Article

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Research on the Mechanism by Which Lysophosphatidylcholine Regulates the Inflammatory Response in Ischemic Stroke Through the NF-κB Signaling Pathway

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Background: Neuroinflammation following ischemic stroke is a significant factor leading to secondary brain damage, but the role of lysophosphatidylcholine (LPC) in this process remains unclear. This study sought to determine whether LPC participates in the post-ischemic inflammatory process and to evaluate if this effect is mediated through the nuclear factor-κB (NF-κB) signaling pathway.

Methods: For the *in vivo* study, male C57BL/6 mice aged 8–10 weeks were randomly assigned to a sham group (n = 10) and a middle cerebral artery occlusion model group (MCAO, n = 10). The suture occlusion method was applied to induce MCAO, with 60 min of ischemia followed by 24 h of reperfusion. LPC concentrations in serum and brain tissue were measured using ELISA. The protein levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in cerebral tissue, together with the expression of nuclear factor-κB (NF-κB) pathway-associated proteins (p-p65, p65), were evaluated using ELISA and Western blotting. TTC staining was performed to determine infarct size, while neurological deficits were scored with the modified neurological severity score (mNSS). For the *in vitro* study, BV2 microglial cells were subjected to oxygen-glucose deprivation/reperfusion (OGD/R) and allocated to four groups: Control, OGD, OGD+LPC, and OGD+LPC+BAY 11-7082. The concentrations of inflammatory mediators in the culture supernatant were measured using ELISA, and the expression of NF-κB pathway-related proteins was examined by Western blotting.

Results: In the *in vivo* study, relative to Sham controls, the MCAO group exhibited markedly elevated brain LPC concentrations and reduced serum LPC levels ($p < 0.05$). Tissue and serum contents of TNF-α, IL-1β, and IL-6 were also significantly increased in the MCAO group ($p < 0.05$), along with a higher p-p65/p65 ratio in brain tissue ($p < 0.05$). Moreover, infarct size and mNSS scores were significantly augmented ($p < 0.05$). These observations suggest elevated brain LPC levels are accompanied by increased neuroinflammation and NF-κB pathway activation in the MCAO model. In the cell-based experiments, OGD/R exposure led to significant elevations in supernatant TNF-α, IL-1β, and IL-6 levels and in the p-p65/p65 ratio versus the Control group ($p < 0.05$). Addition of LPC further increased these parameters in OGD/R-treated cells ($p < 0.05$), whereas co-treatment with BAY 11-7082 significantly reversed these effects ($p < 0.05$).

Conclusion: *In vitro* findings suggest that LPC can promote the expression of inflammatory factors in microglia following ischemic stroke, potentially through activating the NF-κB signaling pathway. While *in vivo* data show a correlation between elevated brain LPC levels and post-stroke inflammation, further studies with direct LPC intervention are required. The LPC-NF-κB axis may represent a candidate pathway for future therapeutic investigation.

Keywords: LPC; ischemic stroke; NF-κB signaling pathway; inflammatory markers

Introduction

Among all neurological disorders, ischemic stroke carries one of the highest burdens of mortality and long-term disability worldwide [1]. Although intravenous thrombolysis and endovascular reperfusion therapy have significantly improved the acute prognosis of some patients, the secondary neuroinflammatory response induced by cerebral ischemia-reperfusion remains one of the important mechanisms leading to persistent brain tissue damage and deterioration of neurological function [2,3]. A consensus has emerged that after ischemic brain injury, resident microglia and peripheral immune cells become activated and release multiple inflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β). These inflammatory mediators can exacerbate disruption of the blood-brain barrier, worsen cerebral edema, and promote neuronal apoptosis, all of which compromise neurological recovery and long-term outcomes [4,5,6].

Lysophosphatidylcholine (LPC) is an important component of oxidized low-density lipoprotein (ox-LDL) and a lipid mediator that is significantly elevated in various cardiovascular and cerebrovascular diseases [7]. Previous studies have shown that LPC participates not only in the development of atherosclerosis and vascular endothelial dysfunction but also in the regulation of inflammatory responses by modulating immune cell activation and inflammatory signaling pathways [8]. In neurological diseases, some clinical studies have found that the plasma lipid metabolism profile of patients with ischemic stroke is significantly altered, among which LPC levels may be associated with inflammatory status and disease severity [8,9]. Among the transcriptional regulatory networks governing inflammation, the nuclear factor-κB (NF-κB) signaling pathway plays a particularly crucial role [10,11]. In the context of ischemic brain injury, NF-κB can be activated by a variety of stimuli, including oxidative stress, inflammatory cytokines, and lipid mediators. Once activated, it drives the transcriptional upregulation of downstream effectors such as TNF-α, IL-1β, and IL-6, which in turn sustains and intensifies the inflammatory cascade [12,13,14]. According to previous findings, LPC is capable of triggering NF-κB pathway activation in both vascular endothelial cells and immune cells, which in turn stimulates the production of inflammatory mediators. This evidence points to a potential role for LPC as a key lipid signaling molecule involved in the modulation of inflammatory responses [15,16]. Despite its well-established functions in other settings, whether LPC acts through the NF-κB pathway to regulate inflammatory mediators and exacerbate cerebral tissue injury following ischemic stroke has not been systematically investigated.

To investigate whether LPC can activate the NF-κB pathway and thereby promote the expression of inflammatory factors in ischemic stroke, the present study was designed. This work may offer a new theoretical foundation and identify potential targets for post-stroke anti-inflammatory therapy.

Materials and Methods

Experimental Animals and Model Establishment

This study was conducted in accordance with the ARRIVE Guidelines 2.0 and approved by the Kongjiang Hospital of Yangpu District Animal Ethics Committee (Ethics Approval No. LL-2026-KY-24). SPF-grade male C57BL/6 mice, aged 8 to 10 weeks and weighing 22–25 g, were supplied by Beijing Vital River Laboratory Animal Technology Co., Ltd. (Certificate No.: SCXK (Beijing) 2021-0006). The animals were maintained in the SPF laboratory of Kongjiang Hospital and given one week to acclimatize prior to the start of the study.

Randomization of the animals was performed via a random number table, with 10 mice allocated to the Sham group and 10 mice to the MCAO group. The MCAO model was generated by the modified Longa filament occlusion method. Before the incision, the mice received anesthesia, were placed supine, and the cervical skin was prepared with standard disinfection. Inhaled isoflurane (induction at 3%–4%, maintenance at 1.5%–2%) was administered via a vaporizer coupled to an anesthesia circuit (RWD Life Science, Shenzhen, China) to anesthetize the mice, as previously described for C57BL/6 male mice. Once deep anesthesia was verified, the animals were placed supine, and the cervical skin was prepared with antiseptic. An anterior midline neck incision roughly 1.5 cm in length was created, and blunt dissection of the subcutaneous tissue and fascia permitted visualization of the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA). Following ligation of both the proximal and distal portions of the ECA, a small opening was created between the two knots on the ECA. A blunt-tipped nylon suture, prepared by heat cauterization, was inserted via the ECA stump and carefully advanced through the ICA. When mild resistance was felt (approximately 9–10 mm from the bifurcation), the filament had reached and occluded the origin of the middle cerebral artery. Reperfusion was initiated after 60 min by gently removing the suture. The ECA stump was subsequently ligated, and the cervical incision was closed in layers. In the Sham group, the CCA, ECA, and ICA were similarly exposed, but no filament was introduced. The intraluminal filament MCAO method used in this study has been established in the literature [17]. A previous comparison study demonstrated that the surgical success rate for mouse MCAO models is approximately 85% [18].

