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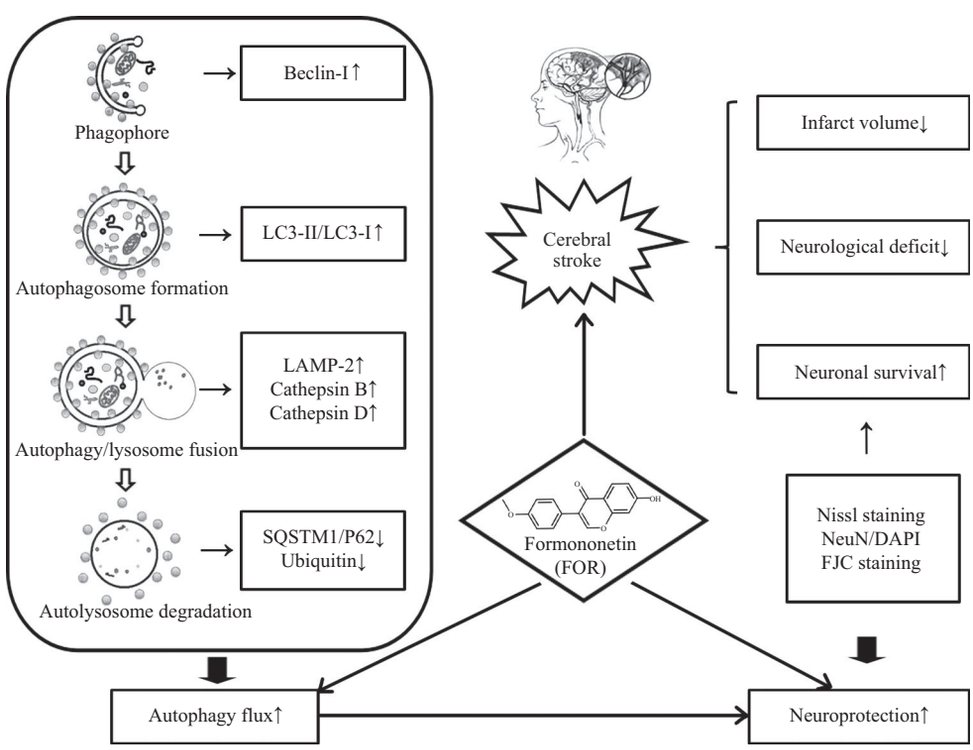
Formononetin Enhances Autophagy Flux in The Penumbra of Cerebral Ischemia and Improves Nerve Damage*

GUO Tao[1,2], ZUO Han-Jun[1], SHI Jin-Sha[1], SHI Hao-Long[1], WANG Zhao[1], CHEN Bo-Lin[1], Li Juan-Juan[1]**

([1]*Faculty of Basic Medical Sciences, Kunming Medical University, Kunming* 650500, China;
[2]*Faculty of Basic Medical Sciences, Kunming University of Science and Technology, Kunming* 650500, China)

Graphical abstract



Abstract **Objective** Formononetin (FOR), a traditional Chinese medicine, has been widely used for nerve protection and nerve function rehabilitation after cerebral stroke. However, the role of FOR in autophagic lysosome function in cerebral ischemia-

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** Corresponding author.
Tel: 86-15808712057, E-mail: lijuanjuan@kmmu.edu.cn
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reperfusion damage has not been investigated. This study aimed to explore whether the therapeutic benefits of FOR were influenced by the regulation of autophagy flux. **Methods** Male Sprague-Dawley rats were separated into sham, model, and MCAO+FOR (30 mg/kg) groups after undergoing middle cerebral artery occlusion (MCAO) and ischemia-reperfusion (I/R). Then, the brain tissues in the ischemic penumbra were obtained to detect the proteins in autophagic/lysosomal pathway with antibodies of Beclin-1, LC3, SQSTM1/P62, Ubiquitin, LAMP-2, Cathepsin B (CTSB) and Cathepsin D (CTSD) by Western blot and immunofluorescence, respectively. Meanwhile, the therapeutic effectiveness was evaluated by measuring infarct volume, neurological impairments, and neuronal necrosis. **Results** The findings of this study demonstrate that FOR treatment exhibits a dual effect by enhancing the autophagic activities of Beclin-1 and LC3 in neurons, while simultaneously improving the autophagic clearance function, as evidenced by reinforced lysosomal activities of LAMP-2, CTSB, and CTSD, as well as reduced autophagic accumulation of Ubiquitin and P62 in the MCAO+FOR group compared to the MCAO group. Additionally, 7 d of FOR treatment dramatically reduced neurological deficits, infarct volume, and neuronal death caused by cerebral ischemia. **Conclusion** These findings suggest that the neuroprotective mechanism of FOR therapy in accelerating recovery from ischemic stroke may involve the increase of autophagy flux in the penumbra.

Key words cerebral ischemia, formononetin (FOR), autophagy flux, penumbra, neuroprotection

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Cerebral stroke is a prevalent disease of the central nervous system and ranks as the second leading cause of death and permanent disability worldwide[1]. Annually, China witnesses approximately 2.5 million new cases, with ischemic stroke accounting for around 70% of all stroke patients[2-3]. Ischemic stroke is characterized by insufficient blood flow to the brain, leading to rapid depletion of oxygen and glucose in brain tissue[4]. Currently, tissue plasminogen activator (tPA) is the only Food and Drug Administration (FDA)-approved thrombolytic medication for ischemic stroke treatment. It functions by activating a proteolytic enzyme to dissolve thrombi[5]. However, the therapeutic use of thrombolytic drugs faces challenges including a limited treatment window and associated side effects such as severe hemorrhage. Moreover, the restoration of blood flow following ischemia, known as cerebral ischemia-reperfusion injury, can cause significant damage to neurons and ultimately result in neuronal apoptosis. Therefore, it remains crucial to explore drugs that offer high safety profiles and broad clinical applicability for effective recovery from cerebral stroke.

