Original Article

Blockage of cytosolic phospholipase A2 alpha by monoclonal antibody attenuates focal ischemic brain damage in mice

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Summary	The purposes of the current study were to investigate the effects of a monoclonal antibody (mAb) on cytosolic phospholipase A2 alpha (cPLA2α) in mice with cerebral ischemia-reperfusion (IR) injury and to ascertain the potential mechanisms of those effects. This study evaluated whether the use of anti-cPLA2α mAb could reduce stroke injury in a mouse model of cerebral IR injury. The expression/activity of cPLA2α and cPLA2α-derived proinflammatory lipid mediators such as prostaglandin E2 (PGE2), leukotriene B4 (LTB4), lysophosphatidylcholine (LPC), and free fatty acids (FFA) was assessed. This study also evaluated neurological deficits, motor function, pathological changes, apoptosis, and the area of infarction in the injured mice. Mice treated with anti-cPLA2α mAb recovered neurological function and their condition improved, apoptosis in the brain decreased and infarct volume decreased, and expression of cPLA2α, 5-LOX, COX-2, FFA, LPC, PGE2, and LTB4 was attenuated. Our findings indicate that cPLA2α plays a key role in cerebral IR injury and that treatment with anti-cPLA2α mAb after cerebral IR injury helps to reduce levels of proinflammatory cytokines, alleviate tissue damage, and reduce levels of deleterious lipid mediators. Thus, anti-cPLA2α mAb treatment has the potential to treat ischemic brain damage.
	Keywords: Cerebral ischemia-reperfusion (IR) injury, neurological deficit score (NDS), 5-lipoxygenase (5-LOX), cyclooxygenase-2 (COX-2)

1. Introduction

Cerebral ischemia is a result of a complex process, mainly including excitotoxicity, peri-infarct depolarization, inflammation, and apoptosis (*1*). The damage cascade is the recognized theory of the physiological and pathological mechanism of cerebral ischemia. Cerebral ischemia is associated with neuronal injury (*2-4*), where neuronal death occurs in the ischemic core as a result of failure to maintain membrane ion gradients in neurons, excitotoxicity due to elevated glutamate levels, and disruption of the blood-brain barrier (BBB). Oxidative stress and inflammatory factors including lipoxygenases (LOXs), phospholipases (PLAs), and mitogen-activated protein kinases (MAPKs) are factors contributing to the pathology following cerebral ischemia. Several studies have found that group IVA cytosolic phospholipase A2 (cPLA2α) is a key component in the pathway of stroke injury (*3,5*). cPLA2α has been found to play a significant role in causing neurological injury following ischemic brain injury, and inhibition of cPLA2α may reduce stroke injury (*6*). Preventing the overexpression of cPLA2α may protect neuronal tissue from ischemic injury.

Phospholipases A2 (PLA2) are key enzymes of phospholipid degradation and crucial to maintaining and determining membrane composition. A number of mammalian PLA2 isotypes have been identified, and they are divided into three major subfamilies known as secretory PLA2 (sPLA2), cytosolic Ca^{2+}-dependent PLA2 (cPLA2), and Ca^{2+}-independent PLA2 (iPLA2). cPLA2α is a member of the cPLA2 class, and it has unique characteristics that include its preference for arachidonic acid (AA) at the sn-2 position of phospholipid substrate

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molecules (*7*). The enzyme catalyzes the hydrolysis of membrane phospholipids to release AA, which is subsequently metabolized into eicosanoids through cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP) pathways (*8,9*). Metabolism of AA and the resulting eicosanoid products, including prostaglandin E2 (PGE2), leukotriene B4 (LTB4), and 20-hydroxyeicosatetraenoic acid (20-HETE), have been implicated in the mechanism of injury (*4*). AA itself can cause BBB dysfunction and subsequent brain edema (*10,11*). cPLA2α activity results in the production of proinflammatory lipid mediators, which play an important role in acute inflammatory responses and oxidative stress associated with neurological diseases, and blocking any of key pathways in the aa-metabolic network might inhibit progression of tissue injury. Several studies have suggested that cPLA2α plays a key role in apoptosis and tissue injury (*4,5,12,13*).

The current study used monoclonal antibodies to study the effects of inhibiting the activity of cPLA2α *in vivo* in a mouse model.

2. Materials and Methods

2.1. *Materials*

Anti-cPLA2α mAb used in an animal model was originally prepared and provided by Detai Biologics Co., Ltd. The primary antibodies used for western blotting, such as cPLA2α antibody, 5-LOX antibody, and β-actin antibody, were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). COX-2 antibody was purchased from Sigma (St. Louis, MO, USA). The cPLA2α Assay Kit was purchased from Cayman Chemicals (Interchim, Montlucon, France). TRIzol reagent was purchased from Invitrogen (USA). The PrimeScript Reverse Transcriptase Kit and the SYBR Premix Ex TaqTM II PCR Kit were purchased from Takara (Japan). Leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) ELISA kits were purchased from Cayman Chemical (Michigan, USA). The In Situ Cell Death Detection Kit was purchased from Roche (USA).

2.2. *Animals and treatment*

Male Swiss CD-1 mice (12-16 weeks, body weight = 25.57 ± 1.85 g) were used. All mice were housed in a temperature-controlled facility with a 12-h dark/light cycle and had free access to water and food except when otherwise specified. All procedures related to the care of animals were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The mice were randomly divided into a sham-operated group, a model group, a negative control (NC) group, and an mAb group. One hour before cerebral ischemia-reperfusion (IR) injury, freshly prepared anti-cPLA2α mAb (20 μg) or class-matched control mAb (IgG2a) against keyhole limpet hemocyanin was intravenously administered to the mAb group and NC group. The sham-operated and model groups received only the vehicle solution. The mAb and vehicle solution were similarly administered every 24 h.

