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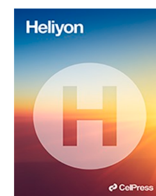

The neuroprotection of controlled decompression after traumatic epidural intracranial hypertension through suppression of autophagy via PI3K/Akt signaling pathway

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A R T I C L E I N F O

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A B S T R A C T

Acute intracranial hypertension (AIH) is a common and tricky symptom that inflicts upon patients after traumatic brain injury (TBI). A variety of clinical options have been applied for the management of AIH, such as physiotherapy, medication, surgery and combination therapy. Specifically, controlled decompression (CDC) alleviates the extent of brain injury and reduces the incidence of a series of post-TBI complications, thereby enhancing the prognosis of patients suffering from acute intracranial hypertension. The objective of the present project is to illuminate the potential molecular mechanism that underlies the neuroprotective effects of CDC in a rat model of traumatic epidural intracranial hypertension (TEIH). Herein, we observed the functional recovery, the degree of brain edema, the level of apoptosis, the expressions of neuronal cell autophagy-related signaling pathway proteins (including Akt, *p*-Akt, LC3 and Beclin-1) in rat TEIH model at 24 h post-surgery. The results showed in comparison with rapid decompression (RDC), CDC reduced the degree of brain edema, diminished the level of cellular apoptosis and enhanced neurological function, and whereas the neuroprotective effect of CDC could be reversed by rapamycin (Rap). The expressions of Beclin-1 and LC3 in CDC group were significantly lower than those of RDC group, and the expression levels of these two proteins were significantly elevated after the addition of Rap. The expression of *p*-Akt in CDC group was considerably enhanced than RDC group. After the addition of LY294002, a PI3K/Akt pathway inhibitor, *p*-Akt protein expression was reduced, and the neuroprotective effect of the rats was markedly inhibited. Taken together, our data demonstrate the superior neuroprotective effect of CDC with regard to alleviating early brain edema, improving the neurological status, suppressing apoptosis and inhibiting neuronal autophagy via triggering PI3K/Akt signaling pathway.

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1. Introduction

Acute intracranial hypertension (AIH) is a grave clinical problem that frequently afflicts upon patients with traumatic brain injury (TBI), and over 50 million patients experience TBI on a worldwide scale each year [1], in China, the majority of TBI patients are men between 18 and 65 years old, mostly caused by traffic accidents, the mortality rate of TBI patients, especially those with life-threatening AIH, is amount to 5 % [2,3]. Therefore, the prompt and effective management to alleviate the symptom of AIH could, to a great extent, improve TBI patients' poor prognosis [4].

Decompression craniectomy (DC) with debridement has long been considered as a rapid and effective approach to reduce the level of intracranial pressure (ICP) in patients with severe traumatic brain injury (sTBI) combined with refractory AIH [5,6]. In a randomized controlled trial (RCT), Cooper DJ et al. reported that DC significantly reduced ICP and shortened the duration of admission in the ICU for patients suffering from sTBI and refractory AIH. However, the application of DC proved somewhat futile to improve the extended score of Glasgow Outcome Scale (GOSE) at 6 months post-injury [7]. Therefore, more effort should be made to investigate how to relieve AIH and how to improve patients' long-term prognosis. Recent clinical study indicated that the intraoperative application of controlled decompression (CDC) could alleviate acute brain swelling and reduce delayed hematoma by reducing ischemia-reperfusion injury (IRI), ultimately decreasing the all-cause mortality rate at 30 days and improving GOS-E scores of patients at 6 months [8,9]. A preclinical study using a rat epidural extreme intracranial hypertension (EEIH) model concluded that CDC exerts a cerebral protective effect by attenuating the disrupted function of blood-brain barrier, inhibiting inflammatory responses and ROS generation [10]. While the interaction between ROS and autophagy through a variety of pathways has been revealed [11,12], it remains obscure as to the possible association between CDC and autophagy.

Autophagy is a procedure of intracellular degradation in which components such as organelles and intracellular macromolecules are wrapped by autophagic vesicles for degradation under certain stressful physiological conditions to help with the recycling of materials and reuse of energy [13]. Autophagy rarely occurs in normal organismal metabolism and can only be abnormally activated by external factors, such as hypoxia, ischemia, nutritional deficiencies, excessive concentration of growth factors, or by intracellular stimuli, such as aging or organelle breakdown, protein misfolding induced by metabolic stress [14]. Autophagy is particularly closely related to neural apoptosis and is deeply implicated in the pathogenesis of brain injury after TBI [15,16]. The peaked expression of autophagy-related marker LC3 at 24 h after TBI has been reported [17], yet the specific role of autophagy in craniocerebral trauma remains controversial [18]. It has been suggested that thwarting of autophagy after severe TBI may lead to neuronal death [19]. Some other researchers implied that the suppression of autophagy could diminish brain edema, exert neuroprotective effects and improve cognitive function of TBI patients [20,21]. Therefore, the pathological process of autophagy after TBI warrants more insight into the mechanisms of neuronal death after TBI.

Phosphatidylinositol-3-kinase (PI3K) is a complex and large family of enzymes that become 3′phosphatidylinositol lipids upon activation by various cellular target proteins as second messengers [22], thereby stimulating the downstream protein kinase B (AKT) and other downstream effector pathway to regulate autophagy via inducing phosphorylation of mTOR in mammals [23]. Since PI3K/AKT signaling pathway is deeply involved in the regulation of a vast variety of cellular functions, such as proliferation, survival, growth, metabolism, transcription and protein synthesis, it has become an important hotspot in preclinical research [24]. PI3K/Akt signaling pathway also plays a vital role in mediating neuroprotection via boosting cellular proliferation, thwarting apoptosis and autophagy to sustain the survival of cells after TBI [25]. Hence, in the case of AIH induced by TBI, the plausible regulatory effect of CDC on neuronal autophagy to achieve neuroprotective effects should be investigated.

The present study aims to explore the correlation between CDC's potent neuroprotective effect and neuronal autophagy, and to explore the molecular mechanism that underlines the neuroprotective effect of CDC from the perspective of PI3K/Akt pathway regulation and autophagy inhibition in the rat model of TEIH.

2. Materials and methods

2.1. Animals

Male SD rats, weighted from 250 to 300 g, were purchased from Zhenlin Biology (Jiangsu province, China), and were housed in a controlled environment animal care facility throughout the experiment in a 12 h light-dark cycle at a constant temperature of 20 ± 2 °C and a humidity of 55 ± 5 %. All rats were granted free access to water and fodder for the experimental period. The animal experiment design of the study has been approved by the Ethics Committee of Wuxi Clinical College of Anhui Medical University (Wuxi; Jiangsu; China) and the experimental procedures was in strict accordance with the National Institutes of Health (NIH) guidance for the use of laboratory animals.