Formononetin (FOR, $C_{16}H_{12}O_4$) is a member of the 7-hydroisoflavones class with substitution of a methoxy group at position 4. It has a molecular mass of 268.268 g/mol. The chemical structure of this isoflavone, depicted in **Graphical abstract**, features O-methylation and can be found in various legumes, particularly red clovers and the traditional Chinese herb *Astragalus membranaceus*[6]. FOR has been discovered to possess a range of pharmacological effects, such as antioxidant, anti-inflammatory, and anticoagulant activities[7]. These effects hold great significance for the prevention and treatment of ischemic stroke. Experimental studies have demonstrated that FOR can safeguard brain cells against ischemic injury by inhibiting cell apoptosis and inflammatory reactions, while enhancing antioxidant capacity and improving cerebral blood flow[8]. In clinical research involving patients with acute ischemic stroke, FOR administration led to considerable improvements in neurological deficits and cerebral blood flow perfusion, consequently reducing brain tissue damage[9]. Furthermore, FOR is capable of expediting cell differentiation and promoting cell apoptosis through the regulation of signal pathways such as PI3K, Akt, MAPK, *etc*. [10] These biological characteristics have facilitated the extensive use of FOR in cancer treatment and stem cell research[11-12]. Overall, as a natural plant extract, FOR has made notable strides in medical research. However, current investigations into the autophagy pathway of FOR remain relatively limited, necessitating further exploration of its mechanism.

Autophagy is a catabolic mechanism that involves the delivery of damaged organelles, long-lived proteins and cytoplasmic contents to lysosomes for degradation[13]. It plays a critical role in maintaining cellular homeostasis in the brain under normal physiological conditions. Meanwhile,

autophagy also serves to protect cells from injury by eliminating pathogens, toxins, and abnormal cytoplasmic components during pathological conditions[14]. The activation of autophagy has been observed in response to ischemic cerebral stroke, but it remains uncertain whether autophagy plays a cytoprotective or cytotoxic role in this context. Studies have shown that enhancing autophagy can alleviate neurological damage caused by cerebral ischemia. However, other research has revealed that autophagy activation can worsen ischemic lesions and even lead to autophagic cell death[15-16]. As a result, autophagy is considered to be a double-edged sword in the pathological processes of cerebral stroke[17]. In fact, an integral autophagy consists of several consecutive processes, including autophagosome formation, fusion of autophagosomes with lysosomes, and degradation of autophagosomes in lysosomes[18-19]. The integrity of autophagic processes termed autophagy flux is crucial for restoring cellular homeostasis. In order to fulfill autophagy's fundamental role in metabolic clearance, the contents of autophagosomes must be properly digested in lysosomes[20]. Therefore, the breakdown of autophagic cargo in lysosomes represents an important step in the complete removal of autophagic payloads, highlighting the key role of lysosomal function in autophagy[21]. Consequently, it is necessary to evaluate both lysosomal function and autophagosome biogenesis when assessing the contributions of autophagy. In our investigation, the expressions of LC3, LAMP-2, P62, Ubiquitin, Cathepsin B (CTSB) and Cathepsin D (CTSD) were examined as indicators of lysosomal function.

It has been confirmed that FOR possesses a neuroprotective function in ischemic stroke, and autophagy is also involved in this function[22]. In order to investigate the relationship between these three factors, this study focuses on discussing how the therapeutic effect of FOR on ischemic stroke is achieved through regulating the autophagy flow mechanism and further activating the autophagy/lysosome pathway. Therefore, we first examined the effect of FOR on autophagy expression following cerebral ischemia, and then explored its protective effect against nerve injury. The results demonstrated that FOR could significantly reduced nerve injury after ischemic stroke by 7 d of treatment. In addition, FOR treatment not only promoted the autophagy activity of neurons in the ischemic penumbra but also restored the lysosome dysfunction caused by ischemic stroke, hence reducing autophagy accumulation. Based on these findings, we can conclude that FOR improves cerebral ischemia/reperfusion injury by increasing autophagy flux.

1 Materials and methods

1.1 Experimental animals

66 adult male Sprague-Dawley (SD) rats were purchased from the Hunan Slac Laboratory Animal Corporation (laboratory animal certificate: SCXK2023-0002) for this study. The rats were selected to ensure uniform age (8−10 weeks) and weight (250−280 g). All rats were housed under identical conditions, including a room temperature of 20−25℃, a humidity level of 60%±5%, free access to food and water, and a standard 12-h light-dark cycle. The Experimental Research Center of Kunming Medical University supervised and approved all animal experimentation procedures (No. kmmu31960194).

1.2 Middle cerebral artery occlusion model

The middle cerebral artery occlusion (MCAO) model was established using the modified Zea Longa method[23], following intraperitoneal administration of 1% sodium pentobarbital (50 mg/kg) to induce unconsciousness in the rats. The surgical procedure was performed under a controlled temperature of 37℃. Briefly, the left common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed. To induce occlusion, a 4-0 nylon monofilament (Beijing Xinong Biotechnology Co., Ltd, Beijing, China) with a thread length of 18−20 mm was inserted into the ICA, thereby blocking the left middle cerebral artery. After 90 min of occlusion, the surgical sutures were removed to allow reperfusion. With the exception of MCAO, the sham group just administered anesthesia and exposed the CCA.

1.3 FOR drug intervention

Based on the experimental targets and the principle of randomization, all the rats were randomly divided into three groups: the sham group, the ischemia-reperfusion group (MCAO), and the FOR intervention group (MCAO+FOR). High-purity FOR (≥98%) was obtained from Hebei Shuoxing Biotechnology Co., Ltd, China and dissolved in 10%

dimethyl sulfoxide (DMSO). The rats were received intraperitoneal injections once daily for 7 d, starting at the onset of reperfusion. The dosage and administration method were determined based on a study[24], by which demonstrated that intraperitoneal treatment with 30 mg/kg significantly reduces inflammatory damage caused by cerebral ischemia-reperfusion. In the MCAO or sham group, the animals were given the same volume of DMSO instead of FOR injection.

