Review Article

The Roles of Autophagy and Inflammatory Responses in Acute Ischemic Stroke

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Abstract

Autophagy and inflammation play a key role in the pathogenesis of ischemic stroke; the latest research shows that autophagy and inflammation are seen as a double-edged sword. Insufficient or excessive autophagy will cause nerve damage, promote cell death, while the moderately autophagy has a neuroprotective effect. After the occurrence of ischemic stroke, the inflammatory response activates anti-inflammatory mediators and proinflammatory mediators, breaking the dynamic balance between proinflammatory response and anti-inflammatory response, causing detriment to the brain, affecting brain function recovery. The main purpose of this article is to systematically summarize the recent studies on autophagy and inflammatory responses in acute ischemic stroke, a brief analysis the roles of autophagy and inflammatory responses in the pathophysiology of ischemic stroke, investigate the correlation between both. To build a foundation for research on how to make autophagy and proinflammatory and anti-inflammatory responses achieve the desired balance at different stages of the stroke, in order to provide research directions for acute ischemic stroke and to form new and effective therapeutic strategies.

ABBREVIATIONS

IPC: Ischemic Preconditioning; 3-MA: 3-Methyl Adenine; OGD: Oxygen Glucose Deprivation; MCAO: Middle Cerebral Artery Occlusion; TGF-β: Transforming Growth Factor-beta; MMPs: Matrix Metalloproteinase's; IL-10: Interleukin-10; IGF-1: Insulin-like Growth Factors-1

INTRODUCTION

Stroke is one of the major causes of death and disability, seriously affecting public health [1-3]. Ischemic stroke accounts for 60% to 80% [4] of total strokes. The main effective treatment for acute ischemic stroke is use thrombolytic therapy within 4.5 hours in the pathogenesis of ischemic stroke, but limited by the time window, thrombolytic therapy for most patients still has great limitations [5]. Therefore, exploring new drugs, new therapeutic target for the treatment of acute ischemic stroke has become a new hot research target worldwide. Many scholars currently are exploring a series of new treatments strategies based on cytokines, growth factors, stem cells, a high concentration of atmospheric oxygen, metal ions, cell metabolism, small molecule modulators of hypoxia inducible factor, tissue kallikrein and cell tumor protein, etc. for the treatment of ischemic cerebrovascular disease [6].

In recent years, autophagy become a hot research, although much evidence indicates that autophagy may become a new therapeutic strategy of stroke treatment, but studies show that excessive autophagy will cause nerve damage, while moderately autophagy will have the neuroprotective effect, autophagy in ischemic stroke is a double-edged sword [7]. In the pathogenesis of ischemic stroke inflammation plays an important role, more and more of the evidence also indicates that the inflammatory response in the pathogenesis of ischemic stroke is also as a double-edged sword, it can cause secondary brain damage in the acute phase of stroke, while beneficial in brain function recovery after the stroke [8]. The main purpose of this article is to systematically summarize the recent studies on autophagy and inflammatory responses in acute ischemic stroke, a brief analysis the roles of autophagy and inflammatory responses in the pathophysiology of ischemic stroke, as well as the correlation between them.
THE ROLE OF AUTOPHAGY IN ISCHEMIC BRAIN INJURY

In recent years, the effect of autophagy and its participation in cerebral ischemia has gradually been taken seriously. Numerous studies indicate that autophagy plays an important role in ischemic brain injury. Acute cerebral ischemia caused by excessive autophagy can lead to cell death, and in some specific aspects of the pathology of cerebral ischemia, moderate autophagy has also a positive neuroprotective effect. Autophagy plays a dual role in the protection and damage in ischemic stroke, it is like a "double-edged sword" [7].

The protective effect of autophagy in brain ischemia

Autophagy, by clearing rubbish intracellular, maintains a relatively stable internal environment and is a biological mechanism to promote cell survival, it is essential for cell survival [9]. Autophagy is the main degradation pathway to clear the abnormal protein aggregation and damaged organelles within the neuron cell after cerebral ischemia. Protein aggregation after the cerebral ischemia is at least partly due to injury to the autophagy mechanism. Excess protein aggregates can cause a variety of organelles damage, and ultimately lead to delayed neuronal death [10]. Ischemic Preconditioning (IPC) can significantly reduce ischemic brain damage caused by middle cerebral artery occlusion through activating autophagy, autophagy inhibitor 3-methyl adenine (3-MA) and Bafilomycin A1 (Baf A1) to inhibit ischemic preconditioning neuroprotection effect, indicating that autophagy involved in ischemic preconditioning-induced neuroprotection, and autophagy inducer Rapamycin (Rapa) preconditioning can reduce infarct size caused by occlusion of the middle cerebral artery, decrease the levels of cerebral edema and neurologic dysfunction [11]. When brain damage induced by ischemia and hypoxia, the level of nerve cells autophagy increased, autophagy rapamycin will further raise the level of autophagy in order to reduce necrotic cell death and decrease brain injury [12]. Wang et al. [13], reported apply ribose phosphate during cerebral ischemia to signal TSC2-mTOR-S6K1 pathway then induce autophagy which may promote the survival of neurons.

