Propofol Reduces Inflammatory Brain Injury after Subarachnoid Hemorrhage: Involvement of PI3K/Akt Pathway

Hua-bin Zhang, MD, Xian-kun Tu, MD, PhD, Quan Chen, MD, and Song-sheng Shi, MD, PhD

Background: Our previous study showed that propofol, one of the widely used anesthetic agents, can attenuate subarachnoid hemorrhage (SAH)-induced early brain injury (EBI) via inhibiting inflammatory and oxidative reaction. However, it is perplexing whether propofol attenuates inflammatory and oxidative reaction through modulating PI3K/Akt pathway. The present study investigated whether PI3K/Akt pathway is involved in propofol's anti-inflammation, antioxidation, and neuroprotection against SAH-induced EBI. *Materials and methods:* Adult Sprague-Dawley rats underwent SAH and received treatment with propofol or vehicle after 2 and 12 hours of SAH. LY294002 was injected intracerebroventricularly to selectively inhibit PI3K/Akt signaling. Mortality, SAH grading, neurological scores, brain water content, evans blue extravasation, myeloperoxidase, malondialdehyde, superoxide dismutase, and glutathione peroxidase were measured 24 hours after SAH. Immunoreactivity of p-Akt, t-Akt, nuclear factor- kappa B (NF-κB) p65, nuclear factor erythroid-related factor 2 (Nrf2), NAD(P)H:quinone oxidoreductase (NQO1), and cyclooxygenase-2 (COX-2) in rat brain was determined by western blot. Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) in rat brain were examined by ELISA. *Results:* Propofol significantly reduces neurological dysfunction, BBB permeability, brain edema, inflammation, and oxidative stress, all of which were reversed by LY294002. Propofol significantly upregulates the immunoreactivity of p-Akt, Nrf2, and NQO1, all of which were abolished by LY294002. Propofol significantly downregulates the overexpression of NF-κB p65, COX-2, TNF-α, and IL-1β, all of which were inhibited by LY294002. *Conclusion:* These results suggest that propofol attenuates SAH-induced EBI by inhibiting inflammatory reaction and oxidative stress, which might be associated with the activation of PI3K/Akt signaling pathway.

Key Words: Subarachnoid hemorrhage—early brain injury—inflammation—oxidative stress—propofol—PI3K/Akt

Introduction

At present although subarachnoid hemorrhage (SAH) only accounts for 5% of strokes,[1] but it is associated with high disability and mortality. Traditionally, cerebral vasospasm and delayed cerebral ischemia were considered as the most important causes of the poor outcomes after SAH onset.[2] Considerable progress in diagnostic techniques, endovascular treatment, surgical, and perioperative management paradigms has been made, but the outcome in patients suffering from SAH was still extremely poor. On the other side, accumulated evidence showed that early brain injury (EBI) is possibly the primary cause of high disability and mortality in SAH patients within 24-72 hours.[3] Therefore, treatment of EBI has been considered as the major goal in SAH patients. The potential pathophysiological processes involved in EBI included inflammatory reaction, oxidative stress, and the subsequent blood-brain barrier (BBB) disruption, vascular brain

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edema, and intracranial pressure.[4] Therefore, inflammatory reaction and oxidative stress have been considered as the important therapeutic targets for SAH-induced EBI.[4,5]

Propofol, one of anesthetic agents for maintenance of anesthesia for surgical procedures, has been world-widely used in clinic. Previous study from our laboratory demonstrated that propofol improves neurological scores, reduces vascular brain edema and BBB disruption in rats of SAH.[6] We also showed propofol attenuates EBI in rats of SAH partly associated with the inhibition of nuclear factor- kappa B (NF-κB) inflammatory pathway, including downstream cyclooxygenase-2 (COX-2), tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and the activation of nuclear factor erythroid-related factor 2/antioxidant-response element (Nrf2/ARE) antioxidative pathway, however, more mechanisms remain unknown until now. As a neuroprotective agent, propofol might be a promising drug for the treatment of SAH, therefore, it is necessary to further explore the potential mechanisms.

Phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signaling pathway, is a significant survival pathway in neurons.[7] PI3K/Akt signaling plays a critical role in promoting neuronal survival not only in ischemic stroke,[8] but also in traumatic brain injury,[9] Parkinson's disease,[10] Alzheimer's disease,[11] intracerebral hemorrhage,[12] and SAH.[13] The neuroprotective effect of PI3K/Akt signaling has been well demonstrated in the antiapoptotic action[14] and antioxidative action,[15] but rarely in anti-inflammatory mechanism. Our previous study showed that PI3K/Akt signaling pathway can also mediate the anti-inflammatory reaction through inhibiting the NF-κB/COX-2/TNF-α/IL-1β inflammatory signaling pathway in experimental ischemic stroke.[16] PI3K/Akt signaling pathway was also demonstrated to be involved in the neuroprotection of propofol against cerebral ischemic injury[17] and spinal ischemic injury.[18] In the present study, we demonstrated whether PI3K/Akt signaling pathway was involved in propofol's neuroprotection against SAH brain injury.

Materials and Methods

Animals and Animal Model

All animal experiments were conducted according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee of Fujian Medical University. Adult male Sprague-Dawley rats weighed 250-300 g were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Sciences. Animals were housed in a colony room under controlled temperature (22 °C), and a 12:12 light-dark cycles, with food and water available. The rat SAH model was induced by endovascular perforation described in the previous study,[6] with minor modifications. Briefly, rats were anesthetized with an intraperitoneal injection of chloral hydrate (300 mg/kg), and the right common carotid artery, external carotid artery, and internal carotid artery were carefully exposed through a ventral midline neck incision, a blunted 4-0 monofilament nylon suture was inserted into the internal carotid artery until resistance was felt (18-20 mm). The suture was carefully pushed approximately 3 mm further to perforate the artery wall to create a SAH. Sham-operation rats were manipulated in the same way without perforation.

Drug Administration and Experimental Groups

Propofol (50 mg/kg) was dissolved in normal saline and then administered to rats by intraperitoneal injection twice after 2 and 12 hours of SAH according to our previous report,[6] in which showed that the propofol at 50 mg/kg could produce the best protective effect on the brain of rats subjected to SAH. Rats were divided into 4 groups as follows: (1) sham group (n = 20), which underwent sham operation and received vehicle; (2) SAH+vehicle group (n = 20), which was subjected to SAH and received vehicle; (3) SAH+propofol group (n = 20), which was subjected to SAH and treated with propofol 50 mg/kg; (4) SAH+propofol+LY294002 group (n = 20), which was subjected to SAH, treated with propofol 50 mg/kg and received intracerebroventricular injection of LY294002. Rats were treated with normal saline as the vehicle control at the same volume and time point as propofol.

Intracerebroventricular (ICV) Injection

To investigate whether PI3K/Akt signaling mediates the propofol's neuroprotection, LY294002 was injected intracerebroventricularly 15 minutes before SAH described in previous report.[16,19] In brief, LY294002 was dissolved in dimethyl sulfoxide (DMSO) and diluted with PBS to 10 μM. With use of a stereotaxic device (Reward, Shenzhen, China), 10 μL of LY294002 solution was injected into the right ventricle of rats. The stereotactic ICV injection site was chosen at the following sites from bregma: anteroposterior, .8 mm; lateral, 1.5 mm; depth, 3.5 mm. The sham operation, SAH+vehicle and SAH+propofol groups were intracerebroventricularly injected with same volume of DMSO diluted in PBS as control (final concentration of DMSO is 14.1 μM), and the duration of ICV injection is 10 minutes.

Assessment of SAH Severity

The SAH severity of all rats was quantified after sacrificed as reported previously.[20] The scale was based on the amount of subarachnoid blood in 6 segments of basal cistern: Grade 0, no subarachnoid blood; Grade 1, minimal subarachnoid blood; Grade 2, moderate blood clot with recognizable arteries; and Grade 3, blood clot obliterating all arteries within the segment. A total score ranging from 0 to 18 was obtained by adding the scores from all 6 segments.

