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Research article

Artesunate improves learning and memory impairment in rats with vascular cognitive impairment by down-regulating the level of autophagy in cerebral cortex neurons

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ABSTRACT

Background: Vascular cognitive impairment (VCI) is the second leading cause of dementia. Cognitive impairment is a common consequence of VCI. However, there is no effective treatment for VCI and the underlying mechanism of its pathogenesis remains unclear. This study to investigate whether artesunate (ART) can improve the learning and memory function in rats with VCI by down-regulating he level of autophagy in cerebral cortex neurons.

Methods: The models for VCI were the rat bilateral common carotid artery occlusion (BACCO), which were randomized into three groups including the sham operation group (Sham), model + vehicle group (Model) and model + ART group (ART). Then the animal behaviors were recorded, as well as staining the results of cortical neurons. Western blot was performed to determine the protein expressions of LC3BII/I, p-AMPK, p-mTOR, and Beclin-1.

Results: Behavioral outcomes and the protein expressions in Model group were supposedly affected by the induction of autophagy in cerebral cortex neurons. Compared to the Model group, ART improved memory impairment in VCI rats. And the expression of LC3BII/I, p-AMPK/AMPK, Beclin-1 is significant decreased in the ART group, while significant increases of p-mTOR/mTOR were showed. These results suggest that ART improved learning and memory impairment in VCI rats by down-regulating the level of autophagy in cerebral cortex neurons.

Conclusion: The results suggest that autophagy occurs in cerebral cortex neurons in rats with VCI. It is speculated that ART can improve learning and memory impairment in VCI rats by down-regulating the level of autophagy in cerebral cortex neurons.

1. Introduction

Vascular cognitive impairment (VCI), the second most prevalent cause of dementia after Alzheimer's disease (AD), encompasses a

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Abbreviations

WM	White matter
CCH	chronic cerebral hypoperfusion
AMPK	AMP-activated protein kinase
mTOR	Mammalian target of rapamycin
ART	Artesunate
3-MA	3-Methyladenine
Rapa	Rapamycin
BACCO	Bilateral Common Carotid Arterial occlusion
NOR	novel object recognition
MWM	Morris water maze
H&E	hematoxylin & eosin
CV	Cresyl violet

range of conditions in which vascular factors either induce or contribute to severe neuronal damage and cognitive decline [1]. VCI is characterized by cerebral infarction, white matter (WM) lesions, myelin loss, and amyloid angiopathy [2]. Similar to other cognitive impairments, VCI is primarily caused by neuronal damage to the cortex [3]. Research has indicated that chronic cerebral hypoperfusion (CCH) plays a vital role in the pathophysiology of VCI, affecting the supply of oxygen and glucose to the brain and frequently causing metabolic changes and oxidative stress [4]. AMP-activated protein kinase (AMPK) phosphorylation, which serves as a cellular energy status sensor, guides metabolic adjustments to promote cellular growth and survival following CCH [5]. The mammalian target of rapamycin (mTOR) kinase also participates in diverse cellular processes and neurotransmission and is important for neuronal development [6]. Overexpression of mTOR has been shown to be an early feature of AD associated with synaptic loss and cognitive decline [7].

Autophagy is a fundamental cellular process that plays a crucial role in maintaining neuronal homeostasis and function [8]. Autophagy is regulated by a complex network of signaling pathways and molecular machinery [9]. The key proteins involved include Beclin-1 [10], which initiates autophagosome formation, and LC3, which is lipidated to LC3-II. In addition, mTOR acts as a central regulator of autophagy under nutrient-rich conditions. Autophagy is presumed to contribute to metabolic adaptation and plays a role in neuronal deterioration and cognitive decline following CCH [5,11,12]. Sustained ischemia and hypoxia lead to hyperactivated autophagy, which initiates pathological processes in the brain resulting in neuronal cell damage. Several studies [13–15] have reported increased expression of the autophagy-related marker Beclin-1, which can significantly exacerbate the pathophysiological processes of vascular dementia. Due to the complexity of the pathological mechanisms involved in the occurrence and development of cognitive impairment, there are currently no effective drugs for the prevention or treatment of this neurological disorder. Herein, we propose that reducing neuronal damage by inhibiting excessive autophagy in the pathogenesis of VCI, thus reducing cognitive decline, could be a potential therapeutic strategy.

Artesunate (ART), a water-soluble derivative of artemisinin, has been extensively used in the treatment of malaria [16].According to existing research, ART can maintain a high concentration in the brain and exert neuroprotective effects against brain injury through its anti-inflammatory and anti-oxidative stress mechanisms [17]. ART has also been shown to regulate autophagy [18]. As such, this study explored whether ART could downregulate autophagy in cortical neurons to improve learning and memory impairments in VCI rats.

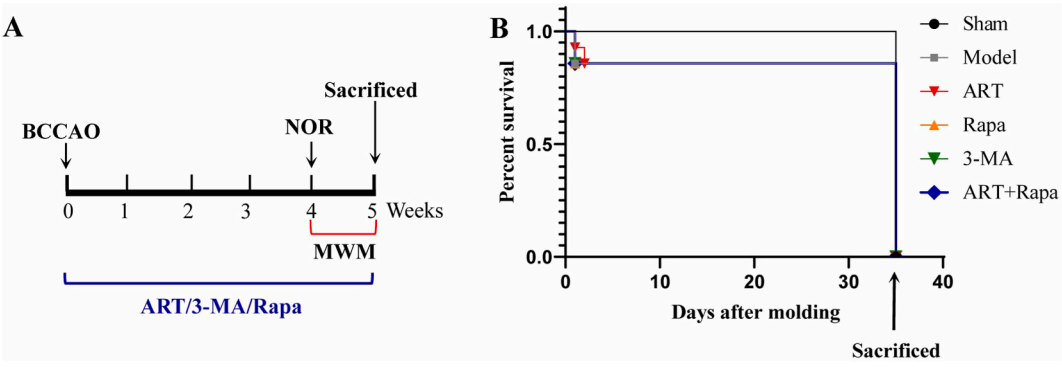

Fig. 1. A: Experimental procedure. (BCCAO: Bilateral Common Carotid Arterial occlusion; NOR: Novel object recognition; MWM: Morris water maze); B: Survival curve of each group.

