1 Introduction

Ischemic stroke has a very complex pathophysiology, not only involving irreversible neuronal damage but also triggering cellular processes for neuronal repair. In addition to apoptosis and necrosis, two vital types of cell death in ischemic brain injury, autophagic cell death has recently been proposed as the third type of cell death in ischemic tissue. Under certain physiological or pathophysiological conditions, autophagy is a homeostatic degradative pathway for intracellular recycling and autodigestion of bulk proteins and aging organelles. It has been described as a physiological and dynamic process that is essential for cellular health and survival. Moderate autophagy protects cells against various stresses by providing them with free fatty acids and amino acids and removing damaged organelles. However, long-term up-regulation of autophagy can trigger cell death by excessive degradation of cellular contents. Recently, autophagy in cerebral ischemic injury has been increasingly reported, however its role in neuronal death is still controversial. With new focus on the mechanisms of autophagy, progress has been made in the use of traditional Chinese medicine (TCM) in adjusting autophagy. In this review, we summarize the role of autophagy in cerebral ischemia and the effects of TCM in modulating its activity.
in neuronal death after cerebral ischemia, and the effects of TCM on cerebral ischemia autophagy.

2 The process and the molecular basis of autophagy

Autophagy depends on the interaction of different complexes, each composed of several different autophagy-related (Atg) proteins. The complete cycle of autophagy is a highly regulated multi-step process which, in general, can be divided into four stages: initiation, vesicle nucleation, vesicle expansion and completion, and vesicle fusion and autophagosome degradation. Transmission electron microscopy is the predominant method for the differentiation of these phases, and diagnosis of the condition.

2.1 Initiation

Autophagy can be induced by a variety of stimuli (e.g., nutrient deprivation, hypoxia, cytokines, hormones and DNA damage). In most cases, it is associated with the mammalian target of rapamycin complex 1 (mTORC1)\(^{[8–11]}\). The yeast serine/threonine protein kinase Atg1 and its mammalian homologs, UNC-51-like kinase-1 (ULK1) and ULK2, play a critical role in initiation of autophagy\(^{[13,14]}\). Under nutrient-rich conditions, mTORC1 inhibits the initiation of autophagy by phosphorylating ULK1 (or ULK2) and hyperphosphorylating mAtg13; in nutrient-deprived conditions, mTORC1 dissociates from the ULK1 complex, freeing it to induce autophagy\(^{[8,9]}\).

2.2 Vesicle nucleation

The activation of the phosphatidylinositol 3-kinase (PI3K) complex is an essential step in vesicle nucleation\(^{[12]}\). In mammals, the PI3K complex is divided into three classes: I, II and III. Class I and III PI3K complexes function as negative and positive regulators of autophagy, respectively. The class III PI3K complex consists of Beclin-1 (a homolog of Atg6) and its mammalian homologs, Vps30-1 like kinase-1 (ULK1) and ULK2, play a critical role in initiation of autophagy\(^{[8–11]}\). Under nutrient-rich conditions, mTORC1 inhibits the initiation of autophagy by phosphorylating ULK1 (or ULK2) and hyperphosphorylating mAtg13; in nutrient-deprived conditions, mTORC1 dissociates from the ULK1 complex, freeing it to induce autophagy\(^{[8,9]}\).

2.3 Vesicle expansion and completion

Two ubiquitin-like conjugation systems, the Atg12-Atg5-Atg16-like 1 (Atg16L1) complex and the microtubule-associated protein 1 light chain 3 (LC3) (Atg8 homolog)-phosphatidylethanolamine (PE) conjugate are involved in vesicle expansion and completion\(^{[17,18]}\). The Atg12-Atg5-Atg16L1 directs the complex to autophagic isolation membrane. LC3 (Atg8 homolog)-PE converts the soluble Atg8 (mammalian LC3-I) into a membrane bound, autophagosome-associated form (mammalian LC3-II), which represents an important biomarker of autophagy. LC3-II, the lipidated form of microtubule-associated protein 1 LC, recruits lipid molecules to expand the autophagosome membrane\(^{[19,20]}\).

2.4 Vesicle fusion and autophagosome degradation

Vesicle fusion requires the involvement of lysosomal membrane proteins such as lysosomal-associated membrane protein 1 (LAMP-1), LAMP-2 and the small GTPase Rab7, but the mechanism is less well characterized\(^{[21,22]}\). After fusion, autophagosome degradation depends on a series of lysosomal/vacuolar acid hydrolases including cathepsins B, D and L. The breakdown products are released by lysosomal permeases back into the cytosol, where nutrients and energy are recycled.

