP3 pathway is a key signaling pathway regulator of pyroptosis (31). A previous study has indicated that activation of the Nrf2/NLRP3 signaling pathway can suppress the expression of pyroptosis-associated proteins in neuronal cells of the brain in the cerebral I/R injury model (32). The present study demonstrated that adenosine A1R agonist not only led to the activation of the Nrf2/NLRP3 pathway but also the inhibition of pyroptosis. However, in contrast with a previous study (33), after the co-administration of adenosine A1R agonist and the Nrf2-specific inhibitor ML385, the effect of adenosine A1R agonist in inhibiting pyroptosis was reversed, thereby demonstrating that the inhibition of pyroptosis by the adenosine A1R agonist following cerebral I/R injury is dependent on the Nrf2/NLRP3 signaling pathway. To the best of our knowledge, the present study demonstrates for the first time that adenosine A1R agonist can alleviate cerebral ischemia/reperfusion injury by inhibiting Nrf2/NLRP3 signaling-mediated pyroptosis.

In conclusion, the present study demonstrated that adenosine A1R agonist can improve cerebral I/R injury, potentially by inhibiting pyroptosis mediated by the Nrf2/NLRP3 signaling pathway. However, there are certain limitations. Future studies should examine the active/cleaved forms of certain proteins, such as GSDMD, caspase-1 and IL-1β, to confirm the effect of A1R agonist on pyroptosis. The association between adenosine A1R and the Nrf2/NLRP3 signaling pathway is complex, where other signaling pathways, such as PI3K, may also be involved. Previous studies have shown that adenosine A1R agonist can activate the PI3K/Akt pathway in neurons (34,35). Nrf2 is a key molecule in inhibiting pyroptosis to ameliorate cerebral I/R injury, but it is also a downstream molecule of the PI3K/Akt pathway (36). Therefore, future research will further explore the association between adenosine A1R and the Nrf2/NLRP3 signaling pathway and validate these findings in clinical settings.

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Availability of data of materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

QM and ZL designed the study protocol, performed data analysis and wrote the final manuscript. JT and YL contributed to the study design, data analysis and revised the manuscript. QM and ZL confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Xinxiang Medical University (approval no. K2023-64-01; Xinxiang, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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