2. Method

2.1. Experimental procedure

The experimental setup is shown in Fig. 1A. Sprague-Dawley (SD) rats were systematically assigned to six groups: sham operation (Sham, n = 18), model + vehicle (Model, n = 21), model + ART (ART, n = 14), model+3-Methyladenine (3-MA, n = 7), model + rapamycin (Rapa, n = 7), and model + ART + Rapamycin (ART + Rapa, n = 7). The Novel Object Recognition (NOR) test, Morris Water Maze (MWM) test, and tissue collection procedures were conducted four weeks after bilateral common carotid arterial occlusion (BCCAO) induction. Fig. 1B shows the survival of animals during the experiment.

2.2. Animal

Sprague-Dawley (SD) rats weighing 280–300g were sourced from Shanghai Ji Hui Experimental Animal Breeding Co. Rats were housed in a controlled environment with a 12-h light-dark cycle and were provided ample water and food. All animal experiments adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the 3R principle. All animal experiments were approved by the hospital's institutional committee (protocol approval number: 2022-A004-01).

2.3. Model and drug treatment

A VCI rat model was established using BCCAO [19,20]. The carotid arteries were exposed through a ventral midline incision and ligated using 4-0 silk sutures. Sham animals underwent a similar procedure without ligation. After the operation, the rats were intraperitoneally injected the vehicle, ART (50 mg/kg), 3-MA (30 mg/kg) [21] and rapamycin (10 mg/kg) [22] every day.

2.4. MWM test

The MWM experiments comprised three stages: adaptive swimming with a visual platform, place navigation trials, and spatial probe trials. The entire experiment was recorded and analyzed as previously described [23].

2.5. NOR test

The NOR experiment, which was designed to assess learning and memory in rats, comprised three stages [24]: habituation, familiarization, and testing. Memory ability was evaluated using the recognition index: TB/(TA + TB) × 100 %, where TA is the time spent with a familiar object and TB is the time spent with a new object.

2.6. Staining of brain and electron microscopy

Paraffin sections of rat brains were stained with hematoxylin & eosin (H&E) and cresyl violet (CV). After fixing with glutaraldehyde, the rat brain cortex was sliced and photographed using an electron microscope.

2.7. Immunohistochemistry

After the relevant treatment, the paraffin brain sections of the rats were incubated with the primary antibody anti-phospho (p)-ampk (#50081, Cell Signaling Technology). Subsequently, the sections were stained with DAB and hematoxylin. Finally, sealed slices were observed and photographed.

2.8. Western blot

Rat cortex tissue stored at −80 °C was thawed, homogenized, and subjected to protein concentration determination and protein denaturation. Subsequently, protein samples (30 μg) were loaded 10 % SDS-PAGE gels. A constant voltage of 100 V was applied for 150 min at low temperature for membrane transfer to a PVDF membrane. After transferring, the PVDF membrane was blocked with 5 % skim milk at room temperature for 1 h, followed by incubation with the following diluted primary antibodies: p-AMPK (#50081, Cell Signaling Technology), AMPK (#2532, Cell Signaling Technology), p-mTOR (#5536, Cell Signaling Technology), mTOR (#5536, Cell Signaling Technology), P62 (#23214, Cell Signaling Technology), Beclin-1 (#3495, Cell Signaling Technology), LC3B (#83506, Cell Signaling Technology), and β-actin (#4970, Cell Signaling Technology). The membrane was washed with TBST and incubated with the appropriate secondary antibodies. After ECL chemiluminescence treatment, the results were captured using a Gel Imaging System. The gray values were subsequently quantified and analyzed using ImageJ software (NIH).

2.9. Immunofluorescence

Rat brain frozen sections (25 μm thickness) were treated with normal goat serum and Triton X-100 in PBS, followed by overnight incubation with primary antibodies against LC3B (1:400; #83506, Cell Signaling Technology) and NeuN (1:100; ab104224, Abcam) at