2.2. Establishment of TEIH rat model

The balloon system is consisted of an intervention catheter (MAGIC, Montmorency, France), fitted with the embolization balloon (Balt 2; France), and connected to a pressure pump (#IN4130, Merit Basix Touch Inflation Device, USA). The ICP monitor (Johnson & Johnson, #82–6635, USA) was zeroed in advance before the operation. SD rats were anesthetized using 5 % isoflurane along with N_2O/O_2 mixture (1:1). The anesthesia of rats was maintained by using mask anesthesia via 2.5 % isoflurane mixed with N_2O/O_2 (1:1). After reaching a depth of anesthesia that was non-responsive to stinging pain, the rats were placed on the prone position on a fixed frame. The fur in the surgical area of the head was removed with an electronic trimmer, followed by local disinfection with 75 %

alcohol. After that, 2 % lidocaine was subcutaneously injected along the midline between the ears and the eyes to prevent the possible skin incision that affects blood pressure. An incision of approximately 2–3 cm along the anesthesia area was made to separate the periosteum and the subcutaneous tissue, thereby exposing the posterior sagittal and anterior sagittal and sutures. Two holes sized of 3.5×3.5 mm were drilled by using the dental drills (JSDA, JD700, China) bilaterally at 0.6 cm behind the anterior-sagittal suture and a distance of 0.5 cm to the midline. The bone fragments were carefully removed to ensure that the dura is intact bilaterally under the bony foramen. The balloon was instantly inserted into the left side of bone foramen toward the frontotemporal side, so as to compress the dura without destroying its integrity and then connect the balloon catheter to the pressure pump. Subsequently, we inserted the ICP probe through the right bone foramen to a depth of approximately 4 mm into the brain, sealed the bilateral bone foramina with dental cement (Dentsply, Tulsa, O.K., USA). We then inflated the balloon using a pressure pump and recorded the initial ICP values. The rats were divided into 3 groups according to different intervention methods: Sham group, rapid decompression (RDC) group and controlled decompression (CDC) group. In the Sham group, the balloon was not inflated after the ICP and balloon were inserted and kept there for 30min. For RDC group and CDC group, the balloon was inflated rapidly until the ICP reached the ultimate compensatory value (40 mmHg) [10], then the pressurization was stopped and the status of TEIH was maintained for 30min. In RDC group, gas in the balloon was rapidly evacuated after 30min. In CDC group, we slowly extracted the gas to control the reduction rate of ICP at 2 mmHg/2 min until it dropped to the initial value. After surgery was completed, the balloon and the ICP probe were removed, the bilateral holes on the bone were resealed using dental cement and then the incisions were sutured. The rats were then raised back into the care facility in the same way as before. The rats were cared for 24 h before being behavioral scoring. Finally, pentobarbital sodium (100 mg/kg) was administered intraperitoneally for execution, collected the required data. Diligence has been taken throughout the experiments to minimize the suffering of the rats and to reduce their mortality.

2.3. *Intracerebroventricular administration of drug*

Intracerebroventricular (*i.c.v.*) administration of drug was performed by following the established protocol [26,27]. The rats were anesthetized using 2 % isoflurane and were placed on the stereotaxic frame. A 10-μL Hamilton syringe was used to pierce through the burr perforated hole into the right lateral ventricle on the skull. These coordinates of the bregma were followed: 1.0 mm lateral, 1.5 mm posterior, and 3.3 mm below the horizontal plane. We controlled the speed of administration at 1 μL/min. For different groups, drugs were administered in the following manner: In CDC + Vehicle (CDC + Veh) group, 5 μL of saline containing the same amount of co-solvent (DMSO) was injected into the right ventricle 40 min prior to TEIH; In CDC + rapamycin (CDC + Rap) group, rapamycin (5 μL, 1 mM, MCE) was injected into the right ventricle 30 min before TEIH [28]; In CDC + LY294002 group, LY294002 (a PI3K/Akt inhibitor, 5 μL, 50 mM, Sigma) was injected into the right ventricle 40 min prior to TEIH [29].

2.4. *Neurological deficits measurement*

mNSS (Modified Neurological Severity Score) was used to measure the neurological function of rats in each group at 24 h after induction of TEIH. mNSS is a multifunctional evaluation scale that includes rat motion, sensation, reflex and balance tests [30]. Neurological function of the rats was graded on a scale from 0 to 18 (normal score being 0, and maximal deficit score being 18). Specifically, a higher score indicates a severer neurological injury. All scoring procedures were performed by an operator who had no knowledge of the experimental design or the grouping of animals.

2.5. *Measurement of brain water content*

We sacrificed the rats 24 h after successful molding, then removed and divided the entire brain tissue into the left and right hemispheres in a step-wise manner. We used the wet weight and dry weight method to determine the brain water content. The left hemisphere compressed by the balloon was measured as wet weight; after being dried at 100 °C for 24 h, we obtained the value of dry weight. We used the formula to measure the water content of brain tissue: (wet weight - dry weight)/wet weight $\times$ 100 %.

2.6. *TUNEL assay*

The deeply anesthetized rats by using 2 % isoflurane were sacrificed at 24 h after the establishment of TEIH. The rats were perfused intracardially by using 50 ml cold PBS and 60 ml paraformaldehyde (10 %, pH = 7.4). We collected the whole brain to fix it in 10 % paraformaldehyde for 24 h, and then incubated it in 30 % sucrose solution for another 72 h. After the incubation, the brain was cut into coronal sections (thickness = 10 μm). The left temporal brain cortex with the pressured balloon was assessed. Three vision fields (40 $\times$) of the oppressed area in the selected section were chosen randomly to count the number of apoptotic indexes. 4 sections for each rat were collected for quantification to obtain the final average number of TUNEL-positive cells. ImageJ software was applied to measure the percentage of TUNEL-positive neurons. These procedures were performed by two investigators without knowledge of the grouping of animals.

2.7. *IF staining*

Under deep anesthesia, rats were perfused intracardially with 50 ml of cold PBS and 60 ml of paraformaldehyde (10 %, pH = 7.4), the brain tissues were removed and fixed with 10 % paraformaldehyde for 24 h and then paraffin-embedded. In each brain tissue of

each group, a coronal section (thickness = 4 μm) was made in the central region of the balloon compression. All brain sections were, at first, treated with 0.1 % Triton X-100, and were blocked by using 5 % BSA. We then incubated the samples with anti-NeuN primary antibody (1; 500, #ab104224, Abcam), anti-LC3 primary antibody (1; 100, #AF5402, Affinity) overnight at 4 °C. The slides were washed with PBST in triplicate, and were then incubated with fluorescence-conjugated secondary antibodies for 2 h at room temperature. Afterwards, the sections were rinsed with PBS and incubated with DAPI at room temperature for 15 min. Finally, the slides were sealed with mounting liquid containing the anti-fluorescence quencher. One field was selected randomly for analysis in the left cortex of each coronal section (n = 4). The region of interest (ROI) (320 × 250 μm^2 per field) is at approximately 200 μm adjacent to the boundary of the lesion in the brain lesions. The number of cells expressing LC3 protein, as well as their co-localization with NeuN staining, were calculated by using ImageJ 1.8.0 (National Institutes of Health, USA).

2.8. Western blot assay

Western blot assay was conducted following the standard protocol [31]. The brain samples of left cerebral cortical tissues were collected from TEIH rats, and were homogenized in RIPA and were then centrifuged at 10,000 r per min for 30 min. We mixed the supernatant with the loading buffer and then boiled it for 10 min before use. 50 μl protein was loaded into each lane of the SDS-PAGE gel for electrophoresis. The separated proteins were then transferred onto nitrocellulose membranes, which were blocked by using 5 % non-fat milk in TBST for 1 h at RT, and were then incubated overnight, separately with the following primary antibodies at 4 °C: LC3 (#AF5402, Affinity, USA), Beclin-1 (#AF5128, Affinity, USA), Akt (#AF6261, Affinity, USA), *p*-Akt (#AF0016, Affinity, USA) at a

