Original Article
Curcumin protects against cerebral ischemia-reperfusion injury by activating JAK2/STAT3 signaling pathway in rats

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Abstract: Curcumin (Cur) is the active component in *Curcuma longa*, and it has been reported to exhibit a variety of biological effects such as anti-inflammation and anti-oxidation. This study aims to investigate the effect of Cur on cerebral ischemia-reperfusion injury and whether the Janus kinase 2 and signal transducer and activator 3 of transcription (JAK2/STAT3) signaling pathway is involved in the neuroprotective effects of Cur. Rats were subjected to 2 h transient middle cerebral artery occlusion (MCAO), followed by 24 h reperfusion. Rats were randomly assigned into sham group, MCAO group, MCAO + Cur group. AG490, a JAK2 inhibitor, was utilized to throw light upon the underlying mechanism. The results suggested that compared to MCAO, Cur attenuated neurological deficits, reduced cerebral infarction area and lowered brain water content. In addition, Cur reduced the activity of IL-1β and IL-8. The results of western blot indicated that Cur enhanced the expression of p-JAK2 and p-STAT3, which was abolished by AG490 administration. Our results suggested that Cur protects effects against cerebral I/R injury through the activation of JAK2/STAT3 signaling pathway.

Keywords: Curcumin, cerebral ischemia, neuroprotection, JAK2/STAT3 signaling

Introduction

Ischemic stroke is the third leading cause of death and its complications include hemiplegia, coma and even death [1, 2]. The middle cerebral artery is often involved in ischemic stroke. Although clinical experience with clot-lysing drugs has confirmed that early reperfusion ameliorates clinical outcome, the efficacy is limited due to the narrow therapeutic window [1, 3]. Cerebral ischemia triggers a series of damages, including cell necrosis, cell apoptosis and brain edema [4]. In addition, reperfusion triggers ischemia-reperfusion injury, which in turn aggravates brain injury, such as reactive oxygen species (ROS) overproduction, neuroinflammation and neuronal apoptosis [5, 6]. Therefore, novel therapeutic agents and targets need to be explored.

The Janus kinase2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling pathway is suggested to play a critical role in a variety of physiological processes, such as cell development and differentiation [7]. It has been demonstrated that JAK2/STAT3 is involved in cardioprotection against ischemia-reperfusion injury [8, 9]. In addition, SMND-309, a novel derivative of salvianolic acid B, reportedly protects ischemia and reperfusion injury by activating JAK2/STAT3 pathway in rat brain [10]; indicating JAK2/STAT3 activation is associated with protection against cerebral ischemia-reperfusion injury.

Curcumin (Cur) is a natural polyphenolic component in *Curcuma longa*. It has been suggested to exhibit a wide range of biological activities, such as anti-inflammation, anti-oxidation, anti-apoptosis and anti-tumor [11-16]. Additionally, Cur is reported to be neuroprotective in neuro-degenerative disorders and cerebral ischemia [17-22]. However, whether Cur confers neuroprotection against cerebral is chemia-reperfusion injury via JAK2/STAT3 signaling remains unclear. This study, therefore, aims to investi-
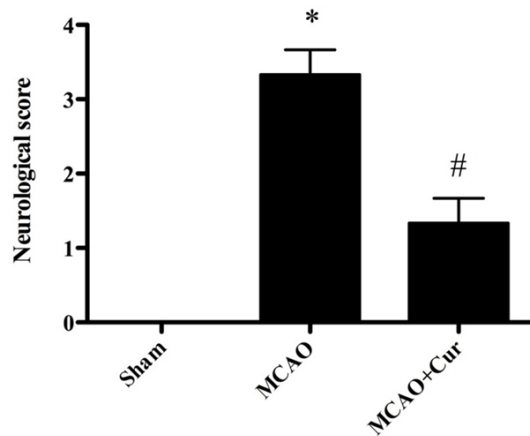
Figure 1. Effect of curcumin on neurological deficit. Curcumin (Cur) significantly attenuates neurological deficit compared with that in the MCAO group. Data were expressed as mean ± S.E.M. (n=10 in each group); *P < 0.05 versus thesham group, #P < 0.05 versus theMCAO group.

gate the role of JAK2/STAT3 signaling in the neuroprotective effect of Cur.