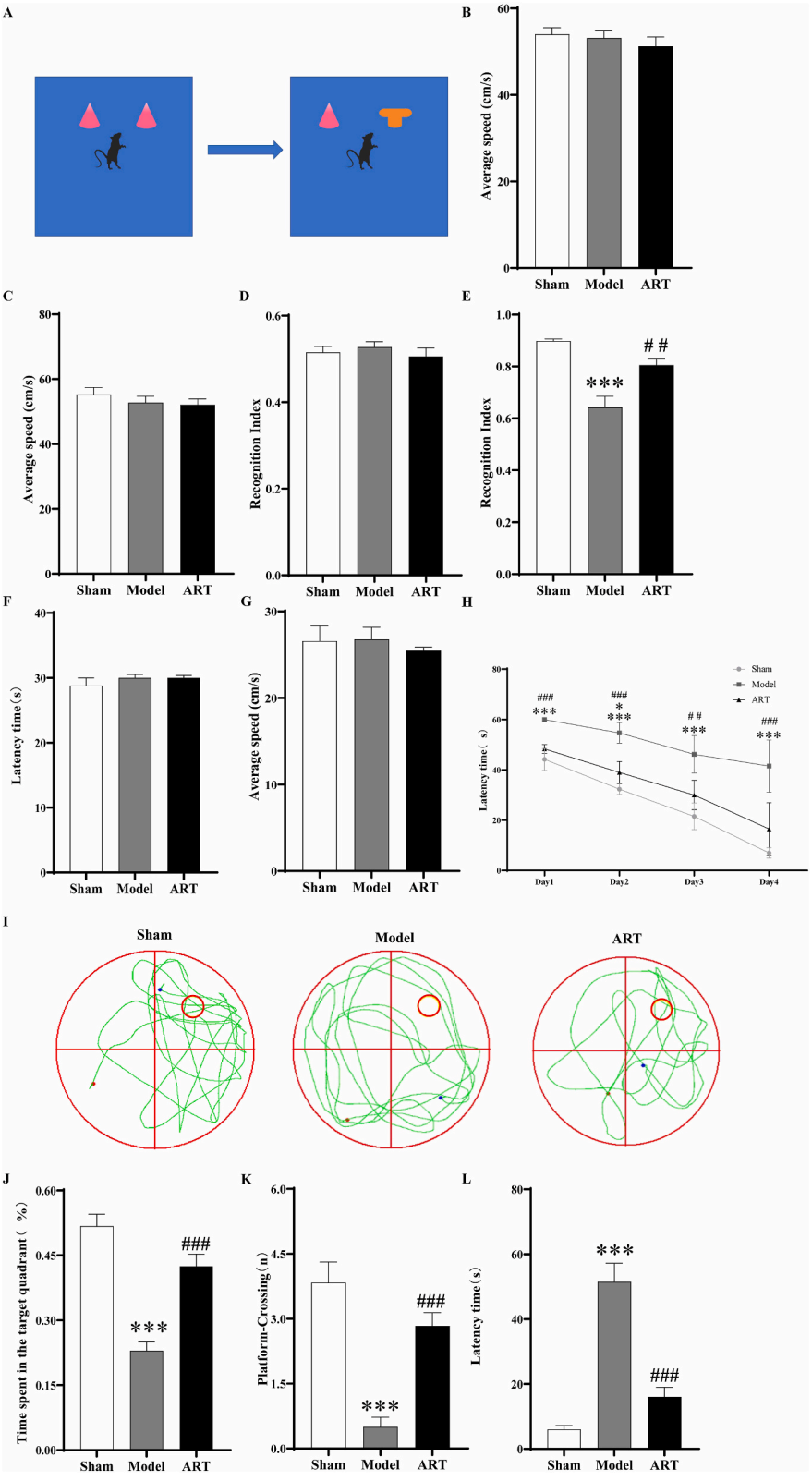

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Fig. 2. A: Pattern diagram of the NOR; B: Movement speed of rats during the NOR experiment's familiarization period; C: Movement speed of rats during the NOR experiment's test period; D: Rats' movement bias time towards object A2 recognition during the familiarization period of the NOR experiment; E: Rats' movement bias time towards object B recognition during the test period of the NOR experiment. F, G: In the visual plateau stage, the latency of finding the platform and swimming speed of rats in each group were observed; H: Latency period for finding the platform during the navigation trial in three groups. I：Representative swimming trajectories of rats in each group during the exploration experiment in the water maze; J, K, L: Percentage of time spent in the platform quadrant, number of crossings over the platform location, and latency period for finding the platform location during the Morris water maze spatial probe trail. ***$p < 0.001$ vs. Sham; $^{\#\#}p < 0.01$ vs. Model; $^{\#\#\#}p < 0.001$ vs. Model.