Assessment of Neurological Scores

Neurological scores of all rats were evaluated after 24 hours of SAH according to the scoring system of Garcia[21] with minor modification. Briefly, the neurobehavioral study consisted of 6 tests, including spontaneous activity, symmetry in the movement of 4 limbs, forepaw outstretching, climbing, body proprioception, and response to vibrissae touch. A lower score represents serious deficit of neurological function. Neurological scores were assessed by a "blinded" co-worker.

Assessment of Brain Edema

Rats (n = 5) were killed 24 hours after SAH, and the brains were promptly removed. The whole rat brains were gently blotted with filter-paper and weighed on electronic balance, as the wet weights (WW), and then dried for 24 hours in 100 °C vacuum oven to obtain the dry weights (DW). Cerebral water content was calculated according to the following formulation: H_2O (%) = (WW − DW)/WW × 100%.

Measurement of BBB Permeability

BBB permeability (n = 5) was assessed by measurement of evans blue (EB) extravasation. Under anesthesia, EB dye (2% in saline) was injected into the left jugular vein 23 hours after SAH, and rats were transcardially perfused with PBS (250 ml) to remove the intravascular dye. The whole brains were removed and homogenized in a ten-fold volume of 50% trichloroacetic acid solution to precipitate protein and centrifuged for 10 minutes at 3000 r/minutes. The supernatant was diluted with ethanol (1:3), and its fluorescence was measured at 610 nm for absorbance of EB. The results were expressed as μg/g tissue.

Biochemical Analysis

Animals (n = 5) were sacrificed to determine the biochemical analysis. Neutrophil infiltration was estimated by measuring the enzymatic activity of myeloperoxidase (MPO). MPO activity in rat brain was detected according to the manufacturer's instructions from the assay kit (Nanjing Jiancheng Bioengineering Institute, China). The results were expressed as U/g tissue.

Lipid peroxidation was evaluated by measuring the formation of malondialdehyde (MDA). The content of MDA in rat brain was measured according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China). The results were expressed as μmol/g protein.

Superoxide dismutase (SOD) content in rat brain was assessed by a commercially available kit (Nanjing Jiancheng Bioengineering Institute) based on the auto-oxidation of hydroxylamine. The development of blue color was measured at 550 nm and the results were expressed as U/mg protein.

Glutathione peroxidase (GSH-Px) activity was determined by the velocity method using a GSH-Px kit (Nanjing Jiancheng Bioengineering Institute). The reaction was initiated by the addition of H2O2. A series of enzymatic reactions was activated by GSH-Px in the homogenate which subsequently led to the conversion of GSH (reduced glutathione) to oxidized glutathione (GSSG). The change in absorbance during the conversion of GSH to GSSG was recorded spectrophotometric absorbance at 412 nm and the results were expressed as U/mg protein.

Western Blot

Animals (n = 5) were sacrificed to extract the protein and mRNA of brain samples (bilateral temporal cortex). The protein (n = 5) was extracted using protein extraction kit (Beyotime Biotech. CO., China) according to the manufacturer's instructions. Protein samples (50 μg) were separated on 10% SDS polyacrylamide gels, transferred to nitrocellulose membranes. The membranes were respectively incubated at 4 °C for 2 hours with a mouse monoclonal antibody against p-Akt (ser473) (1:1000, Cell Signaling Technology), monoclonal antibody against t-Akt (1:1000, Cell Signaling Technology), mouse monoclonal antibody against Nrf2 (1:500, Abcam), rabbit polyclonal antibody against NQO-1 (1:1000, Abcam), mouse monoclonal antibody against p-NF-κB p65 (1:200, Santa Cruz), and rabbit polyclonal antibody against COX-2 (1:500, Santa Cruz). The nitrocellulose membranes were incubated with horseradish-peroxidase conjugated secondary antibodies (1:3000, Zhongshan Biotechnology CO. LTD, Beijing) for 2 hours at 25 °C and developed with an enhanced chemiluminescence detection system. GAPDH was used as a loading control. The optical densities of protein bands were analyzed by the Quantity one software (Bio-Rad).

ELISA Assay

Twenty-four hours after the induction of SAH, the frozen brain samples (200 mg) were homogenized with a glass homogenizer in cell lysis buffer containing 1 mM phenylmethanesulfonylfluoride (PMSF), 20 mM Tris pH7.5, 150 mM NaCl, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM EDTA, 1% Na_3VO_4, and .5 μg/ml leupeptin and centrifuged at 14,000×*g* for 20 minutes at 4 °C. The contents of cytokines (TNF-α and IL-1β) in the brain tissue were measured using a rat TNF-α or IL-1β immunoassay enzyme-linked immunosorbent assay (ELISA) kits (Boster Biological Technology, LTD, Wuhan, China) according to the manufacturer's instructions. The results were expressed as pg/g protein.

Statistical Analysis

Experimental data were presented as mean ± SD. Statistical analysis was performed using ANOVA followed

by LSD and Bonferroni test for individual comparisons between group mean. The analysis of mortality was done with χ^2 test. A value P less than .05 was considered statistically significant.

Results

Mortality

No rats died in the sham group (n = 20), 3 of 23 rats (13.04%) died in the SAH group, 4 of 24 rats (16.67%) died in the propofol group, and 3 of 23 rats (13.04%) died in the LY294002 group. No significant difference was observed for mortality among the vehicle-treated, propofol-treated, and LY294002-treated groups.

LY294002 Reduces the Neuroprotection of Propofol on Early Brain Injury after SAH

There was no significant difference in SAH grading between SAH+vehicle group, SAH+propofol group, and SAH+propofol+LY294002 group ($P > .05$, Fig 1A and B). Propofol significantly improved neurological scores after 24 hours of SAH in rats ($P < .01$), which was abolished by LY294002 ($P < .05$, Fig 1C), demonstrating that PI3K/Akt was possibly involved in propofol's neuroprotection against SAH-induced EBI.

Measurement of cerebral water content was used to assess the extent of brain edema. Propofol significantly reduced cerebral water content after 24 hours of SAH in rats ($P < .01$), which was abolished by LY294002 ($P < .05$, Fig 1D), suggesting that PI3K/Akt was possibly involved in effect of propofol on brain edema.

Propofol significantly improved BBB integrity after 24 hours of SAH in rats ($P < .01$), which was abolished by LY294002 ($P < .05$, Fig 1E), indicating that PI3K/Akt was possibly involved in effect of propofol on BBB permeability.

LY294002 Reverses the Inhibitory Effect of Propofol on Neutrophil Infiltration and Lipid Peroxidation

Measurement of MPO activity was performed to reflect the extent of inflammatory reaction in brain after SAH. MPO activity was markedly elevated after 24 hours of SAH in rats. Propofol significantly decreased MPO activity ($P < .05$), which was abolished by administration of LY294002 ($P < .05$, Fig 2A). MDA content was markedly elevated after 24 hours of SAH in rats. Propofol significantly decreased MDA content ($P < .01$), which was abolished by LY294002 ($P < .01$, Fig 2B). These results demonstrated that propofol attenuates inflammation and oxidative stress possibly through PI3K/Akt pathway.

Effect of LY294002 on PI3K/Akt in SAH

Immunoreactivity of p-Akt and t-Akt in rat brain was detected by western blot. Results showed that p-Akt in rat brain was downregulated after 24 hours of SAH, propofol significantly upregulated the immuoreactivity of p-Akt ($P < .01$), which was abolished by LY294002 ($P < .01$, Fig 3), suggesting that PI3K/Akt could be inhibited by ICV injection of LY294002 in rats of SAH. No significant change of t-Akt was observed in different groups.

LY294002 Inhibits the Effect of Propofol on Nrf2 and NQO1

Expression of Nrf2 and NQO1 in rat brain was also detected by western blot. Results showed that Nrf2 and NQO1 were obviously decreased in rat brain after 24 hours of SAH, propofol significantly increased the expression of Nrf2 and NQO1 ($P < .01$), which were abolished by LY294002 ($P < .05$ and $P < .01$, Fig 4), suggesting that propofol upregulates the expression of Nrf2 and NQO1 possibly through PI3K/Akt pathway.