Mice were sacrificed at different time points after reperfusion. Some mice were anesthetized and perfused through the left ventricle with 0.01 M phosphate buffered saline. Brains were removed from the skull and prepared for examination. The rhinencephalon, cerebellum, and brain stem were removed, and the tissues from the coronal brain region were cut into slices (about 2 mm). Coronal sections of the brain were subjected to TTC staining, and 2 slices were fixed in 4% paraformaldehyde in PBS. The brain was directly removed from the remaining mice.

2.3. *Focal cerebral ischemia in mice*

Transient focal ischemia was induced using the intraluminal occlusion technique as described by Zhang J *et al* (*6*). Focal brain ischemia was produced by occlusion of the right middle cerebral artery (MCA) for 1 h, followed by reperfusion. Mice were anesthetized with 2% halothane in a mixture of 50% N_2O and 50% O_2 using a face mask. A filament (nylon monofilament 6/0, Suturas Aragó, Spain) was introduced (11 mm) through the external carotid artery to the level where the MCA branches out. Mice were allowed to recover from the anesthesia, and the mice were anesthetized again 5 min before reperfusion. The filament was carefully removed, and the cerebral blood flow was restored 1 h after MCA occlusion. Sham surgery was performed with a vertical cervical incision.

2.4. *Determination of cerebral blood flow*

In order to monitor the cerebral blood flow (CBF) in mice after surgery, a laser-Doppler flow probe was secured on the skull. The scalp was incised at the midline and the skull was exposed. The probes were affixed with glue to the skull surface 2 mm posterior and 3 mm lateral to the bregma and 1 mm caudal to the coronal suture on exposed temporal bone of the ipsilateral hemisphere. This location corresponds to the region supplied by the MCA that becomes severely ischemic upon occlusion (*14*). Occlusion of flow was indicated by a decrease in the laser-Doppler flow greater than 70% of the baseline. CBF signals from both hemispheres were simultaneously monitored throughout the surgery.

2.5. *Evaluation of neurological deficits*

Neurological deficits were evaluated using two

methods. Functional stroke injury was evaluated with the neurological deficit score (NDS) immediately after reperfusion and before sacrifice. Neurological impairment was assessed using an NDS between 0 and 4 points, where 0 points = no neurological deficits; 1 point= forelimb weakness; 2 points= circling to the affected side; 3 points= falling to the affected side; and 4 points= unable to walk spontaneously (*15*). Animals with a score of 1-3 points after reperfusion were used in the following experiment, and if necessary, were used to randomly supplement the number of laboratory animals. The mice were subsequently scored 12 h, 24 h, 48 h, and 72 h after reperfusion.

The rotarod test was used to analyze the motor coordination and resistance to fatigue of the mice. The initial rotating speed and accelerating speed of the rotarod machine (Med Associates) can be adjusted. The parameters used in this study were the latency time to the first fall and the number of falls in five minutes. Mice were trained at 2-20 rpm in the days prior to testing. Motor ability was tested 6 h, 12 h, 24 h, 48 h, and 72 h after surgery.

2.6. Determination of water content in the brain

Mice were sacrificed 24 h after reperfusion and their brains were removed and divided into ipsilateral and contralateral hemispheres. The brains were wrapped in tin foil and weighed and dried for 24 h at 100ºC. Water content in the brain was calculated as (wet weight – dry weight) / wet weight × 100%.

2.7. Triphenyltetrazolium chloride (TTC) staining

The area of infarction was evaluated with TTC staining as described previously (*16*). Two-mm brain sections were incubated with 2% TTC at 37°C for 30 min with gentle shaking and then fixed with 10% formalin in PBS. The stained slices were photographed, and the size of the area of infarction was determined by subtracting the area of the non-infarcted ipsilateral hemisphere from that of the intact contralateral hemisphere. The percentage of the infarct volume was calculated by dividing the sum of the area from all sections of infarction by the total of that of the contralateral hemisphere to avoid the influence of tissue edema (*17*).

2.8. Histological examination

Fixed 2-mm brain sections were embedded in paraffin, sliced into 5-μm sections, and then dewaxed and stained with hematoxylin and eosin (H&E). The sections were histologically examined under a microscope.

2.9. Western blotting

Total proteins were extracted from frozen brain tissue (*n* = 3 in each group). Brain tissue lysates were prepared as described previously (*18*). Briefly, brain tissue was homogenized and centrifuged, and the supernatant was collected. Bradford's method was used to determine the protein concentrations (*19*), and bovine serum albumin (BSA) standard protein was used as standard. Western blotting was performed using standard protocols. The proteins were separated with SDS-PAGE and then transferred and blocked. The primary antibodies were cPLA2α antibody, 5-LOX antibody, COX-2 antibody, and β-actin antibody. Protein bands were quantified using Quantity One software (Bio-Rad).

2.10. Measurement of PLA2 activity

In order to measure the inhibition of cPLA2α by anti-cPLA2α mAb, the cPLA2α Assay Kit was used to determine cPLA2α activity from protein extracts, as previously described (*20,21*). The results are expressed as μmol/min/μg of protein.