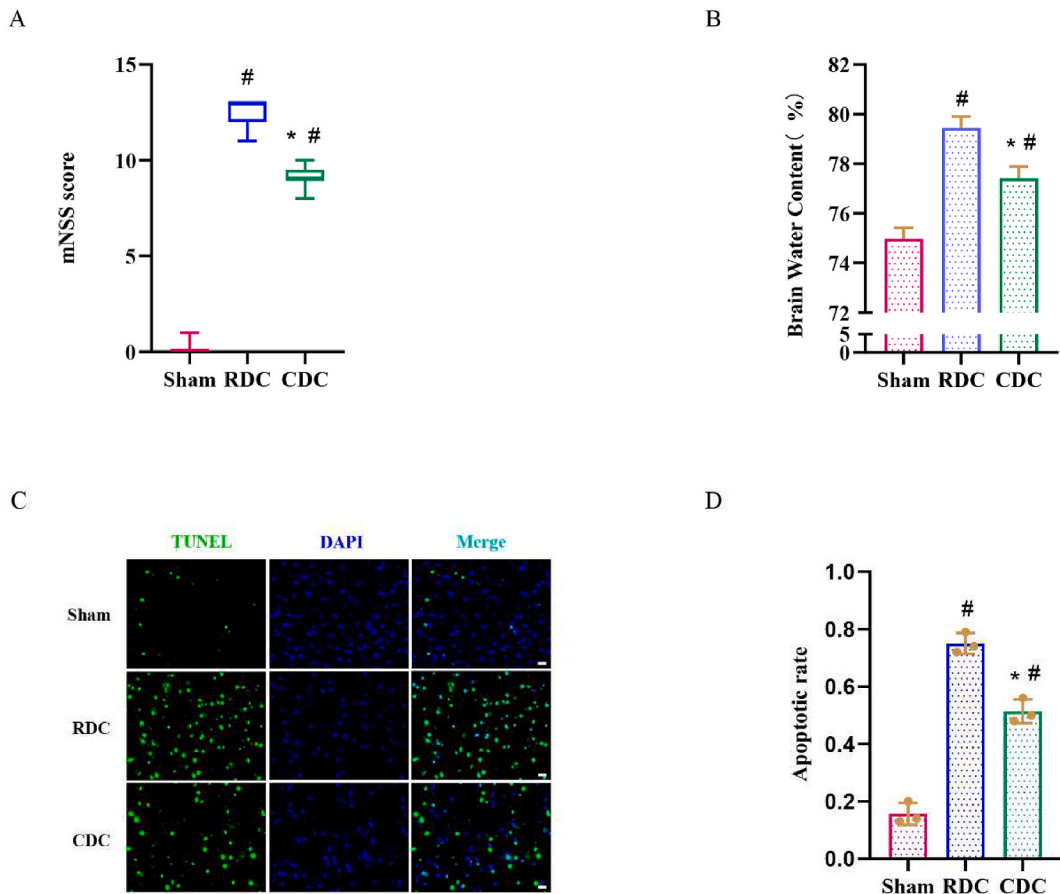

Fig. 1. CDC could reduce early brain damage after TEIH.
(A) The mNSS scores at 24 h postoperatively. mNSS scores were higher in the CDC and RDC groups than in the Sham group and lower in the CDC group than in the RDC group ($n = 9$ for each group).
(B) The brain water content at 24 h postoperatively. Brain water content was higher in the CDC and RDC groups than in the Sham group and lower in the CDC group than in the RDC group ($n = 6$ for each group).
(C) (D) TUNEL staining of the cerebral cortex (C) and quantitative analysis (D) showed that the apoptotic index was higher in the CDC and RDC groups than in the Sham group and lower in the CDC group than in the RDC group ($n = 3$ for each group).
Scale bar represented 20 μm. Data are represented as mean ± SEM. #$p < 0.05$ vs Sham group; *$p < 0.05$ vs RDC group; ns, not statistically significant.

1:10000 dilution ratio and β-actin (#AF7018, Affinity, USA) at a dilution ratio of 1:5000. After that, the PVDF membrane was washed using TBST for 15 min in triplicate for the incubation with HRP-conjugated secondary antibody (1:10000, CST, USA). Finally, the membranes were washed 20 min with TBST. The bands of interested protein were visualized using ECL (Merck Millipore, USA) and were exposed on the X-ray film. The intensity of the signals was quantified by using Image J software.

2.9. Statistical analysis

All data were displayed in the form of mean ± SEM. For inter-group comparison, One-way analysis of variance (ANOVA) was used, followed by Bonferroni's multiple comparison as post hoc test. A P value of no larger than 0.05 indicates statistical significance. SPSS (21.0, IBM, Chicago, USA) was used for statistical analysis.

3. Result

3.1. CDC diminished the extent of brain damage after TEIH

To confirm the neuroprotective effect of CDC in TEIH, we measured brain water content, neurological function score, and cortical apoptosis level in rats. The results showed that the neurological function score at 24 h postoperatively was significantly higher in TEIH

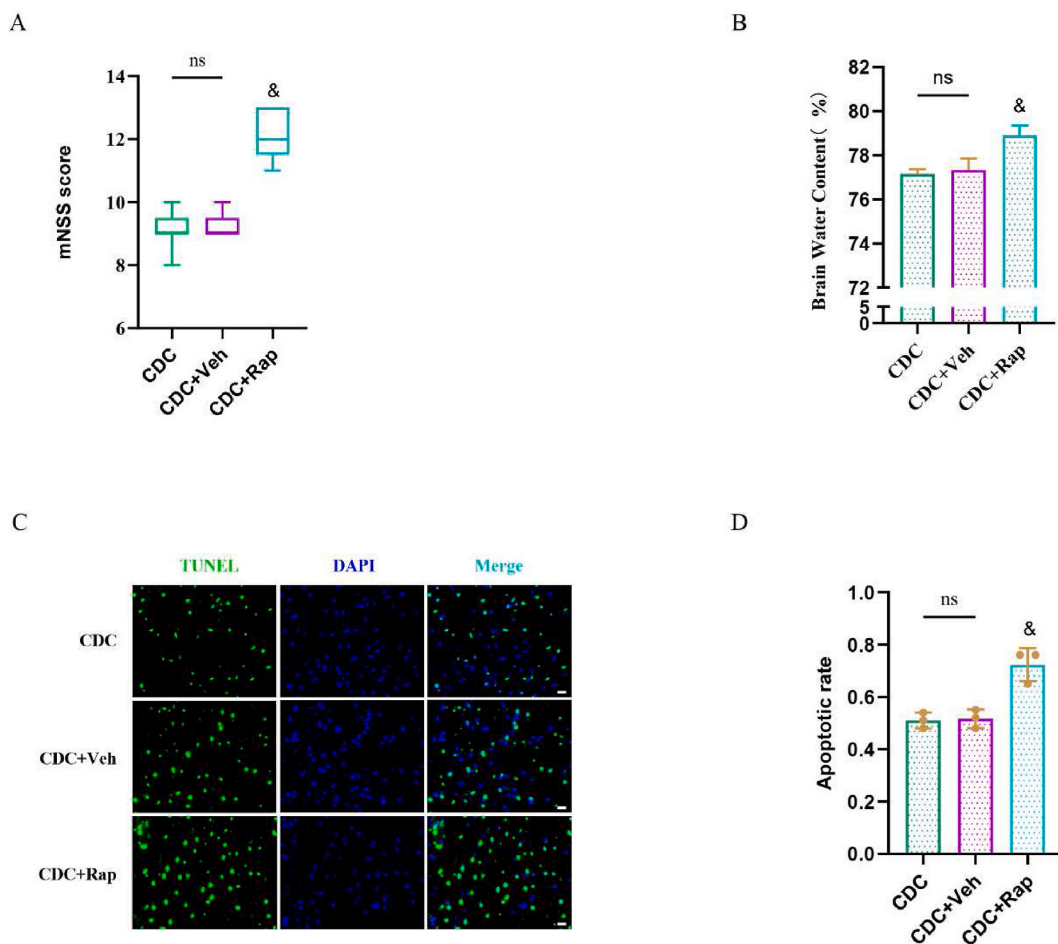

Fig. 2. The neuroprotective effect of CDC could be reversed by rapamycin.
(A) The postoperative mNSS scores at 24 h. The mNSS score increased in CDC + Rap group compared to CDC + Veh group, with no significant difference between the CDC and CDC + Veh groups (*n* = 9 for each group).
(B) The brain water content at 24 h postoperatively increased in CDC + Rap group compared to CDC + Veh group, with no significant difference between the CDC and CDC + Veh groups (*n* = 6 for each group).
(C) (D) TUNEL staining of the cerebral cortex (C) with quantitative analysis (D) showed that CDC + Rap reduced neuronal cell loss at 24 h compared to CDC + Veh group, with no significant difference between the CDC and CDC + Veh groups (*n* = 3 for each group).
Scale bar represented 20 μm. Data are represented as mean ± SEM. &$p < 0.05$ vs CDC + Veh group; ns, not statistically significant.

rats, compared with that of the Sham group. Moreover, the neurological function of CDC group was significantly lower than that of RDC group (Fig. 1A). Brain water content at 24 h postoperatively was significantly lower in Sham group than that of CDC group and RDC group. Consistently, brain water content of CDC group was significantly different from that of RDC group (Fig. 1B). TUNEL staining found that the level of apoptosis of CDC group and RDC group was significantly increased than that of Sham group. Meanwhile, the level of apoptosis of CDC group was significantly lower than that of RDC group (Fig. 1C and D). These results suggest that CDC reversed cellular apoptosis, improved neurological function and alleviated the damage of brain edema in TEIH rats.