Body temperature was maintained throughout the surgical procedure and the subsequent recovery period. After surgery, each mouse was housed separately with unrestricted access to food and water. In each group, 10 mice were used. After the 24-hour reperfusion period, neurological function was assessed in all mice using the mNSS. Mice were then euthanized by cervical dislocation. In each group, 7 mice were randomly assigned to TTC staining for infarct volume measurement, and the remaining 3 mice were used for molecular analyses (ELISA and Western blotting). For the 3 mice allocated to molecular analyses, the ischemic hemispheric cortex was dissected on ice, snap-frozen in liquid nitrogen, and stored at −80 °C until further processing. The same subset of 3 animals per group was used across all molecular assays (LPC, inflammatory cytokines, and NF-κB pathway proteins) to maintain data consistency. For the 7 mice allocated to TTC staining, brains were processed as described in TTC Staining and Measurement of Cerebral Infarction Volume. Blood was collected by enucleation from all 10 mice per group, allowed to clot at 4 °C for 2 h, and centrifuged at 4000 r/min (radius 13.5 cm) for 20 min. The resulting serum was aliquoted and stored at −80 °C. For serum ELISA analyses, samples from the same 3 mice used for brain molecular analyses were assayed.

Animal Experimental Index Detection

LPC Level Detection

Serum samples obtained from section Experimental Animals and Model Establishment were thawed at 4 °C. Brain tissues were weighed and homogenized in PBS (pH 7.4) at a tissue-to-buffer ratio of 1:9 (w/v) on ice, followed by centrifugation at 3000 r/min for 20 min at 4 °C (H1650R centrifuge, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.). The resulting supernatants were collected for analysis. LPC concentrations in both serum and brain homogenates were determined using a commercial ELISA kit specific for lysophosphatidylcholine, with all procedures conducted according to the manufacturer's protocol. The assay setup included blank control wells, calibrator wells (each containing 15 µL of standard), and sample wells (each loaded with 15 µL of serum or tissue supernatant). Except for the blank wells, 100 µL of Assay Diluent was added to each well, and the plate was incubated at 37 °C for 60 min (DNP-9162E incubator, Shanghai Jinghong Experimental Equipment Co., Ltd.). The contents were then removed, the plate rinsed three times with wash buffer, and 100 µL of SA-HRP was dispensed to every well (excluding blank wells), followed by a 30-min incubation at 37 °C and three additional washes. Next, 100 µL of TMB substrate was introduced into each well, and the reaction was allowed to proceed in the dark at 37 °C for 15 min. The reaction was halted with 100 µL of stop solution, and the absorbance was measured at 450 nm (CMaxPlus, Molecular Devices, Sunnyvale, CA, USA). Sample concentrations were calculated by interpolating optical density values against a standard curve generated from the calibrators. Final LPC levels were expressed as ng/mL for serum samples and ng/mL for brain tissue homogenate samples.

ELISA Detection of Inflammatory Factor Protein Expression

Brain tissue homogenate supernatant and serum samples obtained from Experimental Animals and Model Establishment was subjected to ELISA for quantification of TNF-α, IL-1β, and IL-6 using commercial kits (IL-1β: Cat. No. JYM0531Mo; IL-6: Cat. No. JYM0012Mo; TNF-α: Cat. No. JYM0218Mo; all from Wuhan Genemei Biotechnology Co., Ltd., Wuhan, China) according to the manufacturer's protocol. The plate was set up with standard wells (prepared by serial dilution as instructed, with IL-1β ranging from 90 to 7.5 pg/mL, IL-6 from 120 to 10 pg/mL, and TNF-α from 480 to 40 pg/mL), blank wells, and sample wells. For brain tissue homogenate, each sample well received 10 µL of supernatant and 40 µL of diluent. For serum samples, each well received 10 µL of serum and 40 µL of diluent (a 5-fold dilution), and was gently mixed. Subsequently, 50 µL of enzyme-labeled reagent was added to all wells except the blank wells. The plate was sealed and incubated at 37 °C for 30 min, after which the liquid was discarded, and the wells were washed five times with buffer and dried by gently tapping the plate. Colorimetric reagents A and B (50 µL each) were then added in sequence, and the plate was incubated at 37 °C in darkness for 10 min. The reaction was stopped by adding 50 µL of stop solution, and absorbance at 450 nm was recorded. Concentrations of both brain tissue homogenate and serum samples.

Assessment of NF-κB Pathway Protein Expression in Brain Tissue by Western Blotting

Cryocortical tissue from the ischemic side, as described in Experimental Animals and Model Establishment, was collected and lysed in Western and IP cell lysis buffer (Cat. No. P0013J, Shanghai Beyotime Biotechnology Co., Ltd, Shanghai, China). Western lysis buffer containing 1 mM PMSF (Cat. No. ST506, Beyotime) and IP cell lysis buffer were added. The mixture was homogenized on ice and lysed on ice for 10 min. Following centrifugation at 12,000 g for 10 min at 4 °C, the supernatant was collected, and protein levels were measured using a BCA kit (Beyotime). The protein lysates were then mixed with 5× loading buffer (P0015, Beyotime) at a ratio of 4:1 ratio, heated to 100 °C for 5–10 min in a metal bath, and stored at −20 °C. Equal amounts of protein (~40 µg/lane) were separated by 10% SDS-PAGE gels (G2043, Servicebio, Wuhan Servicebio Technology Co., Ltd., Wuhan, China) at a constant voltage of 200 V (JY300E, Beijing Junyi Dongfang Electrophoresis Equipment Co., Ltd., Beijing, China) until the dye front reached the bottom. Proteins were transferred to PVDF membranes by wet electroblotting at 400 mA for 40 min (JY-ZY5, Beijing Junyi Dongfang).

Membranes were blocked with 5% non-fat dry milk for 1 h at room temperature and then incubated overnight at 4 °C with gentle shaking using the following primary antibodies: rabbit anti-mouse p-p65 (1:1000, HA723223, HuaBio), rabbit anti-mouse p65 (1:1000, 80979-1-RR, Proteintech), and mouse anti-GAPDH (1:5000, 60004-1-Ig, Proteintech). After three washes with 1× TBST (10 min each), the membranes were incubated for 1 h at room temperature with HRP-conjugated goat anti-rabbit IgG (1:2000, ZB-2301) or goat anti-mouse IgG (1:2000, ZB-2305, both from Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., Beijing, China), followed by another three TBST washes. Protein bands were detected with ECL chemiluminescent substrate (Immobilon Western HRP Substrate Luminol Reagent, WBKLS0500, Millipore) and imaged on a Tanon-4600 system (Beijing Yuanpinghao Biotechnology Co., Ltd.). Band intensities were measured using ImageJ (ImageJ, National Institutes of Health, Bethesda, MD, USA), and NF-κB pathway activation was assessed based on the p-p65/p65 ratio. For each target protein, the integrated optical density of the specific band was quantified and normalized to the corresponding GAPDH band intensity from the same sample. The relative expression of p-p65 and p65 was expressed as the ratio of the target protein to GAPDH. NF-κB pathway activation was assessed by cal-

culating the p-p65/p65 ratio. Data from three independent experiments were used for statistical analysis and presented as mean ± SD in bar graphs.

TTC Staining and Measurement of Cerebral Infarction Volume

At 24 h post-reperfusion, deep anesthesia was induced with inhaled isoflurane (3%–4% induction, 1.5%–2% maintenance) as detailed in Experimental Animals and Model Establishment. After the absence of the pedal reflex was confirmed, mice were decapitated, and the brains were rapidly collected. For the 7 animals assigned to TTC staining, the olfactory bulbs, cerebellum, and lower brainstem were excised, and the remaining cerebrum was rapidly frozen at −20 °C for 20 min. Serial coronal slices (2 mm thick) were cut from the frontal to the occipital pole. Brain sections were immersed in 2% TTC solution (2,3,5-triphenyltetrazolium chloride, Shanghai Yuanye Biotechnology Co., Ltd.) and incubated at 37 °C for 20 min in the dark with gentle agitation. The stained slices were then fixed overnight in 4% paraformaldehyde. Digital photographs were taken, and infarct areas were quantified with ImageJ. Infarct volume percentage was computed as: (area of the contralateral hemisphere − area of the non-infarcted ipsilateral region) / area of the contralateral hemisphere × 100%.