LY294002 Reverses the Effect of Propofol on the Activity of SOD and GSH-Px

Measurement of enzymatic activity of SOD and GSH-Px was performed to reflect the ability of scavenge oxidative stress in brain after SAH. Enzymatic activity of SOD and GSH-Px was significantly descended after 24 hours of SAH in rats. Propofol significantly increases the activity of SOD and GSH-Px ($P < .01$), which was abolished by LY294002 ($P < .05$ and $P < .01$, Fig 5), suggesting that propofol elevates the antioxidative property possibly through PI3K/Akt pathway.

LY294002 Inhibits the Effect of Propofol on Phosphorylated NF-κB p65

Phosphorylated NF-κB p65 in rat brain was also detected by western blot. Phosphorylated NF-κB p65 was obviously increased in rat brain after 24 hours of SAH. Propofol significantly decreased the overexpression of p-NF-κB p65 ($P < .01$), which was abolished by LY294002 ($P < .01$, Fig 6), suggesting that propofol inhibits p-NF-κB p65 expression possibly through PI3K/Akt pathway.

LY294002 Inhibits the Effect of Propofol on the Expression of COX-2

Expression of COX-2 in rat brain was detected by western blot. Expression of COX-2 was obviously increased in rat brain after 24 hours of SAH. Propofol significantly decreased the overexpression of COX-2 in rat brain ($P < .01$), which was abolished by LY294002 ($P < .01$, Fig 7), suggesting that propofol downregulates the overexpression of COX-2 possibly through PI3K/Akt pathway.

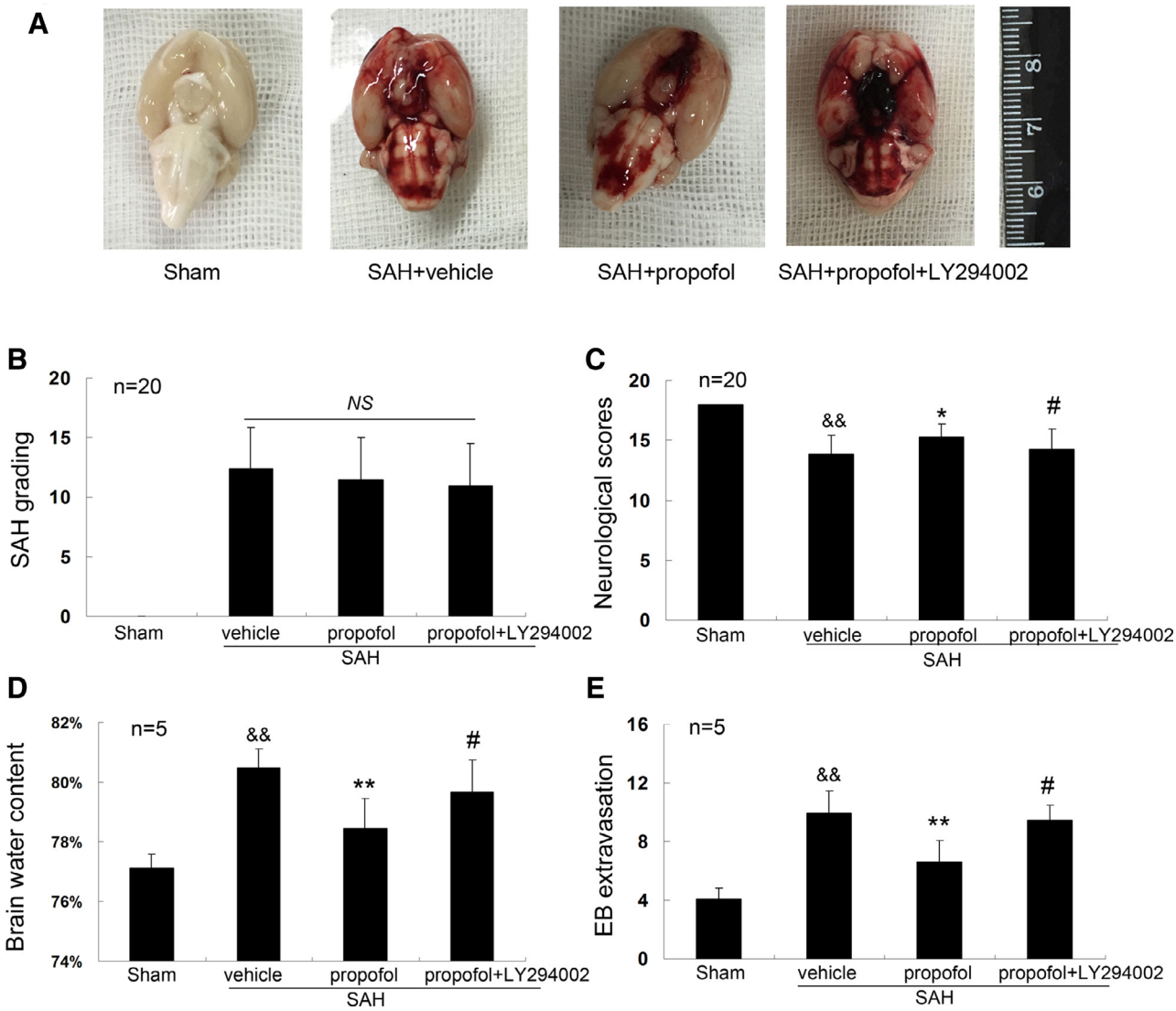

Figure 1. *LY294002 reduces the neuroprotection of propofol on SAH-induced neurological dysfunction, cerebral water content and BBB permeability. SAH grading score (A, B), neurological scores (C), cerebral water content (D) and BBB permeability (E) 24 hours after SAH. The upper panel showed representative macroscopic pictures of brain base 24 hours after SAH (A), and the SAH scores were summarized in (B). Subarachnoid blood clots exist equally at the ventral surface of the brain and brain stem between the SAH groups. Propofol improved neurological scores, and reduced cerebral water content and EB extravasation 24 hours after SAH in rats, all of which were significantly abolished by administration of LY294002. &&P < .01 compared to sham group, *P < .05 and **P < .01 compared to SAH+vehicle group, #P < .05 compared to SAH+propofol group.* NS *means not significant.*

LY294002 Inhibits the Effect of Propofol on the Expression of TNF-α and IL-1β

Contents of TNF-α and IL-1β in brain were increased after 24 hours of SAH. Propofol significantly decreased the expression of TNF-α and IL-1β in rats brain subjected to SAH ($P < .01$), which was abolished by LY294002 ($P < .01$ and $P < .05$, Fig 8), suggesting that propofol decreases the expression of TNF-α and IL-1β possibly through PI3K/Akt pathway.

Discussion

Previous studies from our laboratories demonstrated that propofol, one of general anesthetics, reduces brain injury in experimental cerebral ischemia[22] and SAH.[6] Further investigation found that propofol attenuates inflammatory reaction and oxidative stress, ameliorates cerebral edema and BBB permeability in rats of cerebral ischemia[22] and SAH.[6] In SAH, the propofol's anti-inflammatory and antioxidative effects against brain injury might be associated with the inhibition of NF-κB signaling pathway and activation of Nrf2/ARE signaling pathway. PI3K/Akt signaling pathway was reported to play an important role in promoting neuronal survival following SAH.[23] Propofol was demonstrated to protect cerebral ischemia-reperfusion injury in rats via the activation of PI3K/Akt signaling pathway.[17] Propofol pretreatment attenuates lipopolysaccharide-induced acute lung injury in rats by activating the PI3K/Akt pathway.[24] Recently, propofol was also shown to protect spinal cord ischemic

