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Amifostine ameliorates cerebral ischaemia-reperfusion injury *via* p38-mediated oxidative stress and mitochondrial dysfunction

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Abstract

Amifostine is a cytoprotective compound that is beneficial in ischaemic stroke cases. However, the neuroprotective effect of amifostine on ischaemia/reperfusion (I/R)-induced brain injury and its underlying mechanism are still poorly understood. Herein, we constructed an animal model of middle cerebral artery occlusion and reperfusion (MCAO/R) injury and an in vitro model of oxygen and glucose deprivation and reperfusion (OGD/R) injury. After administration of amifostine, we found significant improvements in neurological deficits, infarct size, and cerebral oedema. Moreover, amifostine alleviated histopathological alteration and increased the number of surviving neurons. Biochemical analysis showed that treatment with amifostine obviously improved the brain damage of MCAO/R mice, as manifested by a decrease in reactive oxygen species (ROS) and malondialdehyde (MDA) generation, and an increase in superoxide dismutase (SOD) activity. Moreover, amifostine decreased the mitochondrial membrane potential ($\Delta\Psi m$) loss, and cytochrome c escaping to cytoplasm, but increased the ATP level. In vitro, amifostine also showed an antioxidant effect, which was reflected by the reduced ROS generation, decreased mitochondrial superoxide generation, increased total SOD, SOD1 (Cu/Zn SOD, cytoplasmic SOD), and SOD2 (mitochondrial SOD) activities, and decreased $\Delta\Psi m$ loss. Furthermore, amifostine suppressed neuronal apoptosis, accompanied by the reduction of Bax, cleaved caspase-9, cleaved caspase-3, and Bcl-2 upregulation. Amifostine also reduced the expression of p-p38 (Thr 180/Tyr 182) in vivo and in vitro. In short, amifostine exhibits a protective effect on cerebral I/R damage through modulating p38-related oxidative stress, mitochondrial dysfunction, and apoptosis.

Key words: *amifostine, ischaemia-reperfusion injury, brain, mitochondrial dysfunction, neuron.*

Introduction

Cerebral ischaemia is the third major cause of death in the world, following cardiovascular diseases and tumours, in terms of morbidity and mortality [22,32]. At present, early blood supply or reperfusion is generally considered to be the most effective treatment for cerebral ischaemia in the clinic. However, reperfusion following ischaemia may result in severe brain failure [9]. In the past few decades, investigators have discovered that the underlying mechanisms of ischaemia-reperfusion (I/R) injury are closely connected with the generation of oxygen-free radicals, subsequent oxidative stress response, inflammatory damage, and eventually apoptosis [15,19,22]. In detail, ischaemia injury is caused by pathological conditions, including the production of a large amount of

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reactive oxygen species (ROS) and the activation of metabolic enzymes, respiratory chain, and adenosine triphosphate (ATP) synthase, eventually leading to cerebral infarct, neurological deficit, and neuronal death [10]. Therefore, it is imperative to find effective drugs to reduce the process of cerebral I/R injury.

Amifostine, formerly known as WR-2721, chemically known as ethanethiol, 2-[(3-aminopropyl) amino]-dihydrogen phosphate, is an inorganic thiophosphate with the potential of being a cytoprotectant and therapeutic agent [30]. It can scavenge free radicals and donate hydrogen to protect against radiation in animals and cells [28]. Increasing studies have shown that amifostine reduces the neurotoxicity induced by paclitaxel [35], cisplatin [40], cyclophosphamide [21], and platinum [27] *in vitro*. Moreover, amifostine alleviates hippocampal neurogenesis and brain recognition memory impairment [18]. More importantly, amifostine has been found to ameliorate ischaemic injury. One previous study described preconditioning with amifostine in prevention of heart I/R damage, depending on its antioxidant property [39]. Similarly, the protective effect of amifostine is also found in kidney [20] and spinal cord [7] with I/R injury. Additionally, amifostine has an anti-apoptotic effect, which is related to its regulation in apoptosis-related proteins [39].

In our study, we focused mainly on whether amifostine has antioxidant and anti-apoptotic effects on brain I/R injury, which might provide a candidate drug for minimising I/R injury.

Material and methods

Animal model

Male C57BL/6 mice weighing approximately 24 g (10~12 weeks old) were acquired from Liaoning Changsheng Biotechnology Co., Ltd (Liaoning, China). They were kept at the room temperature (25 ±1°C) and humidity of 45-55%. Before the operation, the mice were free to receive food and water, and were fully adapted to their surroundings. All animal experiments are approved by the Animal Care Committee of the Second Hospital of Shanxi Medical University.

The establishment of the MCAO model in mice was based on the description in previous studies [5,13]. After ischaemia, the nylon thread in the external carotid artery was carefully drawn out for reperfusion. The control animals were also given the above surgical procedure without carotid ligation. For amifostine administration, the experimental animals were intraperitoneally injected with amifostine at a dose of 200 mg/kg or 400 mg/kg [2,17] (HY-B0639, MedChemExpress [MCE], New Jersey, USA) 30 min before ischaemia operation. After 1 h of ischaemia, the reperfusion was administrated for 24 h. Then, the brains of each group of mice were collected and evaluated.

Oxygen and glucose deprivation/reoxygenation

Oxygen and glucose deprivation/reoxygenation (OGD/R) in primary hippocampal neurons was achieved according to the previous method [43]. The hippocampal tissues were minced, trypsinised, and then kept in DMEM (12100-46, Gibco, New York, USA) containing additional 10% foetal bovine serum (FBS; 04-001-1ACS, BI, Israel). After centrifugation for 5 min, the neurons were mixed and maintained in DMEM supplemented with 2% B27 (17504044, ThermoFisher, Waltham, USA) and 0.5 mM glutamine (HY-B0639, MCE) until the final cell density was 90%.