1.4 Brain infarct volume

The brain was placed in a brain trough after the completion of neurological deficiency evaluation. It was promptly frozen at −20℃ for 20 min, and then sliced into coronal slices with a thickness of 2 mm using a blade. There were a total of 5 slices. Before fixed with 4% paraformaldehyde for 12 h at room temperature, the brain tissue slices were stained with 2% 2, 3, 5-triphenyl tetrazolium chloride (TTC, Solarbio Science & Technology Co., Ltd, Beijing, China) for 30 min at room temperature in the dark. The healthy brain tissues were displayed as red, while the dead part was shown as pale. Image J software was used to calculate the cerebral infarct area. The result was determined by the infarction rate=(infarct volume/ipsilateral hemisphere volume)×100%.

1.5 Modified neurological severity score

In a previous study, the assessment of neurological impairment was measured using a modified neurological severity score (mNSS) test after 7 d of FOR treatment[25]. The mNSS test consists of four sections: sensory function, which evaluates deficits in proprioceptive; tactile, visual perception and reflex assessment; motor function, which assesses abnormal movements and muscular status; and a test of balancing ability. There was no neurological deficit scored 0, and the maximum score was 18. Consequently, a higher score indicated a neurological deficiency that was more serious.

1.6 Nissl staining

Brain sections were mounted on 1% thiopurine staining solution (Solarbio, Beijing, China) for 5 min and subsequently washed 3 times with ultrapure water for 10 s each. Afterwards, they were successively submerged in 95% and 75% ethanol for 10 s each. Next, the sections were dehydrated in 100% ethanol after being permeabilized with xylene. Finally, the damaged neurons in the rat brain tissue were examined under a microscope. Two people independently counted the number of Nissl bodies from each slice in a double-blind manner, and the average value was recorded.

1.7 Fluoro−Jade C staining

The Fluoro-Jade C (FJC) staining was employed to measure neuronal loss. Firstly, The frozen sections were prepared following the staining instructions provided with the FJC kit (Biosensis, California, USA). Briefly, brain slices were consecutively treated with 1% NaOH, 70% ethanol, distilled water and 0.06% potassium permanganate. Subsequently, the sections were washed with distilled water and stained using a 0.000 1% FJC working solution. Afterward, the sections were thoroughly rinsed, air-dried in complete darkness, cleaned with xylene, and mounted on slices. The FJC staining images were captured using a fluorescence microscope (model 200; Nikon, Tokyo, Japan). Five slices were randomly selected from each sample for analysis of neuronal loss.

1.8 Western blot

Samples of the ischemic penumbra were taken from the ischemic hemisphere and stored at −80℃. The total protein content in the brain tissue was extracted using cold lysis buffer solution and the protein concentration was assessed using the BCA kit (Beyotime Biotechnology Co., Ltd, Beijing, China). The gel was transferred to a PVDF membrane (Millipore, Billerica, MA, USA) following SDS-PAGE. Rabbit antibodies against rat β-actin, LC3, Beclin-1 and SQSTM1/P62 (1∶1 000 in PBST, Cell Signaling Technology, Danvers, MA, USA), CTSB and CTSD (1∶1 000, Santa Cruz Biotechnology, Dallas, TX, USA), LAMP-2 and Ubiquitin (ABclonal Technology, Wuhan, China, 1∶800) were incubated with primary antibody in 4℃ overnight, respectively. Following this, the PVDF membranes were blocked with 5% nonfat milk at room temperature for 1 h and then washed 3 times with PBST (PBS containing 0.1% polysorbate 20). An electrochemiluminescence (ECL) instrument was used to develop the membrane. Finally, images were collected and analyzed by Image J software.

1.9 Immunofluorescence

The brains were coronally cut into pieces with a freezing microtome (SLEE, Mainz, Germany) after dehydration in a 30% sucrose solution. The brain slices were washed with PBS and permeabilized with

Triton X-100 for 10 min. Subsequently, fetal bovine serum was utilized to block the antigen for 1 h. An appropriate amount of primary antibody was added, including rabbit antibodies against rat LC3 and SQSTM1 (1∶400, Cell Signaling Technology, Danvers, MA, USA), mouse antibody against rat LAMP-2 (1∶200, ABclonal Technology, Wuhan, China), CTSD, and NeuN (1∶400, Santa Cruz Biotechnology, Dallas, TX, USA). These antibodies were incubated overnight at 4℃, respectively. The slices underwent three PBS washes after each incubation. The Alexa Fluor-594-conjugated anti-rabbit IgG and Alexa Fluor-488-conjugated anti-mouse IgG (1∶800, Invitrogen, Shanghai, China) were both labeled for 2 h in the dark, respectively. Following washing, the 4',6-diamidino-2-phenylindole (DAPI, 1∶2 000, Cell Signaling Technology) was added for counterstaining for 5 min in the dark. A fluorescence inverted microscope (Nikon Instruments Co., Ltd., Tokyo, Japan) was used to observe and image the location and fluorescence intensity of the proteins in each section (under high magnification, ×400). The percentages of positive cells were employed to represent the results.

1.10 Statistical analysis

Statistical differences were analyzed with GraphPad Prism 8 (GraphPad Software, California, USA). Data were presented as mean±standard error of the mean (SEM). Statistical differences were assessed through one-way analysis of variance (ANOVA) followed by Tukey's test. $P<0.05$ was considered statistically significant.