3.2. *Rapamycin reverses the neuroprotective effects of CDC*

Rapamycin was added as an intervention variable to further explore the mechanism that underlies the neuroprotective effect of CDC. As shown in Fig. 2A and B, we observed the neurological function scores of rats was aggravated and the brain water content was increased in CDC + Rap group compared to that of CDC + Veh group. Nevertheless, the difference between CDC group and CDC + Veh groups could not reach the level of significance. TUNEL staining revealed that the apoptotic index of CDC + Rap group was

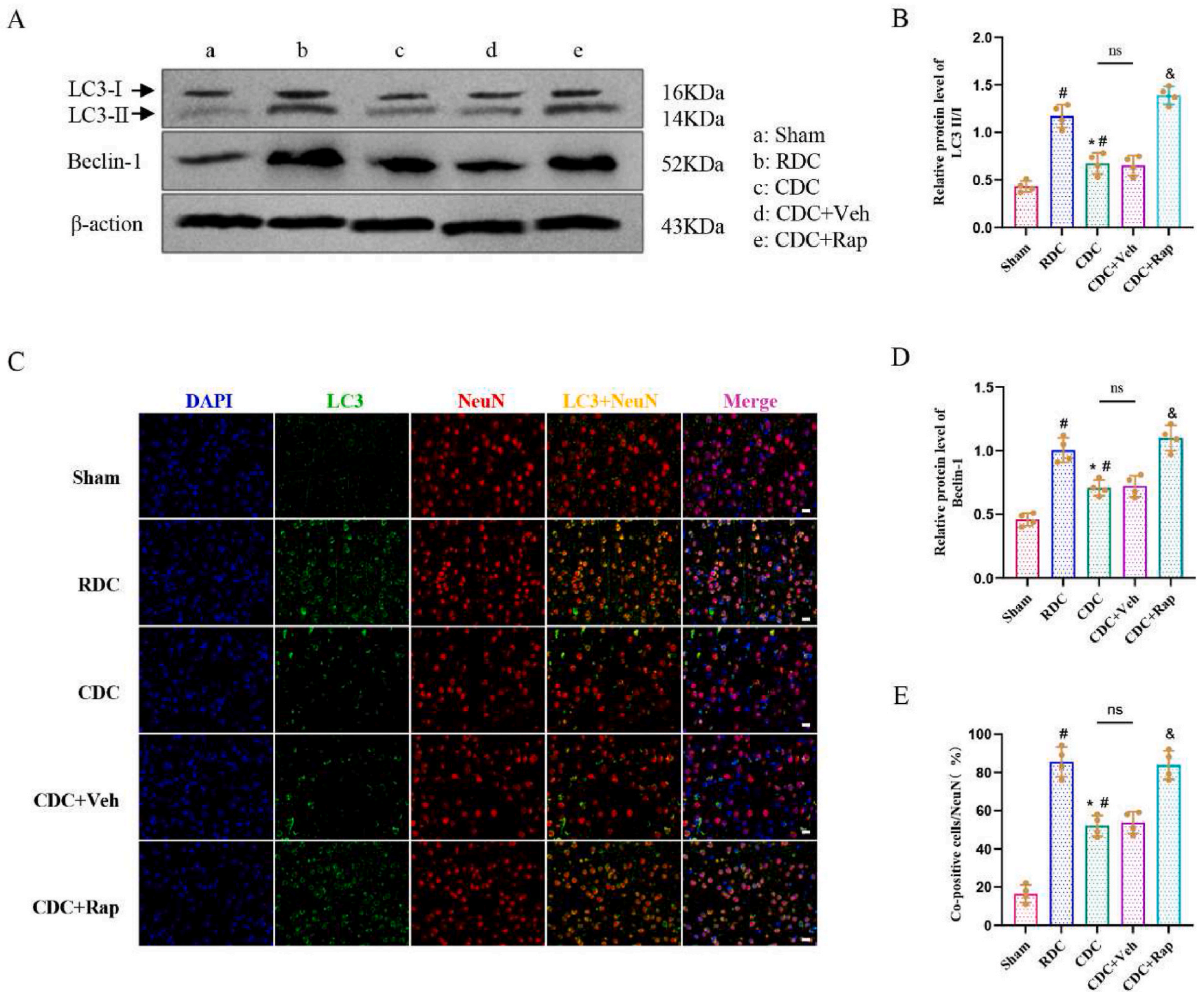

Fig. 3. CDC inhibits the expression level of neuronal autophagy.
(A) Representative Western blot showing LC3-I, LC3-II and Beclin-1 expression levels, where LC3-II/I and Beclin-1 levels were reduced after TEIH in CDC group and this effect could be inhibited by rapamycin.
(B) Relative expression levels of autophagy-related protein LC3-II/I (n = 4 for each group).
(C) Representative images of LC3 and NeuN double staining in brain cortex after TEIH.
(D) Relative expression levels of autophagy-related protein Beclin-1 (n = 4 for each group).
(E) Quantitative analysis of co-positive cells by NeuN-positive cells (n = 4 for each group).
Scale bar represented 20 μm. Data are represented as mean ± SEM. #$p < 0.05$ vs Sham group; *$p < 0.05$ vs RDC group; &$p < 0.05$ vs CDC + Veh group; ns, not statistically significant.

significantly enhanced than that of CDC + Veh group (Fig. 2C and D), whereas no significant difference in apoptotic index between the CDC and CDC + Veh groups could be observed. These results consistently showed that autophagy activator rapamycin reversed the neuroprotective effect of CDC in TEIH rats.

3.3. CDC reduced cortical neuronal autophagy, rapamycin inhibited this function

To explore the level of autophagy, we examined the level of autophagy-related proteins, including LC3 and Beclin-1, in the damaged cortex at 24 h postoperatively. Notably, the expression of LC3II/I and Beclin-1 in Sham group were significantly lower than those in RDC group and CDC group. The expression of Beclin-1 and LC3II/I in CDC group was lower than RDC group. However, the differences in the expression level were not significantly different between CDC group and CDC + Veh group. The intervention of rapamycin led to significantly higher expression of Beclin-1 and LC3 in CDC + Rap, as compared with CDC + Veh group (Fig. 3A, B, D). The results of IF assay showed that neuronal expression of LC3 in the cerebral cortex after being compressed by the balloon was upregulated compared to the Sham group. LC3 expression was notably lower in the damaged cortex of CDC group than RDC group, however, the lower expression of neuronal autophagy in CDC + Rap group was elevated gain compared with that of CDC + Veh group (Fig. 3C, E).