After pretreatment with 50 µM or 100 µM amifostine [39], the primary hippocampal neurons were cultured in glucose-free DMEM (95% N_2/5% CO_2, 37°C) to initiate OGD for 2 h, and subsequently incubated in a 95% O_2/5% CO_2-mixed medium with high glucose for 24 h. The cells were harvested for subsequent experiments.

Neurological deficit scores

The neurological deficits of animals were assessed and scored in accordance with the 5-point scale method of Nishimura *et al*. [23]. The evaluation criteria were as follows: 0 – normal score and no deficit, 1 – mild injury, the trunk and forelimbs were bent when the whole mouse was lifted by the tail, 2 – moderate neurological deficit, the contralateral of mouse was circled to the left, but the state of immobility was normal, 3 – severe neurological deficit, the mouse leaned to the contralateral side when resting, 4 – more serious neurological deficit, lack of spontaneous motor activity or unconsciousness.

Measurement of infarct size

After that, the mice were deeply anaesthetised and euthanised. Infarction size was evaluated and

visualised using the 2,3,5-triphenyltetrazolium chloride (TTC; 603R302, Solarbio, Beijing, China) staining method. Brain slices (1 mm) were stained with 1% TTC at room temperature for 10-15 min and then photographed. The clearly visible red area was non-ischaemic, and the white area was infarcted. Then, Image J software was performed to quantify the size of cerebral infarction.

Determination of brain oedema

Brains were removed at 24 h post-reperfusion and weighed for wet weight. Then, they were dried and weighed. The degree of brain oedema was determined using the following formula: water content (%) = ([wet weight – dry weight]/wet weight) × 100% [41].

Haematoxylin and eosin staining

Brain tissues collected were fixed and embedded. After deparaffinisation and rehydration, the sections of 5 µm were stained with haematoxylin (H8070, Solarbio) and eosin (A600190, Sangon, Shanghai, China). The mounted slides were photographed using a DP73 Olympus microscope at 200× and 400× magnification.

Nissl staining

The 5 µm brain slices were then set for Cresyl violet staining. Morphological alteration of CA1 in hippocampus was observed by the fluorescence microscope (200× and 400×). Finally, the number of Nissl bodies was counted from 5 randomly selected fields and the mean value was calculated.

Measurement of ROS, MDA, SOD, and ATP

Reactive oxygen species, malondialdehyde (MDA), SOD, and ATP levels in brain tissues and cultured neurons were assessed and quantified using the appropriate reagent kits following the instructions of Nanjing Jiancheng Bioengineering Institute.

MitoSOX Red staining

After 24 h of OGD/R induction, the mitochondrial superoxide levels in primary cultured hippocampal neurons were measured with MitoSOX Red reagent (M36008, MKBio, Shanghai, China), and photographed under a 400× fluorescence microscope.

Determination of mitochondrial membrane potential ($\Delta\Psi$m)

$\Delta\Psi$m in hippocampus and primary cultured hippocampal neurons was determined by the recommended assay kit (C2006, Beyotime, Shanghai, China; M8650, Solarbio). The samples were incubated (JC-1 staining solution) and analysed. The results were observed using fluorescence microscopy.

Western blotting analysis

Protein extractions were quantified using BCA assay. The separation of specified proteins was achieved by SDS-PAGE (P0015, Beyotime), and the proteins were electrotransferred to a PVDF membrane (IPVH00010, Millipore, Billerica, USA). After blocking with 5% (M/V) nonfat dry milk, the membranes were incubated with primary antibodies against cytochrome c, Bax, Bcl-2, cleaved caspase-9, cleaved caspase-3, p38, p-p38, COX IV and β-actin at 4°C. On the second day, the bands were incubated with horseradish peroxidase (HRP)-labelled secondary antibodies and visualised by an enhanced chemiluminescence system (ECL, P0018, Beyotime). Image analysis of protein blots was measured by the Gel-Pro-Analyzer software. The information of antibodies is described in Table I.

TUNEL-NeuN staining

Localisation of apoptotic neurons in brain tissues was measured by the fluorescent TUNEL/NeuN method [11]. Briefly, the slices were pretreated with Triton X-100 (ST795, Beyotime) and incubated in the TUNEL reaction mixture (11684817910, Roche, Basel, Switzerland) at room temperature. Sections were then cultured with NeuN primary antibody (ab104224, 1 : 400, Abcam, Cambridge, UK) overnight in a humid chamber, followed by the incubation of secondary antibody (A0521, Beyotime). Finally, the sections were counterstained with DAPI (C1002, Biosharp, Hefei, China) and observed using a DP73 microscopy at 200× and 400× magnification. The number of surviving neurons and apoptotic cells were counted, respectively.

TUNEL staining

Apoptotic neurons were measured with In Situ Cell Death Detection Kit (11684795910, Roche) in accordance with the description recommended by

Table I. Antibody information and incubation conditions

Primary antibodies	Dilution ratio	Incubation condition	Secondary antibodies	Dilution ratio	Incubation condition	Manufacturer	Number
Bcl-2 antibody	1 : 1000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#3498
Bax antibody	1 : 1000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#5023
Cleaved caspase-3 antibody	1 : 1000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#9661
Cleaved caspase-9 antibody	1 : 500	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#9509
p-p38 antibody	1 : 1000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#4511
p38 antibody	1 : 2000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#9212
Cytochrome c antibody	1 : 1000	4°C overnight	anti-rabbit IgG-HRP	1 : 5000	37°C 45 min	CST	#11940

the manufacturer. The slices were treated with 0.1% Triton X-100 (ST795, Beyotime) and then stained by TUNEL staining followed by DAPI counterstaining (D106471-5 mg, Aladdin). The apoptotic neurons were counted from 400× microscopic field in each section.