2 Results

2.1 FOR dramatically reduced the infarct volume and neurological impairments after ischemic stroke

To examine the effects of FOR on neurological outcomes after ischemic stroke, the brain infarct volume and neurological score were assessed by TTC staining (Figure 1a, b) and mNSS test (Figure 1c), respectively. Brain infarct was observed in the MCAO rats compared to the sham surgery animals. However, after 7 d of FOR therapy, the infarction was clearly mitigated in MCAO+FOR group compared to the MCAO group. The mNSS test was assessed to investigate the effect of FOR therapy on neurological damage after MCAO. Both infarction and

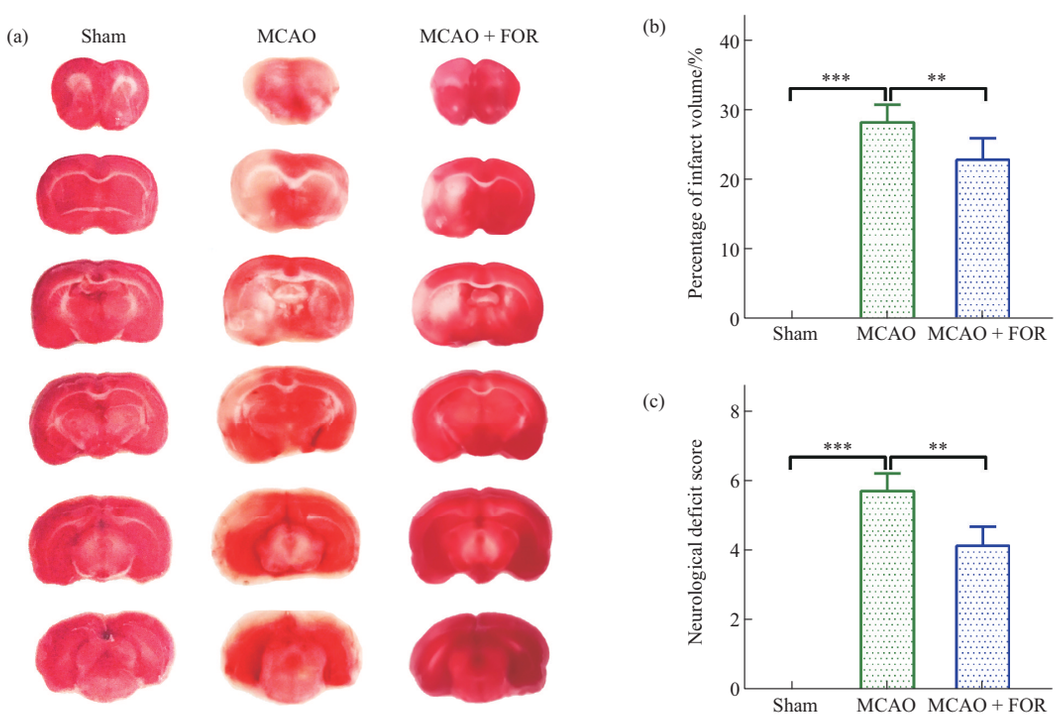

Fig. 1 The TTC staining and mNNS test were assessed to evaluate the infarct volume and neurological score at the penumbra after MCAO

(a, b) After 7 d of FOR therapy, the infarct volume was significantly alleviated in MCAO+FOR group, compared with that in MCAO group. (c) FOR atlenuated the neurological deficit of MCAO. n=6, **P<0.01, ***P<0.001.

neurological impairment were assessed as similar outcomes. In the sham group, there was no infarction or neurological deficit was observed.

2.2 FOR obviously promoted improved neuron survival at the penumbra

To study the effect of FOR therapy on neuronal damage after 7 d of ischemic stroke, the neuronal survival at the penumbra was assessed by Nissl staining (Figure 2a), immunofluorescence (Figure 2c) and FJC staining (Figure 2e), respectively. The findings demonstrated that FOR treatment significantly increased the number of viable neurons (Figure 2b) and NeuN-positive cells (Figure 2d) in the MCAO+FOR group compared to the MCAO group. Meanwhile, FOR therapy clearly decreased the number of FJC-positive cells (Figure 2f). These results suggest that FOR treatment could increase neuronal survival after ischemic stroke.