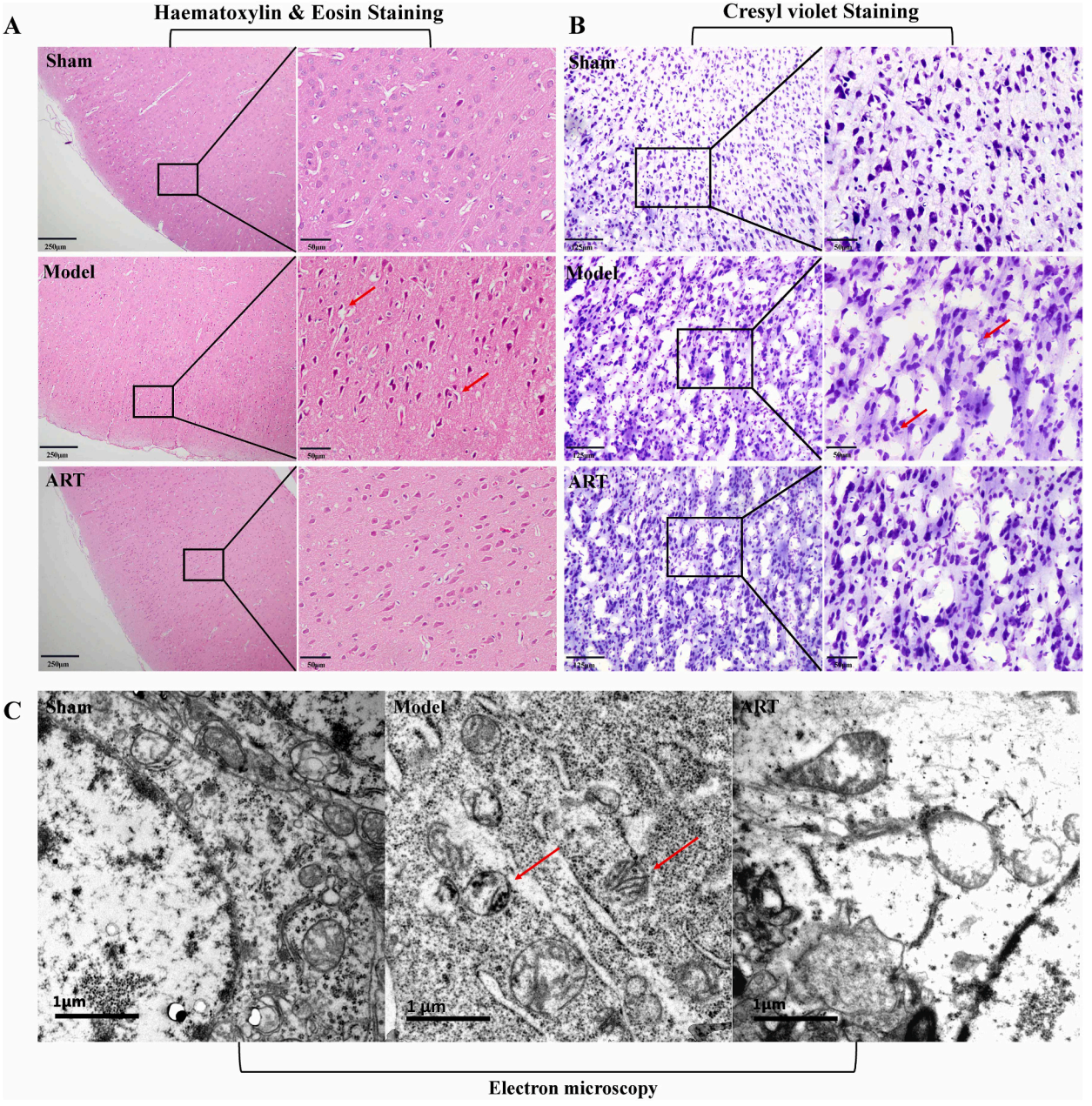

Fig. 3. A: HE staining(A), CV staining(B) and Electron microscopy(C) results of brain tissue in the Sham, Model and ART rat groups.

4 °C. After washing with PBS, sections were incubated with Alexa Fluor secondary antibodies. Imaging was performed using a Leica SP5II laser scanning confocal microscope after the final washing.

2.10. Statistical analysis

Data are presented as the mean ± standard deviation (Mean ± SD), and statistical analyses were conducted using SPSS 21.0. One-way analysis of variance was used to compare multiple groups, while the independent samples t-test was used to compare two groups.

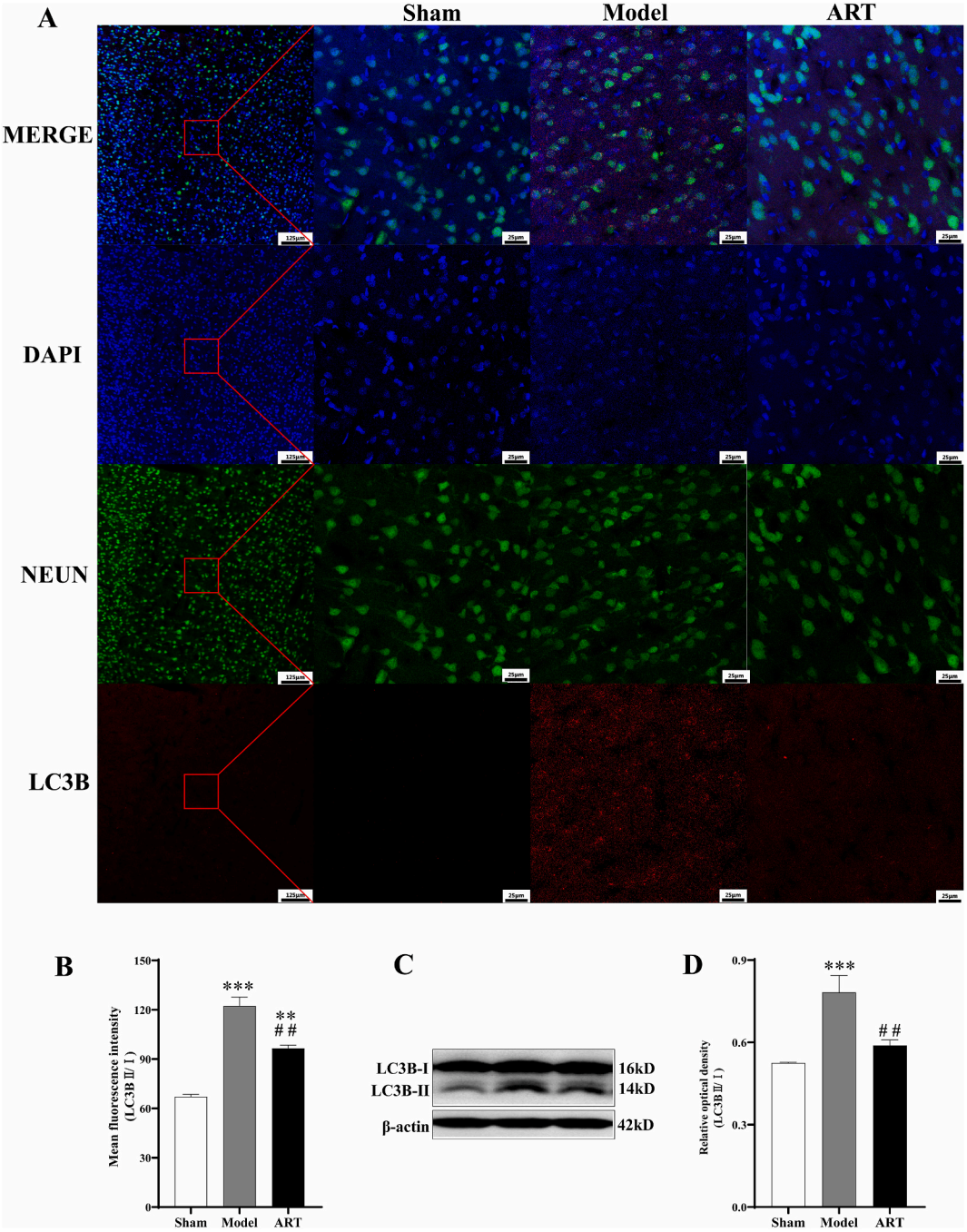

Fig. 4. A:Expressions of LC3B (red) and NEUN (green) in brain cortex neurons of rats in Sham group, Model group and ART group were observed by confocal microscopy(200x; 600x); B：Mean fluorescence intensity of LC3B; C：Western blot analysis of LC3B; D: Gray level analysis of LC3BII/I, **p < 0.01 vs. Sham; ***p < 0.001 vs. Sham;##p < 0.01 vs. Model.