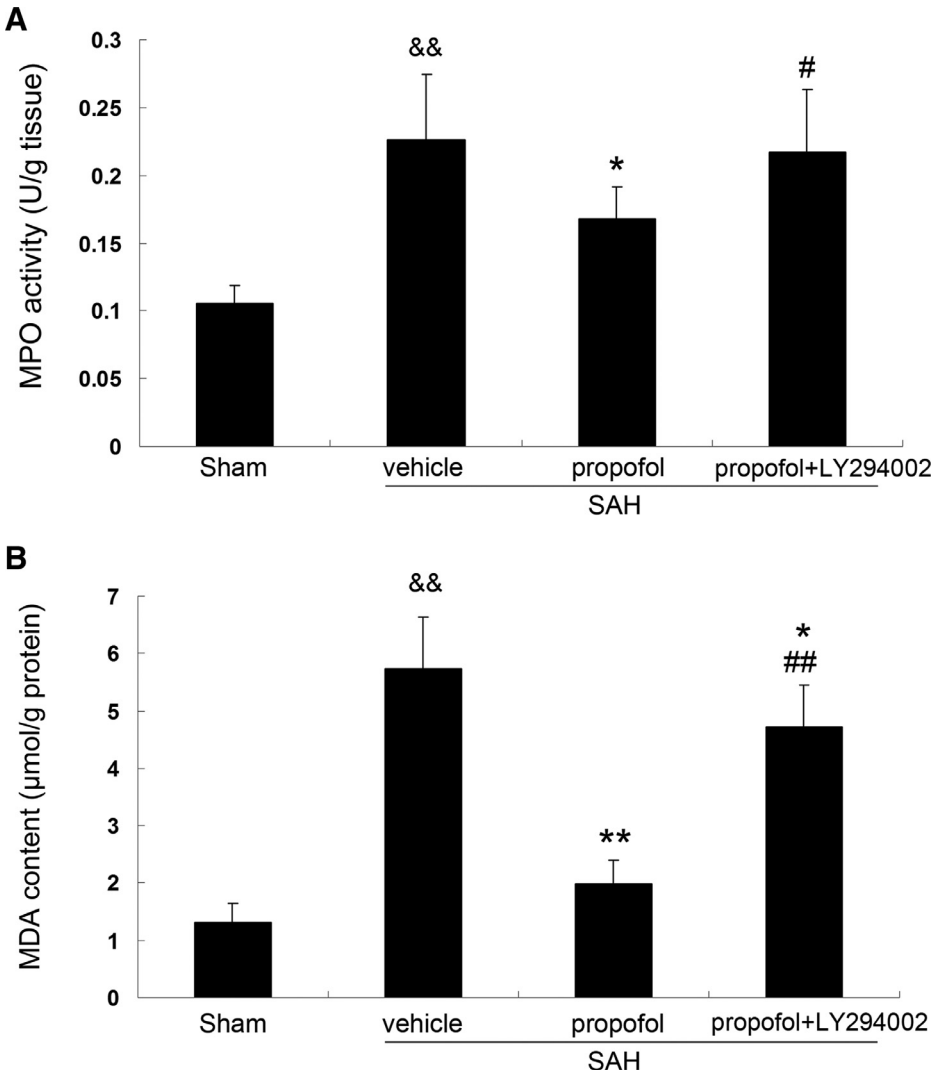

Figure 2. *LY294002 inhibits the effect of propofol on the enzymatic activity of MPO and MDA content. SAH caused an obvious elevation of MPO activity (A) and MDA content (B) in brain after 24 hours of SAH. Propofol significantly reduced the MPO activity and MDA content, both of which were abolished by LY294002. n = 5, &&P < .01 compared to sham group, *P < .05 and **P < .01 compared to SAH+vehicle group, #P < .05 and ##P < .01 compared to SAH +propofol group.*

reperfusion injury in rabbits possibly associated with the activation of PI3K/Akt signaling.[18] However, it is unknown whether PI3K/Akt signaling pathway was involved in propofol's neuroprotective, anti-inflammatory, and antioxidative roles against experimental SAH.

The main findings of the present study are as follows: (1) propofol reduces EBI after SAH possibly through the activation of PI3K/Akt signaling pathway; (2) propofol attenuates inflammatory and oxidative reactions possibly through the activation of PI3K/Akt signaling pathway; (3) propofol inhibits the expression of inflammatory mediators (NF-κB/COX-2/TNF-α/IL-1β pathway) possibly through the activation of PI3K/Akt signaling pathway; (4) propofol promotes the expression of oxidative mediators (Nrf2/NQO-1/GSH-Px/SOD pathway) possibly through the activation of PI3K/Akt signaling pathway.

The PI3K/Akt signaling is well known as a major upstream element that plays a critical role in controlling the balance between survival and cell damage.[25] Akt is activated by phosphorylating, which promotes cell survival and prevents oxidative injury and inflammatory reaction by modulating the downstream targets, including NF-κB and Nrf2 signalings.[26,27] Sugawara et al[28] showed intraperitoneal injection of simvastatin (20 mg/kg) reduces neurological dysfunction and cerebral vasospasm after SAH in rats, and intravenous injection of PI3K/Akt inhibitor wortmannin (15 μg/kg) abolished the above-mentioned neuroprotection of simvastatin, which demonstrated that PI3K/Akt signaling mediates the neuroprotection against SAH and its cerebral vasospasm. Hao et al[29] demonstrated that intraperitoneal injection of ghrelin (80 μg/kg) alleviates EBI (neurological deficit and brain edema) after SAH in rats, which was reversed by intracerebroventricular injection of PI3K/Akt inhibitor LY294002. Furthermore, ghrelin also reduces neuronal apoptosis and cleaved caspase-3 expression in