3.4. LY294002 reverses CDC's neuroprotective effects

To analyze the mechanism of CDC's inhibitory effect on autophagy, LY294002 was used to inhibit PI3K/Akt signaling pathway. It

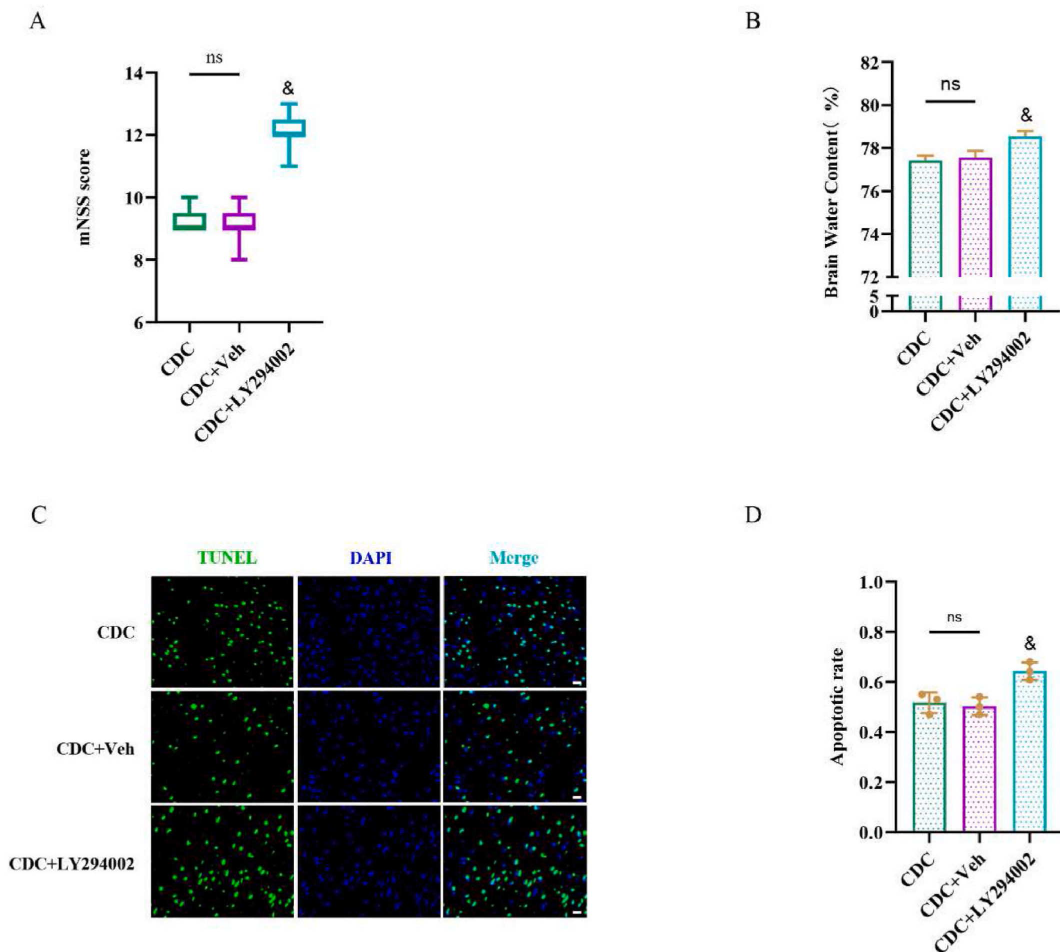

Fig. 4. The influence of LY294002 on the neuroprotective effect of CDC.
(A) The mNSS score was increased at 24 h after LY294002 intervention ($n = 9$ for each group).
(B) The brain water content increased after LY294002 intervention compared to CDC + Veh group (n = 6 for each group).
(C) (D) Cortical TUNEL staining (C) with quantitative analysis (D) showed that compared to CDC + Veh group, neuronal cell loss was increased in CDC + LY294002 group at 24 h (n = 3 for each group).
Scale bar represented 20 μm. Data are represented as mean ± SEM. &$p < 0.05$ vs CDC + Veh group; ns, not statistically significant.

was observed that CDC + LY294002 group had aggravated neurological function scores (Fig. 4A) and more pronounced cerebral edema (Fig. 4B) than CDC + Veh group. The results of TUNEL staining showed increased apoptosis level in CDC + LY294002 group than that of CDC + Veh group (Fig. 4C and D). As shown in Fig. 5A, the levels of autophagy-related proteins, including Akt, *p*-Akt, LC3-I, LC3-II and Beclin-1, were detected in western blotting assay. The results showed that the expression of Akt was not significantly different among these groups (Fig. 5B). *p*-Akt expression was markedly lower in CDC group and RDC group, compared to Sham group. *p*-Akt level in CDC group was considerably higher compared with RDC group. The difference in the expression level of *p*-Akt between CDC group and CDC + Veh group was not significant (Fig. 5C). Compared with CDC + Veh group, CDC + LY294002 group had lower *p*-

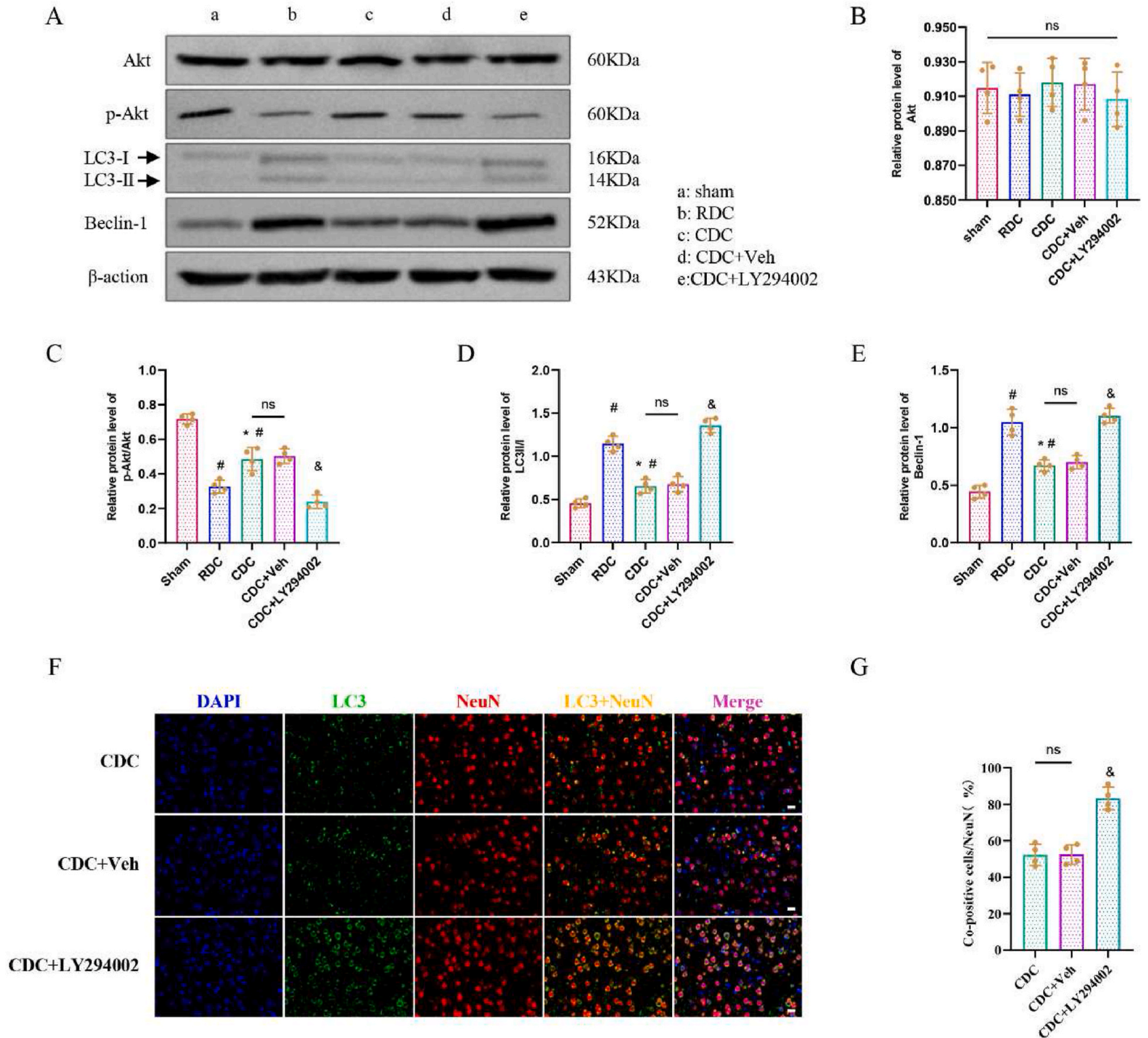

Fig. 5. Effect of PI3K/Akt inhibitor LY294002 on protein expression of autophagy-related signaling pathways.