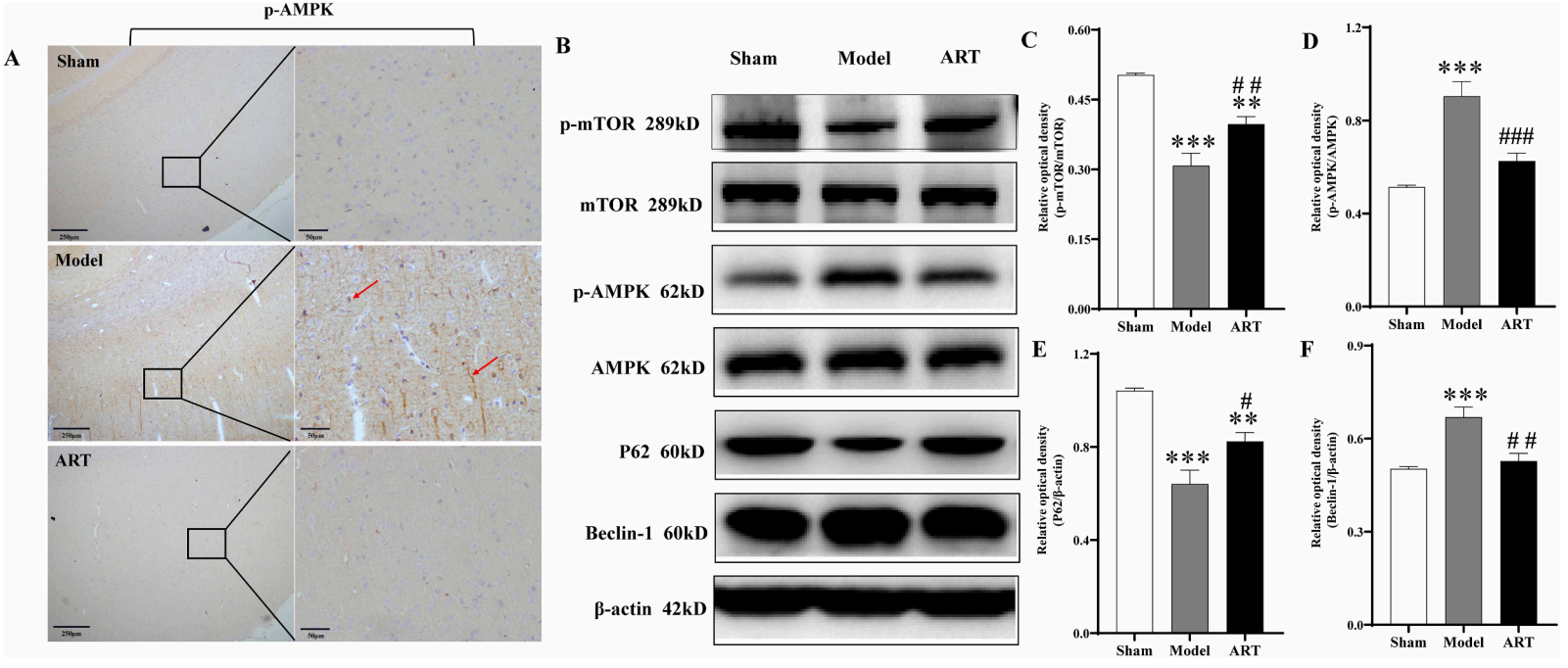

Fig. 5. A:Immunohistochemical results of p-AMPK in brain cortex cell of rats in Sham group, Model group and ART group; B：Western blot analysis of p-mTOR, mTOR, p-AMPK, AMPK, P62, Beclin-1,β-actin; C: Gray level analysis of p-mTOR, mTOR, p-AMPK, AMPK, P62, Beclin-1. **p < 0.01 vs. Sham; ***p < 0.01 vs. Sham;#p < 0.01 vs. Model; ##p < 0.01 vs. Model; ###p < 0.001 vs. Model.