Statistical analysis

Data of all at least three samples were expressed as mean ± standard deviation (SD). Their difference analysis was shown using GraphPad Prism 8.0 software. Generally speaking, Student's *t*-tests were used to analyse the differences between pairs, and one-way analysis of variance (ANOVA) was performed for statistical significance of difference among groups. *P*-values < 0.05 were considered statistically significant.

Results

Amifostine restored brain functional outcome in MCAO/R mice

To reveal the positive effect of amifostine on I/R injury in brain, we observed and analysed the brain functional outcome after reperfusion. The neurological deficit scores of the mice were significantly increased after reperfusion. Strikingly, amifostine treatment markedly reduced neurological deficits and appeared to be dose-dependent (Fig. 1A). Next, TTC staining revealed the brain infarct volume after injury, and the areas of ischaemic necrosis were stained white (Fig. 1B). The percentage of brain infarct area was obviously enlarged in the MCAO/R group, while amifostine treatment apparently diminished it, particularly in the MCAO/R + H group (Fig. 1C). Also, there was a significant increase in brain water content of MCAO/R mice, which could be relieved by amifostine treatment (Fig. 1D). Furthermore, H&E staining displayed the histopathological and morphological alteration of hippocampus CA1 (Fig. 1E). In the sham group, the neuron cell structure was normal, the edge was clear, and the nuclei and intercellular substance were stained evenly. In contrast, the neurons in the MCAO/R group exhibited a disordered arrangement with obvious oedema and necrosis. Importantly, treatment of amifostine significantly attenuated the extent of brain oedema and neuronal necrosis. By using Nissl staining, the results showed that the number of stained Nissl's bodies was significantly declined in mice of the MCAO/R group. After amifostine treatment, its number was increased significantly (Fig. 1F). Our findings provided evidence that amifostine could prevent brain failure caused by I/R.

Amifostine attenuated MCAO/R-induced oxidative stress and mitochondrial damage

To determine the effect of amifostine on oxidative stress and mitochondrial damage, we performed subsequent experiments. As demonstrated in Figure 2A-C, E, I/R injury significantly induced ROS and MDA levels, and reduced SOD activity and ATP level. Using flow cytometry analysis, we found a obvious decline in fluorescence intensity in the hippocampus of MCAO/R mice, indicating the depolarization of mitochondria (Fig. 2D). Western blotting analysis demonstrated that I/R injury upregulated cytochrome c expression in cytoplasm and