The damage effect of autophagy in cerebral ischemia

Despite the large number of pharmaceutical research showed that after the function of autophagy damage which can cause autophagy body, the accumulation of abnormal cells and cell death, it verified autophagy's pro-survival role under pathological conditions. However, from the observation, a phenomenon of the increased autophagy and lysosomal autophagy in the dying cells may indicate that the presence of an autophagy can cause cell death mechanism, that is, autophagic cell death [14]. Autophagy promotes cell death hypothesis that autophagy is activated by excessive degradation of protein components inside the cell or activation of apoptosis-related enzymes mediating cell death [15], Koike et al. [16], found on autophagy-related genes Atg7 deficient mice that the number of nerve cells deaths was reduced after ischemic brain damage, it led to the theory that autophagy can aggravate the nerve cell damage from ischemic brain damage. Activate ischemia induced autophagic lysosomal pathway can lead to damaged astrocytes.

Autophagy inhibitor 3-MA significantly reduced OGD (Oxygen Glucose Deprivation) induced astrocyte cell death [17]. During Ischemic, the neurons autophagy lysosome pathways are activated and cause ischemic neuronal damage [18], inhibition of autophagy have neuroprotective effect [19]. RNA interference (RNAi) mediated Beclin1 suppressing inhibition of autophagy can reduce infarct size of brain damage which caused by middle cerebral artery occlusion (MCAO) model and decrease the levels of brain histological damage and neurological deficits [20]. Wen et al. [21], studied the MCAO model uses autophagy inhibitor 3-MA suppress ischemia-induced up regulation of LC3-II, which will significantly reduce infarct size, cerebral edema, and motor impairment. Xin et al. [22], found that after ischemia by inhibiting autophagy might prevent death of vertebral neurons.

The role of autophagy in ischemic brain injury depends on the levels of autophagy

The level of autophagy cerebral ischemia decided the fate of cells, Kang [23] believes that the level of autophagy is very crucial to cell damaged or cell death. Physiological level of autophagy promotes survival, while insufficient or excessive autophagy will promote death. Autophagy-deficient cells are more sensitive to external stimuli stress reaction, however, long-term excessive activation of autophagy may cause cells completely self-degradation, activation of autophagy is beneficial or harmful, depending on the intracellular substrate load and autophagy mechanism, it somehow indicates the levels of autophagy determined the extent of cell survival or death [24-26]. The activation of autophagy after cerebral ischemia may use as a protection mechanism to clear or recycle damaged organelles, may also lead to pro-death process as the final disintegration of cells [27].

In addition, after the cerebral ischemia, the time of induce-autophagy determines its role, Ravikumar et al. [28], found that in ischemic preconditioning autophagy has protective effect, once the ischemia/reperfusion occurs, the autophagy effect will change. He et al. [29], found in studies that Autophagy has different effect at different stages of cerebral ischemia, at different stages, the time of intervention related to the level of autophagy. In short, cerebral ischemia can cause changes in the autophagy mechanism body, due to lack of specific methods to verify whether autophagy has a protective effect or pro-death effect, also the different ischemic models were used by researchers, the body autophagy with protective effect or injury effect remains highly controversial.

THE ROLE OF INFLAMMATORY RESPONSES IN THE PATHOGENESIS OF ISCHEMIC STROKE

Inflammatory response play an important role in the pathogenesis of ischemic stroke, but its mechanism affect is still not fully understood [30-32]. Once ischemic stroke occurred, inflammation response first activates microglia, thereby activating anti-inflammatory mediators and proinflammatory mediators, break the balance between proinflammatory and anti-inflammatory responses. Related research shows that in preclinical stroke inhibit the inflammatory response that could reduce brain damage and improve the outcome of neuron damage [33], while systemic inflammation will also
affect patient’s susceptibility to illness and prognosis after stroke [34,35]. However, in ischemic stroke, the inhibition of the inflammatory response will have an impact on brain repair and long-term function rehabilitation. Inflammatory response in ischemic stroke is also a double-edged sword. So to maintain dynamic equilibrium between proinflammatory and anti-inflammatory responses after the stroke is the key to reduce brain damage.