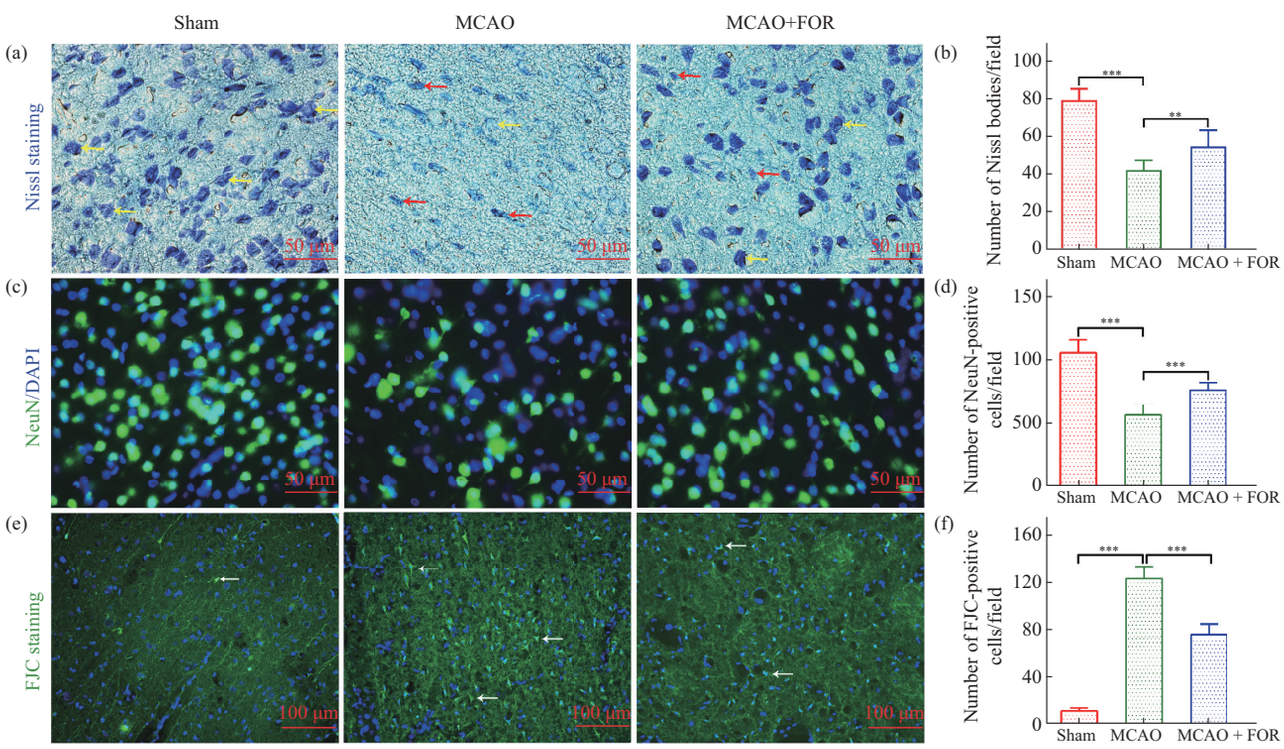

Fig. 2 Nissl staining, immunofluorescence and FJC staining show significantly promoted neuron survival at the penumbra after MCAO

(a) Nissl staining images of pyknotic neurons (red arrows) and viable neurons (yellow arrows). (c) Immunofluorescence images of NeuN (green)/DAPI (blue) co-localization. (e) FJC staining images of degenerative neurons. (b, d, f) The number of viable neurons, NeuN-positive cells and FJC-positive cells were statistically analysized. n=6, $^{**}P$<0.01, $^{***}P$<0.001.

2.3 FOR significantly promoted autophagic activity at the penumbra

The rats were subjected to immediate treatment with FOR for 7 d following MCAO. The brain tissues were obtained to detect autophagic activity by western blot analysis with antibodies of Beclin-1 (Figure 3a, b) and LC3 (Figure 3a, c) in the penumbra, respectively. The results demonstrated that both Beclin-1 expression and ratio of LC3-II/LC3-I were obviously elevated in the MCAO group compared to the Sham group. Moreover, FOR treatment led to a marked increase in Beclin-1 and LC3 levels compared to the MCAO group. Immunofluorescence staining further supported Western blot findings that the FOR drug intervention increased the expression of LC3 (Figure 3d, e).

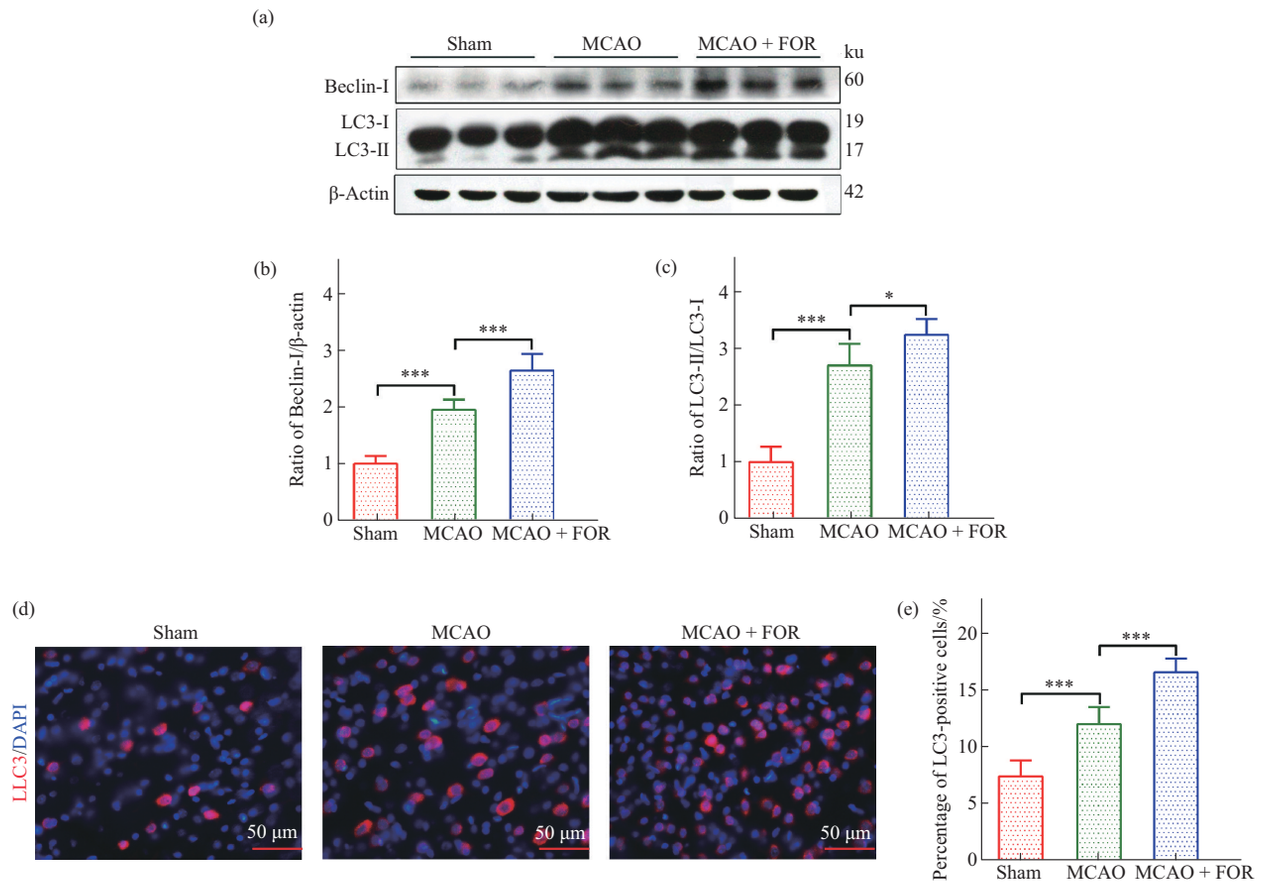

Fig. 3 Western blot and immunofluorescence were performed to investigate the effect of FOR treatment on autophagic activity at the penumbra after MCAO

(a−c) Both Beclin-1 expression and the ratio of LC3-II/LC3-I in MCAO+FOR group were significantly higher than those in MCAO group or in sham group. (d, e) The percentage of autophagy marker LC3 positive cells detected by immunofluorescence assay. n=6, *P<0.05, ***P<0.001.