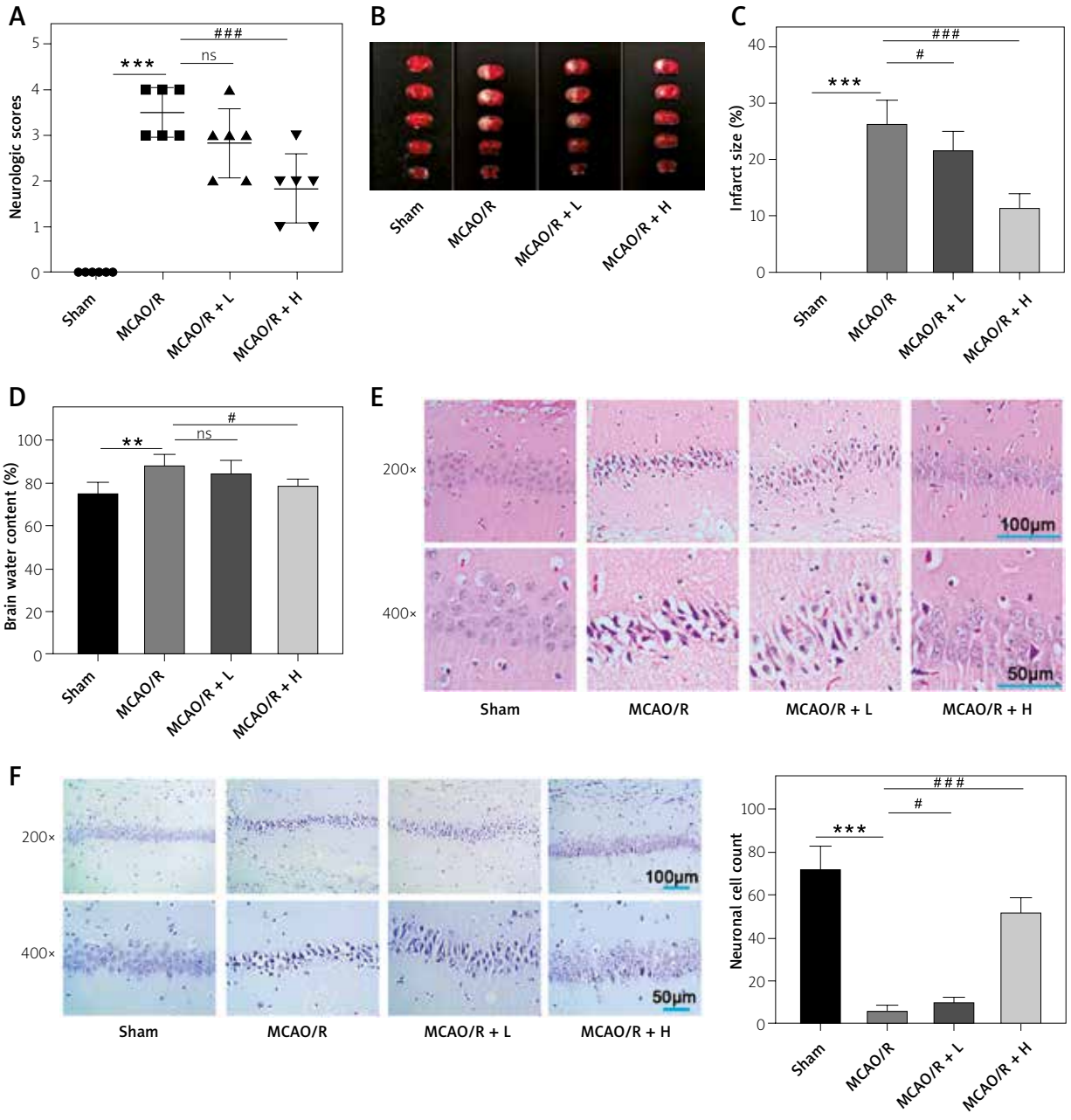

Fig. 1. Amifostine protected against ischaemia and reperfusion injury in brain. Male C57BL/6 mice were intraperitoneally injected with amifostine (200 mg/kg or 400 mg/kg) before MCAO/R surgery. **A**) Assessment of neurological deficit scores at 24 h after reperfusion. **B, C**) TTC staining was used to evaluate the cerebral infarct area, followed by quantification of infarct volumes. **D**) Brain water content = ([wet weight – dry weight]/wet weight) × 100. **E**) H&E staining showed the pathological alterations of neurons in hippocampus CA1 region. **F**) Nissl staining revealed the surviving neurons in hippocampus, followed by quantification. $^{**}p < 0.01$, $^{***}p < 0.001$ vs. sham group; $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$ vs. MCAO/R group.

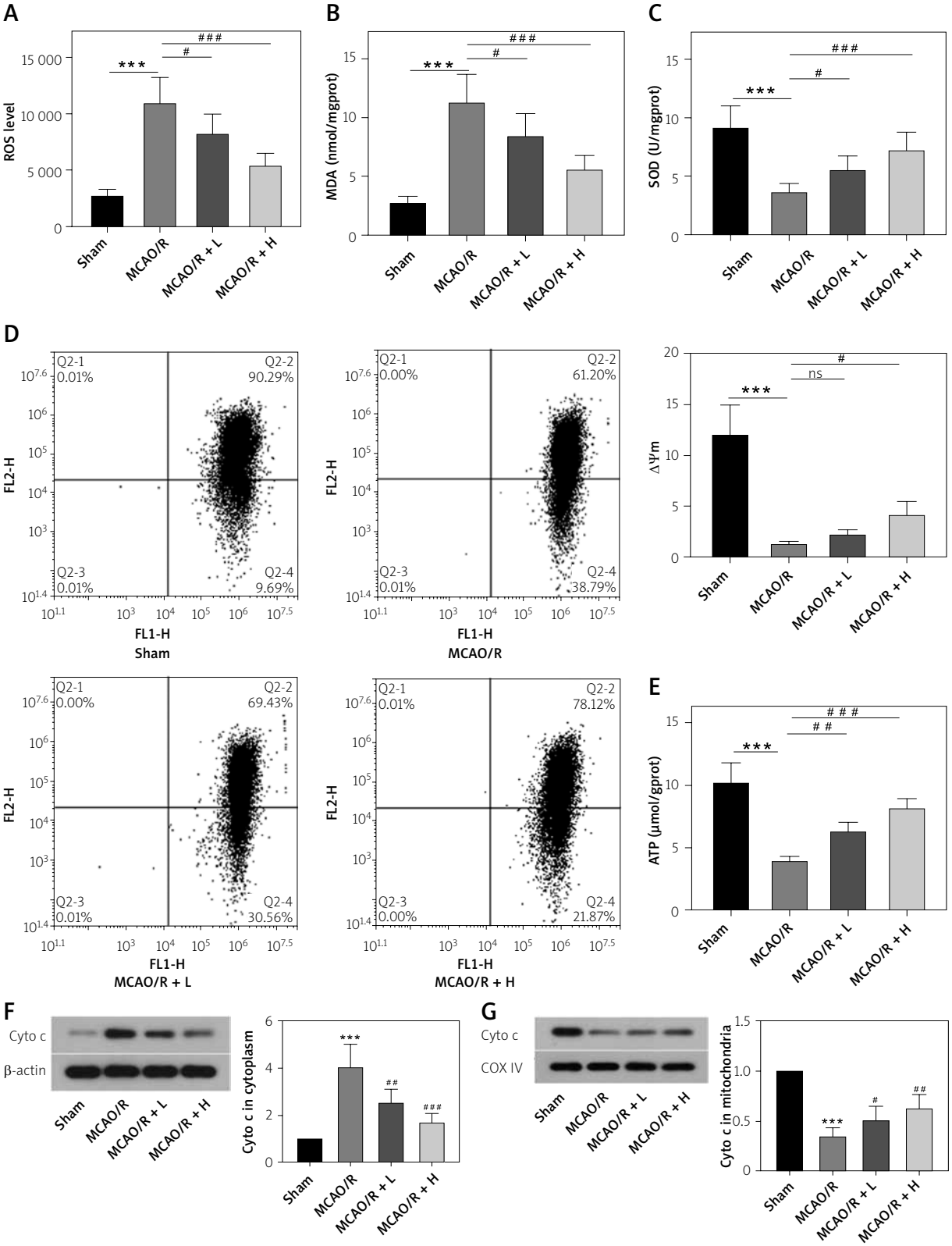

Fig. 2. Amifostine attenuated oxidative and mitochondrial damage after reperfusion. **A-C**) At 24 h post-reperfusion, the ROS level, MDA content and SOD activity in hippocampus were measured by the kits. **D**) Mitochondrial membrane potential changes (ΔΨm) in hippocampus were determined using flow cytometry. **E**) ATP level was determined by the ATP assay kit. **F, G**) Cytochrome c (Cyto c) expression levels in cytoplasm and mitochondria were analyzed using western lightning plus-ECL. ***$p < 0.001$ vs. Sham group; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ vs. MCAO/R group.

downregulated cytochrome c in mitochondria (Fig. 2F, G). However, amifostine treatment obviously diminished the above-mentioned results induced by MCAO/R, indicating that amifostine could attenuate I/R-caused mitochondrial damage in the brain.

