Review Article

Neuroprotective Effect of Electroacupuncture Against Acute Ischemic Stroke via PI3K-Akt-mTOR Pathway-Mediated Autophagy

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Abstract

Acupuncture has been used to treat ischemic stroke, and mounting evidence demonstrates the neuroprotective and autophagy-mediated actions of electroacupuncture (EA). This review summarizes the evidence that EA affects different stages of autophagy, its neuroprotective action, and effects of different acupoints in treating ischemic stroke. Systematic searches were conducted on English and Chinese databases (MEDLINE, Elsevier, ScienceDirect, Cochrane Library, and China National Knowledge Infrastructure). Studies published up until February 2021 were considered for inclusion, and a final of fifty articles were included in this review. There is evidence that acupuncture promotes neuroprotection by modulating autophagy, and the treatment effectiveness is related to the acupoint selection and timing of treatment administration.

Keywords: Acupuncture, autophagy, ischemic stroke, nerve regeneration, neuroprotection

Introduction

Stroke, one of the leading causes of death and disability, has significantly affected public health. Of the different types of strokes, ischemic stroke accounts for 60%–80% of all strokes, with escalating mortality rates over the years.[1-3] Alarmingly, about 795,000 people in the late 20s suffer from this disease annually.[1,4] Causes of ischemic stroke include thrombosis, embolism, systemic hypoperfusion, and cerebral venous sinus thrombosis.[5] These abnormalities deplete nutrient supply and damage the neuron cells, which then impair the neurological and motor function.[6] Ischemic stroke is further classified into small-vessel occlusion (20%–30%), large artery intracranial or extracranial atherosclerosis (30%–40%), and cardio-embolism (20%–30%).[1,2,7] The main effective treatment for acute ischemic stroke is to administer thrombolytic therapy within 4.5 h of development of symptoms. However, most patients miss this narrow time window for thrombolytic therapy and are left with limited life-saving treatment options.[3] Therefore, exploring novel therapeutic option for acute ischemic stroke has become the focus of current research worldwide.

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The optimum intervention time for ischemic stroke is within 60 min from the initial attack, the disease starts to deteriorate afterward, and autophagy is activated in the brain to maintain cellular homeostasis.[8] "Golden hour" which refers to the 60 min after the initial attack, has becoming a research interest owing to its high potential of recovery and prevention of cell death.[9] Autophagy serves as a double-edged sword; autophagy can save the neuron cells from cellular death but also cause fatalities.[10] During the early stage of reperfusion, autophagy still functions to clear the damaged cells or proteins. However, the continuous autophagy will cause excessive cell death. In the case of late management of ischemic stroke, the chance of recovering from the progressive ischemic stroke

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is hypothesized to <60 min. It has been proposed that the autophagy cell death mechanism takes over during this period and disrupts cell survival.[8]

Emerging evidence has suggested that autophagy is reversible, and regulating autophagy provides neuroprotection, making them a potential therapeutic target and strategy.[11,12] Autophagy is associated with 5' AMP-activated protein kinase (AMPK) pathway and markers, such as beclin-1 and light chain 3 (LC3). Studies suggested that acupuncture potentially regulates autophagy to prevent further progression of ischemic stroke.[13] In treating ischemic stroke, acupuncture evidently enhances cognitive activity, promotes endogenous neurogenesis, inhibits apoptosis, and improves inflammatory response.[13,14] In the field of traditional and alternative medicine, acupuncture has been an effective treatment, especially in treating pain and motor dysfunction.[15,16] Electroacupuncture (EA), a modified needling technique that applies frequent electrical stimulation, is more stable and effective than the conventional acupuncture.[17]

Providing EA stimulation to rats with cerebral ischemic injury has been shown to markedly restore the blood supply, decrease cerebral infarction, and improve neurological functions.[18] These findings are garnering global interest and corroborating acupuncture as an effective treatment option. This review summarizes the effects of acupuncture treatment to ischemic stroke and the reversal of autophagy function from causing autophagy-dependent apoptotic death of neuron cells to neuroprotection. Reversing the autophagy function can potentially treat ischemic stroke, preventing further deterioration of neuron cells that ultimately provides neuroprotection.