2.11. RNA extraction and qPCR

Total RNA extraction, RT-PCR, and real-time PCR were performed as described previously (*22*). Total RNA (*n* = 3 in each group) was extracted from tissues or cells using TRIzol reagent according to the manufacturer's instructions. First-strand cDNA was synthesized using PrimeScript reverse transcriptase and oligo (dT). Quantitative real-time PCR was performed with the SYBR Premix Ex TaqTM II PCR kit according to the manufacturer's instructions. Relative levels of cPLA2α mRNA were calculated on the ABI Prism 7300 Sequence Detection System (Applied Biosystems, CA, USA) using SYBR green and the DDCt method with β-actin as the endogenous reference gene amplified from the samples. The forward and reverse primers were as follows: cPLA2α, 5'-GGTGGGAGAGAAGAAAGAAGTC-3' and 5'-AGGGATTTTGGATTGTGCGACC-3'; COX-2, 5'-CCCTTCTGCCTGACACCTTTC-3' and 5'-AACCCTGCCAGCAATTTGC-3'; 5-LOX, 5'-TGCTGAGCGCAACAAGAAGA-3' and 5'-ACACCTGCCAGGCAGGA-3'; β-actin, 5'-GCTATGCTCTCCCTCACGCCAT-3' and 5'-TCACGCACGATTTCCCTCTCAG-3'. Relative quantification was performed using the $2^{-\Delta\Delta Ct}$ method. β-actin served as an appropriate reference gene in this experiment.

2.12. Measurement of LTB4 and PGE2

The levels of leukotriene B4 (LTB4) and prostaglandin E2 (PGE2) were examined using commercially available ELISA kits. The experiment need 50 μL serum samples from mice, and all operations were performed according to the manufacturer's instructions.
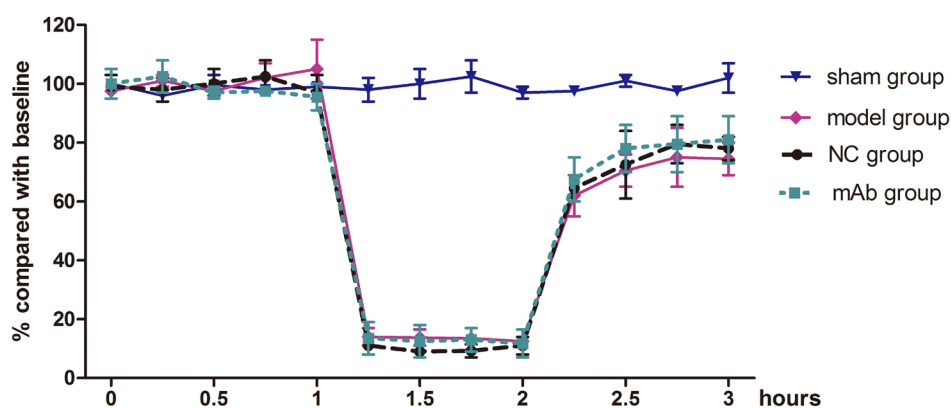
Figure 1. The effect of anti-cPLA2α mAb on blood flow in the brains of model mice. Laser-Doppler flow measurement of cerebral blood flow was performed (as described in the Methods) before surgery (0-1 h), during the surgery (1-2 h), and after reperfusion (2-3 h). Results are the mean ± SD for 30 mice. Anti-cPLA2α mAb treatment did not alter the relative volume of cerebral blood flow.

2.13. *Determination of FFA and LPC*

FFA and LPC were determined and quantified using HPRLC. The needed lipids samples were extracted from brain tissue using the Folch method (*23*). FFA was determined and quantified using high-performance thin layer chromatography (HPTLC) plates (*24*). Quantification of LPC was performed using one-dimensional HPTLC as previously described (*25*).

2.14. *TUNEL staining*

To evaluate the degree of apoptosis, TUNEL staining was performed on brain sections using an In Situ Cell Death Detection Kit, POD (Roche). Tissues were prepared as paraffin sections and dewaxed before the TUNEL assay; all procedures were done in accordance with the manufacturer's instructions. Results were recorded using a fluorescence microscope equipped with AxioVision software. Four brains samples from each group were analyzed. At least 4 microscopic fields within the ischemic penumbra of each brain sample were photographed, and TUNEL-positive cells were counted in a double-blinded manner. An average of the TUNEL-positive cells counted from these areas was calculated to represent each brain section.

2.15. *Statistical processing*

All values are presented as the mean ± SD. Each value is the mean for each group from at least three separate experiments. Data were analyzed by comparing two groups using the Student's *t*-test and *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered to indicate significant differences.

3. Results

3.1. *Effects of anti-cPLA2α mAb on the expression and activity of cPLA2α*

The role of cPLA2α in ischemic brain injury has been determined in cPLA2α-knockout (cPLA2α-/-) mice, which are partially protected from transient focal cerebral ischemia. The current study evaluated whether the use of anti-cPLA2α mAb could reduce stroke injury in a mouse model of cerebral IR injury. In a separate group of mice, laser-Doppler flowmetry was initiated prior to treatment and continued until 1 h after reperfusion (Figure 1). The relative blood flow did not change as a result of anti-cPLA2α mAb treatment during the period of observation (Figure 1). Anti-cPLA2α mAb effectively decreased cPLA2α expression in mice with ischemic damage 6 h, 12 h, 24 h, 48 h, and 72 h after surgery compared to expression in the NC group (Figure 2, $p < 0.05$, $n = 3$). cPLA2α activity was measured in homogenates of ischemic (ipsilateral) hemispheres using an *in vitro* assay. cPLA2α activity was reduced by anti-cPLA2α mAb in comparison to the level of activity in the NC group and the model group 24 h after reperfusion (Figure 2D, $p < 0.05$, $n = 3$).