Neurological Deficit Score

Neurological function was assessed using the modified neurological deficit score (mNSS) by a researcher who was blinded to the experimental groups [19]. The mNSS scoring system includes four items: motor, sensory, balance and reflexes, with a total score of 18 points. 0 points indicates normal neurological function; 1–6 points indicate mild impairment; 7–12 points indicate moderate impairment; and 13–18 points indicate severe impairment.

Cell experiments

Cell Culture and Establishment of OGD/R Model

BV2 murine microglial cells (CL-0493, Procell Life Science & Technology Co., Ltd., Wuhan, China) were cultured in DMEM high-glucose medium containing 10% fetal bovine serum and 1% penicillin-streptomycin (all supplied by Gibco, USA) at 37 °C with 5% CO_2. When the cultures reached 80%–90% confluency, they were passaged with 0.25% trypsin, and cells in logarithmic growth were used for subsequent assays. Prior to experimentation, the BV2 cell line was authenticated by short tandem repeat (STR) profiling and confirmed to be free of mycoplasma contamination using a PCR-based detection kit (Cat. No. BPM-Pro50, Shanghai Biowing Applied Biotechnology Co., Ltd., Shanghai, China).

An oxygen-glucose deprivation/reperfusion (OGD/R) protocol was employed to simulate *in vivo* ischemia-reperfusion conditions. Once cells reached approximately 80% confluence, the original medium was discarded, and the cells were washed twice with PBS before being transferred to glucose-free DMEM. The cultures were placed in a tri-gas incubator (1% O_2, 94% N_2, 5% CO_2, 37 °C; Forma Series II, Thermo Fisher Scientific, USA) and subjected to glucose deprivation for 4 h. After OGD, the medium was replaced with normal high-glucose DMEM supplemented with 10% FBS, and the cells were moved back to a normoxic incubator (95% air, 5% CO_2, 37 °C) for 24 h of reoxygenation. The chosen OGD/R settings were supported by published studies; Chen et al. [19] likewise applied 4 h of OGD followed by 24 h of reoxygenation to induce inflammatory gene upregulation in BV2 cells [20]. Cells in the Control group were maintained under standard culture conditions with normal medium and a normoxic atmosphere through the experimental period.

Cell Experiment Grouping and Drug Treatment

BV2 cells in logarithmic growth phase were randomly divided into the following 4 groups: (1) Control group: Normal culture, without any drug intervention or OGD treatment. (2) Model group (OGD group): Only receiving OGD/R treatment. (3) LPC intervention group (OGD + LPC group): Following 4 h of OGD, cells were incubated in normal culture medium containing 20 μmol/L LPC (1-palmitoyl-2-hydroxy-sn-glycero-3-phosphocholine, 16:0 LPC; Cat. No. 855675P, Avanti Polar Lipids, USA; dissolved in ethanol to prepare a stock solution and diluted with culture medium before use, with the final ethanol concentration <0.1%) during the 24-h reoxygenation period. (4) OGD + LPC + BAY group: During the 4-h OGD period, cells were pretreated with 5 μmol/L BAY 11-7082 (Cat. No. S2913, Selleck, USA; dissolved in DMSO, final DMSO concentration <0.1%). Following OGD, cells were incubated in normal culture medium containing 20 μmol/L LPC and 5 μmol/L BAY 11-7082 during the 24-h reoxygenation period. All cell experiments were independently repeated three times, with three replicate wells per group in each experiment.

Cellular Experimental Index Detection

Cell culture supernatant was collected, and the levels of TNF-α, IL-1β, and IL-6 were determined using the ELISA method described in ELISA Detection of Inflammatory Factor Protein Expression. The results are expressed as pg/mL. Cell pellets were collected, and the expression levels of p-p65 and p65 proteins were analyzed using the Western blotting described in Assessment of NF-κB Pathway Protein Expression in Brain Tissue by Western Blotting.

Statistical Analysis

GraphPad Prism 9.0 was employed for all statistical analyses and graphical presentations. Data distribution normality was evaluated using the Shapiro–Wilk test, and ho-

Table 1. Comparison of mNSS scores between the two groups.

Group	Sham Group	MCAO Group	Statistic	*p*
mNSS	1.00 (0.50, 1.00)	13.00 (11.50, 15.50)	Z = –3.11	0.002

mogeneity of variance was assessed using Levene's test. Normally distributed variables are expressed as mean ± SD ($\bar{x} \pm s$). Comparisons between two groups were performed using the independent samples *t*-test; for multiple groups, one-way ANOVA was applied, with post hoc pairwise comparisons conducted using the LSD-t test when variances were homogeneous or Dunnett's T3 test when variances were unequal. Non-normally distributed data are summarized as median with interquartile range M (IQR). The Mann–Whitney U test was used to compare two groups, and the Kruskal-Wallis H test was used for multi-group comparisons. A two-tailed *p* value below 0.05 was considered statistically significant.

Results

Establishment and Validation of the MCAO Model

TTC staining revealed uniformly red-stained brain sections without obvious infarct lesions in the Sham group (n = 7), indicating intact tissue morphology. In contrast, the MCAO group (n = 7) exhibited distinct pale-colored infarct regions on the ischemic side, predominantly distributed in the cortex and striatum (Fig. 1). Neurological function evaluated by the mNSS showed that Sham-operated mice exhibited near-normal performance, whereas mice in the MCAO group displayed marked neurological deficits, with significantly higher mNSS scores compared with the Sham group ($p < 0.05$, Table 1). These findings collectively confirm the successful establishment of the MCAO model.

Effects of Ischemia - Reperfusion Injury on Serum and Brain Tissue LPC Levels in Mice

ELISA analysis revealed that, relative to the Sham group (n = 3), the MCAO group (n = 3) exhibited markedly elevated LPC concentrations in brain tissue ($p < 0.05$), whereas serum LPC levels were significantly reduced ($p < 0.05$) (Fig. 2).

Changes in Serum Inflammatory Factor Levels in a Cerebral Ischemia Model

The ELISA assay revealed that the MCAO group (n = 3) exhibited significant increases in brain tissue IL-1β, IL-6, and TNF-α ($p < 0.05$), as well as in serum levels of these three factors ($p < 0.05$) relative to the Sham group (n = 3) (Fig. 3). These results indicate that inflammatory cascades are activated after cerebral ischemia-reperfusion, with elevated TNF-α, IL-1β, and IL-6, and support subsequent examination of whether LPC is involved in inflammatory modulation by enhancing the expression of these mediators.

Activation of the NF- κB Signaling Pathway in a Model of Cerebral Ischemia

Western blot analysis revealed that both p-p65 protein levels and the p-p65/p65 ratio were markedly elevated in the MCAO group relative to the Sham group ($p < 0.05$; Fig. 4A), indicating that NF-κB signaling can be activated following cerebral ischemia-reperfusion. Representative immunoblot images of p-p65, p65, and GAPDH are shown in Fig. 4B. Combined with the LPC accumulation observed in brain tissue in Effects of Ischemia - Reperfusion Injury on Serum and Brain Tissue LPC Levels in Mice, these findings provide a basis for further investigation into whether LPC regulates the inflammatory response through activation of this pathway.