Literature Search Strategy

We performed a systematic search to identify studies applying EA to treat ischemic stroke. The published literature source is collected from the online journal databases – MEDLINE (PubMed), Elsevier, ScienceDirect, Cochrane Library, and China National Knowledge Infrastructure were searched from inception to Febuary 2021. The keywords used in the search were: "acupuncture," "electroacupuncture," "neuroprotection," "neuroprotective," "autophagy," "ischemic stroke," and "ischemia stroke." Studies that fullfiled the following criteria were included: (i) English language journals, (ii) studies conducted on ischemic stroke, (iii) studies that focused on neuroprotection mechanism of autophagy, and (iv) Using acupuncture or any other method of acupuncture as a primary method of intervention. We excluded studies meeting the following criteria: (i) studies that evaluated acupuncture for other conditions, (ii)published studies more than 5 years, (iii) applying Western or nonacupuncture treatment, and (iv) autophagy mechanism that are irrelevant to neuroprotective effect. The search found 1597 studies and screening through title and abstracts narrowed down to 1052 studies [Figure 1]. A total of 83 full-text review studies included were selected through. Of these collected, we excluded 33 studies that were published over 5 years and 50 publications met our inclusion criteria for this literature review.

Role of Autophagy in Ischemia Stroke

Autophagy is a regulated cellular mechanism that is vital under physiological and pathological conditions. This essential catabolic pathway maintains cell survival by eliminating damaged or aged cells, proteins, and organelles. Under stressful conditions, autophagy tries to restore cellular homeostasis. Prolonged stressed condition, however, will activate apoptosis.[19] Activated autophagy recruits lysosomes to initiate cellular degradation.[19,20] Sequentially from the induction of autophagy, phagophore is formed and autophagosome fuses with lysosome, and cellular components are eventually degraded and recycled. The PI3K-Akt-mTOR is one of the major pathways in autophagy, with possible outcomes of either neurodegeneration or neuroprotection.[21,22] Regulators such as AMPK, which is also a potential therapeutic target for ischemic stroke, regulates the inhibition of autophagy and mediates neuroprotection.[23] Such target protein is crucial in the autophagy mechanism and targeting it may hinder further neurological damage.

Figure 2 illustrates the autophagy signaling pathway in response to growth and stress signals. Previous studies have supported that targeting cell death pathway blocks the progression of ischemic stroke. The progression of the disease is reversed and neuroprotection mechanism is switched on. The neuroprotection mechanism secures neurons from further damage due to ischemia, especially during the healing period. [24] The PI3K/Akt/mTOR pathway is a crucial pathway to be targeted as it can affect the cell death pathway.[25,26] In response to nutrient-deprived stimuli, the generation of mammalian target of rapamycin complex1 (mTORC1) initiates autophagy mechanism by dissociating the Unc-51-like-kinase (ULK) complex.[27] Dephosphorylated ULK complex increases the levels of enzymes and proteins such as beclin-1 and P13K.[3,10,22,28] The interaction of ULK complex and beclin-1 inhibits autophagosome formation and degradation of cellular components.[10] During these stages, rapamycin functions as an mTOR mechanistic target to block against stimuli that promote the progression of ischemic stroke.[10,19,27] PI3K signaling and beclin-1 are essential for autophagosome formation; inhibiting PI3K signaling pathway can potentially provide neuroprotection. Another treatment strategy is to induce the interaction of anti-apoptotic Bcl-2 protein with beclin-1, resulting anti-apoptotic activity.[20,29-31] During ischemic stroke, the structures around glia cells, astrocytes, and neurons are primarily affected.[6] Autophagy is a complicated mechanism that involves many other molecular mechanisms. Autophagy induces a neuroprotective mechanism during the early stage of ischemic stroke,[3,32] and activated autophagy is detectable under electron microscopy.[10,32,33] The presence of LC3 proteins, particularly LC3-II and LC3-GFP (green fluorescent protein), indicates the autophagosome activity