Amifostine inhibited MCAO/R-induced apoptosis

Apoptosis is the ultimate and major determinant of brain damage by I/R. Here, TUNEL/NeuN staining revealed that MCAO/R induced a significant decrease in the number of surviving neurons but an increase in the number of apoptotic cells. After treatment of amifostine, the apoptotic cells were dose-dependently decreased (Fig. 3A). Furthermore, protein expression of genes related to apoptosis was estimated by western blotting analysis (Fig. 3B, C). MCAO/R induced the upregulation of Bax, cleaved caspase-9, and cleaved caspase-3 levels but downregulation of Bcl-2. In terms of p38 signalling transduction, MCAO/R increased the p-p38 level but had no impact on p38. Nevertheless, compared with the MCAO/R group, amifostine treatment in part reversed MCAO/R-induced apoptosis and p38 signalling pathway activation. These results showed that amifostine suppressed brain I/R injury-induced neuronal loss, possibly modulated by the p38 signalling pathway.

Amifostine alleviated OGD/R-induced oxidative stress and mitochondrial damage

Based on the protective effect of amifostine against MCAO/R *in vivo*, we further verified its *in vitro* effect on mouse hippocampal neurons. After OGD/R induction, ROS and mitochondrial superoxide levels were increased, but total SOD, SOD1 (Cu/Zn SOD, cytoplasmic SOD), and SOD2 (mitochondrial SOD) activities were decreased. Treatment of amifostine could obviously alleviate the above oxidative stress markers in OGD/R-treated neurons (Fig. 4A-C). In addition, the depolarisation of $\Delta\Psi$m was visualised through a JC-1 fluorescent probe. In general, red fluorescence image presented polarised mitochondria with high $\Delta\Psi$m, and green fluorescence indicated depolarised mitochondria with low $\Delta\Psi$m. JC-1 staining showed that in the control group, the red fluorescence was obvious and the green fluorescence was relatively weak, suggesting a relatively stable $\Delta\Psi$m and normal cellular function. In the OGD/R group, the ratio of red and green fluorescence was significantly decreased, suggesting the obvious decrease in $\Delta\Psi$m. However, amifostine treatment showed enhanced red fluorescence and reduced green fluorescence, indicating that the $\Delta\Psi$m was restored to a stable state after amifostine treatment (Fig. 4D). These findings confirmed the antioxidant activity of amifostine in cerebral I/R injury.

Amifostine alleviated OGD/R-induced neuronal apoptosis

TUNEL staining was carried out for apoptosis analysis 48 h after OGD/R induction. Accordingly, exposure to OGD/R contributed to neuronal apoptosis, which was partially reversed by amifostine pretreatment (Fig. 5A). Moreover, western blotting assay showed that OGD/R induced an increase in Bax, cleaved caspase-9, cleaved caspase-3, and p-p38 and a decrease in Bcl-2. However, these changes induced by OGD/R were blocked by amifostine pretreatment (Fig. 5B, C).

Discussion

Amifostine has been recognised as an effective cytoprotectant drug that plays a role in the prevention of I/R damage [39]. This current work is the first to reveal the role of amifostine in cerebral I/R injury, from aspects of oxidative stress, mitochondrial dysfunction, and apoptosis. In our current study, we demonstrate that amifostine can repair neurological deficits, and reduce infarct area and brain oedema. Moreover, amifostine reduces I/R-induced neuron loss *via* reducing overactive oxidative stress and mitochondria damage. These results indicate the beneficial effect of amifostine in the treatment of ischaemic injury in the brain.

Accumulating research has demonstrated that oxidative stress is the key factor in the pathogenesis of multiple neurodegenerative diseases [3,33]. During I/R injury, excessive oxygen free radicals and ROS will be produced, subsequently activating multiple signals, and leading to the dysfunction of lipids, mitochondria, and other cellular components [16,37]. In the antioxidant system, SODs such as SOD1 and SOD2 are considered as superoxide radical scavengers to prevent ROS attack [1]. MDA is identified as a marker of oxidant stress, indicating the content of lipid peroxidation. High lipid levels and oxygen consumption are responsible for oxidative damage in the brain [25]. Amifostine has been found to scavenge