Effects of LPC on the Secretion of Inflammatory Factors in BV2 Cells After OGD/R Injury

Compared with the Control group, the OGD group exhibited markedly higher concentrations of IL-1β, IL-6, and TNF-α ($p < 0.05$). The addition of LPC to OGD-treated cultures further increased the levels of these three inflammatory mediators relative to the OGD group ($p < 0.05$). In contrast, treatment with BAY 11-7082 in the presence of LPC significantly diminished the levels of IL-1β, IL-6, and TNF-α compared to the OGD+LPC group ($p < 0.05$, Fig. 5). These results imply that BAY 11-7082 can counteract the LPC-driven promotion of inflammatory factor secretion, lending experimental support to the notion that LPC regulates inflammatory response through activation of the NF-κB pathway. Additionally, no significant difference was observed between the OGD+LPC+BAY group and the OGD group, suggesting that the reversal of the LPC effect was incomplete.

Western Blot Results of Cell Experiments

Western blotting revealed that the OGD group displayed a significant upregulation of p-p65 and an elevated p-p65/p65 ratio relative to the Control group. These indicators were further elevated in the OGD+LPC group compared with the OGD group alone. In the presence of BAY 11-7082, however, both p-p65 abundance and the p-p65/p65 ratio markedly declined from the levels observed in the OGD+LPC group (Fig. 6A, $p < 0.05$). Representative immunoblot images of p-p65, p65, and GAPDH are shown in Fig. 6B. Taken together, these results imply that BAY 11-7082 can partly reverse the LPC-driven stimulation of the NF-κB signaling cascade.

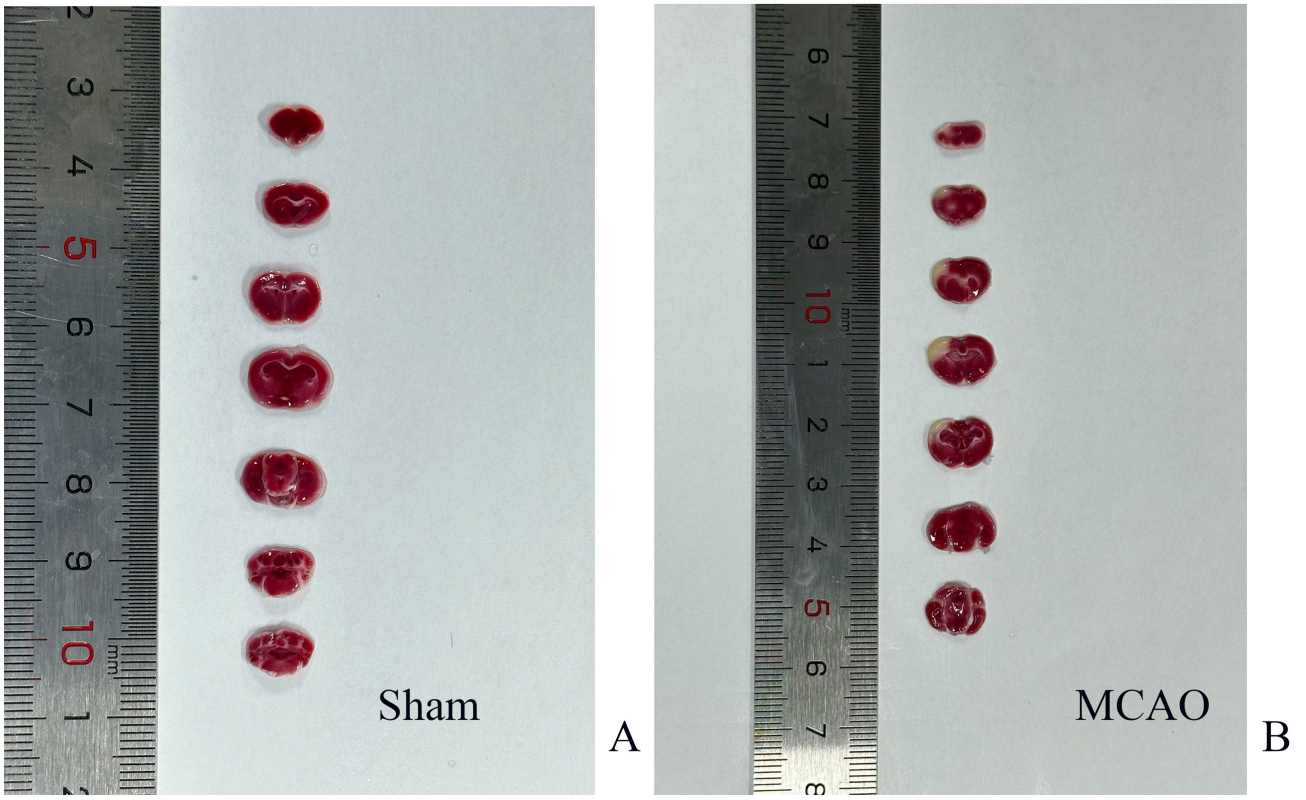

Fig. 1. TTC staining results. (A) Sham group; (B) MCAO group. TTC, 2,3,5-triphenyltetrazolium chloride; MCAO, middle cerebral artery occlusion.

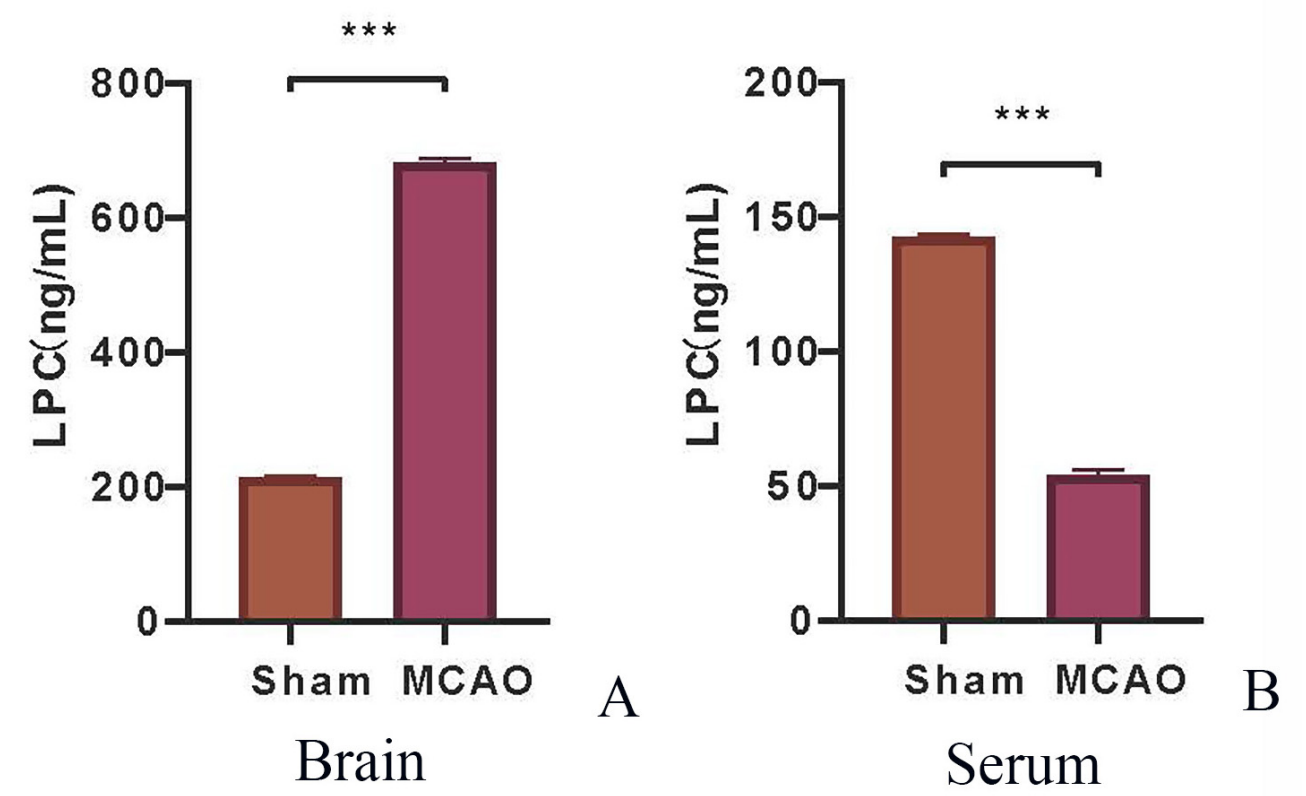

Fig. 2. Serum and Brain Tissue LPC Levels in Mice. (A) Comparison of LPC levels in brain tissue; (B) Comparison of serum LPC levels. n = 3 animals per group. *** $p < 0.001$. LPC, lysophosphatidylcholine.