Materials and methods

Reagents

Cur and triphenyltetrazolium chloride (TTC) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies againstphospho-JAK2, phospho-STAT3, JAK2, STAT3 and β-actin antibodies were purchased from Santa Cruz Company, USA. The rabbit anti-goat, goat anti-rabbit and goat anti-mouse secondary antibodies were purchased from Beyotime, China.

Animals

Male Sprague-Dawley rats (230-280 g), purchased from the Laboratory Animal Center of Shandong University, were kept under a controlled environment (12/12 h light/dark cycle, 60%±5% humidity, 22°C±3°C). And they have free access to water and food. All procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 80-23), revised 1996.

Middle cerebral artery occlusion model and experimental protocol

A middle cerebral artery occlusion (MCAO) model was established as previously described.

Briefly, rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate (350 mg/kg). The right common carotid artery was then exposed and isolated. The MCA was occluded by inserting a monofilament nylon suture with a heat-rounded tip into the internal carotid artery, which was advanced further until it closed the origin of the MCA. Ninety minutes after induction of ischemia, reperfusion was initiated by withdrawal of the monofilament for 24 h. Sham-operated control rats received the same surgical procedure without MCA occlusion. Rectal temperature was maintained at 37°C using a heating blanket and heating lamp during the surgery.

Rats were randomly assigned into three groups: sham group (n=10), MCAO group (n=10) and MCAO+Cur group (n=10). Cur, dissolved in normal saline, was administered at a dose of 80 mg/kg i.p. starting at 0.5 h prior to reperfusion. Sham group and MCAO group rats were injected solely with an equal amount of normal saline. For the mechanism part, rats were administrated with Cur and AG490 (i.p. 3 mg/kg, dissolved in DMSO, n=10) 0.5 h prior to reperfusion.

Neurological score assessment

Neurological deficits were evaluated by an observer blinded to the treatment of animals after 24 h reperfusion according to the methods previously described. Score 0: no neurologic deficit; Score 1: failure to extend left forepaw fully; Score 2: circling to the left; Score 3: falling to the left; Score 4: did not walk spontaneously and had a depressed level of consciousness. Rats that did not show neurological deficits immediately after reperfusion (neurological score=0) were excluded from the groups.

Evaluation of infarct volume

Infarct volume was evaluated by 2,3,5-triphenyltetrazolium chloride (TTC) at 24 h after reperfusion. Animals were euthanized and the brains were quickly removed. Then the brain was sliced into five coronal sections (3 mm thick each) and stained with 2% solution of TTC at 37°C for 20 min, followed by fixation in 4% paraformaldehyde. TTC-stained sections were photographed and the digital images were analyzed using image analysis software (Image-Pro
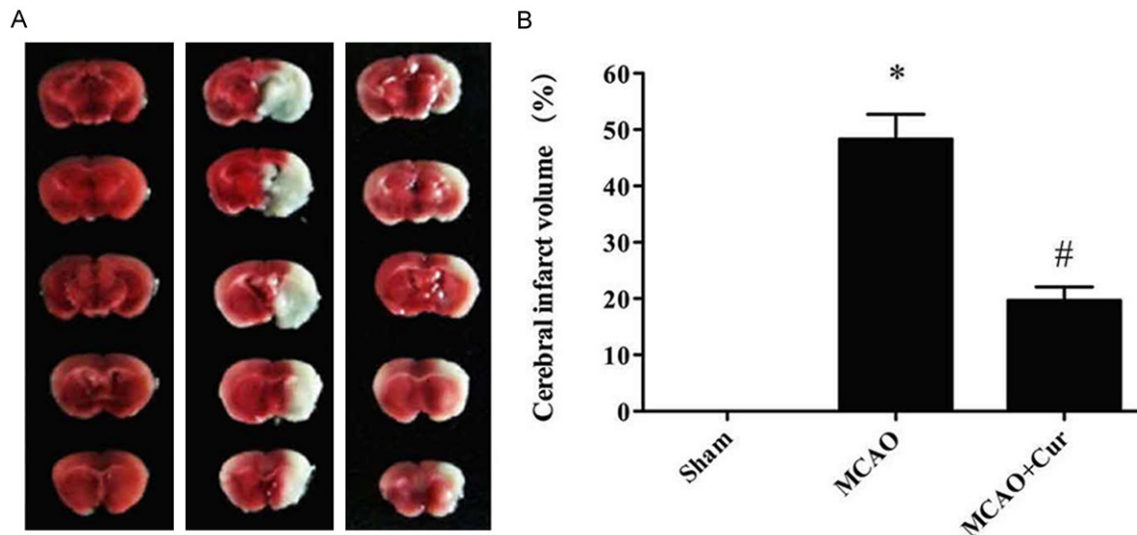
Figure 2. Curcumin (Cur) decreases the infarct size in rats subjected to middle cerebral artery occlusion. A. Representative pictures of stained cerebral sections in each group. The normal tissue stained dark red, while the infarct tissue was white; B. Quantitative analysis of infarct volume. Data were expressed as mean ± S.E.M. (n=10 in each group); *$P < 0.05$ versus thesham group, #$P < 0.05$ versus theMCAO group.