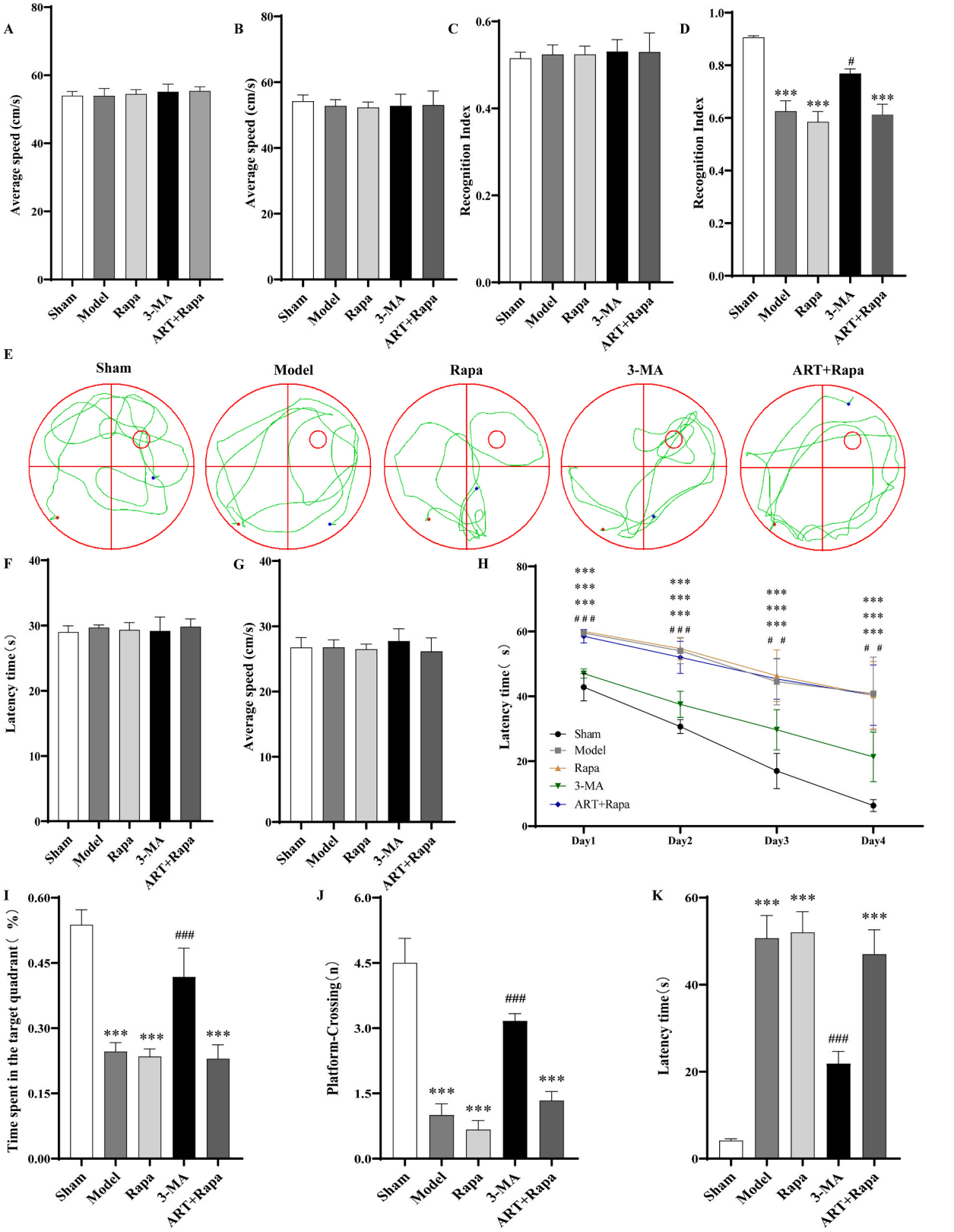

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Fig. 6. A: Movement speed of rats during the NOR experiment's familiarization period; B: Movement speed of rats during the NOR experiment's test period; C: Rats' movement bias time towards object A2 recognition during the familiarization period of the NOR experiment; D: Rats' movement bias time towards object B recognition during the test period of the NOR experiment; E：Representative swimming trajectories of rats in each group during the exploration experiment in the water maze; F, G: In the visual plateau stage, the latency of finding the platform and swimming speed of rats in each group were observed; H: Latency period for finding the platform during the navigation trial in three groups. I, J, K: Percentage of time spent in the platform quadrant, number of crossings over the platform location, and latency period for finding the platform location during the Morris water maze spatial probe trail. ***$p < 0.001$ vs. Sham; $^{\#\#}p < 0.05$ vs. Model; $^{\#\#}p < 0.01$ vs. Model.

$p < 0.05$ was used as the criterion for statistical significance.

3. Results

3.1. ART improves learning and memory impairment in rats with VCI

This study assessed learning and memory in rats using the NOR and MWM tests. In the NOR group, no differences in movement speed were observed (Fig. 2B and C). During familiarization, the recognition biases for objects A1 and A2 were similar (Fig. 2D). During the test period, the model group explored the new object less than the sham group, while the ART group explored it more than the model group ($p < 0.01$, Fig. 2E).

In the visible platform experiment of the MWM test, no significant differences were observed in escape latency or swimming speed between the rat groups. This suggests that there were no abnormal variations in the visual or motor abilities (Fig. 2F and G). In the navigation trials, the model group exhibited a significant decrease in escape latency compared to the sham group ($p < 0.001$, Fig. 2H). However, compared to the model group, rats in the ART group exhibited a significant increase in escape latency ($p < 0.01$; Fig. 2H). Fig. 2I shows the movement patterns in the MWM of the three rat groups. The sham and ART groups both moved predominantly in the quadrant containing the platform, crossing it multiple times. However, the Model group did not concentrate on movement in the platform quadrant.

In the spatial probe trial, rats in the model group exhibited a significant decrease in the percentage of time spent and the number of crossings in the platform quadrant compared to the sham group ($p < 0.001$, Fig. 2J and K). Further, the ART group showed a significant increase compared to the Model group in both percentage of time spent and number of crossings in the platform's quadrant ($p < 0.001$, Fig. 2J and K). The Model group showed increased latency to find the platform compared to the sham group ($p < 0.001$, Fig. 2L), whereas the ART group displayed a significant decrease in latency compared to the model group. These results suggested that artesunate ameliorated memory impairment in rats with VCI.

3.2. ART improves cerebral cortical cell damage and alleviates autophagy levels in rats with VCI

In the cortical neuron staining results, H&E staining revealed that, compared to the Sham group, the Model group rats exhibited increased nuclear condensation, cytoplasmic granulation, and intensified cytoplasmic color, while the ART group showed improvement in all of these symptoms (Fig. 3A). Similarly, CV staining images showed that, compared with the sham group, the model group rats displayed reduced Nissl bodies, disordered cell arrangement, nuclear condensation, and uneven cytoplasmic color, with improvements observed in the ART group (Fig. 3B). Electron microscopy highlighted autophagosomes digesting organelles in cortical neurons of the model group, while no obvious autophagosomes were found in the Sham and ART groups (Fig. 3C).