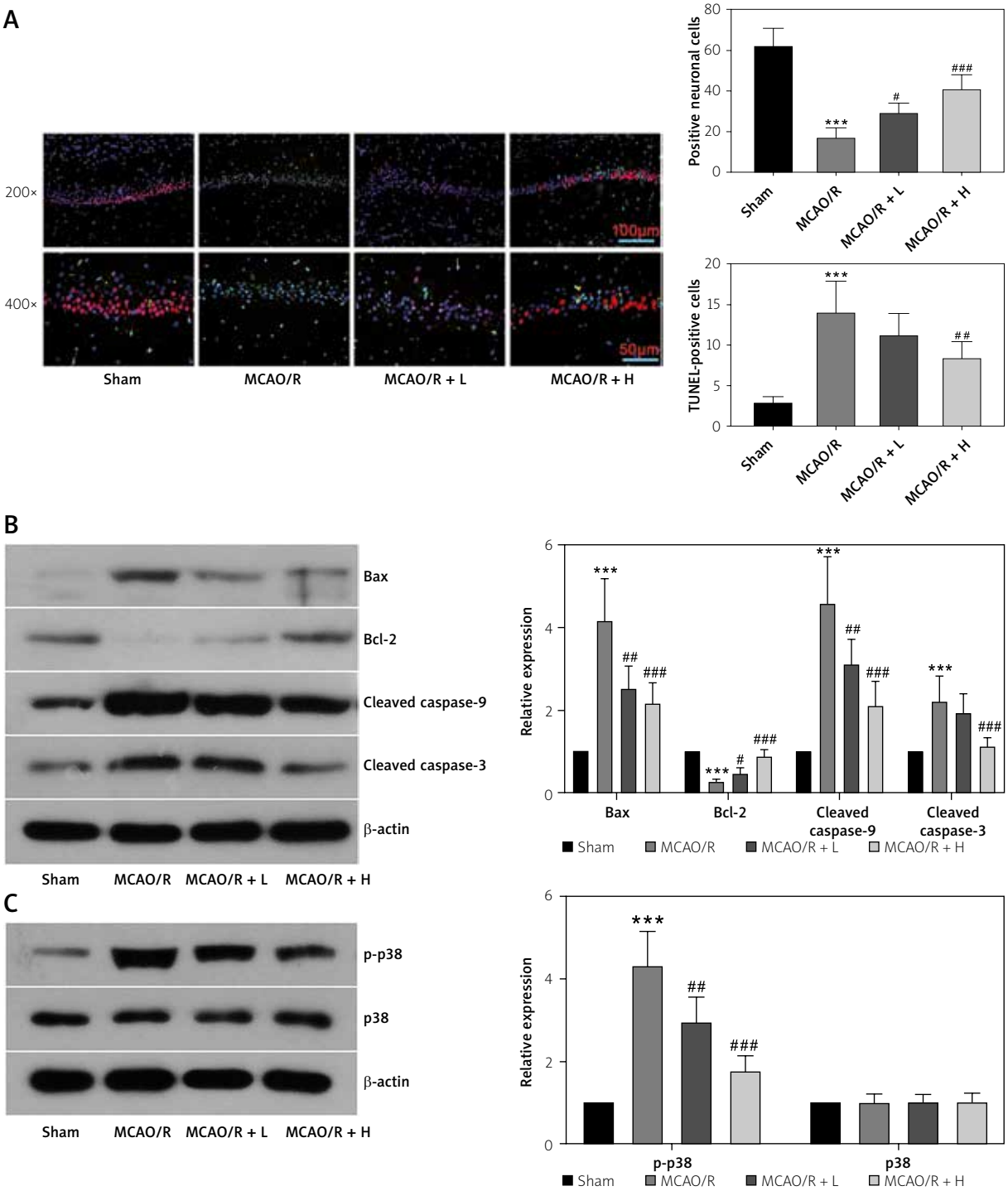

Fig. 3. Amifostine inhibited neuronal apoptosis 24 h after reperfusion. **A**) TUNEL-immunofluorescence assay represented the distribution of neurons and apoptotic cells in hippocampus CA1 region at magnification 200× and 400×. **B, C**) Western blot assay was carried out to detect levels of Bax, Bcl-2, cleaved caspase-9, cleaved caspase-3, p38, and p-p38. ***$p < 0.001$ vs. sham group; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ vs. MCAO/R group.