Plus 5.1). The lesion volume was calculated by multiplying the area by the thickness of slices. The percentage hemisphere lesion volume (% HLV) was calculated by the following formula [23]: % HLV = ([total infarct volume-(the volume of intact ipsilateral hemisphere-the volume of intact contralateral hemisphere)])/contralateral hemisphere volume × 100%.

Nissl staining

After rats were deeply anesthetized, brains were collected for assessing the neuronal damage 24 h after reperfusion. Rats were perfused transcardially with 4% paraformaldehyde in PBS, and the brains were obtained, then post-fixed with 10% formalin for 24 h and embedded in paraffin. Coronal sections at 5-μm thickness were cut and the sections were stained using cresyl violet.

Evaluation of brain edema

As described previously, after the wet weight of the brain tissues was quantified, the red and white parts of these brains were desiccated at 105°C for 48 h until the weight was constant. The total weight of the dried TTC-stained brains was obtained by measuring the desiccated red and white parts of these brains together, and the water content of each brain was calculated as follows: (wet weight-dried weight)/wet weight ×100%.

Detection of inflammatory cytokines

The inflammatory cytokines in brain tissue were measured using commercially ELISA kits for IL-1β and IL-8 (Beyotime, China). All spectrophotometric readings were performed with a microplate reader (Multiskan MK3, Thermo, USA). All procedures were performed according to the instructions.