3 Autophagy in cerebral ischemia

Autophagy can be subclassified into two major forms: basal and induced autophagy. Basal autophagy is considered a “housekeeping” process in neurons\(^{[23]}\), whereas induced autophagy may be a promising neuroprotective strategy in neurodegenerative diseases and ischemic stroke. Signaling pathways regulating autophagy activation in cerebral ischemia include PI3K III-protein kinase B (Akt)-mTOR, AMP-activated protein kinase (AMPK)-mTOR, Beclin-1-Bcl-2 complex, nuclear factor-κ B (NF-κB), mitogen-activated protein kinase (MAPK)-mTOR, peroxisome proliferator-activated receptor-γ (PPAR-γ)-Bcl-2/Bcl-xl, hypoxia-inducible factor 1 (HIF-1) α-adenovirus E1B 19 kD interacting proteins 3 (BNIP3)/p53 and the ERS signaling pathway\(^{[24,25]}\).

3.1 Activation of autophagy in cerebral ischemia protects neurons from death

Carloni et al\(^{[26,27]}\) reported that neonatal hypoxia-ischemia autophagy could be a part of an integrated pro-survival signaling pathway, which included the PI3K-Akt-mTOR axis. When the autophagic or the PI3K-Akt-mTOR pathway is interrupted, cells undergo necrotic cell death. Activation of autophagic pathways represented a potential protective mechanism in the early stage of the brain injury (at 24 h after hypoxia-ischemia). Buckley et al\(^{[28]}\) found that rapamycin (a pharmacological autophagy inducer) not only reduced lesion size by 44% and 50% in the permanent middle cerebral artery ligation (MCAL) and embolic clot middle cerebral artery occlusion (eMCAO) models, respectively, but also improved the neurological score and rate of survival, which may benefit from the induction of autophagy. Zhang et al\(^{[29]}\) found that autophagy was activated in the reperfusion phase, as revealed in both mice with MCAO and oxygen-glucose-deprived cortical neurons in culture. In contrast to permanent ischemia, inhibition of autophagy by 3-methyladenine (3-MA), bafilomycin A1, Atg7 knockdown or in atg5−/−MEF cells reinforced the brain and cell injury, prompted the release of cytochrome c and activated apoptosis after ischemia-reperfusion (I/R). Moreover, MitoTracker Red-labeled...
neuronal mitochondria increasingly overlapped with green fluorescent protein (GFP)-LC3-labeled autophagosomes during reperfusion. The mitophagy inhibitor Mdivi-1 aggrivated the ischemia-induced neuronal injury both in vivo and in vitro, indicating that the protective role of autophagy during reperfusion may be attributable to mitophagy-related mitochondrial clearance and inhibition of downstream apoptosis. Brain microvascular endothelial cell (BMVEC) injury followed by I/R is the initial phase of blood-brain barrier (BBB) disruption, which results in a poor prognosis for ischemic stroke patients. Lì et al. confirmed a beneficial effect of BMVEC autophagy on BBB integrity during I/R injury. LC3 and Beclin-1 are two pacemakers in the autophagic cascade. The autophagic protein p62 is selectively incorporated into autophagosomes through direct binding to LC3 and efficiently degraded by autophagy. The level of p62 inversely correlates with autophagic activity. Wang et al. showed that overexpression of phosphoribosyltransferase (Nampt) increased autophagy (LC3 puncta immunocytochemistry staining, LC3-II/Beclin-1 expression and autophagosome number) both in vivo and in vitro at 2 h after MCAO. This treatment also enhanced neuronal survival via regulating the tuberous sclerosis complex-2 (TSC2)-mTOR-S6K1 signaling pathway during cerebral ischemia. Sheng et al. demonstrated that both IPC and lethal oxygen and glucose deprivation (OGD) up-regulated the LC3-II expression and down-regulated p62 protein levels; IPC treatment ameliorated OGD-triggered cell damage in cortical neurons, whereas 3-MA (5–20 mmol/L) and bafilomycin A1 (75–150 nmol/L) suppressed the neuroprotection induced by IPC. Treatment with rapamycin at 50–200 nmol/L in vitro and 35 pmol/L in vivo 24 h before ischemia relieved endoplasmic reticulum (ER) stress and ischemia-triggered neuronal damage. Jiang et al. found that IPC activated AMPK and aroused autophagy in the brain, which was accompanied by a reduction of infract volume, neurological deficits and cell apoptosis after cerebral ischemia. That neuroprotection of IPC was abolished by compound C (an AMPK inhibitor) or 3-MA, indicated that AMPK-mediated autophagy contributed to the neuroprotection of IPC, and that AMPK was beneficial to stroke prevention and treatment. These studies suggested that autophagy might be a potential target for ischemic neuronal protection.