2.4 FOR therapy markedly facilitated autophagy flux after cerebral ischemia

Western blot analysis was employed to identify proteins involved in the autophagy/lysosome pathway and investigate the impact of FOR intervention on autophagy flux after 7 d. The results (Figure 4a) showed that the expression levels of SQSTM1/P62 (Figure 4b) expression was decreased, while Ubiquitin (Figure 4c), LAMP-2 (Figure 4d), CTSB (Figure 4e) and CTSD (Figure 4f) were elevated after MCAO compared to the Sham group. It implies that autophagic activity is enhanced after cerebral ischemia, leading to an accumulation of autophagic substrates and impaired autophagic flow. Importantly, lysosomal activities of the autophagic pathways LAMP-2, CTSB and CTSD were significantly increased by FOR treatment compared to the MCAO group. In contrast, the accumulation of autophagic substrates of P62 and ubiquitin was markedly decreased. These findings indicate that FOR intervention promotes a smooth autophagic flow.

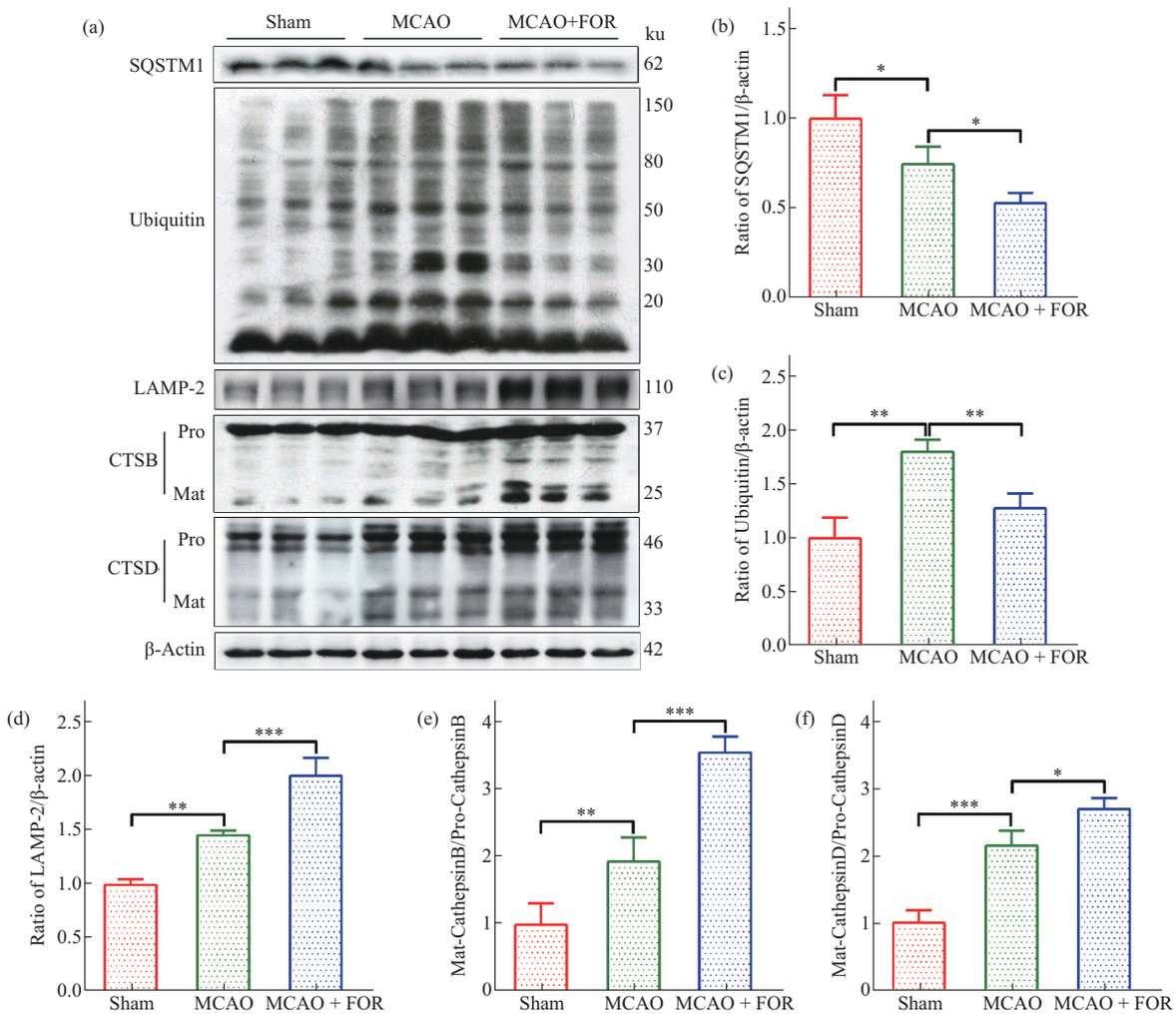

Fig. 4　Western blot was performed to evaluate the efficacy of FOR treatment on autophagy flux at the penumbra after MCAO

(a−c) The autophagic substrates of SQSTM1/P62 and Ubiquitin were attenuated after 7 d of FOR therapy. (a, d−f)The lysosomal activities of LAMP-2, CTSB and CTSD were considerably increased in comparison with those in MCAO group. n=6, * P<0.05, **P<0.01, ***P<0.001. Pro: precursor form, Mat: mature form.