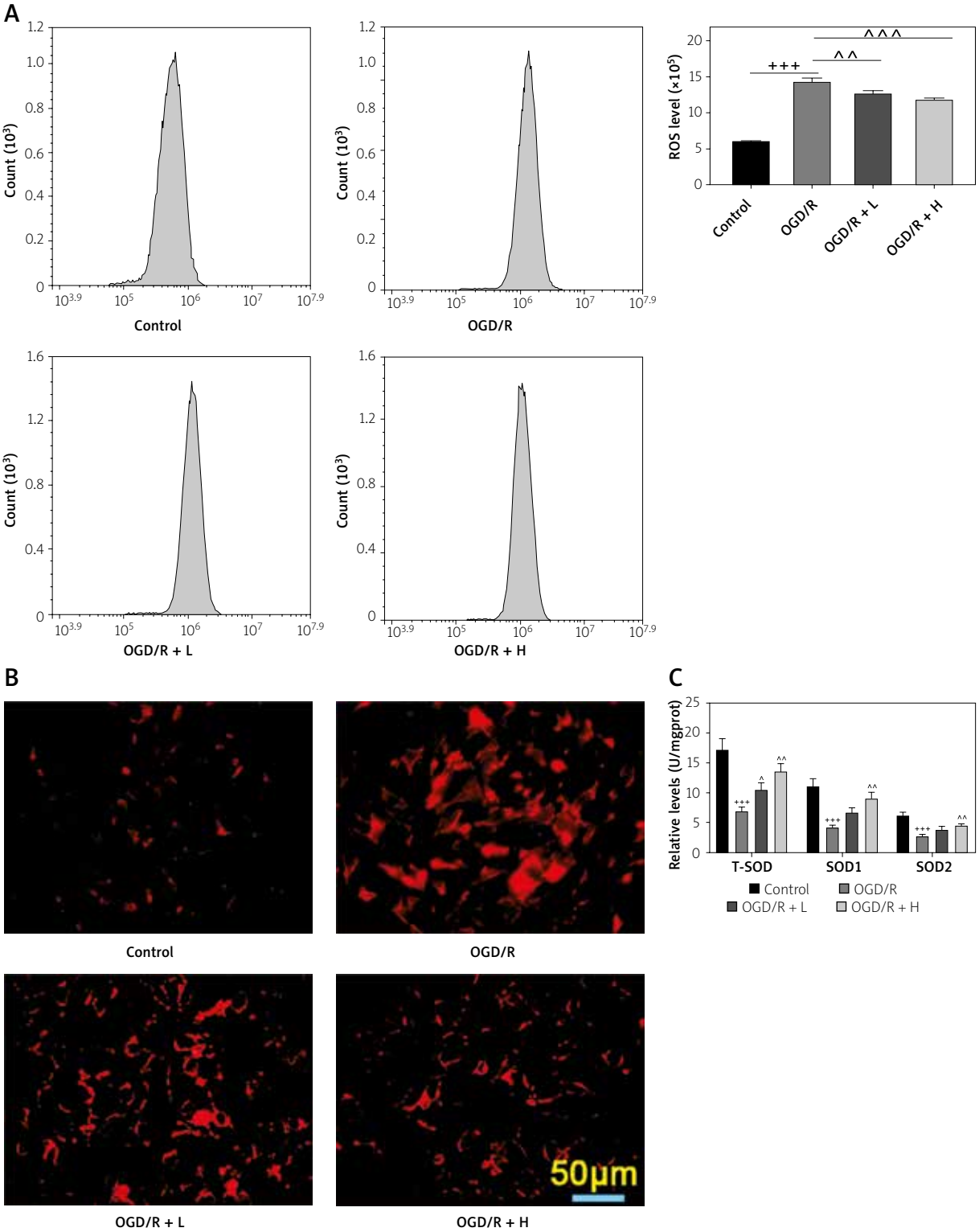

Fig. 4. Amifostine alleviated OGD/R-induced oxidative and mitochondrial damage. After pre-treatment of amifostine for 30 min, the primary cultured mouse hippocampal neurons underwent OGD/R to induce neuronal cell injury. **A**) Flow cytometry was performed to detect ROS level. **B**) MitoSOX Red staining revealed the production of mitochondrial superoxide. **C**) SOD assay kits were used for the determination of the total SOD, cytoplasmic SOD (SOD1) and mitochondrial SOD (SOD2) activities. $^{+++}p < 0.001$ vs. Control group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. OGD/R group.

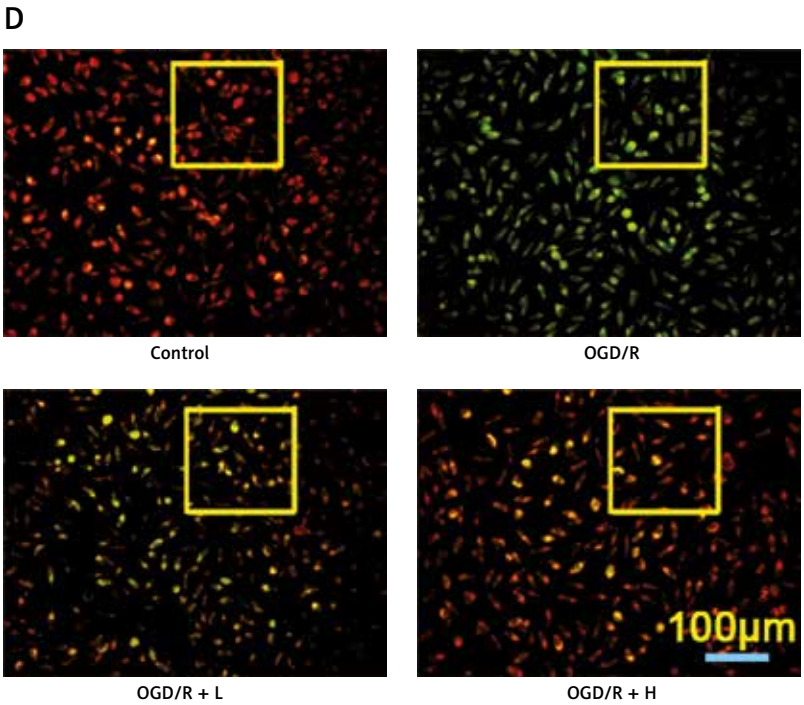

Fig. 4. D) JC-1 fluorescent staining revealed the high or low mitochondrial membrane potential ($\Delta\Psi$m) in hippocampal neurons.

ROS, induce antioxidant enzymatic activities, and reduce oxidative stress in spinal cord and heart after I/R injury [7,39]. In this study, amifostine significantly attenuates the increase of ROS level and MDA content, and the decrease of SOD activity in MCAO/R-induced hippocampal tissues and OGD/R-induced primary cultured hippocampal neurons, which is consistent with the previous results on I/R injury.

Mitochondria form a complex, dynamic, interconnected network that exerts a variety of biological functions. Mitochondrial dysfunction is a common predictor of many human diseases, such as cancer, metabolic disorders, and neurodegenerative diseases [24]. $\Delta\Psi$m is a necessary component that can selectively eliminate dysfunctional mitochondria to maintain mitochondrial homeostasis. Long-term increase or decrease of $\Delta\Psi$m may contribute to various pathologies [45]. In addition, abnormalities in ATP generation are thought to be a core of mitochondrial dysfunction. Impaired mitochondrial function contributes to the reduction of ATP production [6]. Relatively stable $\Delta\Psi$m and ATP level are vital for the maintenance of normal cell function. Moreover, cytochrome c, an important mitochondrial molecule, exhibits peroxidase activity and functions as a scavenger of ROS following neuronal damage [4]. More importantly, previous studies revealed that amifostine induces antioxidant enzymatic activities, and reduces mitochondrial damage and neuronal cell death [12,14]. In line with these observations, we find that the decreased $\Delta\Psi$m and ATP level caused by I/R injury are partially abolished by amifostine treatment. Amifostine treatment also significantly prevents cytochrome c escaping into cytoplasm, along with the increase of cytochrome c in mitochondria. Increasing evidence demonstrates that there are some phenomena, including stable $\Delta\Psi$m, elevated ATP level, and accumulated cytochrome c in mitochondria, indicating improvement of I/R brain injury [8,38]. Overall, these findings indicate that amifostine attenuates brain I/R injury *via* attenuating oxidative stress response and mitochondrial dysfunction.