3.2 Activity of autophagy in cerebral ischemia can have negative effects

Kang and Avery proposed that the degree of autophagy was critical for the survival or death of cells: typical physiological levels of autophagy promote survival, whereas insufficient or excessive levels of autophagy promote death. Additionally, 24 h prior to reperfusion, 3-MA triggered a high rate of neuronal death. However, during 48–72 h of reperfusion, 3-MA markedly protected neurons from death after both primary cortical neurons and SH-SY5Y cells were exposed to OGD for 6 h and reperfusion (RP) for 24, 48 and 72 h, respectively. An increase in autophagy was indicated by the increased ratio of LC3-II to LC3-I and Beclin-1 expression. This increase in autophagy after exposure to OGD/RP was accompanied by increased autophagic cell death, which was reduced by the specific autophagy inhibitor, 3-MA. Autophagy activity was enhanced dramatically in the ischemic brains 3–7 d after injury in a rat model of neonatal cerebral hypoxia/ischemia as shown by increased punctate LC3 staining and Beclin-1 expression, suggesting that excessive activation of autophagy results in neuronal death in cerebral ischemia. Wang et al. showed that the levels of Beclin-1 and LC3-II were markedly increased in a time-dependent manner during the process of ischemia and reperfusion after 90 min of ischemia, while the protein kinases p70S6K and mTOR showed delayed inactivation after reperfusion. Treatment with 3-MA abolished autophagy and reduced the decline of mitochondria function during reperfusion. Qin et al. demonstrated that glial fibrillary acidic protein (GFAP) staining was decreased in the infarct brain areas 3–12 h following permanent MCAO (pMCAO). Cerebral ischemia or OGD induced autophagy in astrocytes. This was shown by the increased formation of autophagosomes and autolysosomes as well as the up-regulation of LC3-II, Beclin-1, LAMP2, and lysosomal cathepsin B proteins and the down-regulation of Bcl-2 in primary astrocytes. 3-MA inhibited the OGD-induced increase of LC3-II and decline of Bcl-2, attenuated the death of astrocytes, and increased the number of GFAP-positive cells and the protein levels of GFAP in the ischemic cortex core 12 h following pMCAO. These results suggested that activation of the ischemia- or hypoxia-induced autophagic/lysosomal pathway might at least partly contribute to ischemic injury of astrocytes. Xu et al. found that the expression of autophagic-related proteins such as LC3-II, Beclin-1, cathepsin-B and LAMP1 increased significantly in the ischemic cortex after cerebral I/R injury. Furthermore, increased punctate LC3 labeling and cathepsin-B staining occurred in neurons. Treatment with PPAR-γ agonist 15d-PGJ2 decreased not only autophagic-related protein expression in the ischemic cortex, but also the immunoreactivity of LC3 and cathepsin-B in neurons. 3-MA decreased LC3-II levels, reduced the infarct volume and mimicked some protective effect of 15d-PGJ2 against cerebral I/R injury. These results indicated that 15d-PGJ2 exerted neuroprotection through blocking neuronal autophagy. Cui et al. established the cell model of OGD for 6 h, and the rat model of ischemia by a transient two-vessel occlusion for 10 min. The results showed that LC3-II, Beclin-1 and PI3K III were increased accordingly, but cytotoxic...
Bcl-2 protein and cell survival were both decreased. The negative effects of OGD and I/R, including the formation of autophagosomes and autolysosomes, the up-regulation of LC3-II, Beclin-1 and class III PI3K expression and the down-regulation of Bcl-2 production, were all reversed by propofol. Zheng et al \[40\] found that intraperitoneal injection of melatonin could ameliorate rat brain injuries such as infarct size, neurological score, serum creatine kinase and lactate dehydrogenase content, as well as pyknotic-positive cells. Further studies revealed that the beneficial effects of melatonin arise from the inhibition of Beclin-1 and conversion of LC3, as well as the activation of PI3K/Akt pro-survival pathway. Gao et al \[41\] established a focal cerebral ischemic model, finding that autophagy is markedly induced with the up-regulation of LC3-II/Beclin-1 and down-regulation of p62 in the penumbra at various time intervals following ischemia. Ischemic post-conditioning (IPC), performed at the onset of reperfusion, simultaneously reduced infarct size, mitigated brain edema, inhibited the induction of LC3/Beclin-1 and reversed the reduction of p62.