2.5　FOR treatment boost autolysosomal function after cerebral ischemia

The cells were subjected to double immunofluorescence labeling with LC3-LAMP-2 (Figure 5a) and SQSTM1-CTSD (Figure 5b) in the cerebral ischemia penumbra. The results revealed that the MCAO group exhibited a higher percentage of LC3-LAMP-2-positive cells (Figure 5c) and SQSTM1-CTSD-positive cells (Figure 5d) compared to the sham group. Notably, the administration of FOR treatment led to a substantial rise in the percentages of LC3-LAMP-2-positive cells and SQSTM1-CTSD-positive cells compared to the MCAO group. This indicated that the autolysosomal function could be enhanced by FOR therapy.

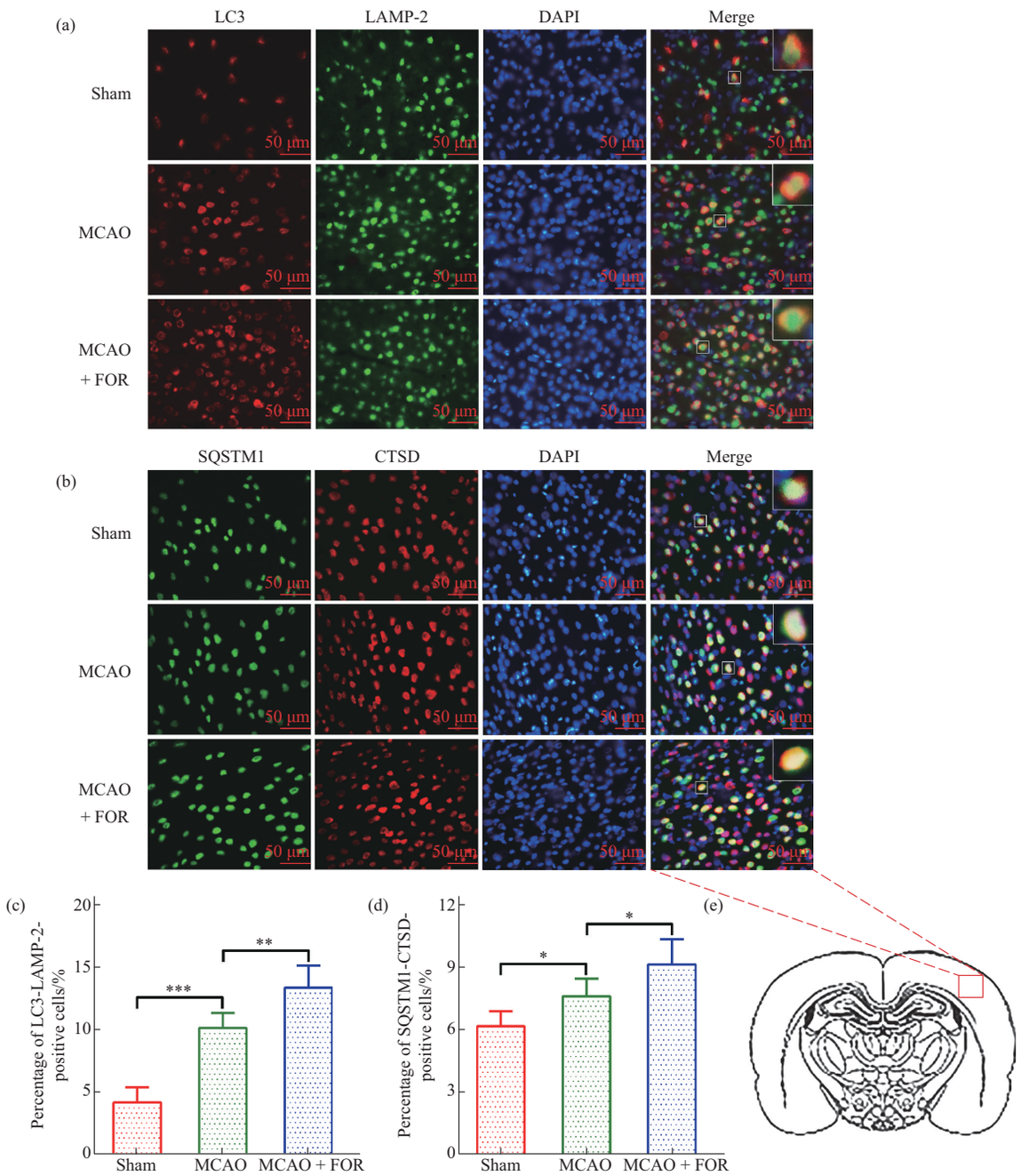

Fig. 5　Double immunofluorescence was performed to evaluate lysosomal function at the penumbra after MCAO

(a−d) The ratios of LC3-LAMP-2 and SQSTM1-CTSD-positive cells in the penumbra were markedly promoted, compared with those in MCAO group. (e) The region of immunofluorescence selection in the penumbra of cerebral ischemia. LC3: a marker of mature autophagosome; LAMP-2: an indicator of lysosomal activation; P62: a recruiting protein of autophagic substrates; CTSB: a main protease of lysosomes. n=6, *P<0.05, **P<0.01, ***P<0.001.

3　Discussion

FOR is a natural compound derived from plants, which has a variety of pharmacological activities and is widely utilized in the treatment of cerebral ischemia[26]. Cerebral ischemia refers to an ischemic disease caused by temporary or permanent interruption of cerebral vascular supply, and it remains one of the primary causes of stroke[27]. Clinical experiments and animal research both

indicated that FOR might produce a discernible neuroprotection against ischemic brain injury. According to earlier research, FOR effectively reduced infarct volume, inhibited apoptosis, improved neurological function, increased antioxidant enzyme levels, mitigated excitotoxic injury, and other factors that contributed to the post-stroke damage[28-29]. However, despite numerous investigations into the role of FOR in cerebral ischemia, further research is still needed to elucidate its therapeutic mechanism and precise efficacy in autophagic flow.