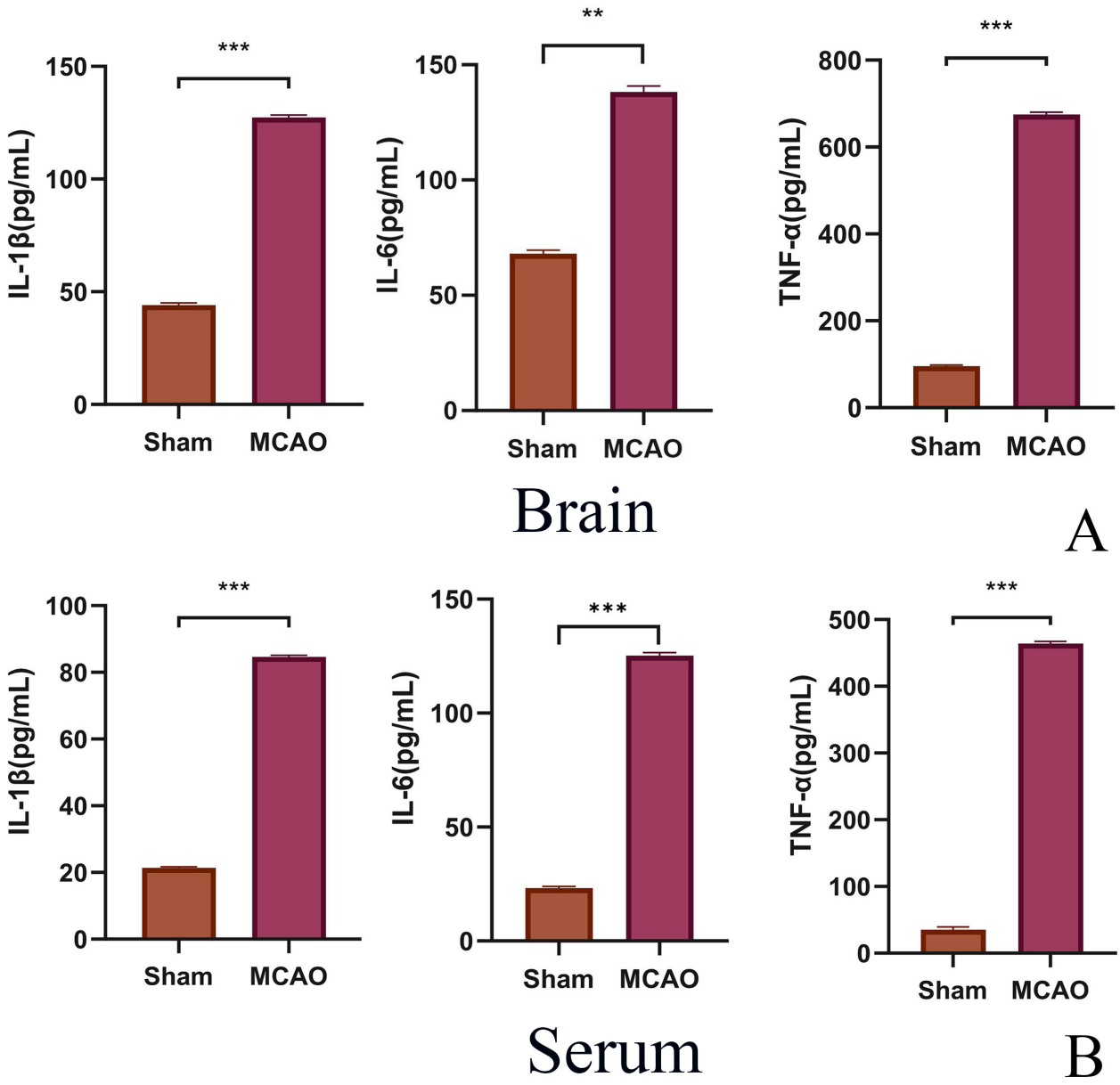

Fig. 3. Serum Inflammatory Factor Levels in a Cerebral Ischemia Model. (A) Comparison of IL-1β, IL-6, and TNF-α levels in brain tissue; (B) Comparison of serum IL-1β, IL-6, and TNF-α levels. n = 3 animals per group. ** $p < 0.01$, *** $p < 0.001$. IL-1β, interleukin-1β; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α.

Discussion

Secondary neuroinflammation following ischemic stroke is an important factor leading to persistent brain damage and deterioration of neurological function, and the interaction between lipid metabolism abnormalities and inflammatory responses has attracted increasing attention in recent years [21]. Lysophosphatidylcholine (LPC), as an important component of oxidized low-density lipoprotein and a lipid mediator significantly elevated in various cardiovascular and cerebrovascular diseases, remains insufficiently characterized regarding its role in regulating inflammation after ischemic stroke [15]. This study focuses on the potential mechanism by which LPC regulates the NF-κB signaling pathway and thus affects the expression of inflammatory factors. Through a combination of *in vivo* and *in vitro* experiments, we have preliminarily explored the role of LPC in the inflammatory response after cerebral ischemia and investigated its potential underlying molecular mechanisms.

The results of *in vivo* experiments showed that LPC levels in the brain tissue of MCAO mice were significantly increased, whereas the levels of serum LPC showed a decreasing trend. This difference in changes between brain