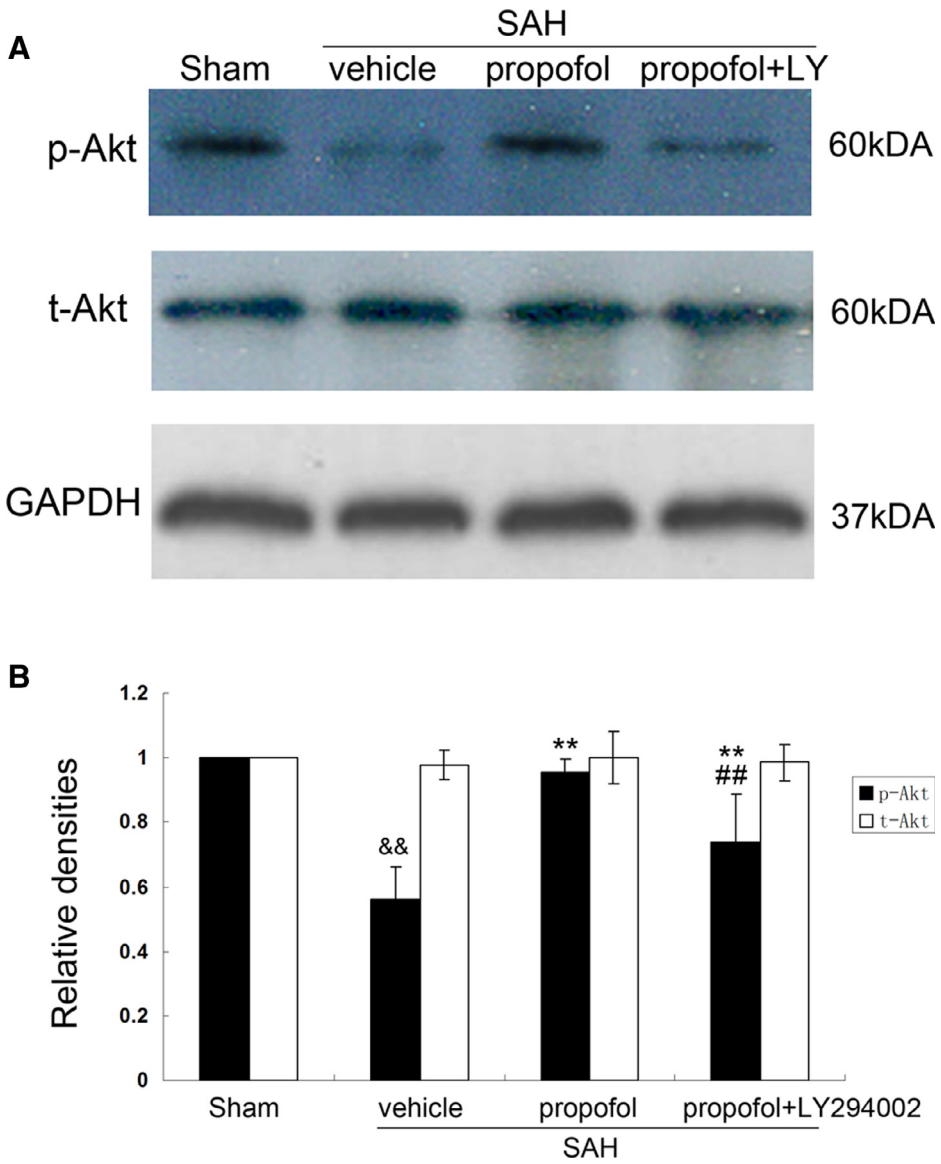

Figure 3. *LY294002 inhibits the activation of PI3K/Akt pathway in rats of SAH. Representative protein bands of p-Akt, t-Akt and GAPDH in rat brains were detected by western blot (A), and the data were summarized in (B). SAH caused the downregulates expression of p-Akt in rats 24 hours after SAH. Propofol upregulated the immunoreactivity of p-Akt in rat brain, which was abolished by LY294002. No significant change was found in the expression of t-Akt between different groups. n = 5, &&P < .01 compared to sham group, **P < .01 compared to SAH+vehicle group, ##P < .01 compared to SAH+propofol group.*

rats of SAH, which were inhibited by LY294002 administration.[29] These results indicate PI3K/Akt signaling is involved in the neuroprotective and antiapoptotic properties against SAH.

Wei et al[30] showed that propofol can protect renal ischemia-reperfusion injury by reducing apoptosis and inflammation partially via the modulation of the PI3K/AKT/mTOR signaling pathway. Wang et al[17] demonstrated propofol postconditioning could provide neuroprotection against cerebral ischemic injury via PI3K/Akt pathway. The present study showed that propofol improves neurological scores, attenuates brain edema and BBB integrity, all of the effects above-mentioned were abolished by LY294002 administration, indicating that propofol reduces EBI after SAH, which is mediated, at least partially through the PI3K/Akt survival signaling pathway. In addition, p-Akt was downregulated after SAH, but recovered by propofol, and LY294002 administration abolished propofol's effect on the Akt phosphorylation, which indicates LY294002 can inhibit PI3K/Akt effectively.

Inflammation and oxidative stress in brain are the main pathological process of EBI after SAH. Agents targeted at brain inflammation and oxidative stress have been considered as the promising therapeutic strategies for SAH. PI3K/Akt signaling pathway was reported to negatively regulate the inflammatory reaction[31] and oxidative stress.[32] NF-κB/COX-2/TNF-α/IL-1β inflammatory pathway was reported to mediate the brain inflammation and EBI following SAH.[33,34] PI3K/Akt signaling was

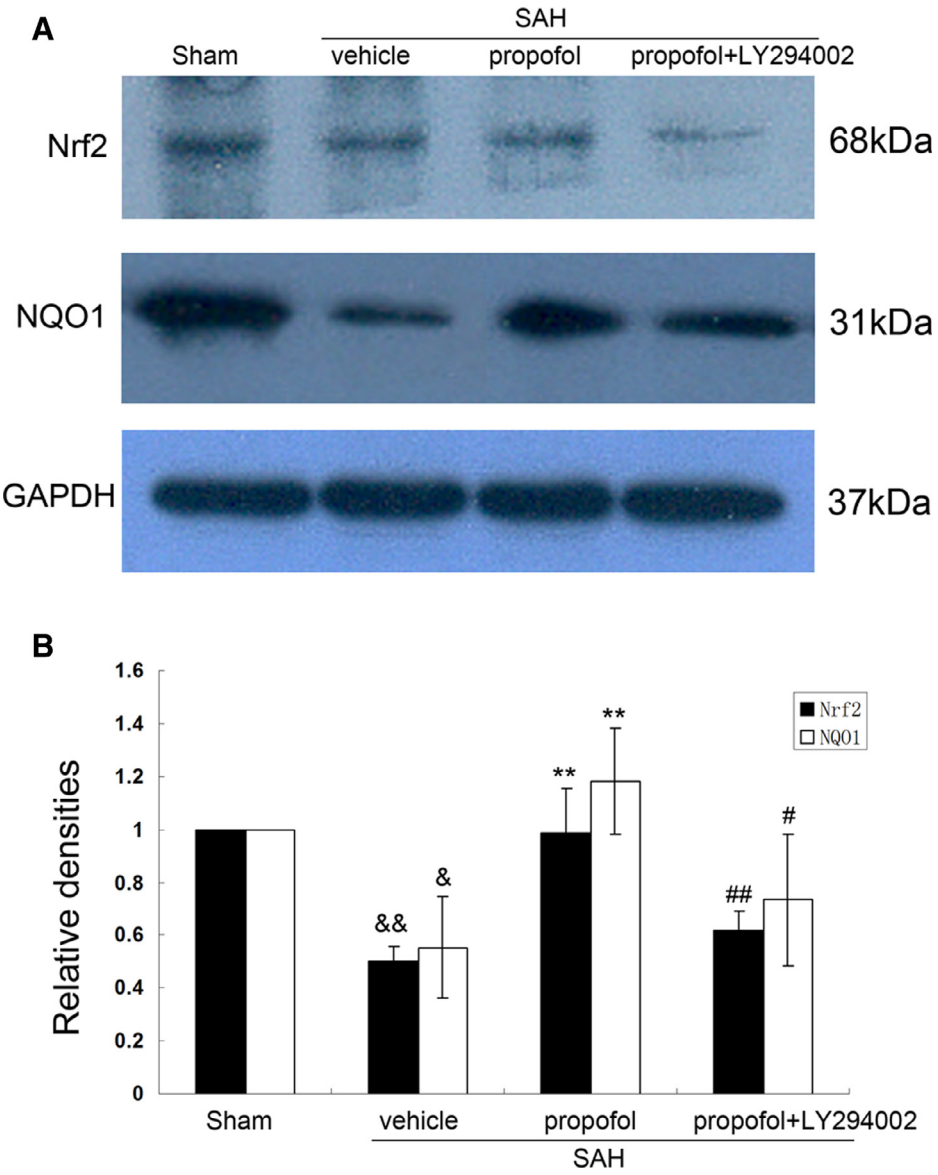

Figure 4. *LY294002 reverses the effect of propofol on Nrf2 and NQO1 expression. Representative protein expression bands of Nrf2, NQO1 and GAPDH in rat brains were detected by western blot (A), and the data were summarized in (B). Propofol upregulated the expression of Nrf2 and NQO1 in rat brain, which was abolished by LY294002. n = 5, &P < .05 and &&P < .01 compared to sham group, **P < .01 compared to SAH+vehicle group, #P < .05 and ##P < .01 compared to SAH+propofol group.*

shown to modulate the inflammatory reaction and the activation of inflammatory pathway (NF-κB/COX-2/TNF-α/IL-1β).[26,35] Propofol was demonstrated to reduce inflammation through suppressing reactive oxygen species (ROS)-regulated Akt/IKKβ/NF-κB signaling pathway.[36] Our previous study showed that propofol reduces brain inflammation and the expression of NF-κB, COX-2, TNF-α, and IL-1β in rats of SAH. The present study demonstrated propofol inhibits neutrophil infiltration and NF-κB/COX-2/TNF-α/IL-1β inflammatory pathway in rats of SAH, but all of which were reversed by intracerebroventricular administration of LY294002. These results indicate propofol reduces brain inflammation and NF-κB/COX-2/TNF-α/IL-1β possibly through the activation of PI3K/Akt pathway.