Western blot

The brain tissue was lysed in ice-cold lysis buffer (50 mMTris-HCl, 1 mM EDTA, 1 mM EGTA, 0.5 mMNa$_3$VO$_4$, 0.1% 2-mercaptoethanol, 1% Triton X-100, 50 mM NaF, 5 mM sodium pyrophosphate, 10 mM sodium β-glyceropyrophosphate, 0.1 mM phenylmethanesulfonyl fluoride and protease inhibitor mixture) for 10 min. The lysates were centrifuged for 15 min at 12,000 g, and the resulting supernatant was collected and boiled. Protein concentrations of the extracts were measured by BCA assay. The whole protein 50 μg was resolved on 8-12% SDS polyacrylamide gel, then transferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat milk in TBST and then incubated with antibodies against phospho-JAK2, phospho-STAT3, JAK2, STAT3 (1:500) and β-actin (1:1000) at 4°C overnight, followed by washes with TBST. The membranes were then probed with the appropriate secondary
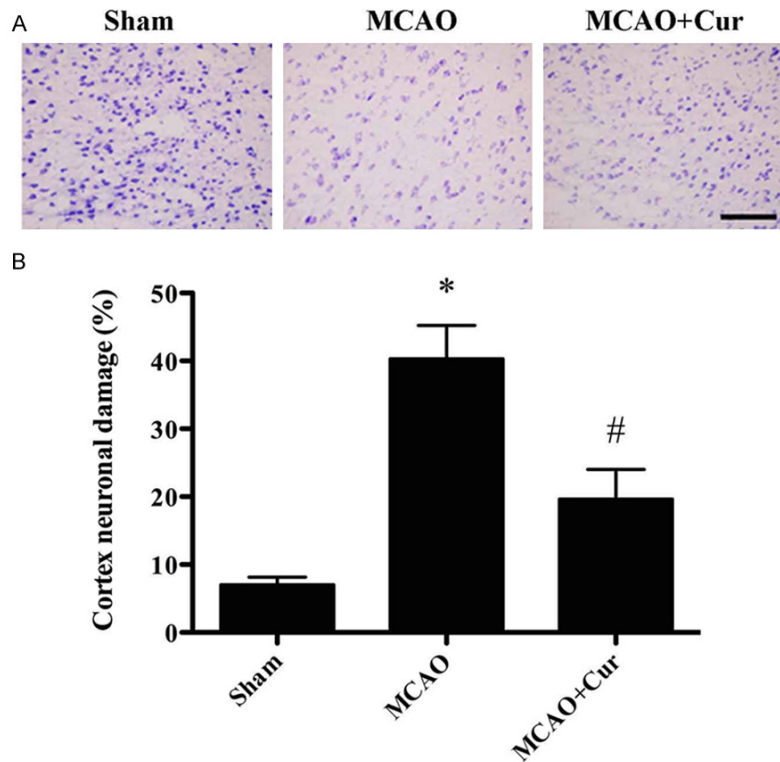
Figure 3. Effect of curcumin on cortec neuronal damage evaluated by Nissl staining. A. Representative pictures of cerebral sections in each group. B. Quantitative analysis of neuronal damage. Data were expressed as mean ± S.E.M. (n=10 in each group); $^*P < 0.05$ versus thesham group, $^\#P < 0.05$ versus theMCAO group.

Figure 4. Effect of curcumin on brain edema. The histogram shows the brain water content of rats. Data were expressed as mean ± S.E.M. (n=10 in

each group); $^*P < 0.05$ versus thesham group, $^\#P < 0.05$ versus theMCAO group.

antibodies (1:5000) at room temperature for 2 h and washed with TBST. The protein bands were detected using a BioRad imaging system (BioRad, Hercules, CA, USA) and quantified using the Quantity One software package (West Berkeley, CA, USA). The value for the MCAO group was defined as 100%.

Statistical analysis

Data were presented as mean ± S.E.M.. One-way analysis of variance (ANOVA) followed by the Dunnett's test was performed to compare the differences of parameters among groups. $P < 0.05$ was considered statistically significant.

Results

Effect of Cur on neurological disorder

The rats in sham group were in good state. 24 h after reperfusion, rats in MCAO group exhibited severe neurological deficits compared with the sham group. Cur administration significantly ameliorated neurological deficits compared with those of rats in the sham group ($P < 0.05$) (**Figure 1**).

Effect of Cur on cerebral infarct volume

The brain in the sham group was in normal state. MCAO resulted in a dramatic infarction in rats 24 h after reperfusion. Cur administration significantly decreased the infarct volume compared with that in the MCAO group ($P < 0.05$) (**Figure 2**).