4 Effects of TCM on autophagy after cerebral ischemia

4.1 TCM can reduce cerebral I/R injury by inducing autophagy

Panax ginseng (Renshen) is a highly valued medicinal herb in Eastern society, which has also been well accepted in the West over the past decades. Ginsenosides, a major group of phytoestrogens, are the main active ingredients of ginseng. Ginsenoside Rb1 (GRb1) is one of the most abundant ginsenosides in ginseng extract, accounting for 27%–42% of the total ginsenosides \[42\]. After being subjected to 1.5 h of MCAO, rats treated with GRb1 or nothing, were killed at 24 h after reperfusion. The results indicated that less infarct volume and more intact neuronal structure were observed in the GRb1 group. GRb1 also restored the elevation of LC3 and Beclin-1, which suggested that Beclin-1-induced autophagy

Qi medicine used for the treatment of ischemic diseases. Carthamus tinctorius L., a traditional Chinese herbal medicine used for the treatment of ischemic diseases. Qi et al \[45\] found that HSYA activated the Akt autophagy pathway in penumbra tissue, which occurred in neuronal-specific cells. Moreover, an inhibitor of the Akt autophagy pathway abolished HSYA-induced neuroprotection, after cerebral ischemia. HSYA may be a promising drug for the treatment of acute ischemic stroke. Huanglian Jiedu Decoction (HLJDD), an ancient antipyretic and detoxifying TCM formula, has been reported to have protective effect on ischemic stroke. A study revealed that HLJDD also notably elevated the levels of LC3, Beclin-1 and other autophagy-related genes (Atgs). This treatment also promoted the activation of extracellular signal-regulated kinases, Akt and 3-phosphoinositide-dependent kinase and inhibited the activation of mTOR, JNK, p38, phosphatase and tensin homolog (PTEN). HLJDD showed neuroprotective effects on ischemic stroke, at least in part due to its role in controlling autophagy via the regulation of MAPK signals \[46\]. Triptolide is one of the major active components of the traditional Chinese herb Tripterygium wilfordii Hook. f., and it has been reported to have potent anti-inflammatory and immunosuppressive properties. Bcl-2, an anti-apoptosis factor, has regulatory effects that interact with Beclin-1 and contribute to the regulation of autophagy and apoptosis. Bcl-2 can inhibit Beclin-1-induced autophagy \[47\]. Further, myeloid cell leukemia-1 (Mcl-1) is an antiapoptosis gene in the Bcl-2 family, whose inhibition promotes cell death. Yang et al \[48\] demonstrated that apoptosis was clearly increased after 24 h of MCAO, while autophagy was down-regulated. In the triptolide treatment group, which had reduced infarction areas, neuroprotection was realized through the down-regulation of apoptosis and up-regulation of autophagy. Immunoblotting analysis revealed increases in Beclin-1 and Mcl-1, as well as a decrease in mTOR in treated rats. The up-regulation of Beclin-1 and Mcl-1 and down-regulation of Bcl-2, caspase-3 and the Bcl-2/Beclin-1 ratio indicated that the neuroprotective effect of TP was a result of its interaction with autophagy and apoptosis pathways. Wang et al \[49\] showed that treatment with resveratrol significantly decreased mortality, neurological deficits, infarction volume and MDA levels while increasing SOD activity. Furthermore, neurocyste apoptosis was alleviated by resveratrol via the increased Bcl-2/Bax ratio, increased LC3II expression and a decreased number of TUNEL-positive neurocytes. It is suggested that resveratrol protection of brain tissue against
ischemic damage was related to the inhibition of apoptosis and induction of autophagy.