**Localized brain cell’s participation in ischemic stroke inflammation**

Microglia are the immune cells of the brain [36], they work as clear cells [37,38] in events of inflammatory, ischemic and neurodegenerative. Once cerebral ischemia occurred, microglia are quickly activated within minutes [39,40]. The activity and appreciation of microglia reach the peak in 2-3 days after the ischemic stroke and last for several weeks after the brain injury [37,38]. Some scholars believe that microglia has a dual role in ischemic stroke, microglia produce inflammatory mediators after activated and will cause cell damage and death, in the same time glia cells can also produce TGF-β 1Transforming Growth factor-beta (a neuroprotective effect [39]. The effect of microglia on nerve whether it is protective effect or damaging effect is mainly related to its time window at the time of activation; early phase activation will be harmful while later phase activation is beneficial [37]. In addition, the different microglia subpopulations have different roles in cerebral ischemia [40]. Astrocytes also play an important role in the pathological process of stroke [41]. The vast majority of astrocytes usually respond to the central regions of the brain injury within four hours, astrocyte’s reaction in ischemic stroke will sustainable 28 days [42]. Some scholars believe that the reaction of astrocytes are activated only in 24 hours after brain injury and reached the peak on the fourth day [43]. Astrocytes produce a series of inflammatory mediators [44,45], through phagocytes lot of histocompatibility complex and costimulatory molecules which can activate the inflammatory response, resulting in paralysis reaction [46]. Like microglial cells, astrocyte also has a dual role, inhibiting gial cell proliferation to improve neurological recovery [47].

**Important inflammatory mediator in ischemic stroke**

After brain injury, up regulated inflammation mediator and immune cell infiltrate in the pathophysiology of cerebral ischemia acts as a complex role. This includes a series of proinflammatory mediators and anti-inflammatory mediators; it has a different role in the occurrence and development of ischemic stroke.

**Proinflammatory mediators**: IL-1α and IL-1β, IL-1α and IL-1β knockout mice in the middle cerebral artery occlusion can reduce brain damage [48]. The main function of IL-1α and IL-1β was achieved by IL-1R1 and IL-1 R2 [49].

**ICAM-1** is one of the members of the immunoglobulin super family is a major proinflammatory cytokines, in stroke-related research, animal experiments showed that increased expression of ICAM-1 is related to the pathogenesis of ischemic lesions [50], while blocking or knockout ICAM-1 will reduce brain damage and improve stroke outcomes [51-54]. Clinical studies have shown that ICAM-1 is also closely associated with stroke; soluble ICAM-1 is up-regulated and reached a peak in acute ischemic stroke within 24 hours [55]. ICAM-1 also significantly up regulated in brain tissue after cerebral ischemia, but the real role of ICAM-1 in stroke still remained controversial [56].

Chemokines Monocyte chemo attractant protein-1 (MCP-1), macrophage inflammatory protein -1(MIP-1α) and chemokine (CX3CL1), these three are the most common proinflammatory chemokines, up regulated in cerebral ischemia animal models [57], ischemic chemokine up regulation is seen as deleterious effect [58]. Related studies have shown that MCP-1, MIP-1α, and CX3CL1 promote stroke pathological process; inhibiting or defect itself can reduce ischemic brain damage [59,60].

MMPs Previous studies showed that matrix metalloproteinases (MMPs) and neurogenic migration are related, inhibition of MMPs activity may reduce brain damage [61]. However, studies show that MMPs may be beneficial in the later phase of cerebral ischemia, with MMPs inhibitors in the treatment of middle cerebral arterial occlusion stroke, inhibiting nerve vascular remodeling and in ceased brain damage [62]. In different MMPs, MMP-9 has the closest relationship with brain damage [63]. When patients with acute ischemic stroke, MMP-9 regulated in brain tissue and serum [64], it is closely related to the damage of Blood Brain Barrier, edema promotion and ischemic stroke progress into hemorrhagic [65,66]. Knockout MMP-9 gene in the early stage of stroke can reduce brain damage [67], therefore, by inhibiting the release of activated MMP-9 through neutrophil, may be an effective treatment for reducing brain injury. In the later phase of stroke due to MMP-9 associated with many growth factors, which are closely related with angiogenesis, therefore it is also beneficial for stroke.