Autophagy may have two distinct effects in cerebral ischemia. Moderate autophagy is beneficial for neuronal survival, while excessive autophagy exacerbates cerebral ischemic injury[30-31]. Therefore, it is crucial to consider the dual impacts of autophagy when investigating and employing it for the treatment of cerebral ischemia to achieve optimal outcomes. However, recent research suggests that the dual effect of autophagy is attributed to the existence of different mechanisms of action during its occurrence[32]. This research focuses on the entire process of autophagy, including phagophore, autophagosome formation, autophagy/lysosome fusion, and autolysosome degradation, which is referred to as “autophagy flow”. The approach differs from the traditional single evaluation of autophagy activity. In recent years, more attention has been given to the roles of autophagy in degradation and clearance[33-34]. Because of this, the autophagy initiation and autophagosome formation were respectively detected with antibodies targeting of Beclin-1 and LC3, the lysosomal activity was evaluated through LAMP-2, CTSB and CTSD, and the content of autophagic cargoes was assessed using SQSTM1/P62 and Ubiquitin in the present study. As a result, the essential autophagic processes may be carefully watched to examine the effectiveness of FOR treatment on the autophagic/lysosomal pathway.

Our research showed that ischemic stroke led to serious neurological deficit, cerebral infarction and neuronal death in MCAO group. Western blot analysis demonstrated that the MCAO group exhibited increased expression of LAMP-2, accompanied by enhanced activities of CTSB and CTSD, compared to the sham group. These results indicate that the activity of lysosomal enzymes was activated after cerebral ischemia. Additionally, the increased lysosomal capacity resulting from ischemic stroke led to the accumulation of autophagic substrates, as evidenced by the upregulation of Beclin-1, LC3, and Ubiquitin. This result was also consistent with the reported studies which suggested the lysosomal dysfunction was an important pathogenesis of ischemic stroke[35]. The neurological harm caused by ischemic stroke was significantly reduced after 7 d of FOR therapy, which is also in line with previous investigations[36].

We subsequently examined the impact of FOR on autophagy flux in order to determine if this neuroprotection was due to modulation of the autophagic/lysosomal pathway. The result showed that the lysosomal dysfunction could be dramatically mitigated, as reflected by increased expressions of LAMP-2, CTSB and CTSD after FOR therapy. Additionally, FOR treatment led to elevated levels of Beclin-1 and LC3 in the penumbral area, while SQSTM1/P62 and Ubiquitin expression levels were decreased compared to the MCAO group. This demonstrated that FOR treatment improved autophagic activity which unavoidably increased the production of autophagic substrates, but these substrates were effectively degraded due to the enhanced lysosomal capacity induced by FOR therapy. Furthermore, double immunofluorescence analysis revealed that the percentages of LC3-LAMP-2 and SQSTM1-CTSB-positive cells were significantly promoted by FOR treatment compared to the MCAO group. These findings further confirmed that FOR therapy restored the lysosomal dysfunction caused by ischemic stroke, as evidenced by enhanced lysosomal activity and decreased accumulation of autophagic substrates.

4 Conclusion

In summary, our data showed that the infarct volume, neurological impairment and neuronal apoptosis were significantly attenuated by 7 d of FOR treatment after ischemic stroke. Meanwhile, the results indicated that FOR treatment might not only stimulate autophagic activity in neurons located at the ischemic penumbra, but also could enhance the autophagic clearance by strengthening the lysosomal function after MCAO. Consequently, we have reached the conclusion that the neuroprotection induced by FOR was achieved through the facilitation of autophagy flux during ischemic stroke.

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刺芒柄花素增强脑缺血半暗带自噬流改善神经损伤*

郭　涛[1,2)]　左涵珺[1)]　石金沙[1)]　石浩龙[1)]　王　朝[1)]　陈柏霖[1)]　李娟娟[1)**]

（[1)] 昆明医科大学基础医学院，昆明 650500；[2)] 昆明理工大学基础医学院，昆明 650500）

摘要　目的　刺芒柄花素（formononetin，FOR）常被用于脑卒中后的神经保护和神经功能康复，其在脑缺血再灌注（ischemia-reperfusion，I/R）损伤中的自噬溶酶体功能尚未见报道。本研究旨在探讨刺芒柄花素的治疗效果是否受到自噬流调节方式的影响。**方法**　雄性Sprague-Dawley（SD）大鼠在接受大脑中动脉闭塞（middle cerebral artery occlusion，MCAO）和I/R后被分为假组、模型组和MCAO+FOR（30 mg/kg）组。然后获得缺血半暗带的脑组织，分别用Beclin-1、LC3、SQSTM1/P62、Ubiquitin、LAMP-2、Cathepsin B、Cathepsin D抗体通过蛋白质印迹法（Western blot，WB）和双重免疫荧光检测自噬/溶酶体通路中的关键蛋白质。同时评估脑梗死体积、神经功能损伤和神经元坏死情况，以评价FOR的干预效应。**结果**　研究表明，与MCAO组相比，FOR干预除了能促进神经元中Beclin-1和LC3的自噬活性外，还能够改善自噬清除功能，如LAMP-2、Cathepsin B、Cathepsin D的溶酶体活性增强，以及Ubiquitin和P62的自噬积累减少。另外，FOR干预7 d后显著减少了脑缺血导致的神经功能缺损、梗死体积以及神经元死亡。**结论**　FOR的干预治疗可能是通过增强自噬流促进脑缺血半暗带恢复的神经保护机制。

关键词　脑缺血，刺芒柄花素，自噬流，半暗带，神经保护

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** 通讯联系人。

Tel：15808712057，E-mail：lijuanjuan@kmmu.edu.cn

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