4.2 TCM can relieve cerebral I/R injury by inhibiting autophagy

Liu et al. found that β-asarone (the active ingredient in *Acorus gramineus* soland) had significant pharmacological effects on the central nervous system and could attenuate the autophagy in a dose-dependent manner. This action was proposed to operate through an initial decrease in JNK and p-JNK levels and increase in Bel-2 level, followed by β-asarone’s interference with the functions of Beclin-1 induced by MCAO. Further, pretreatment with β-asarone (20, 30, or 45 μg/mL) or the calcium channel antagonist nimodipine (10 μmol/L) significantly increased the cell viability and matrix metalloproteinase (MMP), and decreased Beclin-1 expression and [Ca\(^{2+}\)]i in OGD/R-treated PC12 cells. This finding suggested that β-asarone protected PC12 cells against OGD/R-induced injury partly due to attenuation of Beclin-1-dependent autophagy caused by decreasing [Ca\(^{2+}\)]i and increasing MMP [51]. Astragaloside (AST) is the main component of *Astragalus* and is commonly used in the prevention and treatment of cardiovascular and cerebrovascular diseases [52]. Astragaloside (AST) is the main component of *Astragalus* and is commonly used in the prevention and treatment of cardiovascular and cerebrovascular diseases [52]. Zheng et al. found that Weinaokang (WNK) (20 and 10 mg/kg, ig) or its active component bilobalide (10 and 5 mg/kg, ig) could enhance the production of mature neurons at the ischemic site and inhibit expression of autophagy-related gene Beclin-1. These actions reduced the neuronal injury induced by focal cerebral I/R, indicating that WNK and its active component, bilobalide, could prevent neuron autophagy and improve neurogenesis in ischemic peripheral areas. Liu et al. showed that Atg-5 and Beclin-1 protein and mRNA were significantly up-regulated in “stasis heat” syndrome rats with pMCAO; these markers were down-regulated, relative to the control, in groups receiving a moderate dose of Xijiao Dihuang Decoction or positive drug control. The expression of LC-3 was not obvious in the negative control group, but was significantly increased in model group, especially in the 6th day. Low-dose Xijiao Dihuang Decoction had no inhibitory effect, however, the inhibitory effect of the middle dose was better than high dose and reached its peak effectiveness on the 6th day. These findings suggested that Xijiao Dihuang Decoction could protect the brain by significantly reducing the expression of Atg-5 and Beclin-1 mRNA as well as inhibiting the expression of LC-3. Tetrahydrocurcumin (THC) is a major herbal antioxidant and anti-inflammatory agent. In order to determine THC effects in ameliorating autophagy during I/R injury by reducing homocysteinylation of cytochrome c in the hyperhomocysteinemia pathological condition, Tyagi et al. employed 8–10-week-old male cystathionine-β-synthase heterozygote knockout (CBS+/−) mice (genetically hyperhomocystemic mice). THC was injected intraperitoneally once daily for a period of 3 d after 30 min of ischemia. The results showed that brain edema and Evans Blue leakage were relieved in I/R+THC-treated groups relative to sham-operated groups; there was an accompanying decrease in brain infarct size. THC also alleviated oxidative damage and ameliorated the homocysteinylation of cytochrome c through activation of MMP-9, leading to autophagy in I/R groups in contrast to sham-operated groups. Guo et al. found that the combination of Rb1, Rg1, schizandrin and DT-13 (6:9:5:4, called SMXZF) significantly increased cerebral blood flow and reduced the infarct volume, brain water content and the neurological deficits in a dose-dependent manner. Similar to the positive control, SMXZF at 18 mg/kg also notably suppressed autophagosome formation. Immunofluorescence staining and Western blotting demonstrated that SMXZF could significantly decrease the expression of Beclin-1 and LC3; it also inhibited the phosphorylation of AMPK and mTOR, the expression of JNK and its subsequent phosphorylation. This study suggested that SMXZF displayed neuroprotective effect against focal I/R injury, possibly associated with autophagy inactivation through AMPK/mTOR and JNK pathways.

5 Conclusion and perspectives

In neurons, autophagy is important for homeostasis and protein quality control and is maintained at relatively low levels under normal conditions, while it is up-regulated in response to pathophysiological conditions, such as cerebral ischemic injury. Increasing evidence supports the notion that autophagy is a double-edged sword. The mechanisms of autophagy underlying cerebral ischemia and whether autophagy activity is really enhanced in cerebral ischemia should be further investigated. The use of TCM to modulate the role of autophagy may provide potential therapeutic strategies.

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7 Conflict of interests

There is not any actual or potential conflict of interests.

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