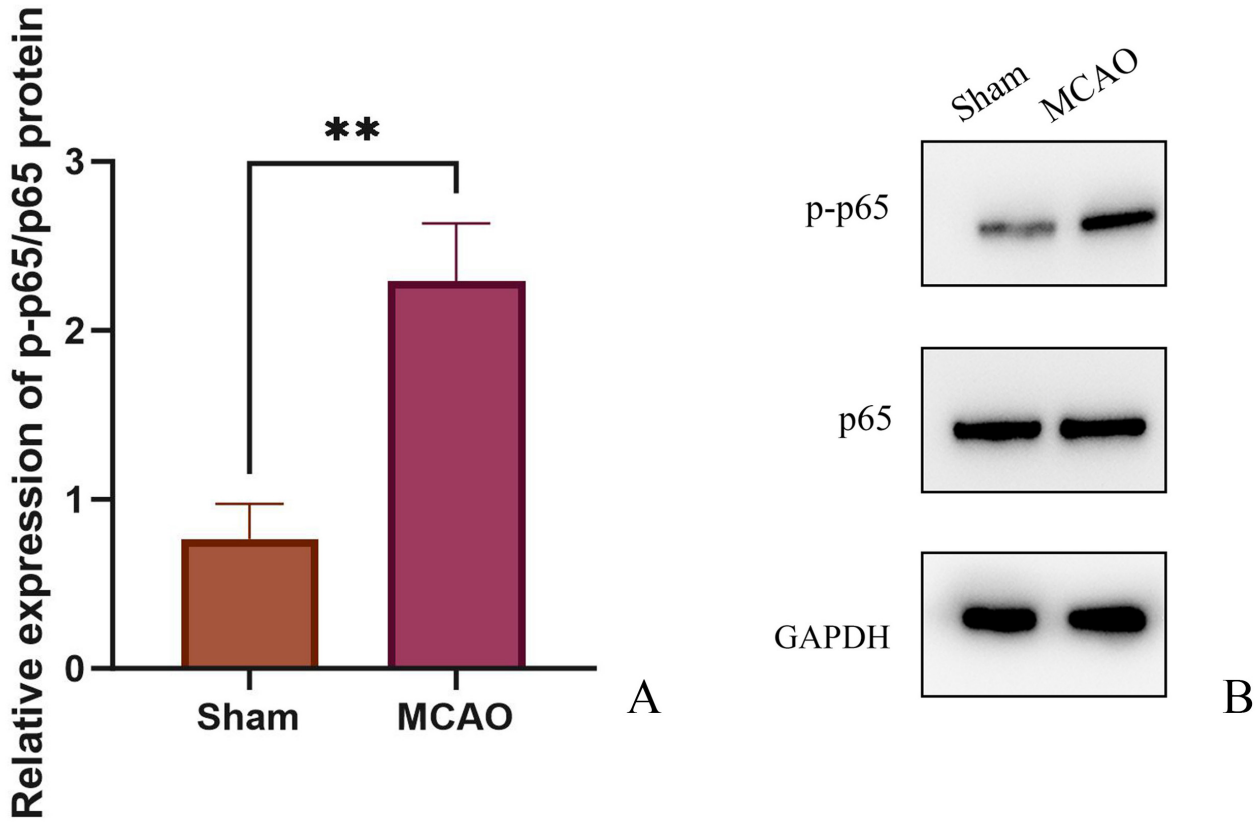

Fig. 4. NF-κB pathway activation in brain tissue after MCAO. (A) Quantitative analysis of the p-p65/p65 ratio in brain tissue. (B) Western blot results. ** $p < 0.01$.

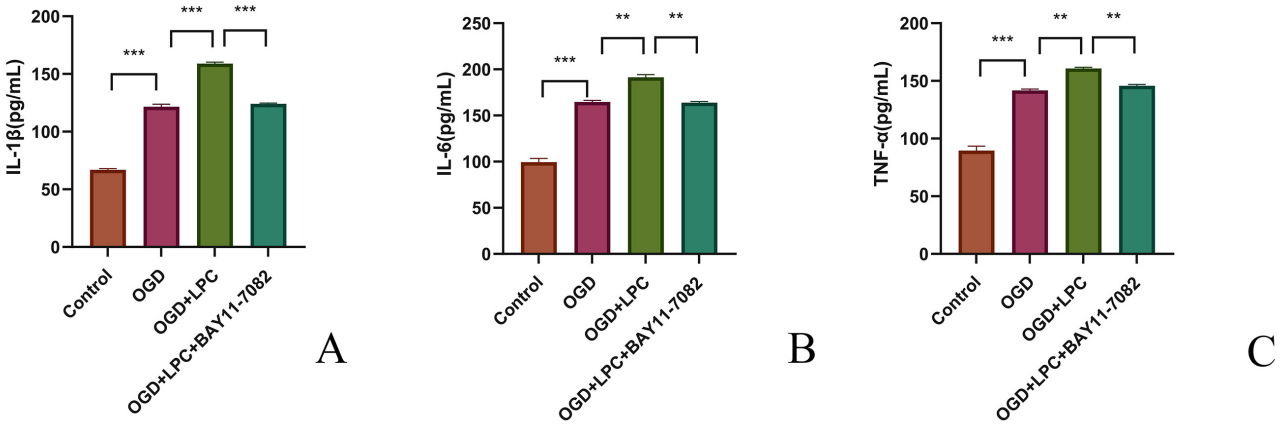

Fig. 5. Effects of LPC and BAY 11-7082 on inflammatory cytokine secretion in BV2 cells after OGD/R. (A) IL-1β; (B) IL-6; (C) TNF-α. Data are presented as mean ± SD from three independent experiments. ** $p < 0.01$, *** $p < 0.001$. OGD/R, oxygen-glucose deprivation/reperfusion.

tissue and peripheral circulation is noteworthy. In a systematic review, Turpin et al. [8] analyzed lipid metabolism data from multiple rodent stroke models and clearly observed a significant difference between plasma and brain tissue LPC levels after stroke; that is, plasma LPC decreased while brain tissue LPC increased, and both were closely related to functional outcomes and mechanistic markers. The review also included experimental evidence that CpG pretreatment reduced LPC accumulation in brain tissue after MCAO, providing a more systematic background for understanding tissue-specific changes of LPC after cerebral ischemia. At the clinical level, Huang et al. [7] performed non-targeted

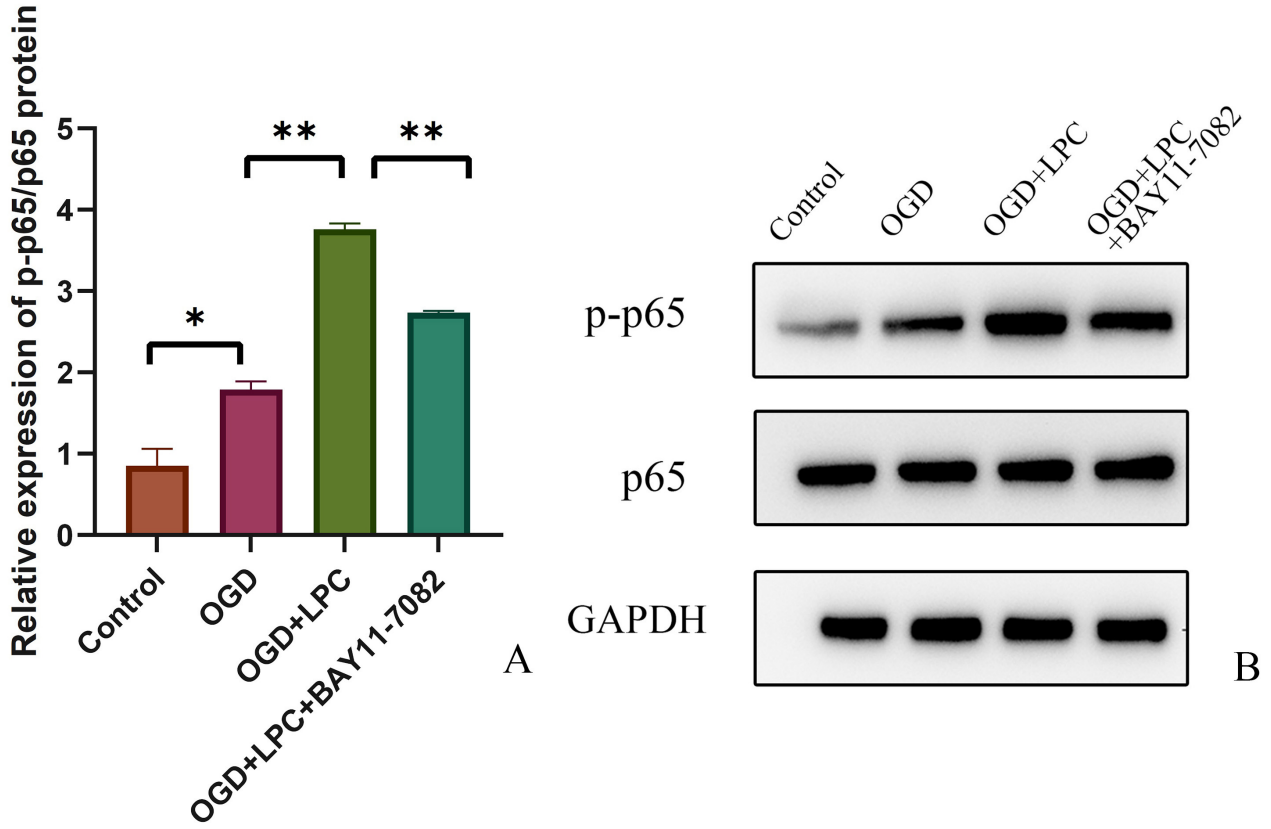

Fig. 6. NF-κB pathway activation in BV2 cells. (A) Quantitative analysis of the p-p65/p65 ratio in BV2 cells. Data are presented as mean ± SD from three independent experiments. * $p < 0.05$, ** $p < 0.01$. (B) Western blot analysis of NF-κB pathway-related protein expression in BV2 cells of each group.