Nuclear factor-E2-related factor 2 (Nrf2) is one of the key regulators of endogenous antioxidant defense. In response to oxidative stress, Nrf2 promotes the expression of a wide range of antioxidant genes through translocating into the nucleus and binding to antioxidant response element (ARE), eventually leading to regulate the transcription of downstream target genes,[37] such as heme oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), GSH-Px, and SOD. Nrf2/ARE antioxidative signaling pathway has been considered to protect brain against cerebral ischemia injury,[37] traumatic brain injury,[38] intracerebral hemorrhage,[39] Pakinson's disease[40], and also SAH.[41] Nrf2 and the downstream target genes like HO-1, NQO1 and glutathione S-transferase-α1 was demonstrated to protect brain subjected to SAH via the

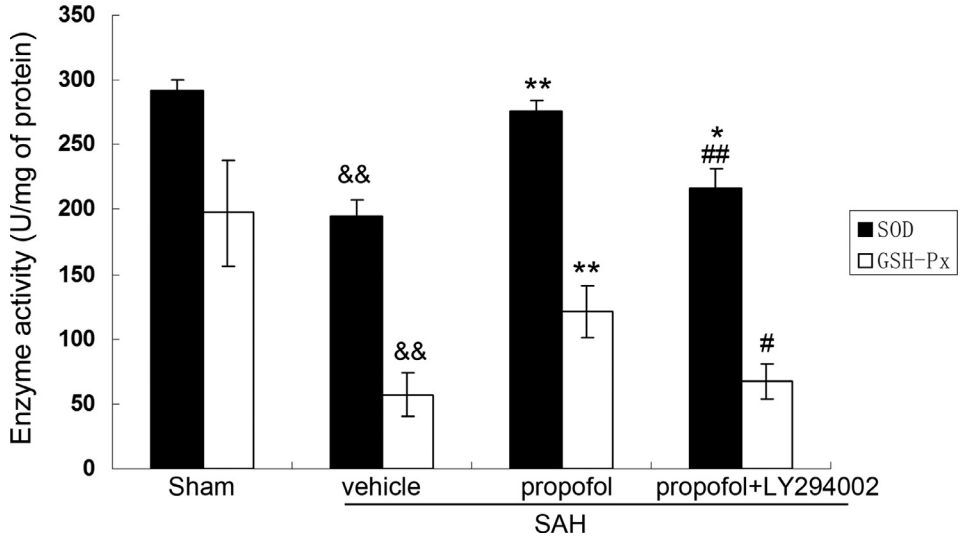

Figure 5. *LY294002 reduces the effect of propofol on the activities of SOD and GSH-Px. SAH caused an obvious attenuation of SOD and GSH-Px activities in brain after 24 hours of SAH. Propofol significantly increased the activities of GSH-Px and SOD, both of which were abolished by LY294002. n = 5, &&P < .01 compared to sham group, *P < .05 and **P < .01 compared to SAH+vehicle group, #P < .05 and ##P < .01 compared to SAH+propofol group.*

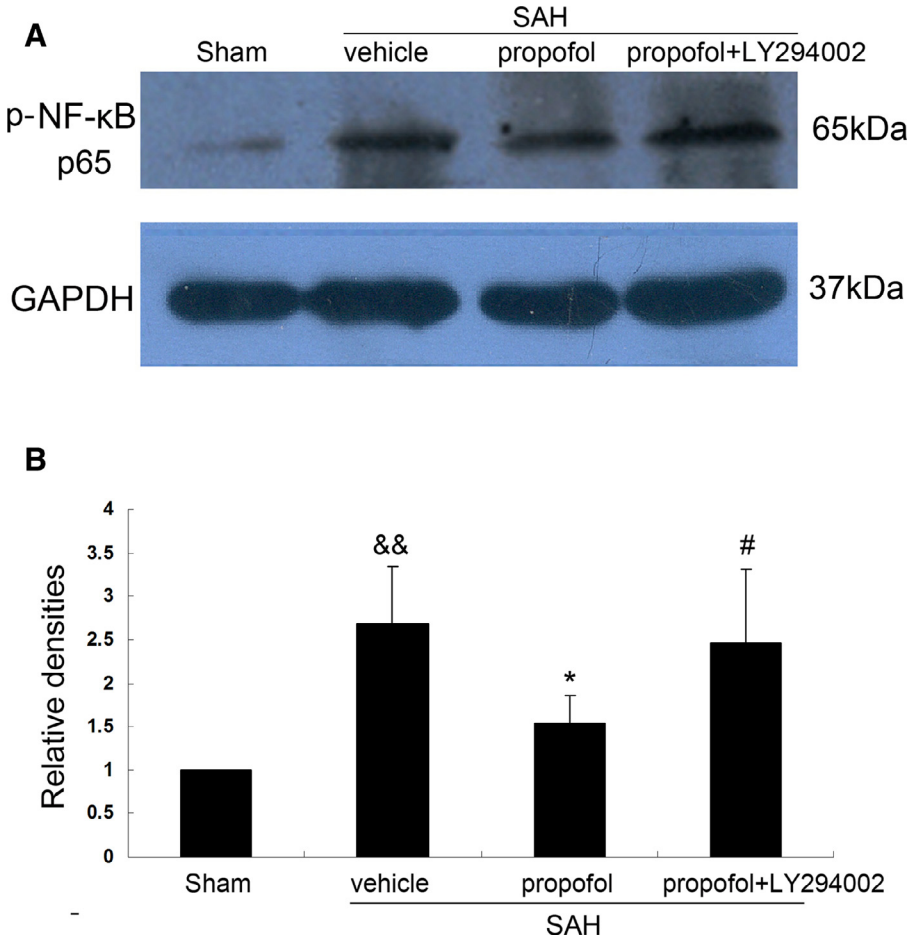

Figure 6. *LY294002 reduces the effect of propofol on the expression of phosphorylated NF-κB p65. Representative protein expression bands of p-NF-κB p65 and GAPDH in rat brains were detected by western blot (A), and the data were summarized in (B). Propofol inhibited the expression of p-NF-κB p65 in rat brain, which was abolished by LY294002. n = 5, &&P < .01 compared to sham group, *P < .05 compared to SAH+vehicle group, #P < .05 compared to SAH+propofol group.*

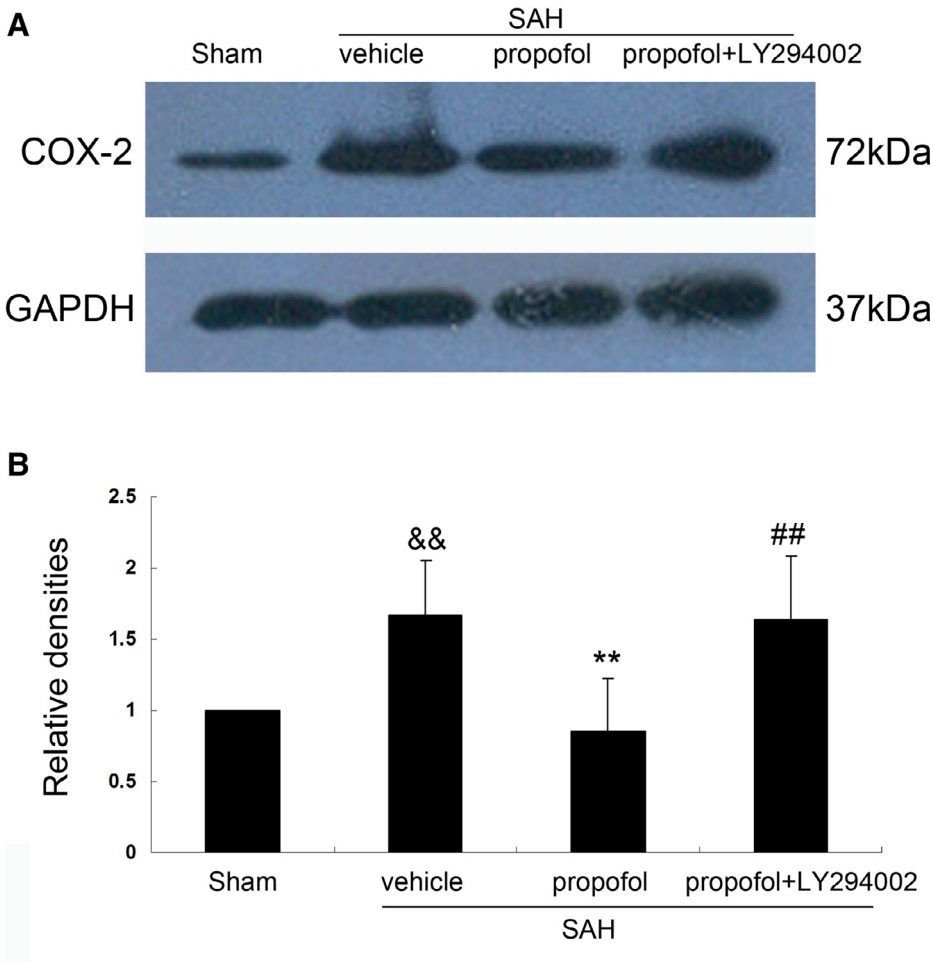

Figure 7. *LY294002 inhibits the effect of propofol on COX-2 expression. Representative bands of COX-2 in rat brain from sham operation, vehicle-treated, propofol-treated and LY294002-treated rats were detected by western blot (A), and the data were summarized in (B). Propofol inhibited the protein expression of COX-2 in rat brain, which was abolished by LY294002. n = 5, &&P < .01 compared to sham group, **P < .01 compared to SAH+vehicle group, ##P < .01 compared to SAH+propofol group.*