3.2. *Effects of anti-cPLA2α mAb on neurological function in animals after ischemic stroke*

To study the effect of cPLA2α on neurological function in ischemic stroke, NDS and rotarod tests were performed 2 h, 12 h, 24 h, 48 h, and 72 h after reperfusion. All of the animals had neurological injury 2 h after reperfusion, which suggests that the surgery was successful and would facilitate further analysis. Neurological deficits were evaluated again 12 h, 24 h, 48 h, and 72 h after reperfusion. The control mice had a higher NDS for up to 72 h; the score for the mAb group decreased in a time-dependent manner and was quite low after 72 h (Figure 3A). Neurological deficits did not differ significantly in animals that underwent surgery 6 h after surgery ($p > 0.05$). The NDS for the mAb group was significantly lower than that for the NC group and the model group 24 h, 48 h, and 72 h after a stroke ($p < 0.05$). Before surgery, the mice did not differ in their
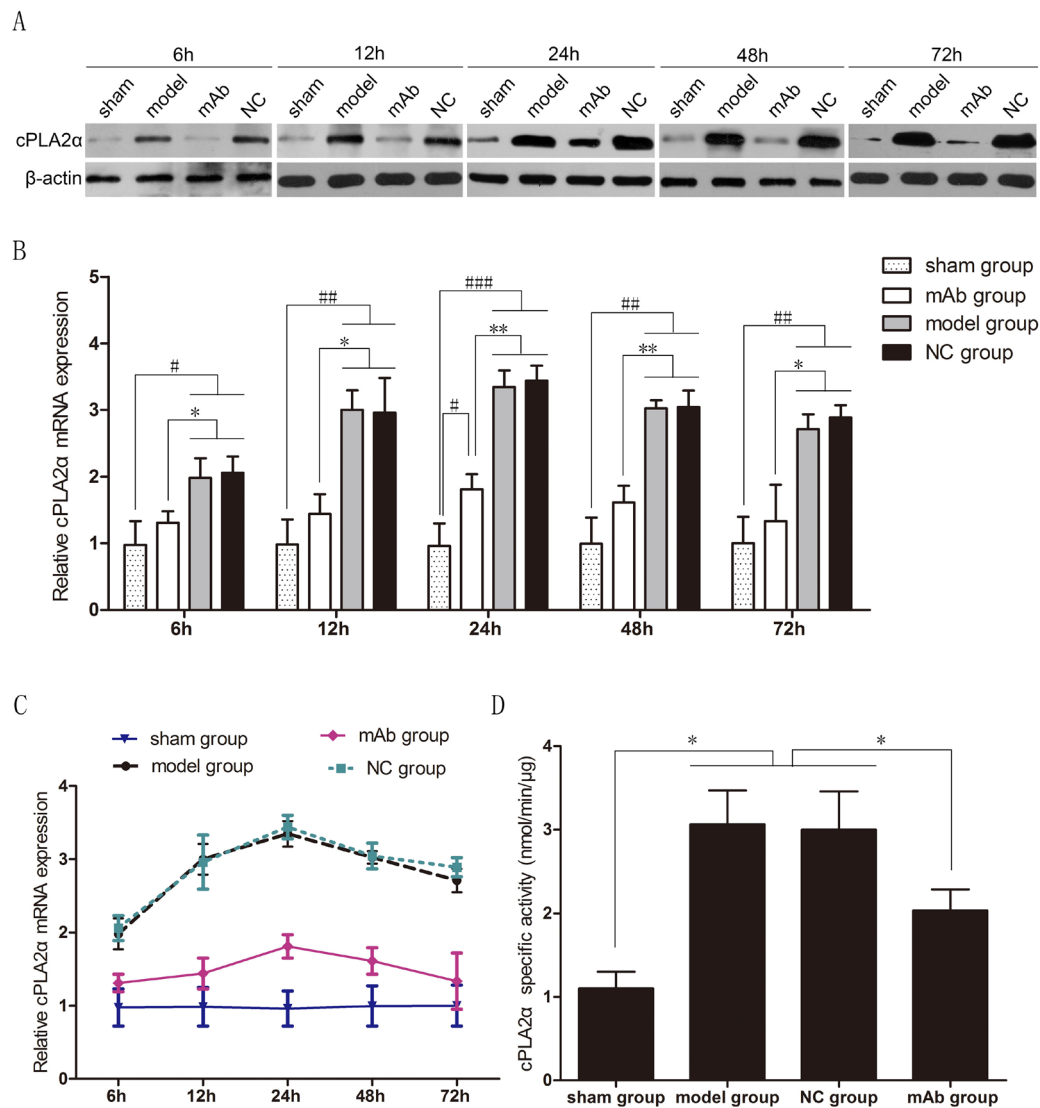
Figure 2. The effect of anti-cPLA2α mAb on the expression and activity of cPLA2α. (Panel A) A representative western blot of cPLA2α protein levels in whole brain lysates of mouse brains 6 h, 12 h, 24 h, 48 h, and 72 h after reperfusion following a stroke **(Panels B and C)**. Levels of cPLA2α mRNA expression 6 h, 12 h, 24 h, 48 h, and 72 h after reperfusion were detected with real-time PCR. Anti-cPLA2α mAb treatment effectively decreased cPLA2α expression in mice with ischemic damage 6 h, 12 h, 24 h, 48 h, and 72 h after surgery compared to the NC group and the model group. **(Panel D)** cPLA2α activity in mice with focal ischemic brain damage 24 h after reperfusion. cPLA2α activity was reduced by anti-cPLA2α mAb treatment in comparison to that in the NC group and the model group 24 h after reperfusion. Data are presented as the mean ± SD of three independent experiments. Statistically significant differences are indicated: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, Student's *t*-test.

performance on the rotarod test (Figure 3B). However, 12 h after a stroke, the measured number of falls during the rotarod tests was significantly higher in mice that underwent surgery compared to sham-operated mice (Figure 3B, $p < 0.001$). Mice in the mAb group appeared to recover completely as assessed by the number of falls 72 h post-stroke, whereas the NC group and the model group still exhibited marked motor dysfunction at that time point. These data suggest that cPLA2α exacerbates stroke-induced motor dysfunction in mice.