lipidomics analysis of plasma samples from stroke patients and found that LPC and phosphatidylcholine were significantly associated with functional recovery after stroke, suggesting that changes in peripheral blood LPC may reflect lipid metabolic reprogramming after brain injury. The findings of the aforementioned studies corroborate those of this study. While the present data do not directly demonstrate a transfer of circulating LPC into the brain, the concurrent increase in brain LPC and decrease in serum LPC is consistent with the possibility that circulating LPCs may redistribute to ischemic brain tissue. Importantly, these results suggest that measuring only peripheral blood LPC levels may not accurately reflect the pathological role of LPCs in the brain parenchyma. Compared to the review perspective of Turpin et al. [8], which focused on LPCs as biomarkers, this study further validated the activation effects of LPC(16:0), one of the most abundant LPC species in plasma and brain tissue, on the NF- κB pathway and their promoting effects on the expression of inflammatory factors in an *in vitro* OGD/R model, providing direct experimental evidence for understanding the functional role of LPCs in the inflammatory response after cerebral ischemia.

Lambertsen et al. [22] systematically described that the overexpression of TNF-α after ischemic stroke can promote neuronal death and inflammatory response through the TNFR1 pathway. Consistent with this framework, both the animal and cellular experiments demonstrated substantial increases in TNF-α, IL-1β, and IL-6 after ischemic-reperfusion injury. More importantly, LPC stimulation following OGD/R enhanced the release of these inflammatory mediators, suggesting that LPC is not merely associated with ischemic injury but may actively drive the amplification of the inflammatory cascade. This finding is uncommon in current stroke research and provides new perspectives on the relationship between aberrant lipid metabolism and exacerbated neuroinflammation. The NF-κB pathway serves as a master regulator of pro-inflammatory transcription. In the present study, OGD/R exposure significantly elevated the p-p65/p65 ratio in BV2 microglia, which was further enhanced by LPC, while the NF-κB inhibitor BAY 11-7082 significantly diminished both p-p65 expression and inflammatory cytokine release. This graded response supports the involvement of LPC in NF-κB-mediated inflammatory activation in BV2 microglia under OGD/R conditions. Xie et al. [16] found in an LPC-induced demyelination model that microglia inflammatory pyroptosis depends on the activation of the NF- κB and ERK1/2 signaling pathways. Halder et al. [23] also showed that under

OGD conditions, astrocytes can promote the expression of IL-6 and TNF-α through NF- κB phosphorylation mediated by TLR4 and TNFR1. In the context of ischemic stroke, our work extends previous knowledge by establishing that LPC(16:0) treatment under OGD/R conditions is accompanied by increased NF-κB pathway activation and inflammatory cytokine production, and that these effects can be attenuated by the NF-κB inhibitor BAY 11-7082. These findings are consistent with a model in which LPC(16:0) acts upstream of NF-κB signaling to promote inflammatory gene transcription, although definitive proof of a direct molecular interaction would require further investigation beyond pharmacological inhibition alone. BAY 11-7082, a well-characterized NF-κB inhibitor, has been repeatedly validated for its anti-inflammatory and neuroprotective properties in cerebral ischemia models. Supporting this, Halder et al. [23] demonstrated in a mouse MCAO model that Thiamet G attenuates post-stroke neuroinflammation through suppression of NF-κB p65 signaling, an effect associated with modulation of microglial and macrophage polarization [24]. The present findings also demonstrate that BAY 11-7082 effectively counteracts LPC-driven inflammatory factor release *in vitro*, providing additional support for the central role of the NF-κB pathway in mediating the pro-inflammatory effects of LPC. These results also indicate that pharmacological blockade of NF-κB may represent a potential strategy for mitigating LPC-related neuroinflammatory injury.

The mNSS data obtained in this study further indicated that LPC accumulation, NF-κB pathway engagement, and elevated inflammatory mediators were accompanied by cerebral infarct development and neurological impairment. A previous study has demonstrated that LPC can stimulate the NF-κB pathway in macrophages through TLR2 and TLR4 [25]. Taken together, the *in vivo* and *in vitro* evidence from this study suggests that post-ischemic LPC accumulation in brain tissue may activate the NF-κB pathway in microglia and infiltrating immune cells, thereby promoting the transcription of inflammatory mediators and intensifying the local inflammatory milieu, which in turn contributes to the propagation of cerebral injury. The above findings provide new experimental evidence for understanding the mechanistic link between abnormal lipid metabolism and neuroinflammation after stroke, while offering preliminary insights into potential intervention strategies targeting the LPC-NF-κB axis.

This study still has certain limitations. First, the current *in vivo* experiments included only the Sham and MCAO groups, and direct LPC intervention at the *in vivo* level has not yet been conducted. Therefore, the causal role of LPC mainly relies on evidence from the *in vitro* OGD/R model. In the *in vitro* experiments, we only evaluated the effect of exogenous LPC supplementation on downstream signaling pathways and did not measure endogenous LPC levels in cells from each group after OGD/R. Therefore, the comparability between the *in vitro* model and the LPC accumulation observed *in vivo* brain tissue needs further confirmation. Future studies should directly intervene in LPC levels in the *in vivo* model and systematically detect LPC content in cells from each group after OGD/R in *in vitro* experiments to further verify the consistency between *in vivo* and *in vitro* experiments. Second, this study only focused on the NF- κB signaling pathway, but LPC may also participate in regulating other inflammation-related pathways such as MAPK/ERK. The interactive regulatory relationships between multiple signaling pathways need further investigation. Regarding pathway validation methods, this study primarily relied on BAY 11-7082 for pharmacological blockade. However, off-target effects of single inhibitors cannot be completely ruled out. In addition, LPC was dissolved in ethanol and BAY 11-7082 was dissolved in DMSO for the cell experiments, whereas solvent vehicle control groups were not included in the current experimental design. Although the final concentrations of ethanol and DMSO in the culture medium were kept below 0.1% to minimize potential cytotoxicity, the influence of the solvents on inflammatory factor secretion and NF-κB pathway activity cannot be entirely excluded. Future studies should combine this approach with complementary methods, such as IκBα-phosphorylation-site mutation vectors or p65 siRNA/CRISPR knockdown in BV2 cells, for cross-validation to further confirm the functional specificity of the NF-κB pathway in mediating the pro-inflammatory effects of LPC. Furthermore, this study only observed the acute phase at 24 hours post-reperfusion. Considering the significant temporal dynamics of post-stroke inflammatory responses, the changes in LPC levels and NF- κB pathway activity at different time points remain to be elucidated. Finally, this study was exploratory in nature, and the limited sample size for the quantitative analysis of some molecular indicators requires further biological replication for validation.

Conclusion

In summary, this study demonstrates that elevated brain LPC levels are accompanied by post-ischemic neuroinflammation *in vivo*. Furthermore, *in vitro* experiments using an OGD/R model provide preliminary evidence that LPC can promote the expression of inflammatory factors in microglia, an effect that may be mediated through the activation of the NF-κB signaling pathway. While the *in vivo* data show a correlation between elevated total brain LPC levels and post-ischemic neuroinflammation, the specific contribution of individual LPC species, including LPC(16:0), to this process requires further investigation. The LPC-NF-κB axis warrants investigation as a potential target for anti-inflammatory intervention.

Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

JLJ and WL designed the research study. JLJ and LF performed the research. WL and LF analyzed the data. JLJ drafted the article and all authors contributed to important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the ARRIVE Guidelines 2.0 and approved by the Kongjiang Hospital of Yangpu District Animal Ethics Committee (Ethics Approval No. LL-2026-KY-24).