Effect of Cur on cortex neuron survival

The results of Nissl staining were shown in **Figure 3**. MCAO resulted in a dramatic loss of neuron in the cortex compared with that in the
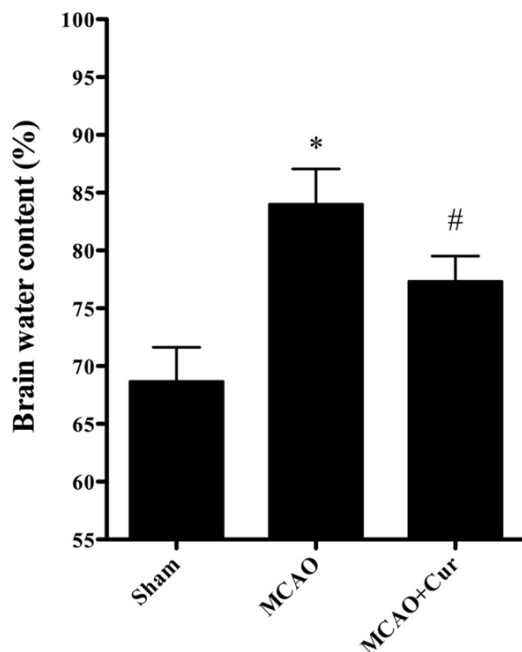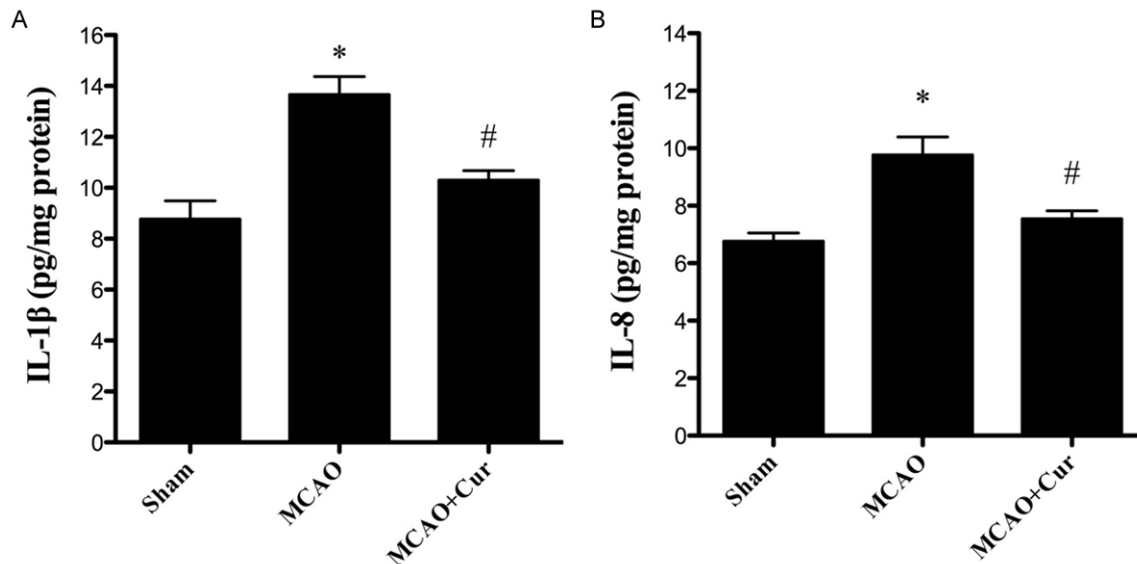
Figure 5. Effect of curcumin on IL-1β and IL-8 activity. A. IL-1β activity. B. IL-8 activity. Data were expressed as mean ± S.E.M. (n=10 in each group); *$P < 0.05$ versus thesham group, #$P < 0.05$ versus theMCAO group.
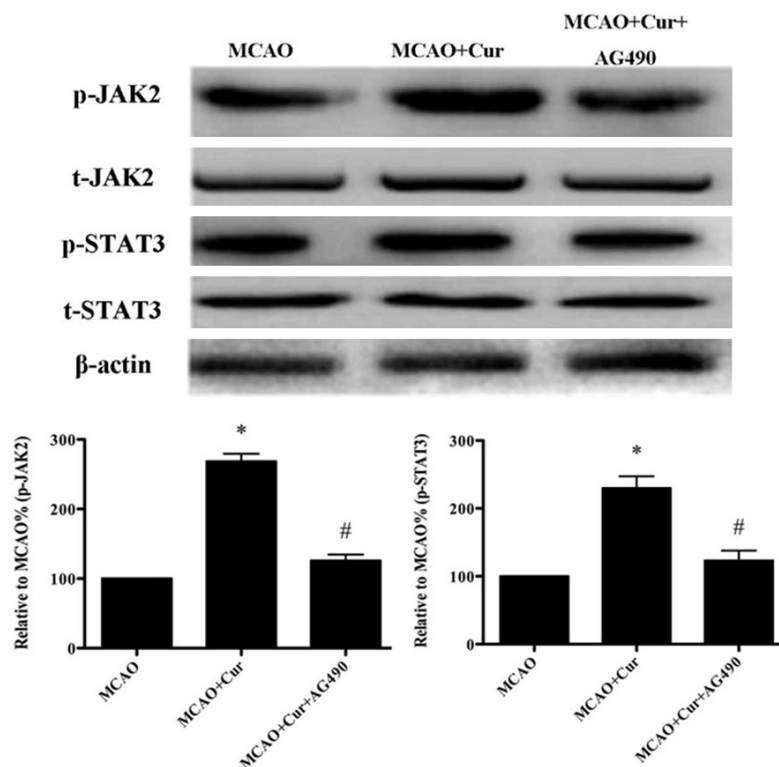