3.3. Effects of anti-cPLA2α mAb on reducing ischemia-induced brain edema

Formalin-fixed, paraffin-embedded archival tissue The

wet-dry method was used to measure water content in the brain. The water content in ipsilateral hemispheres was calculated to be 77.5% ± 2.4% in the sham-operated group compared to 85.9% ± 1.1% in the model group and 85.9% ± 1.2% in the NC group. The water content in the mAb group decreased significantly as a result of anti-cPLA2α mAb treatment (mAb *vs.* model, 82.8% ± 0.8% *vs.* 85.9% ± 1.1%, $p < 0.05$; mAb *vs.* NC, 82.8% ± 0.8% *vs.* 85.9% ± 1.2%, $p < 0.05$).

3.4. Effects of anti-cPLA2α mAb on reducing ischemia-induced brain infarct volume after reperfusion

Brain infarcts were measured 72 h after reperfusion using a standard laboratory volumetric analysis of
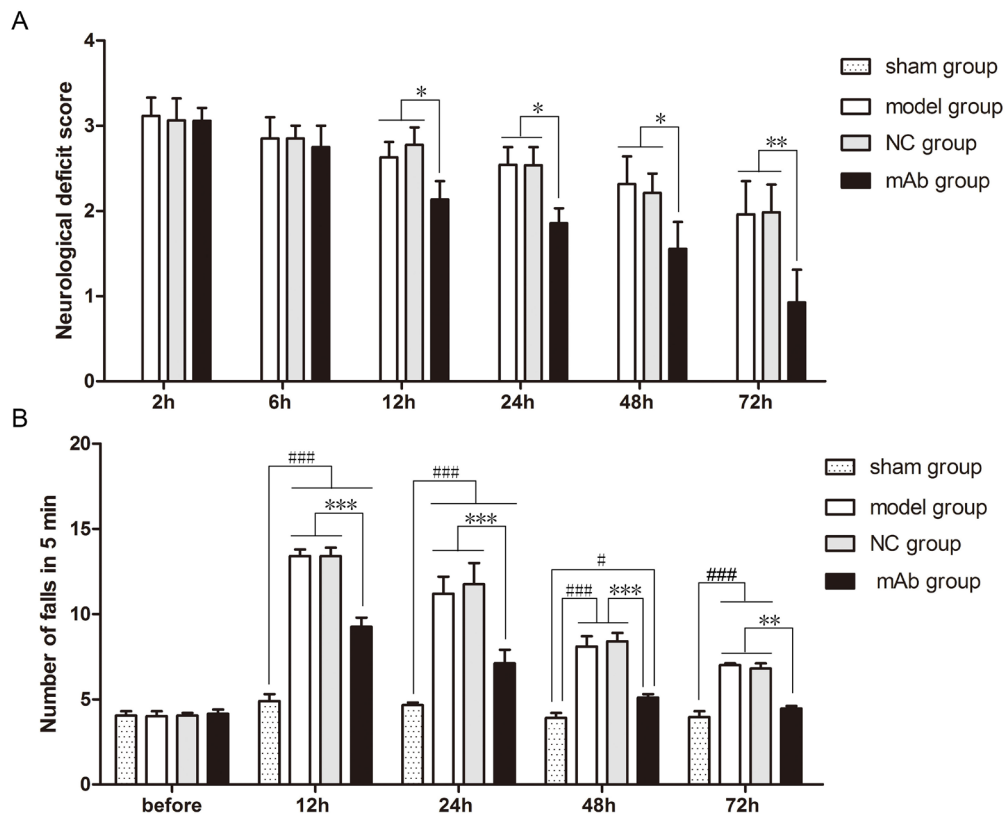
Figure 3. The effect of cPLA2α on neurological function in ischemic stroke. (Panel A) Neurological deficit scores 2 h, 12 h, 24 h, 48 h, and 72 h after reperfusion. Neurological deficits did not differ significantly in animals that underwent surgery 6 h after surgery (*p* >0.05). The neurological deficit score for the mAb group was significantly lower than that for the NC group or the model group 24 h, 48 h, and 72 h after a stroke (*p* < 0.05). **(Panel B)** The average number of falls in 5 min measured during a rotarod test. Before surgery, the mice did not differ in their performance on the rotarod test. However, 12 h after a stroke, the number of falls during the rotarod test was significantly higher for mice that underwent surgery compared to the number for sham-operated mice (*p* < 0.001). Mice in the mAb group appeared to recover completely as assessed by the number of falls at the subsequent time point after a stroke. Data are presented as the mean ± SD. Asterisks denote the following *p*-values: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, Student's *t*-test. Experiments were performed in triplicate.

anterior and posterior views of coronal sections stained with TTC and corrected for swelling. No infarction was observed in the sham-operated group, and an extensive lesion was observed in both the model and NC groups. Anti-cPLA2α mAb treatment effectively decreased cerebral injury with a significant reduction in infarct volume in the ischemic hemisphere in comparison to the NC group and the model group 72 h after reperfusion (Figure 4, *p* < 0.01, *n* = 3).

3.5. Effects of anti-cPLA2α mAb on pathological changes in animals after ischemic stroke

Pathological changes were visually observed using HE staining. An examination revealed that the morphological structures of brain tissue in the sham-operated group were normal (Figure 5). In contrast, the morphological structures of brain tissue in the model group and NC group changed significantly; the tissues were loose and intercellular edema was visible. Similar results were observed in the mAb group: the morphological structures of brain tissue changed, but less markedly so (Figure 5). Such findings suggested that the treatment with anti-cPLA2α mAb ameliorated pathological changes in

animals after ischemic stroke.