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Conflict of Interest

The authors declare no conflict of interest.

References

[1] Pu L, Wang L, Zhang R, Zhao T, Jiang Y, Han L. Projected Global Trends in Ischemic Stroke Incidence, Deaths and Disability-Adjusted Life Years From 2020 to 2030. Stroke. 2023; 54: 1330–1339. https://doi.org/10.1161/STROKEAHA.122.040073

[2] Zhao Y, Zhang X, Chen X, Wei Y. Neuronal injuries in cerebral infarction and ischemic stroke: From mechanisms to treatment (Review). International Journal of Molecular Medicine. 2022; 49: 15. https://doi.org/10.3892/ijmm.2021.5070

[3] Mosconi MG, Paciaroni M. Treatments in Ischemic Stroke: Current and Future. European Neurology. 2022; 85: 349–366. https://doi.org/10.1159/000525822

[4] Zhu H, Hu S, Li Y, Sun Y, Xiong X, Hu X, et al. Interleukins and Ischemic Stroke. Frontiers in Immunology. 2022; 13: 828447. https://doi.org/10.3389/fimmu.2022.828447

[5] Zhao H, Li Y, Zhang Y, He WY, Jin WN. Role of Immune and Inflammatory Mechanisms in Stroke: A Review of Current Advances. Neuroimmunomodulation. 2022; 29: 255–268. https://doi.org/10.1159/000524951

[6] Iordache MP, Buliman A, Costea-Firan C, Gligore TCI, Cazacu IS, Stoian M, et al. Immunological and Inflammatory Biomarkers in the Prognosis, Prevention, and Treatment of Ischemic Stroke: A Review of a Decade of Advancement. International Journal of Molecular Sciences. 2025; 26: 7928. https://doi.org/10.3390/ijms26167928

[7] Huang M, Xu S, Zhou M, Luo J, Zha F, Shan L, et al. Lysophosphatidylcholines and phosphatidylcholines as biomarkers for stroke recovery. Frontiers in Neurology. 2022; 13: 1047101. https://doi.org/10.3389/fneur.2022.1047101

[8] Turpin J, Wadolowski S, Tambo W, Kim D, Al Abed Y, Sciubba DM, et al. Exploring Lysophosphatidylcholine as a Biomarker in Ischemic Stroke: The Plasma-Brain Disjunction. International Journal of Molecular Sciences. 2024; 25: 10649. https://doi.org/10.3390/ijms251910649

[9] Zheng L, Xie C, Zheng J, Dong Q, Si T, Zhang J, et al. An imbalanced ratio between PC(16:0/16:0) and LPC(16:0) revealed by lipidomics supports the role of the Lands cycle in ischemic brain injury. The Journal of Biological Chemistry. 2021; 296: 100151. https://doi.org/10.1074/jbc.RA120.016565

[10] Cao Y, Yue X, Jia M, Wang J. Neuroinflammation and anti-inflammatory therapy for ischemic stroke. Heliyon. 2023; 9: e17986. https://doi.org/10.1016/j.heliyon.2023.e17986

[11] Yan B, Yu X, Cai X, Huang X, Xie B, Lian D, et al. A Review: The Significance of Toll-Like Receptors 2 and 4, and NF-κB Signaling in Endothelial Cells during Atherosclerosis. Frontiers in Bioscience (Landmark Edition). 2024; 29: 161. https://doi.org/10.31083/j.fbl2904161

[12] Kołodziejska R, Pawluk H, Tafelska-Kaczmarek A, Pawluk M, Koper K, Godlewski A, et al. The Role of TNF-α in Ischemic Stroke. International Journal of Molecular Sciences. 2026; 27: 1424. https://doi.org/10.3390/ijms27031424

[13] Liu Y, Zhang Y. The NF-κB-driven inflammatory cascade in ischemic stroke: Linking DAMPs, inflammasomes, and neurovascular dysfunction. Gene. 2026; 999: 150163. https://doi.org/10.1016/j.gene.2026.150163

[14] Lietzau G, Sienkiewicz W, Karwacki Z, Dziewiątkowski J, Kaleczyc J, Kowiański P. The Effect of Simvastatin on the Dynamics of NF-κB-Regulated Neurodegenerative and Neuroprotective Processes in the Acute Phase of Ischemic Stroke. Molecular Neurobiology. 2023; 60: 4935–4951. https://doi.org/10.1007/s12035-023-03371-2

[15] Wang Q, Wang D, Gao Y, Jiang J, Li M, Li S, et al. Impaired membrane lipids in ischemic stroke: a key player in inflammation and thrombosis. Journal of Neuroinflammation. 2025; 22: 144. https://doi.org/10.1186/s12974-025-03464-w

[16] Xie Y, Chen X, Li Y, Chen S, Liu S, Yu Z, et al. Transforming growth factor-β1 protects against LPC-induced cognitive deficit by attenuating pyroptosis of microglia via NF-κB/ERK1/2 pathways. Journal of Neuroinflammation. 2022; 19: 194. https://doi.org/10.1186/s12974-022-02557-0

[17] Chen F, Wang W, Ding H, Yang Q, Dong Q, Cui M. The glucagon-like peptide-1 receptor agonist exendin-4 ameliorates warfarin-associated hemorrhagic transformation after cerebral ischemia. Journal of Neuroinflammation. 2016; 13: 204. https://doi.org/10.1186/s12974-016-0661-0

[18] Lee S, Hong Y, Park S, Lee SR, Chang KT, Hong Y. Comparison of surgical methods of transient middle cerebral artery occlusion between rats and mice. The Journal of Veterinary Medical Science. 2014; 76: 1555–1561. https://doi.org/10.1292/jvms.14-0258

[19] Chen J, Sanberg PR, Li Y, Wang L, Lu M, Willing AE, et al. Intravenous administration of human umbilical cord blood reduces behavioral deficits after stroke in rats. Stroke. 2001; 32: 2682–2688. https://doi.org/10.1161/hs1101.098367

[20] Zhou K, Wu J, Chen J, Zhou Y, Chen X, Wu Q, et al. Schaftoside ameliorates oxygen glucose deprivation-induced inflammation associated with the TLR4/Myd88/Drp1-related mitochondrial fission in BV2 microglia cells. Journal of Pharmacological Sciences. 2019; 139: 15–22. https://doi.org/10.1016/j.jphs.2018.10.012

[21] Luan X, Wang X, Shi Y, Zhang X, Wang Y, Zhou M, et al. Abnormalities of lipid metabolism in the progression and treatment of depression. Frontiers in Psychiatry. 2025; 16: 1589663. https://doi.org/10.3389/fpsyt.2025.1589663

[22] Lambertsen KL, Biber K, Finsen B. Inflammatory cytokines in experimental and human stroke. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2012; 32: 1677–1698. https://doi.org/10.1038/jcbfm.2012.88

[23] Halder A, Yadav K, Aggarwal A, Singhal N, Sandhir R. Activation of TNFR1 and TLR4 following oxygen glucose deprivation promotes mitochondrial fission in C6 astroglial cells. Cellular Signalling. 2020; 75: 109714. https://doi.org/10.1016/j.cellsig.2020.109714

[24] He Y, Ma X, Li D, Hao J. Thiamet G mediates neuroprotection in experimental stroke by modulating microglia/macrophage polarization and inhibiting NF-κB p65 signaling. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism. 2017; 37: 2938–2951. https://doi.org/10.1177/0271678X16679671

[25] Carneiro AB, Iaciura BMF, Nohara LL, Lopes CD, Veas EMC, Mariano VS, et al. Lysophosphatidylcholine triggers TLR2- and TLR4-mediated signaling pathways but counteracts LPS-induced NO synthesis in peritoneal macrophages by inhibiting NF-κB translocation and MAPK/ERK phosphorylation. PloS One. 2013; 8: e76233. https://doi.org/10.1371/journal.pone.0076233