(A) Representative Western blot showed no significant differences in Akt expression among each group, compared to RDC group, CDC group enhanced the expression level of *p*-Akt and this effect was inhibited by LY294002, and the inhibitory effect of CDC group on LC3-II/I and Beclin-1 expression was also reversed by LY294002.

(B) Expression levels of PI3K/Akt signaling pathway protein Akt ($n = 4$ for each group).

(C) Expression levels of PI3K/Akt signaling pathway protein *p*-Akt ($n = 4$ for each group).

(D) Expression levels of autophagy-related protein LC3-II/I ($n = 4$ for each group).

(E) Expression levels of autophagy-related protein Beclin-1 ($n = 4$ for each group).

(F) Representative images of LC3 and NeuN double staining in each group of brain cortex after LY294002 intervention.

(G) Quantitative analysis of co-positive cells with NeuN-positive cells ($n = 4$ for each group).

Scale bar represented 20 μm. Data are represented as mean ± SEM. #$p < 0.05$ vs Sham group; *$p < 0.05$ vs RDC group; &$p < 0.05$ vs CDC + Veh group; ns, not statistically significant.

Akt/Akt expression (Fig. 5C) and higher expression of LC3II/I and Beclin-1, as shown in Fig. 5D and E. IF results revealed that LC3 expression was significantly higher in damaged cortical neurons of CDC + LY294002 group than CDC + Veh group (Fig. 5F and G).

4. Discussion

In the cases of intracranial hypertension after TBI, DC surgery has long been the fastest and efficient way for neurosurgeons to reduce intracranial pressure and to save patients who are suffering from acute, life-threatening IH that can develop into brain herniation at any time [32]. The trials of Decompressive Craniectomy (DECRA) and Randomized Evaluation of Surgery with Craniectomy for Uncontrollable Elevation of Intracranial Pressure (RESCUEICP) found that the application of DC can speed up the recovery of patient in the ICU, reducing mortality and improving their short-term prognosis [7]. However, it was also found that DC increased the vegetative outcome of survivors over a 12-month period [33,34]. Hence, rapid intracranial pressure reduction and restoration of cerebral perfusion induced in DC surgery may not be the most beneficial approach for patients with IH, and the complications that worsen the long-term prognosis of patients are also of grave concern in clinical practice.

Recently, a series of RCT studies revealed that CDC could delay the formation of hematoma in patients with sTBI, alleviate acute intraoperative brain swelling, reduce the incidence of post-traumatic cerebral infarction and delayed hematoma, and improve patients' long-term prognosis [8,9]. Nevertheless, the underlying therapeutic mechanisms of CDC as a novel and promising surgical protocol are yet to be clarified. Some exploration based on animal models revealed the advantages of this surgical approach. Zhang et al. used New Zealand rabbits to construct an AIH model and found that CDC could effectively attenuate oxidative damage and diminish inflammatory responses, thereby exerting as a neuroprotective effect. Specifically, the expression levels of oxidative damage markers, antioxidant enzymes, inflammatory factors, GFAP and UCH-L1 in RDC group were aberrantly increased than those of CDC group. CDC intervention diminished neuronal damage and improved neurological function scores of the animals [35]. Consistently, Qian et al. constructed an EEIH model of rats and reported that the mortality rate was significantly increased when intracranial pressure exceeded 40 mmHg. Moreover, CDC group revealed a more intact structure of BBB, potently alleviated ischemia-reperfusion injury to the brain tissue, significantly lower expression of inflammatory cytokines (TNF-α and IL-1β) in damaged cortex and significantly lower expression levels of proteins associated with programmed necrosis. Consistently, CDC facilitated the recovery of neurological function in rats with EEIH. These findings jointly demonstrate that CDC protects neurological function in rats with EEIH via suppressing inflammatory response and alleviating programmed necrosis [36]. In light of the series of clinical data, we explored the molecular mechanism that explains the neuroprotective effects of CDC on TEIH rats. We confirmed that CDC reduced brain edema and improved neurological functions with regard to the abilities of motor, sensory, reflex and balance of TEIH rats, as compared to RDC. These results concurred with the findings of previous studies which indicated that CDC improves early neurological function in rats [37].

In the current project, we discovered that CDC inhibits neuronal autophagy via reducing the protein expression of LC3 and Beclin-1 in the cerebral cortex of rats at 24 h after TEIH. Autophagy is known as a highly conserved catabolic process in eukaryotic cells that sends intracellular macromolecules and organelles to lysosomes to be degraded under certain specific physiological conditions for the purpose of recycling materials and reusing energy [38]. In the event of triggered autophagy, the non-specific capture of the cytoplasm will lead to irreversible cellular damage. Evidence suggested that autophagy is closely associated with the pathogenesis of brain injury after TBI [39]. Gao et al. found that the intraperitoneal injection of tetrahydrocurcumin in TBI model of rats resulted in elevated expression levels of Beclin-1 and LC3-II and suppressed the cellular apoptosis induced by TBI, thereby presenting the neuroprotective effect [40]. Meanwhile, the expression of Beclin-1 and LC3 was increased in the early cortex of damaged rats in the TBI model, while intraoperative sevoflurane intervention facilitated autophagy and suppressed the excessive apoptosis of neurons to protect the neurological function of rats [41]. However, some studies reported the plausible negative effect of autophagy after the induction of TBI. Sun et al. indicated that miR-27a overexpression could ameliorate brain injury by thwarting FoxO3a-mediated neuronal autophagy after the induction of TBI [42], and that the knockdown of FoxO3a diminished neurological dysfunction through downregulating autophagic flux [43]. Gao et al. constructed the rat model of TBI, and found that activation of IL-33/ST2 axis inhibited autophagy expression, reduced brain edema and improved motor function, memory deficits and spatial learning of rats [44]. These studies featured the duality and complexity of the role of autophagy in the onset and progression of TBI. Our data showed that CDC exerted a neuroprotective effect via suppressing autophagy, as reflected by the down-regulation of LC3 and Beclin-1. Analogously, TUNEL staining found that the apoptosis in the rat cortical brain tissue was diminished upon the intervention of CDC.

While the mechanism that clarifies the role of neuronal autophagy in TEIH remains inconclusive, PI3K/Akt/mTOR pathway, as an important regulator of neuronal cell proliferation, may be regarded as a key factor and thus warrants exceptional exploration [45]. Intraperitoneal injection of NaHS could alleviate the damage of BBB of mice and thwart autophagy via triggering PI3K/Akt/mTOR pathway to promote neurological recovery after the induction of TBI [20]. Gong et al. reported that the intraperitoneal administration of urolithin A, a compound commonly found in fruits and nuts, could improve brain edema after TBI in mice, safeguard the structural integrity of BBB to a certain extent, reduced the rate of neuronal cell death at the injured cortex, and inhibited excessive activation of autophagy, which was closely associated with the inhibition of Akt/IKK/NFκB and PI3K/Akt/mTOR signaling pathways [46]. A line of evidence found that exogenous CGRP can attenuate neuronal apoptosis and autophagy in TBI mice by activating PI3K/AKT/mTOR signaling pathway, thereby protecting brain tissue integrity and blood-brain barrier (BBB), and alleviating the severity of brain edema to exert neuroprotective effects [47]. In addition, the clinical efficiency of some drugs has also been proven in the management of TBI by mediating PI3K/Akt/mTOR signaling pathway. Neuronal autophagy was activated in brain tissue of TBI rats, and that dexmedetomidine promoted the protein expression of Beclin-1 and LC3, reduced brain water content and improved neurological function via activating PI3K/Akt/mTOR pathway. Intriguingly, the use of LY94002 could reverse these therapeutic effect [48]. Since mTOR is

involved in the regulation of cell growth, autophagy and apoptosis after being triggered by upstream signals, we hypothesized that the therapeutic effect of CDC in regulating autophagy is achieved through activating PI3K/Akt pathway. Given that CDC intervention led to enhanced phosphorylation level of Akt at the damaged cortex of TEIH rats, significantly reduced expression of Beclin-1 and lowered LC3I/LC3II ratio, and that these effects could be reversed by LY294002, we conclude that PI3K/Akt signaling pathway plays a key role in mediating CDC's neuroprotective effects.