3.3. ART downregulates the expression levels of LC3B in cortical neurons of rats with VCI

Immunofluorescent double staining of LC3B and NEUN in the rat cortex revealed an increase in LC3B expression in the cortical neurons of the model group compared to the sham group ($p < 0.001$). However, the ART group showed a decrease in LC3B expression compared to that in the model group ($p < 0.01$, Fig. 4A and B). Western blotting results demonstrated a significant up-regulation of LC3BII/I in the model group compared to the sham group ($p < 0.001$), whereas the ART group exhibited a significant downregulation compared to the model group ($p < 0.01$, Fig. 4C and D). These findings suggest that ART alleviates LC3B expression in the cortical neurons of rats with VCI.

3.4. ART downregulates the autophagic levels in cortical neurons of rats with VCI

In immunohistochemical images of cortical neurons in VCI rats, the model group exhibited an upregulation of p-AMPK expression compared to the sham group. However, ART treatment downregulated p-AMPK expression in rat cortical neurons (Fig. 5A). Western blotting results indicated that the model group had significant increases in p-AMPK/AMPK and Beclin-1, along with significant decreases in p-mTOR/mTOR and P62, compared to the sham group ($p < 0.001$, Fig. 5C, D, 5E, 5F). The ART group also showed a significant decrease in p-mTOR/mTOR and P62 ($p < 0.01$; Fig. 5C and E). These findings suggest elevated autophagy in the cortical neurons of VCI rats. However, after ART treatment, compared with the model group, the ART group exhibited significant decreases in p-AMPK/AMPK ($p < 0.001$, Fig. 5D) and Beclin-1 ($p < 0.01$, Fig. 5F), along with significant increases in p-mTOR/mTOR ($p < 0.001$, Fig. 5C) and P62 ($p < 0.05$, Fig. 5E). These results indicate that ART mitigates the increased autophagic levels in the cortical neurons of VCI rats.

3.5. The autophagy agonist rapamycin diminished the beneficial effects of ART in alleviating cognitive impairment in VCI rats

We further examined the role of autophagy in rats with VCI using rapamycin, an agonist of autophagy, and 3-MA, an inhibitor of autophagy. In the NOR test, we found no significant differences in movement speed between the experimental groups (Fig. 6A and B). During the familiarization phase, the interaction between objects A1 and A2 was consistent across all groups (Fig. 6C). However, in the test phase, the Model, Model + Rapa, and Model + ART + Rapa groups all exhibited a significantly lower tendency to explore the novel object than the Sham group ($p < 0.001$). In contrast, the Model+3-MA group showed a significantly higher level of exploration than the Model group ($p < 0.05$). Additionally, there were no statistically significant differences in novel object recognition index among the model, Model + Rapa, and model + ART + Rapa groups (Fig. 6D).

In the visible platform trial of the MWM test, there were no significant differences in escape latency or swimming speed between the rat groups (Fig. 6F and G). In the navigation trials, the Model, Rapa, and ART + Rapa groups all exhibited significantly longer escape latencies than the sham group ($p < 0.001$, Fig. 6H). Conversely, the 3-MA group showed a significant reduction in escape latency compared to the Model group ($p < 0.01$; Fig. 6H). Additionally, there were no statistically significant differences in escape latency among the model, Rapa, and ART + Rapa groups (Fig. 6H). In the spatial probe trial, the Model,Rapa, and ART + Rapa groups all spent significantly less time and made fewer crossings in the platform quadrant than the Sham group ($p < 0.001$, Fig. 6I and J). Conversely, the 3-MA group demonstrated a marked increase in both the percentage of time spent and the number of crossings in the platform quadrant compared to the Model group ($p < 0.001$, Fig. 6I and J). Furthermore, the Model, Rapa, and ART + Rapa groups took significantly longer to locate the platform compared to the Sham group ($p < 0.001$, Fig. 6K), while the 3-MA group exhibited reduced latency compared to the Model group. Notably, there were no significant differences among the Model, Rapa, and ART + Rapa groups in terms of the percentage of time spent in the quadrant, number of crossings in the platform quadrant, or latency to find the platform (Fig. 6I, J, 6K). Fig. 2E illustrates the swimming patterns of the five rat groups in the MWM test. The Sham and 3-MA groups concentrated their movements in the quadrant containing the platform, frequently crossing it. In contrast, the Model, Rapa, and ART + Rapa groups displayed more scattered swimming patterns with fewer crossings in the platform quadrant. These results indicate that the therapeutic effect of ART in reversing VCI in rats was compromised when rapamycin, an autophagy inducer, was administered.

4. Discussion

In clinical settings, the primary symptoms of patients with VCI are cognitive and learning dysfunctions [25]. The mechanisms underlying cognitive and learning impairments, including neuronal damage, glial cell activation, and cholinergic neuronal damage, have been widely reported [26]. Research has further indicated that CCH may lead to neuronal apoptosis, cognitive impairment, and aberrant excessive autophagy in the frontal cortex and hippocampus of rats [4,27]. Additionally, several studies [4,5,28,29] have demonstrated that cortical autophagy precedes hippocampal autophagy in the development of chronic hypoxic-ischemic brain injury, with cortical neurons showing signs of damage earlier than hippocampal neurons. As such, improvements in cognitive and learning abilities are the key outcome indicators of VCI treatment.

In this study, rats were subjected to the MWM and NOR experiments, 4 weeks after BCCAO revealing impairment in learning and memory functions. Concurrently, histopathological staining results using H&E and CV staining showed damage to cortical neurons in rats with VCI. The immunohistochemical results indicated the upregulation of autophagy in the cortical neurons of rats with VCI.