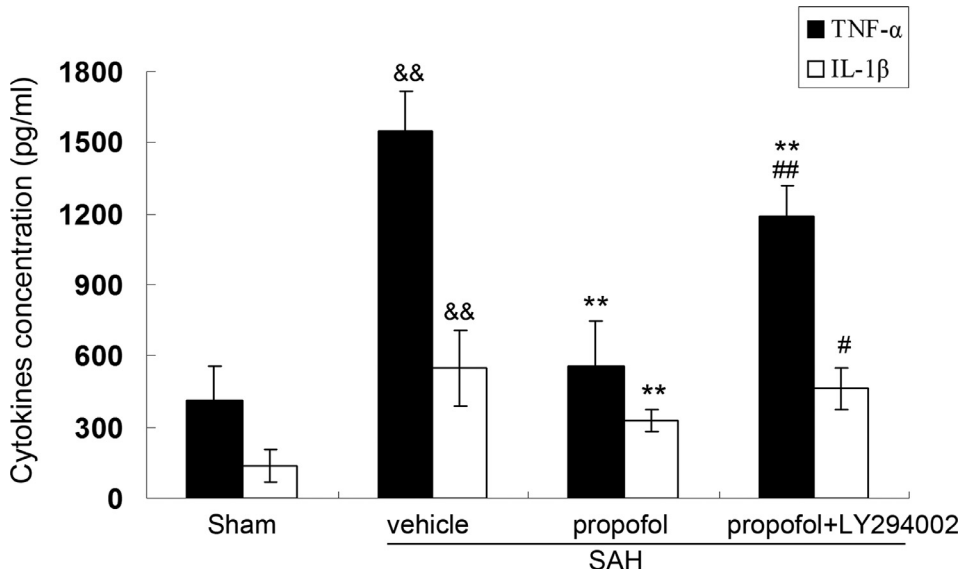

Figure 8. *LY294002 inhibits the effect of propofol on the expression of TNF-α and IL-1β. SAH caused the elevated release of TNF-α and IL-1β in rat brains 24 hours after SAH. Propofol significantly decreased the expression of TNF-α and IL-1β, which was inhibited by LY294002. n = 5, &&P < .01 compared to sham group, **P < .01 compared to SAH+vehicle group, #P < .05 and ##P < .01 compared to SAH+propofol group.*

antioxidative effect.[42,43] PI3K/Akt signaling was reported to positively modulate the nuclear translocation of Nrf2 and the antioxidative pathway and reduce oxidative stress in PC12 cells[27] and B35 neural cells.[44] PI3K/Akt also mediates the neuroprotection against ischemic brain injury through activating the Nrf2/ARE pathway in vitro and in vivo.[45,46] Propofol ameliorates liver oxidative injury through inducing the nuclear translocation of Nrf2 and further upregulates the expression of HO-1 and NQO1,[47] it also reduce acute lung injury in a rat model of liver transplantation via the activation of Nrf2/ARE pathway. The present study showed that propofol attenuates the oxidative stress in rats of SAH, which was reversed by LY294002 administration, indicating that PI3K/Akt signaling mediates propofol's antioxidative effect on SAH. Furthermore, propofol was demonstrated to activate Nrf2/ARE antioxidative pathway (Nrf2/HO-1/NOQ1/SOD/GSH-Px), which was also inhibited by LY294002, suggesting Nrf2/ARE pathway is possibly modulated by activation of PI3K/Akt signaling. Taken together, propofol reduces oxidative stress and activates Nrf2/ARE pathway in rats of SAH, which might be associated with the activation of PI3K/Akt pathway.

Conclusion

Our results demonstrated that propofol reduces EBI, brain inflammation and oxidative reaction in rats of SAH possibly through activating PI3K/Akt signaling pathway. Further investigation showed propofol downregulates the expression of NF-κB/COX-2/TNF-α/IL-1β inflammatory pathway and upregulates the expression of Nrf2/NQO1/GSH-Px/SOD possibly through activating PI3K/Akt signaling pathway. These results suggest that PI3K/Akt pathway-dependent NF-κB inactivation and PI3K/Akt-mediated Nrf2/ARE activation might be a promising therapeutic target for the treatment of SAH in future.

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Declaration of Competing Interest

None declared.

References

1. Chen S, Feng H, Sherchan P, et al. Controversies and evolving new mechanisms in subarachnoid hemorrhage. Prog Neurobiol 2014;115:64-91.
2. Caner B, Hou J, Altay O, et al. Transition of research focus from vasospasm to early brain injury after subarachnoid hemorrhage. J Neurochem 2012;123(Suppl 2):12-21.
3. Fujii M, Yan J, Rolland WB, et al. Early brain injury, an evolving frontier in subarachnoid hemorrhage research. Transl Stroke Res 2013;4:432-446.
4. Ayer RE, Zhang JH. Oxidative stress in subarachnoid haemorrhage_ significance in acute brain injury and vasospasm. Acta Neurochir Suppl 2008;104:33-41.
5. Uekawa K, Hasegawa Y, Ma M, et al. Rosuvastatin ameliorates early brain injury after subarachnoid hemorrhage via suppression of superoxide formation and nuclear factor-kappa B activation in rats. J Stroke Cerebrovasc Dis 2014;23:1429-1439.
6. Shi SS, Zhang HB, Wang CH, et al. Propofol attenuates early brain injury after subarachnoid hemorrhage in rats. J Mol Neurosci 2015;57:538-545.
7. Dudek H. Regulation of neuronal survival by the serine-threonine protein kinase Akt. Science 1997;275:661-665.
8. Huang J, Kodithuwakku ND, He W, et al. The neuroprotective effect of a novel agent N2 on rat cerebral ischemia associated with the activation of PI3K/Akt signaling pathway. Neuropharmacology 2015;95:12-21.
9. Du G, Zhao Z, Chen Y, et al. Quercetin attenuates neuronal autophagy and apoptosis in rat traumatic brain injury model via activation of PI3K/Akt signaling pathway. Neurol Res 2016:1-8.
10. Nakaso K, Tajima N, Horikoshi Y, et al. The estrogen receptor beta-PI3K/Akt pathway mediates the cytoprotective effects of tocotrienol in a cellular Parkinson's disease model. Biochim Biophys Acta 2014;1842:1303-1312.
11. Kong J, Ren G, Jia N, et al. Effects of nicorandil in neuroprotective activation of PI3K/AKT pathways in a cellular model of Alzheimer's disease. Eur Neurol 2013;70:233-241.
12. Cui HJ, Yang AL, Zhou HJ, et al. Buyang huanwu decoction promotes angiogenesis via vascular endothelial growth factor receptor-2 activation through the PI3K/Akt pathway in a mouse model of intracerebral hemorrhage. BMC Complement Altern Med 2015;15:91.
13. Ma J, Wang Z, Liu C, et al. Pramipexole-induced hypothermia reduces early brain injury via PI3K/AKT/GSK3beta pathway in subarachnoid hemorrhage rats. Sci Rep 2016;6:23817.
14. Hong Y, Shao A, Wang J, et al. Neuroprotective effect of hydrogen-rich saline against neurologic damage and apoptosis in early brain injury following subarachnoid hemorrhage: possible role of the Akt/GSK3β signaling pathway. PLoS One 2014;9:e96212.
15. Chen Z, Ma X, Zhu Y, et al. Paeoniflorin ameliorates ANIT-induced cholestasis by activating Nrf2 through an PI3K/Akt-dependent pathway in rats. Phytother Res 2015.
16. Tu XK, Yang WZ, Chen JP, et al. Repetitive ischemic preconditioning attenuates inflammatory reaction and brain damage after focal cerebral ischemia in rats: involvement of PI3K/Akt and ERK1/2 signaling pathway. J Mol Neurosci 2015;55:912-922.
17. Wang HY, Wang GL, Yu YH, Wang Y. The role of phosphoinositide-3-kinase/Akt pathway in propofol-induced postconditioning against focal cerebral ischemia-reperfusion injury in rats. Brain Res 2009;1297:177-184.
18. Yu QJ, Yang Y. Function of SOD1, SOD2, and PI3K/AKT signaling pathways in the protection of propofol on spinal cord ischemic reperfusion injury in a rabbit model. Life Sci 2016;148:86-92.
19. Zhao H, Shimohata T, Wang JQ, et al. Akt contributes to neuroprotection by hypothermia against cerebral ischemia in rats. J Neurosci 2005;25:9794-9806.