**Anti-inflammatory mediators**: IL-10 Interleukin-10 (IL-10) is an anti-inflammatory cytokine, increased in the brain tissue after a stroke [68-70], at the same time plays an important role in the pathogenesis of stroke. Related studies show that IL-10-deficient mice have wider range of brain damage in middle cerebral artery occlusion [71], in animal models, after increased IL-10, the level of brain damage was decreased after the stroke [72], clinical studies have shown that low levels of IL-10 would indicate increased risk of stroke [73,74].

**TGF-β mRNA** increased within 6 hours after stroke, the increasing levels last for 15 days after the stroke [75,76], transforming growth factor-β (TGF-β) has Anti-inflammatory and neuroprotective effect in stroke, which may become effective stroke treatment strategies. TGF-β can reduce ischemic damage and reduce the associated inflammation [77], TGF-β blockade will aggravate ischemic brain damage [78].

**TIEP2** (Tumor necrosis factor-α-induced protein B-like 2) is an anti-inflammatory protein, has an important role in maintaining the steady-state within the immune system [79,80], mice with knockout TIEP2 will induce multi-organ inflammation and splenomegaly, studies show that TIEP2 has high expression in cerebral ischemia, but also has a role in the pathogenesis of stroke, blocking TIEP2 in mice that with middle cerebral artery occlusion can increase infarct size, neurological disorders, inflammatory
cytokine expression, and inflammatory cell permeability [81].

IGF-1 In an animal model, insulin-like growth factors-1 (IGF-1) having a neuroprotective effect after a stroke and can reduce infarct size and improve cell survival [82,83] studies show that high levels of IGF-1 in serum during the early stage of ischemic stroke is closely related to neurological recovery and better functional outcome [84].

Inflammation plays an important role in ischemic brain stroke and other ischemic brain injury, more and more evidence indicate that inflammation has a double-edged sword effect. Inflammation is beneficial or harmful after ischemic stroke; it is likely to depend on different stages and internal environment after the stroke. Nevertheless, it still requires more research to clarify the role of inflammation during the development of stroke. How to make the pro-inflammatory cytokines and anti-inflammatory factor to reach the dynamic equilibrium at different stages of stroke is also the issue that needs an in-depth discussion.

THE ROLE OF AUTOPHAGY IN THE INFLAMMATORY RESPONSE OF ISCHEMIC STROKE

In recent years, evidence for autophagy plays an important role in the regulation of inflammation becomes more and more clear, autophagy affects the effector cells of non-specific and acquired immune mediate inflammatory response, the activation of the effector cells in these cells will affect the promotion of homologous immune defense and the inflammatory process responses [85-87]. Autophagy has the effect in connecting the non-specific immune system with the acquired immune system; autophagy dysfunction has some relevance with inflammation, infection and neurodegeneration [88,89]. Autophagy use the manner of understands endogenous excitatory inflammatory body to regulate the interaction effect of nonspecific immune signaling pathways, and by affecting the pathways that secreting from immune mediator to regulate the inflammatory [90-92].

Autophagy regulate inflammatory cytokines

The inflammatory response occurred in the surrounding area of brain injury, where the focal cerebral ischemia zone has significant increase of autophagic activities [93]. Early stage of cerebral ischemia, activated microglia, astrocytes, and the immersed immune cells release a large number of inflammatory cytokines, not only cause brain damage, but also affect the brain repair [94-96]. Related research development indicates that anti-inflammatory is an important treatment strategy to stroke [97,98]. However, anti-inflammatory treatment result for stroke patients in clinical is still a hot debating topic [99]. Recent studies have shown that use preconditioning treatment with autophagy inhibitor on animal models of either neonatal or adult mice with middle cerebral arterial occlusion can significantly reduce infarct size [100]. In related studies on mice with middle cerebral artery occlusion, the autophagy markers LC3-increased after 12 hours, the autophagy effect is also found in microglial cells; 12 hours, 48 hours, 72 hours after middle cerebral artery occlusion the TNF-α, IL-1β, IL-6 protein levels were significantly increased in mice in vivo. IL-1β, IL-6, TNF-α cause autophagic cell death [101,102]. Mice with cerebral artery occlusion take oral autophagy inhibitor will significantly reduce the inflammatory response and nerve damage; autophagy inducer will significantly promote inflammation and nerve damage. Indicated that autophagy is associated with inflammation of brain and brain damage [103]. It is believed that the autophagy in neurons of the ischemic mice may be the downstream target of inflammatory cytokines [104].