### 3.6. Effects of cPLA2α on brain cell apoptosis in animals after ischemic stroke

TUNEL staining was performed to detect whether anti-cPLA2α mAb treatment would decrease the number of apoptotic cells. The number of TUNEL-positive cells in brain tissue from injured mice increased significantly in comparison to the sham-operated group 6 h, 24 h, 48 h, and 72 h after reperfusion (Figure 6, *p* < 0.001, *n* = 3). The number of TUNEL-positive cells in the mAb group decreased in comparison to that in the NC group and the model group (Figure 6, *p* < 0.01, *n* = 3). Results indicated that anti-cPLA2α mAb may ameliorate pathological changes by inhibiting apoptosis after surgery

3.7. Effects of anti-cPLA2α mAb on the expression of proinflammatory lipid mediators

cPLA2α play an important role in catalyzing the hydrolysis of phospholipids in sn-2, generating FFA and lysophospholipids (*26*). To test whether anti-cPLA2α
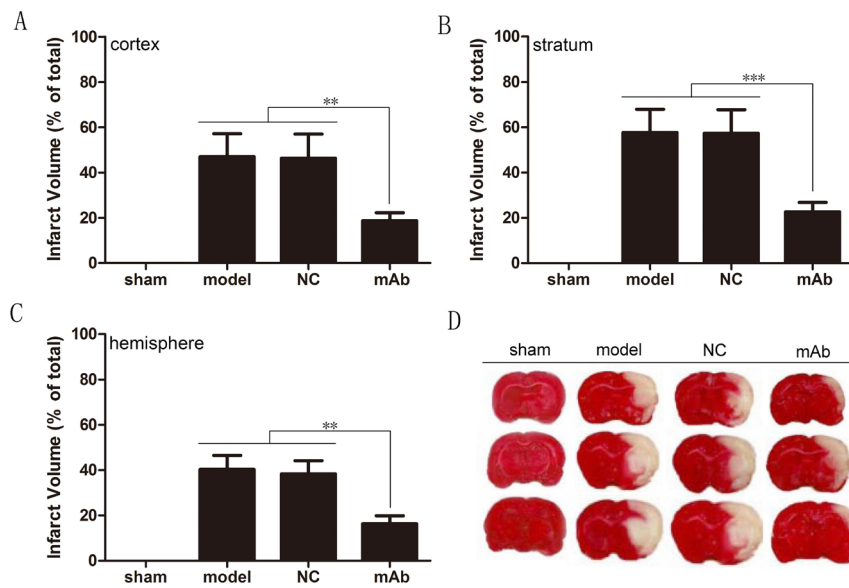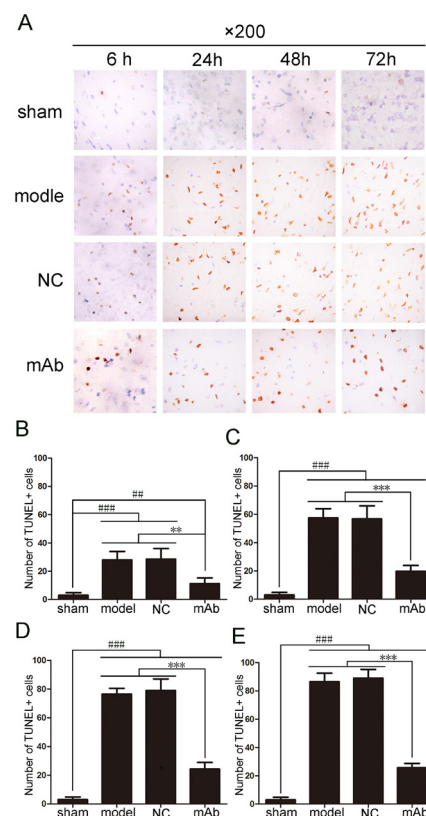
Figure 4. The effect of anti-cPLA2α mAb on reducing ischemia-induced brain infarct volume 72 h after reperfusion. Continuous infusion with anti-cPLA2α mAb reduces ischemia-induced brain infarct volume 72 h after reperfusion. Infarct volume (± SD) in the striatum, cortex, and hemisphere was measured. Cortical and hemispheric infarct volumes were significantly smaller in the mAb group than those in the NC group or the model group. $*p < 0.05$, $**p < 0.01$.
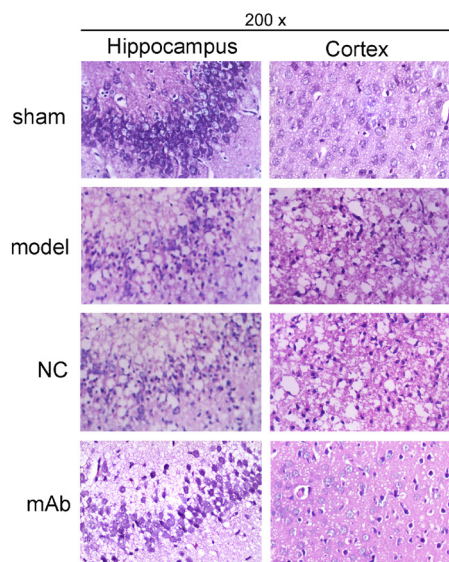
Figure 5. The effect of anti-cPLA2α mAb on pathological changes in the brains of mice after surgery. Pathological changes in hippocampal and cortical tissues as a result of anti-cPLA2α mAb treatment (magnification, ×200). An examination revealed that the morphological structures of brain tissue in the sham-operated group were normal. The morphological structures of brain tissue in the model group and NC group changed significantly; the tissues were loose and intercellular edema was visible. The morphological structures of brain tissue in the mAb group also changed, but less markedly so in comparison to the model group and the NC group.