20. Sugawara T, Ayer R, Jadhav V, Zhang JH. A new grading system evaluating bleeding scale in filament perforation subarachnoid hemorrhage rat model. J Neurosci Methods 2008;167:327-334.
21. Garcia JH, Wagner S, Liu KF, Hu XJ. Neurological deficit and extent of neuronal necrosis attributable to middle cerebral artery occlusion in rats. Statistical validation. Stroke 1995;26:627-634. discussion 635.
22. Shi SS, Yang WZ, Chen Y, et al. Propofol reduces inflammatory reaction and ischemic brain damage in cerebral ischemia in rats. Neurochem Res 2014;39:793-799.
23. Zhuang Z, Zhao X, Wu Y, et al. The anti-apoptotic effect of PI3K-Akt signaling pathway after subarachnoid hemorrhage in rats. Ann Clin Lab Sci 2011;41:364-372.
24. Zhao LL, Hu GC, Zhu SS, et al. Propofol pretreatment attenuates lipopolysaccharide-induced acute lung injury in rats by activating the phosphoinositide-3-kinase/Akt pathway. Braz J Med Biol Res 2014;47:1062-1067.
25. Noshita N, Lewen A, Sugawara T, Chan PH. Evidence of phosphorylation of Akt and neuronal survival after transient focal cerebral ischemia in mice. J Cereb Blood Flow Metab 2001;21:1442-1450.
26. Dai C, Zheng CQ, Meng FJ, et al. VSL#3 probiotics exerts the anti-inflammatory activity via PI3k/Akt and NF-kappaB pathway in rat model of DSS-induced colitis. Mol Cell Biochem 2013;374:1-11.
27. Li C, Pan Z, Xu T, et al. Puerarin induces the upregulation of glutathione levels and nuclear translocation of Nrf2 through PI3K/Akt/GSK-3beta signaling events in PC12 cells exposed to lead. Neurotoxicol Teratol 2014;46:1-9.
28. Sugawara T, Ayer R, Jadhav V, et al. Simvastatin attenuation of cerebral vasospasm after subarachnoid hemorrhage in rats via increased phosphorylation of Akt and endothelial nitric oxide synthase. J Neurosci Res 2008;86:3635-3643.
29. Hao XK, Wu W, Wang CX, et al. Ghrelin alleviates early brain injury after subarachnoid hemorrhage via the PI3K/Akt signaling pathway. Brain Res 2014;1587:15-22.
30. Wei QJ, Zhao JJ, Zhou XG, et al. Propofol can suppress renal ischemia-reperfusion injury through the activation of PI3K/AKT/mTOR signal pathway. Gene 2019;708:14-20.
31. Tu XK, Zhang HB, Shi SS, et al. 5-LOX inhibitor zileuton reduces inflammatory reaction and ischemic brain damage through the activation of PI3K/Akt signaling pathway. Neurochem Res 2016;41:2779-2787.
32. Ayadi AE, Zigmond MJ, Smith AD. IGF-1 protects dopamine neurons against oxidative stress: association with changes in phosphokinases. Exp Brain Res 2016;234:1863-1873.
33. You WC, Wang CX, Pan YX, et al. Activation of nuclear factor-kappaB in the brain after experimental subarachnoid hemorrhage and its potential role in delayed brain injury. PLoS One 2013;8:e60290.
34. Zhang J, Xu X, Zhou D, et al. Possible role of Raf-1 kinase in the development of cerebral vasospasm and early brain injury after experimental subarachnoid hemorrhage in rats. Mol Neurobiol 2015;52:1527-1539.
35. Shalini V, Pushpan CK, G S, et al. Tricin, flavonoid from Njavara reduces inflammatory responses in hPBMCs by modulating the p38MAPK and PI3K/Akt pathways and prevents inflammation associated endothelial dysfunction in HUVECs. Immunobiology 2016;221:137-144.
36. Hsing CH, Lin MC, Choi PC, et al. Anesthetic propofol reduces endotoxic inflammation by inhibiting reactive oxygen species-regulated Akt/IKKbeta/NF-kappaB signaling. PLoS One 2011;6:e17598.
37. Zhang R, Xu M, Wang Y, et al. Nrf2-a promising therapeutic target for defensing against oxidative stress in stroke. Mol Neurobiol 2016.
38. Li X, Wang H, Gao Y, et al. Protective effects of quercetin on mitochondrial biogenesis in experimental traumatic brain injury via the Nrf2 signaling pathway. PLoS One 2016;11:e0164237.
39. Sukumari-Ramesh S, Alleyne Jr. CH. Post-injury administration of tert-butylhydroquinone attenuates acute neurological injury after intracerebral hemorrhage in mice. J Mol Neurosci 2016;58:525-531.
40. Lv E, Deng J, Yu Y, et al. Nrf2-ARE signals mediated the anti-oxidative action of electroacupuncture in an MPTP mouse model of Parkinson's disease. Free Radic Res 2015;49:1296-1307.
41. Liu Y, Qiu J, Wang Z, et al. Dimethylfumarate alleviates early brain injury and secondary cognitive deficits after experimental subarachnoid hemorrhage via activation of Keap1-Nrf2-ARE system. J Neurosurg 2015;123:915-923.
42. Chen G, Fang Q, Zhang J, et al. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. J Neurosci Res 2011;89:515-523.
43. Wu Q, Zhang XS, Wang HD, et al. Astaxanthin activates nuclear factor erythroid-related factor 2 and the antioxidant responsive element (Nrf2-ARE) pathway in the brain after subarachnoid hemorrhage in rats and attenuates early brain injury. Marine Drugs 2014;12:6125-6141.
44. Xu XH, Li GL, Wang BA, et al. Diallyl trisufide protects against oxygen glucose deprivation -induced apoptosis by scavenging free radicals via the PI3K/Akt -mediated Nrf2/HO-1 signaling pathway in B35 neural cells. Brain Res 2015;1614:38-50.
45. Gao Y, Xu X, Chang S, et al. Totarol prevents neuronal injury in vitro and ameliorates brain ischemic stroke: potential roles of Akt activation and HO-1 induction. Toxicol Appl Pharmacol 2015;289:142-154.
46. Li L, Yang N, Nin L, et al. Chinese herbal medicine formula tao hong si wu decoction protects against cerebral ischemia-reperfusion injury via PI3K/Akt and the Nrf2 signaling pathway. J Nat Med 2015;69:76-85.
47. Ge M, Yao W, Wang Y, et al. Propofol alleviates liver oxidative stress via activating Nrf2 pathway. J Surg Res 2015;196:373-381.