Autophagy regulate NF-kB pathway

Inflammation has a crucial role in the pathophysiology of ischemic stroke [105]. Ischemia activates NF-kB pathway and promote its DNA binding activity, NF-kB activation induced tumor necrosis factor-a (TNF-α) and interleukin-6 (IL-6) expression [106], which will serve as proinflammatory cytokines and will aggravate ischemic injury [107]. Microglia as cerebral partial inflammatory cells was also activated by NF-kB, at the same time through the secretion of proinflammatory cytokines, chemokines and adhesion molecules aggravate ischemic injury [108,109]. On the other hand the NF-kB regulates many inflammatory processes, and NF-kB's function is controlled by other processes. Beclin1 is a key component of the formation of autophagy; it is associated with the NF-kB pathway [110]. Previous studies indicate that autophagy regulates inflammation by activating NF-kB pathway [111]. NF-kB pathway and the central inflammation regulation are closely related [112]; NF-kB pathway is more complex, NF-kB promotes neuronal death in cerebral ischemia [113]. In cerebral ischemia NF-kB expression up regulated, the activation of NF-kB will become the agonists of TNF-α, IL6 - [114]. Inhibition of autophagy can reduce the activity of NF-kB pathway [115], blocking the formation of autophagy small bodies and inhibition of lysosomal proteases, will reduce IL8a and NF-kB target gene expression [116]. Therefore, by autophagy degradation and inhibiting the phosphorylation to regulate I KK - b activity [117]. The similarity such as TNF-α, IL-1β and other cytokines were regulated by autophagy and produced during the inflammatory process [118]. Beclin1, Beclin2 interacting protein is a key component in PI3K, it is very crucial during the formation of autophagic bodies [119]. In Jiang Y’s studies [115] believe that tetracycline inhibit ischemic brain tissue autophagic activity, on this basis further to suppress the inflammatory process by reducing NF-kB pathways.

Autophagy regulate other pathways

Recent studies have shown that autophagy pathway play an important role in the immunity and inflammatory. Fujishima et al. [120], demonstrated in intestinal epithelial cells autophagy reduces endotoxin which induced by inflammatory response. Lee et al. [121], studies have shown that autophagy negatively regulated the inflammatory response of keratinocytes through scaffolding proteins p62 / SQSTM1. Collar A [122] uses intraventricular injection of SB216763 on an animal model with ischemic injury, which significantly reduced the damage-induced inflammatory response, at the same time after the SB216763 treatment, the levels of autophagy significantly increased, SB216763 activates autophagy and inhibit the inflammatory response within the cultured microglia cells, the importance is after the SB216763 treatment, microglial cells inhibited the autophagy and increased inflammatory through Beclin1-siRNA. So it is believed that after ischemic injury, GSK-3 inhibitor
SB216763 increasing autophagy activity to inhibit damage-induced neuro inflammatory.

Therefore, by regulating autophagy to suppress inflammation may be the new effective strategies for the cerebral ischemia treatment that being seeking for.

CONCLUSIONS

Autophagy and inflammatory response come along with ischemic stroke occurrence and its development process. What kind of role that the autophagy and the inflammatory response should play in the pathophysiology of the ischemic stroke often depends on its autophagy and the degree of the inflammatory responses, as well as the occurrence of the disease, the stage of the development process and internal environment and other factors. Autophagy and inflammatory response act as a double-edged sword, with neuroprotective and neurotoxic dual roles. How to make autophagy and proinflammatory cytokines to reach a dynamic balance with anti-inflammatory cytokines at different stages of stroke are the issues that need an in-depth exploration, which are also the new therapeutic strategies for the ischemic stroke treatment.

AUTHORS’ CONTRIBUTIONS

Jun Wang, Jian Pei and Jie Chen herein have the same contribution.

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REFERENCES


17. Qin AP, Liu CF, Qin YY, Hong LZ, Xu M, Yang L, et al. Autophagy was activated in injured astrocytes and mildly decreased cell survival following glucose and oxygen deprivation and focal cerebral ischemia. Autophagy. 2010; 6: 738-753.


23. Kang C, Avery L. To be or not to be, the level of autophagy is the question: dual roles of autophagy in the survival response to starvation. Autophagy. 2008; 4: 82-84.