Furthermore, ROS overproduction can activate mitochondrial apoptotic pathway, eventually leading to cell death [44]. Inhibition of apoptosis is the key step for the therapy of ischaemic stroke [34]. It is generally known that the ratio of anti-apoptotic proteins to pro-apoptotic proteins is crucial for the modulation of cellular survival/death. Bcl-2, an anti-apoptotic protein, can act as an effective free

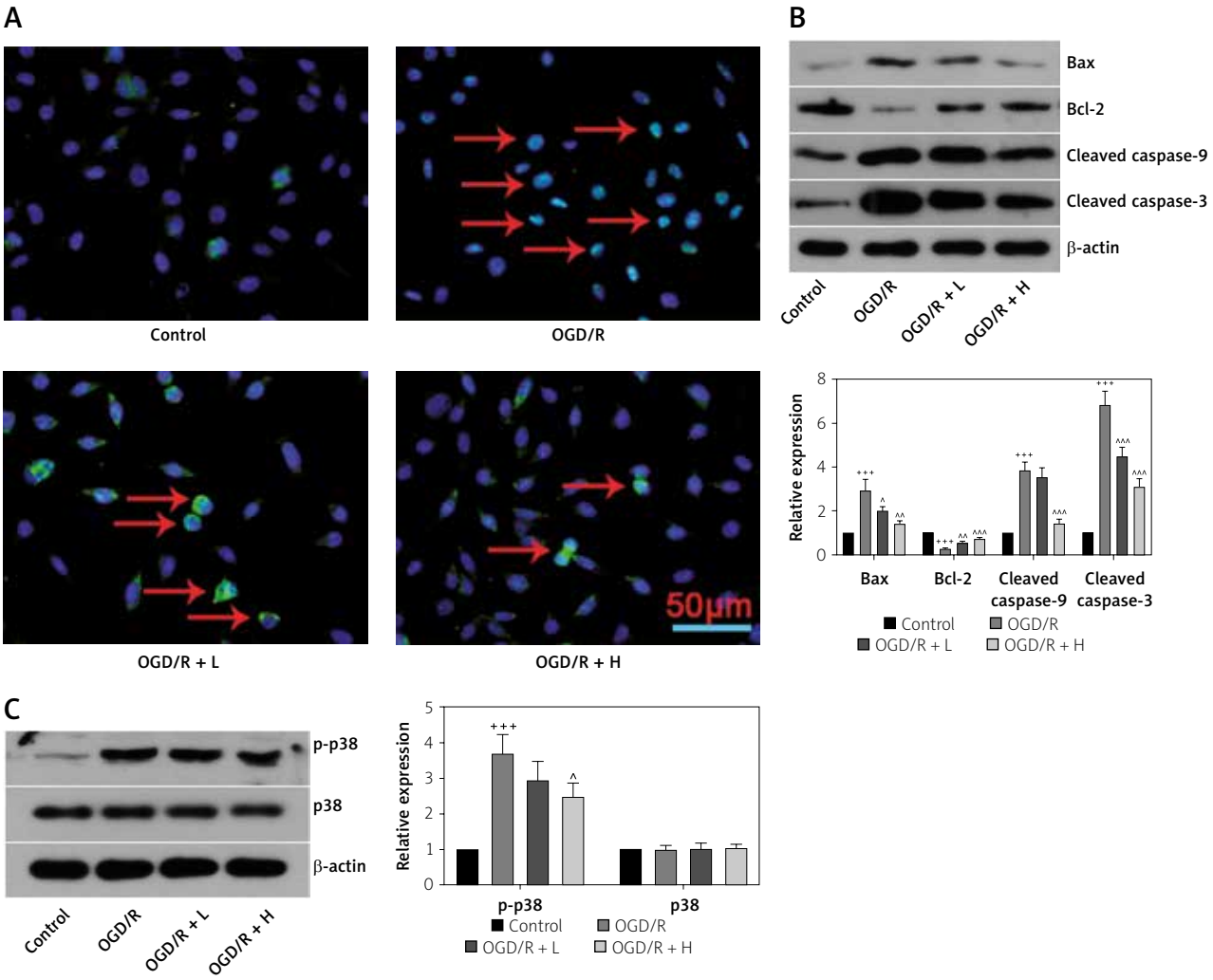

Fig. 5. Amifostine suppressed OGD/R-induced neuronal apoptosis. **A**) Cell apoptosis was determined by TUNEL staining. **B, C**) Relative levels of Bax, Bcl-2, cleaved caspase-9, cleaved caspase-3, p38 and p-p38 were measured through western blot analysis. $^{+++}p < 0.001$ vs. control group; $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ vs. OGD/R group.

radical scavenger to eliminate excessive ROS generation [26]. Conversely, Bax is a pro-apoptotic protein that can cause $\Delta\Psi$m loss and the release of cytochrome c [42]. Then, cytochrome c in the cytoplasm induces Apaf-1 activation and forms apoptosome of cytochrome C/Apaf-1/pro-caspase-9. It can cleave pro-caspase-9 into cleaved caspase-9 and then cleave downstream pro-caspase 3 into cleaved caspase-3, thereby activating the apoptotic signalling pathway [29]. After brain I/R injury, pro-apoptotic proteins are highly expressed, while anti-apoptotic proteins are expressed lowly [36]. Our data show that OGD/R stimulates neuronal apoptosis, accompanied by the elevation of Bax, cleaved caspase-9, cleaved caspase-3, and the decline of Bcl-2. Interestingly, amifostine treatment efficiently ameliorates apoptosis induced by OGD/R. In addition, a study has demonstrated that the anti-apoptotic effect of amifostine is regulated by p38 signalling pathway [31]. When extracellular stimuli occur, p38 can be phosphorylated and can activate transcription factors, ultimately initiating cell apoptosis. Amifostine markedly inhibits phosphorylated p38, thereby inhibiting cell death [31]. In our study, p-p38 expression was upregulated by OGD/R, while it was remarkably repressed by amifostine pretreatment, indicating that amifostine may suppress the activation of p38 apoptosis signalling. All the above data indicate that amifostine may suppress neuronal apoptosis by modulating the p38 signalling activation.

In summary, our findings indicate that amifostine may serve a neuroprotective role in cerebral-neuron injury caused by I/R. Notably, this protective effect is probably realised by attenuating p38-associated oxidative stress, mitochondrial dysfunction, and neuronal apoptosis. All results indicate that amifostine can be used as a candidate agent for the treatment of cerebral I/R injury, providing a new insight for the therapy of I/R brain injury.

Disclosure

The authors report no conflict of interest.

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