Figure 6. The effect of anti-cPLA2α mAb on apoptosis in brain cells of mice after surgery. (Panels B, C, D, E) The number of TUNEL-positive cells was counted 6 h, 24 h, 48 h, and 72 h after reperfusion. The number of TUNEL-positive cells in the ischemia reperfusion group increased significantly compared to that in the sham-operated group 6 h, 24 h, 48 h, and 72 h after reperfusion. The number of TUNEL-positive cells in the mAb group was smaller than that in the NC group or the model group. Data are presented as the mean ± SD. Statistically significant differences are indicated: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, mAb group *vs.* the NC group or model group; $^{#}p < 0.05$, $^{###}p < 0.001$, sham-operated group *vs.* the model group, NC group, or mAb group.

mAb decreased the levels of phospholipid degradation products, cPLA2-derived injurious lipid mediators and the expression of 5-LOX and COX-2 in brain tissue were assessed. Anti-cPLA2α mAb treatment significantly reduced the levels of 5-LOX and COX-2 in brain tissue (Figure 7B and D, $p < 0.01$, $n = 3$). Anti-cPLA2α mAb treatment decreased FFA and LPC compared to levels in the NC group and the model group (Figure 7E and

G, $p < 0.05$, $n = 3$). The mAb group had significantly lower levels of PGE2 in serum than the NC group or the model group 24 h after surgery (Figure 7H, $p < 0.01$, $n = 3$). Similarly, the levels of LTB4 in serum were lower in the mAb group than those in the NC group or the model group (Figure 7F, $p < 0.001$, $n = 3$).
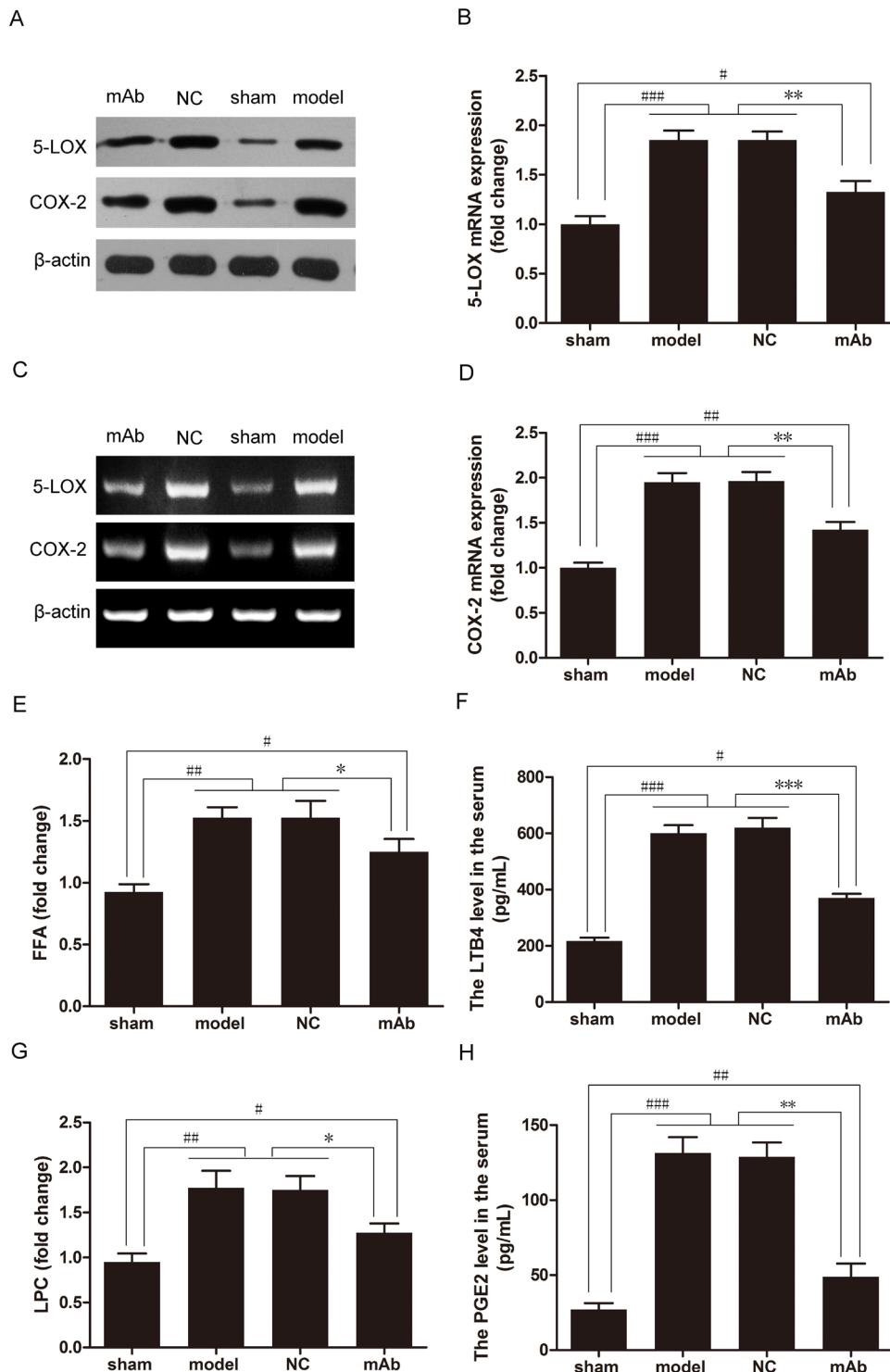
Figure 7. The effect of anti-cPLA2α mAb on the expression of 5-LOX, COX-2, FFA, LPC, PGE2, and LTB4. (Panel A) A representative western blot of 5-LOX and COX-2 protein levels in whole brain lysates of mice 24 h after reperfusion following a stroke. **(Panels B, C, and D)** Levels of 5-LOX and COX-2 mRNA expression in brain tissue from mice 24 h after surgery. Anti-cPLA2α mAb treatment significantly reduced the levels of 5-LOX and COX-2 in brain tissue. **(Panel C) (Panels E and G)** Levels of FFA and LPC in brain tissue from mice 24 h after surgery. Anti-cPLA2α mAb treatment decreased FFA and LPC compared to levels in the NC group and the model group. **(Panels F and H)** Levels of LTB4 and PGE2 in serum from mice 24 h after surgery. Animals in the mAb group had significantly lower levels of LTB4 and PGE2 in serum than did the NC group or the model group 24 h after surgery. Anti-cPLA2α mAb treatment decreased the levels of FFA, LPC, PGE2, and LTB4. Data are presented as the mean ± SD of three independent experiments. Statistically significant differences are indicated: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, mAb group *vs.* the NC group or model group; #$p < 0.05$, ###$p < 0.001$, sham-operated group *vs.* the model group, NC group, or mAb group.