Figure 6. Effect of curcumin and AG490 on the expression of p-JAK2 and p-STAT3. Representative images of the Western blot results are shown. Data were expressed as mean ± S.E.M. (n=10 in each group); *$P < 0.05$ versus thesham group, #$P < 0.05$ versus theMCAO group.

the survival of neuron ($P < 0.05$) (**Figure 3**).

Effect of Cur on brain edema

The brain water content increased significantly in rats subjected to MCAO compared with that in the sham group. However, Cur administration dramatically lowered brain water content in MCAO + Cur group (**Figure 4**).

Effect of Cur on IL-1β and IL-8 activity

The activity of IL-1β and IL-8 increased dramatically in MACO group. However, Cur administration significantly decreased both of them compared with those in MCAO + Cur group (**Figure 5**).

Effect of Cur on p-JAK2 and p-STAT3 expression

The results of western blot indicated that JAK2 and STAT3 phosphorylation was enhanced with

sham group. Cur administration significantly attenuated the neuronal damage and increased

Cur administration. However, these increases were abolished by the AG490 treatment (**Figure 6**).

Discussion

The major observations in the present study are: (1) Cur mitigates cerebral ischemia-reperfusion injury by increasing neuron survival rate. (2) Cur alleviates cerebral ischemia-reperfusion injury by decreasing inflammatory cytokine activity. (3) The JAK2/STAT3 signaling activation is associated with the neuroprotective effect of Cur.

Cur has been reported to exert a variety of pharmacological and biological properties, such as anti-inflammation and anti-oxidation [8]. In addition, Cur is known to protect against cerebral ischemia. It is suggested that Cur protects rat brain against focal ischemia via up-regulating transcription factor Nrf2, HO-1 expression [23]. Furthermore, Cur is reported to attenuate glutamate neurotoxicity in the hippocampus by suppressing ER stress-associated TXNIP/NLRP3 inflammasome activation [24]. In agreement, our results suggest that Cur protect against cerebral ischemia by enhancing neuronal survival and decreasing inflammatory cytokine activity.

The JAK-STAT pathway, which is comprised of a family of receptor-associated cytosolic tyrosine kinases (JAKs) that activate signal-dependent transcription factors (STATs), can be activated by ischemic injury [25]. Additionally, the JAK/STAT pathways have been regarded as critical membrane-to-nucleus signaling pathways for a variety of stress or injury, such as ischemia, hypoxia and oxidative stress [26-28]. As for the ischemia-reperfusion injury, JAK2/STAT3 signaling pathway has been recognized as a key survival pathway that protects against myocardial ischemia-reperfusion injury [29]. More intriguingly, JAK2/STAT3 signaling activation is also involved in protection against cerebral ischemia [10, 30]. In the present study, we found that the expression of p-JAK2 and p-STAT3 were dramatically higher in the MCAO + Cur group compared with that in the MCAO group. However, the expression of p-JAK2 and p-STAT3 were significantly decreased by AG490 administration, suggesting that Cur protects brain ischemic injury via the phosphorylation of JAK2 and STAT3.

In summary, the neuroprotective effect of Cur is associated with the JAK2/STAT3 signaling activation. The administration of AG490 abolished the protective effects of Cur, suggesting that the phosphorylation of JAK2/STAT3 closely associated with the neuroprotective-signaling pathway activated by Cur. Our study suggests that Cur may be a potential therapy to combat lethal cerebral ischemia-reperfusion injury in clinical scenarios.

Disclosure of conflict of interest

None.

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