5. Conclusions

In summary, CDC intervention could alleviate early brain edema, improve neurological status, reduce the level of neuro-apoptosis and inhibit autophagy in the rat TEIH model. The neuroprotective effect of CDC could be attributable to the activation of PI3K/Akt signaling pathway (Fig. 6). These findings reveal the potential mechanism of neuroprotective effect of controlled decompression in traumatic epidural intracranial hypertension, which could provide new potential basis and therapeutic ideas for the treatment of traumatic epidural intracranial hypertension.

Data availability statements

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics statement

This study was reviewed and approved by the Ethics Committee of Wuxi Clinical College of Anhui Medical University (Wuxi; Jiangsu; China), with the approval number: 20220211.

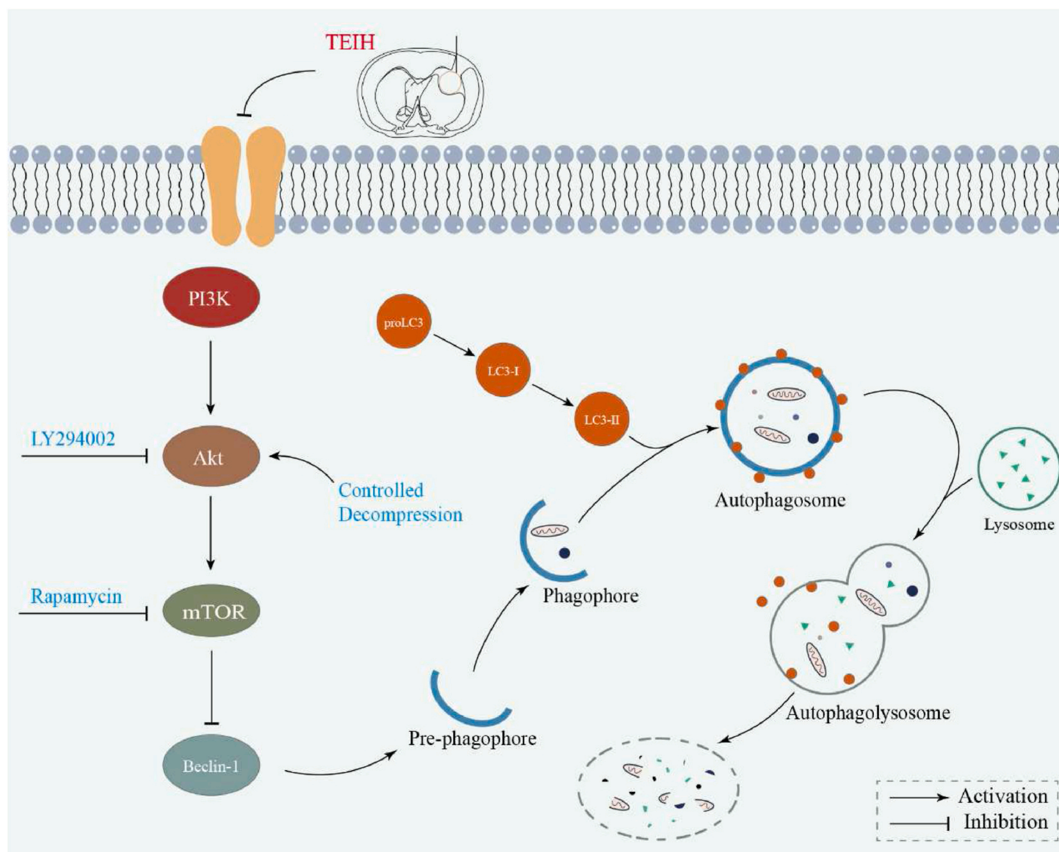

Fig. 6. Molecular mechanism of neuroprotective effect of CDC on traumatic brain injury. CDC regulates the expression of autophagy and reduces nerve cell death by inhibiting PI3K/Akt signaling pathway.

CRediT authorship contribution statement

Yuanyuan Che: Conceptualization, Visualization, Writing – original draft. **Wei Wu:** Formal analysis. **Xiao Qian:** Investigation, Visualization. **Zhengwei Sheng:** Investigation, Resources. **Wang Zhang:** Data curation. **Jie Zheng:** Data curation. **Junhui Chen:** Methodology, Validation, Software, Supervision. **Yuhai Wang:** Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23753.

References

[1] A.I.R. Maas, D.K. Menon, P.D. Adelson, et al., Traumatic brain injury: integrated approaches to improve prevention, clinical care, and research, Lancet Neurol. 16 (12) (2017) 987–1048.
[2] G. Gao, X. Wu, J. Feng, et al., Clinical characteristics and outcomes in patients with traumatic brain injury in China: a prospective, multicentre, longitudinal, observational study, Lancet Neurol. 19 (8) (2020) 670–677.
[3] Y.D. Zhao, W. Wang, Neurosurgical trauma in People's Republic of China, World J. Surg. 25 (9) (2001) 1202–1204.
[4] N. Carney, A.M. Totten, C. O'reilly, et al., Guidelines for the management of severe traumatic brain injury, Neurosurgery 80 (1) (2017) 6–15. Fourth Edition.
[5] H.I. Cushing, Subtemporal decompressive operations for the intracranial complications associated with bursting fractures of the skull, Ann. Surg. 47 (5) (1908) 641–644.1.
[6] J.Y. Jiang, W. Xu, W.P. Li, et al., Efficacy of standard trauma craniectomy for refractory intracranial hypertension with severe traumatic brain injury: a multicenter, prospective, randomized controlled study, J. Neurotrauma 22 (6) (2005) 623–628.
[7] D.J. Cooper, J.V. Rosenfeld, L. Murray, et al., Decompressive craniectomy in diffuse traumatic brain injury, N. Engl. J. Med. 364 (16) (2011) 1493–1502.
[8] J. Chen, M. Li, L. Chen, et al., The effect of controlled decompression for severe traumatic brain injury: a randomized, controlled trial, Front. Neurol. 11 (2020) 107.
[9] Y. Wang, C. Wang, L. Yang, et al., Controlled decompression for the treatment of severe head injury: a preliminary study, Turk Neurosurg 24 (2) (2014) 214–220.
[10] X. Qian, C. Zhang, Z. Zhou, et al., Controlled decompression attenuates brain damage in a rat model of epidural extreme intracranial hypertension: partially via inhibiting necroptosis and inflammatory response, Neurochem. Int. 153 (2022), 105257.
[11] L. Li, J. Tan, Y. Miao, et al., ROS and autophagy: interactions and molecular regulatory mechanisms, Cell. Mol. Neurobiol. 35 (5) (2015) 615–621.
[12] R. Scherz-Shouval, Z. Elazar, Regulation of autophagy by ROS: physiology and pathology, Trends Biochem. Sci. 36 (1) (2011) 30–38.
[13] J. Kim, D.J. Klionsky, Autophagy, cytoplasm-to-vacuole targeting pathway, and pexophagy in yeast and mammalian cells, Annu. Rev. Biochem. 69 (2000) 303–342.
[14] D.J. Klionsky, S.D. Emr, Autophagy as a regulated pathway of cellular degradation, Science 290 (5497) (2000) 1717–1721.
[15] L.K. Feldmann, F. Le Prieult, V. Felzen, et al., Proteasome and autophagy-mediated impairment of late long-term potentiation (l-LTP) after traumatic brain injury in the somatosensory cortex of mice, Int. J. Mol. Sci. 20 (12) (2019).
[16] F. Zhang, H. Dong, T. Lv, et al., Moderate hypothermia inhibits microglial activation after traumatic brain injury by modulating autophagy/apoptosis and the MyD88-dependent TLR4 signaling pathway, J. Neuroinflammation 15 (1) (2018) 273.
[17] C.L. Liu, S. Chen, D. Dietrich, et al., Changes in autophagy after traumatic brain injury, J. Cerebr. Blood Flow Metabol. 28 (4) (2008) 674–683.
[18] J. Wu, M.M. Lipinski, Autophagy in neurotrauma: good, bad, or dysregulated, Cells 8 (7) (2019).
[19] M.M. Lipinski, J. Wu, A.I. Faden, et al., Function and mechanisms of autophagy in brain and spinal cord trauma, Antioxidants Redox Signal. 23 (6) (2015) 565–577.
[20] K. Xu, F. Wu, K. Xu, et al., NaHS restores mitochondrial function and inhibits autophagy by activating the PI3K/Akt/mTOR signalling pathway to improve functional recovery after traumatic brain injury, Chem. Biol. Interact. 286 (2018) 96–105.
[21] L. Zhao, Q. Liu, S. Ma, et al., TPEN attenuates neural autophagy induced by synaptically-released zinc translocation and improves histological outcomes after traumatic brain injury in rats, Ann. Clin. Lab. Sci. 48 (4) (2018) 446–452.
[22] R. Katso, K. Okkenhaug, K. Ahmadi, et al., Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer, Annu. Rev. Cell Dev. Biol. 17 (2001) 615–675.
[23] J. Karar, A. Maity, PI3K/AKT/mTOR pathway in angiogenesis, Front. Mol. Neurosci. 4 (2011) 51.
[24] C.W. Lindsley, The Akt/PKB family of protein kinases: a review of small molecule inhibitors and progress towards target validation: a 2009 update, Curr. Top. Med. Chem. 10 (4) (2010) 458–477.
[25] M. Nakamura, R. Raghupathi, D.E. Merry, et al., Overexpression of Bcl-2 is neuroprotective after experimental brain injury in transgenic mice, J. Comp. Neurol. 412 (4) (1999) 681–692.
[26] C. Tang, Y. Shan, Y. Hu, et al., FGF2 attenuates neural cell death via suppressing autophagy after rat mild traumatic brain injury, Stem Cell. Int. 2017 (2017), 2923182.