Autophagy is a self-degradation process crucial for balancing energy sources which involves the lysosome-dependent cycling, synthesis, and degradation of cellular components, thus maintaining the stability of the internal environment [30]. However, excessive autophagy can be harmful because it enhances brain damage through excessive cytoplasmic degradation and/or induces cell apoptosis or necrosis [31]. Many studies in the literature have shown that autophagy is associated with various neurodegenerative diseases, in particular, CCH disrupts the autophagic machinery by altering the expression of autophagy-specific proteins [32,33]. Dysregulation of the autophagic pathway leads to neuronal loss, hippocampal atrophy, and the eventual loss of synaptic plasticity. In the present study, electron microscopy revealed that autophagosome digestive organelles appeared in the mitochondria of cortical neurons in the model group. Our results are consistent with those of a previous study [34], showing that autophagy is activated in the cortical regions of rats with VCI. We also found that the addition of the autophagy inhibitor 3-MA resulted in a significant improvement in learning and memory compared to the model group. These findings strongly indicate that autophagy plays a crucial role in the pathogenesis of VCI and influences cognitive function. As such, it is necessary to explore the specific molecular mechanisms involved and the clinical significance of autophagy in VCI treatment strategies.

LC3B, Beclin-1 and P62 are major autophagy related proteins. Beclin-1 triggered autophagy, LC3B formed autophagosomes, and P62 was negatively correlated with autophagy activity [35]. LC3B is an RNA-binding protein that triggers rapid mRNA degradation during autophagy [36]. Conversion of LC3B–I to LC3B-II is essential for the formation of autophagosomes [37]. The LC3II level is proportional to the number of autophagosomes formed by nerve cells in the brain, and is a marker of autophagy activation [14]. In this study, it was observed that compared to the sham group, the expression of LC3B and LC3BII/I in cortex neurons was significantly up-regulated in the model groups. Beclin1 is a central regulatory factor in the early stages of autophagy which serves as a key regulator of autophagy, apoptosis, and inflammatory responses [38,39]. Beclin1 and other proteins associated with LC3 play essential roles in the initiation of autophagic processes [40,41]. mTOR regulates downstream signaling pathways primarily by inhibiting the formation and activation of the ULK1 complex and suppressing the promoter activity of protein synthesis, thus preventing the occurrence of autophagy. This establishes mTOR as a crucial intracellular inhibitor of autophagy [42,43]. P62, also known as sequestosome1 (SQSTM1), has been shown to be critical in regulating mitochondrial autophagy, and has further been implicated in various neurodegenerative diseases, such as Parkinson's disease and AD [44]. Several studies [45,46] have further shown that a lack of p62 promotes

the developmental process of AD, which is different from our results, however, it has also been found that excessive autophagy leads to synaptic degeneration and axonal degeneration [47]. Therefore, P62 has the potential to be an effective molecular target for the therapeutic treatment of neurodegenerative diseases; however, further studies on P62 are needed.

AMPK is believed to be an important molecule that promotes hyper-autophagy following CCH. The AMPK family preserves the equilibrium between ATP production and consumption in eukaryotic cells. AMPK further regulates autophagy by phosphorylating ULK1 and inhibiting mTOR [48], representing a potential mechanism of early brain injury [49]. Our findings indicate a significant decrease in P-AMPK/AMPK levels in the ART treatment group. Additionally, the expression of Beclin-1, LC3B, and the LC3BII/I ratio in cortical neurons were all down-regulated, while there was a notable increase in p-mTOR/mTOR levels. Electron microscopy results also showed that the number of autophagosomes in neurons was reduced after ART administration. The behavioral results of this study demonstrated that, compared to the model group, ART improved memory impairment in VCI rats. These results suggest that ART alleviates neuronal autophagic damage following ischemia-hypoxia by inhibiting AMPK activation. Moreover, following the administration of the autophagy inducer rapamycin, we observed a decrease in the therapeutic effect of ART on VCI rats, which further supports the view that ART improves learning and cognitive function in VCI rats by inhibiting autophagy. These findings highlight the critical role of autophagy regulation in mediating the effects of ART.

Previous research findings have indicated that ART, with its numerous complex symptoms and pathophysiological mechanisms, may be a potential candidate drug for the treatment of central nervous system diseases [50]. Studies have also reported that ART can penetrate the blood-brain barrier (BBB) and maintain a relatively high concentration in brain tissue, making it a potential candidate drug for managing brain diseases [51]. Based on previous research findings and our experimental results, we expect that ART could be a potential therapeutic drug which could be applied to improve the learning and memory function in VCI, and research on its pharmacological mechanism will be an important part of our future research.

This study had some limitations. First, our focus on cortical neurons limited the generalizability of our findings to other brain regions and cell types affected by VCI. Second, although our study implicated AMPK-mediated autophagy activation as a potential mechanism underlying the therapeutic effects of ART, the precise molecular pathways involved remain unclear. The translational potential of ART in clinical practice requires further validation through large-scale clinical trials.

In conclusion, our results suggest that autophagy occurs in the cerebral cortex neurons of rats with VCI. ART may improve learning and memory impairment in VCI rats by downregulating autophagy in the cerebral cortex neurons.

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Ethics statements

The animal study was reviewed and approved by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (protocol approval number: 2022-A004-01).

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Honqiao Wei: Writing – original draft, Funding acquisition. **Xiaokun Wang:** Writing – original draft, Methodology, Data curation. **Hequan Zhong:** Methodology. **Xiangyu Kong:** Methodology. **Jie Zhu:** Supervision, Resources, Data curation. **Bing Li:** Writing – review & editing, Conceptualization.

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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Not Applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33068.

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