4. Discussion

Cerebral ischemia injury is a common clinical condition that poses a substantial burden to a patient's family and society. The goal of clinical treatment of cerebral ischemia is to restore blood flow and oxygen supply, suppress inflammation of the ischemic area, and maintain the integrity of the neuronal structure and function. A growing body of evidence has demonstrated that cPLA2α contributes to the biosynthesis of eicosanoids by releasing AA and that it is associated with various inflammatory diseases (*2,5-7,27,28*). The current study provides evidence that cPLA2α is essential for speedy recovery from cerebral ischemia and that it is closely related to inflammation and neurological deficits.

cPLA2 are a group of hydrolytic enzymes that act on membrane phospholipids to release free unsaturated fatty acids. The activation of cPLA2 contributes to an increase in AA, and followed by the generation of FFA and lysophospholipids upon injury. AA itself can cause BBB dysfunction and subsequent brain edema (*10,11*) and can converted into lipid mediators *via* COX and LOX pathways (*8,9*). The final products are prostaglandins, thromboxanes, and leukotrienes, which are members of a group of biologically active oxygenated fatty acids. These oxygenated fatty acids are important modulators of pain in inflammation and immunomodulatory agents. cPLA2 plays an important role in several physiological and pathophysiological processes, including immunity, reproduction, cancer, and inflammation (*2,12,26,29-34*). Inhibition of the expression of cPLA2 has been proven to improve functional recovery in stroke, Alzheimer's disease, and multiple sclerosis (*35,36*). The current study and other studies have found that cPLA2 signaling is predominant in cerebral ischemia, suggesting that upregulation of this enzyme after trauma may be responsible for secondary injury and thus inflammation, nociception, and functional deficits (*6*).

Patients with ischemic brain damage are prone to partial or complete loss of motor function, clinically known as paralysis. Seventy-five percent of patients with cerebral ischemia have varying degrees of neurological deficits (*37*), and motor function deficits are an important manifestation of neurological deficits (*38*). Neurological deficits are a characteristic of cerebral ischemia. Motor function recovery is an index to determine the therapeutic efficacy of a drug. cPLA2α plays a significant role in causing neurological injury following ischemic brain injury. In the current study, a significant neurological deficit was observed 2 h after cerebral IR injury surgery, validating the cerebral ischemia model. The mAb group had a lower NDS than the NC group and the model group, corroborating the contention that cPLA2α contributes to functional injury (Figure 3).

A previously suggested mechanism is one whereby BBB disruption in mice after cerebral IR injury surgery is associated with increased cPLA2α activity 24 h after reperfusion (*39*). The current study evaluated whether the use of anti-cPLA2α mAb would reduce stroke injury in this mouse model. The expression of cPLA2α peaked 24 h after reperfusion (Figure 2C), and cPLA2α activity decreased markedly as a result of anti-cPLA2α mAb at the same time point. Treatment with anti-cPLA2α mAb significantly reduced the expression of cPLA2α for up to 72 h after reperfusion. cPLA2 plays an important role in physiological and pathophysiological processes. The current results indicate that anti-cPLA2α mAb may ameliorate pathological changes in animals after ischemic stroke by inhibiting apoptosis after surgery (Figures. 5 and 6), and anti-cPLA2α mAb treatment effectively decreased cerebral injury caused by cerebral IR injury surgery with a significant reduction in infarct volume in the ischemic hemisphere (Figure 4).

Inhibition of cerebral ischemia by cPLA2α treatment confers anti-inflammatory and neuroprotective effects, leading to increased functional recovery. Many mediators of inflammation are mediated by COX and LOX cascades. cPLA2α plays an important role in the release of AA from membrane phospholipids linked to eicosanoid production in various pathological states. FFA are the product at the start of the COX and LOX cascades, and FFA are subsequently metabolized to PGE by COX-2 and to LTB4 by 5-LOX. PGE are potent vasodilators that contribute to the increased blood flow in inflamed areas. LTB4 is a chemoattractant for leukocytes and may initiate monocyte recruitment. LPC is a signaling molecule involved in chronic inflammation and tissue damage (*40*). The current study found that inhibition of cPLA2 decreased the expression and production of both COX-2 and 5-LOX and that anti-cPLA2α mAb treatment effectively reduced the expression of those proinflammatory lipid mediators (Figure 7), indicating that both originated from cPLA2. PLA2-derived proinflammatory lipid mediators such as PGE2, LTB4, LPC, and FFA are implicated in ischemic brain damage, and anti-cPLA2α mAb treatment may reduce COX-2 and 5-LOX-derived inflammatory mediators in ischemic brain damage.

Together, the current findings suggest that cPLA2α plays a significant role in effectively and efficiently protecting cells from adverse effects of a stroke and that cPLA2α inhibition may ameliorate pathological changes by reducing inflammation and inhibiting apoptosis after cerebral IR injury. The beneficial effects of cPLA2α treatment may be primarily due to the inhibition of cPLA2 and the reduction of proinflammatory lipid mediators originating from the activation of cPLA2. The neuroprotection conferred by anti-cPLA2α mAb treatment has noteworthy implications that since mAb against cPLA2α may one day be used as a treatment for stroke in clinical settings.

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