[27] J. Wang, X. Fu, D. Zhang, et al., Effects of crenolanib, a nonselective inhibitor of PDGFR, in a mouse model of transient middle cerebral artery occlusion, Neuroscience 364 (2017) 202–211.
[28] H. Xue, Y.H. Zhang, Q.S. Gao, et al., Sevoflurane post-conditioning ameliorates neuronal deficits and axon demyelination after neonatal hypoxic ischemic brain injury: role of microglia/macrophage, Cell. Mol. Neurobiol. 41 (8) (2021) 1801–1816.
[29] P. Li, G. Zhao, F. Chen, et al., Rh-relaxin-2 attenuates degranulation of mast cells by inhibiting NF-κB through PI3K-AKT/TNFAIP3 pathway in an experimental germinal matrix hemorrhage rat model, J. Neuroinflammation 17 (1) (2020) 250.
[30] S.F. Chen, C.W. Hsu, W.H. Huang, et al., Post-injury baicalein improves histological and functional outcomes and reduces inflammatory cytokines after experimental traumatic brain injury, Br. J. Pharmacol. 155 (8) (2008) 1279–1296.
[31] Z. Wang, J. Li, A. Wang, et al., Sevoflurane inhibits traumatic brain injury-induced neuron apoptosis via EZH2-downregulated KLF4/p38 Axis, Front. Cell Dev. Biol. 9 (2021), 658720.
[32] A.G. Kolias, P.J. Kirkpatrick, P.J. Hutchinson, Decompressive craniectomy: past, present and future, Nat. Rev. Neurol. 9 (7) (2013) 405–415.
[33] D.J. Cooper, J.V. Rosenfeld, L. Murray, et al., Patient outcomes at twelve months after early decompressive craniectomy for diffuse traumatic brain injury in the randomized DECRA clinical trial, J. Neurotrauma 37 (5) (2020) 810–816.
[34] P.J. Hutchinson, A.G. Kolias, I.S. Timofeev, et al., Trial of decompressive craniectomy for traumatic intracranial hypertension, N. Engl. J. Med. 375 (12) (2016) 1119–1130.
[35] C. Zhang, X. Qian, J. Zheng, et al., Controlled decompression alleviates brain injury via attenuating oxidative damage and neuroinflammation in acute intracranial hypertension, BioMed Res. Int. 2022 (2022), 1936691.
[36] X. Qian, C. Zhang, Z. Zhou, et al., Controlled decompression attenuates brain damage in a rat model of epidural extreme intracranial hypertension: partially via inhibiting necroptosis and inflammatory response, Neurochem. Int. 153 (2022), 105257.
[37] T. Chen, X. Qian, J. Zhu, et al., Controlled decompression attenuates compressive injury following traumatic brain injury via TREK-1-mediated inhibition of necroptosis and neuroinflammation, Oxid. Med. Cell. Longev. 2021 (2021), 4280951.
[38] N. Mizushima, B. Levine, A.M. Cuervo, et al., Autophagy fights disease through cellular self-digestion, Nature 451 (7182) (2008) 1069–1075.
[39] L. Zhang, H. Wang, Autophagy in traumatic brain injury: a new target for therapeutic intervention, Front. Mol. Neurosci. 11 (2018) 190.
[40] Y. Gao, J. Li, L. Wu, et al., Tetrahydrocurcumin provides neuroprotection in rats after traumatic brain injury: autophagy and the PI3K/AKT pathways as a potential mechanism, J. Surg. Res. 206 (1) (2016) 67–76.
[41] H. He, W. Liu, Y. Zhou, et al., Sevoflurane post-conditioning attenuates traumatic brain injury-induced neuronal apoptosis by promoting autophagy via the PI3K/AKT signaling pathway, Drug Des. Dev. Ther. 12 (2018) 629–638.
[42] L. Sun, M. Zhao, Y. Wang, et al., Neuroprotective effects of miR-27a against traumatic brain injury via suppressing FoxO3a-mediated neuronal autophagy, Biochem. Biophys. Res. Commun. 482 (4) (2017) 1141–1147.
[43] L. Sun, M. Zhao, M. Liu, et al., Suppression of FoxO3a attenuates neurobehavioral deficits after traumatic brain injury through inhibiting neuronal autophagy, Behav. Brain Res. 337 (2018) 271–279.
[44] Y. Gao, M.Y. Zhang, T. Wang, et al., IL-33/ST2L signaling provides neuroprotection through inhibiting autophagy, endoplasmic reticulum stress, and apoptosis in a mouse model of traumatic brain injury, Front. Cell. Neurosci. 12 (2018) 95.
[45] V. Kumar, M.X. Zhang, M.W. Swank, et al., Regulation of dendritic morphogenesis by Ras-PI3K-Akt-mTOR and Ras-MAPK signaling pathways, J. Neurosci. 25 (49) (2005) 11288–11299.
[46] Q.Y. Gong, L. Cai, Y. Jing, et al., Urolithin A alleviates blood-brain barrier disruption and attenuates neuronal apoptosis following traumatic brain injury in mice, Neural Regen Res 17 (9) (2022) 2007–2013.
[47] J. Tian, L. Yang, P. Wang, et al., Exogenous CGRP regulates apoptosis and autophagy to alleviate traumatic brain injury through akt/mTOR signalling pathway, Neurochem. Res. 45 (12) (2020) 2926–2938.
[48] M. Shen, S. Wang, X. Wen, et al., Dexmedetomidine exerts neuroprotective effect via the activation of the PI3K/Akt/mTOR signaling pathway in rats with traumatic brain injury, Biomed. Pharmacother. 95 (2017) 885–893.