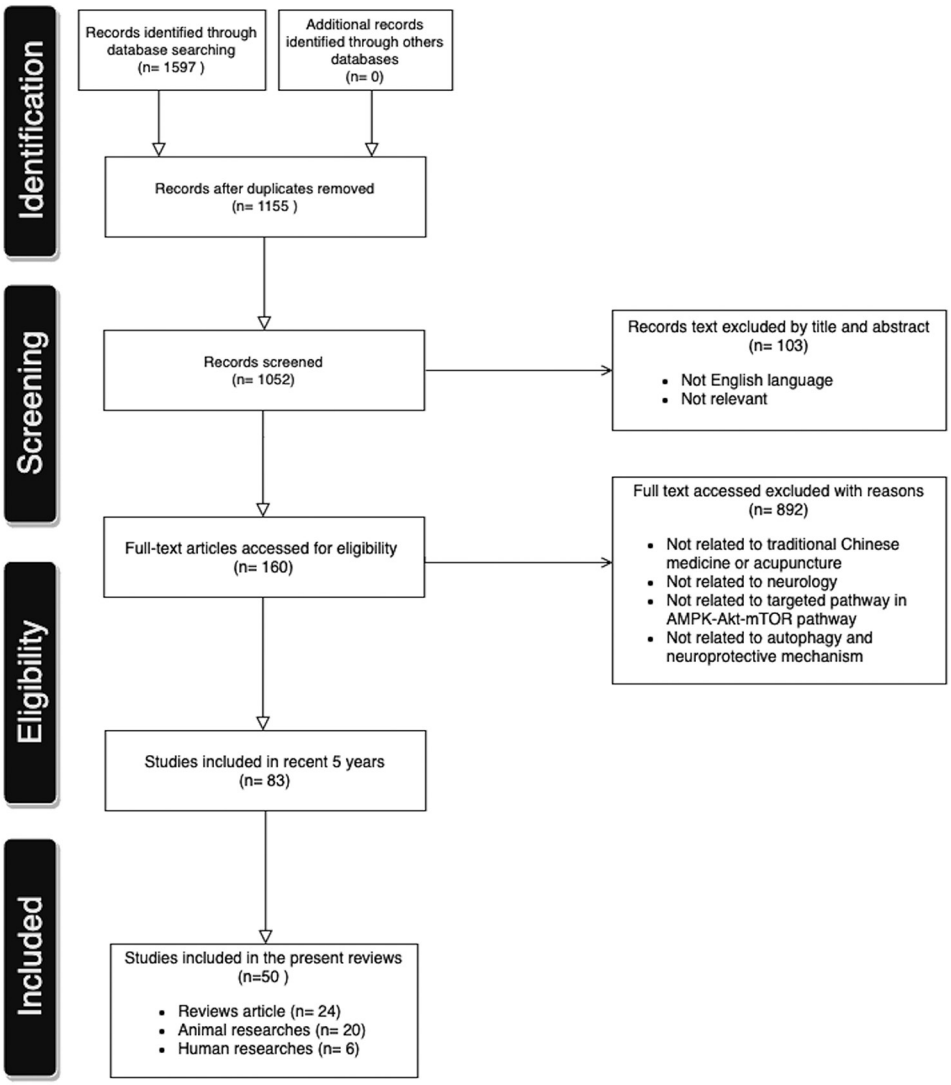

Figure 1: Study flow diagram

whereas intensifying fluorescent signals of LC3 are emitted from the primary neuronal cells.[28,32,34] LC3 proteins mediate the expansion of autophagosome membrane by conjugating with lipid, which then support further expansion of the membrane. Thus, LC3 protein is considered a biomarker that indicates macroautophagy activity.[10] However, during the pathological conditions, autophagosomes are formed, and axons accumulated, leading to cell death.[10,19]

Modulation of Autophagy for Neuroprotection

LC3 and beclin-1 are the two key regulators of autophagy. Beclin-1 involves in the initiation of autophagosomes and autolysosome formation, whereas the increased intensity of the LC3 signal indicates that autophagy activity is present.[19,35] Rapamycin, a mTOR selective inhibitor, activates autophagy and sequentially upregulates beclin-1 in the brain region, decreases necrotic cell death of neuron cells, and reduces damaged brain tissue.[3] Neuroprotective action of autophagy is best activated during the reperfusion stage.[36,37] Rapamycin downsized the lesion in the cerebral ischemic models, i.e., permanent middle cerebral artery ligation and embolic clot middle cerebral artery occlusion (eMCAO) models by 44% and 50%, respectively.[38] Rapamycin also improved the neurological functions and survival rate in both models.[28] Worthy to note, excessive activation of autophagy, in turn, causes cell death, especially under stress condition.[20]

In response to cerebral ischemic, autophagy and PI3K-AKT-mTOR pathways are activated to protect neuron cells from necrotic cell death. The neuroprotective autophagy pathway is initiated during the initial phase of the hypoxic-ischemic brain injury.[28] The first 60 min poststroke assault is crucial to alter the autophagy intracellular cell recycling mode and achieve high recovery rate.

The best timing to perform ischemic preconditioning (IPC) is when ischemic/reperfusion initiates, which then prevents autophagy cell death, and even mitigates the neuron cell

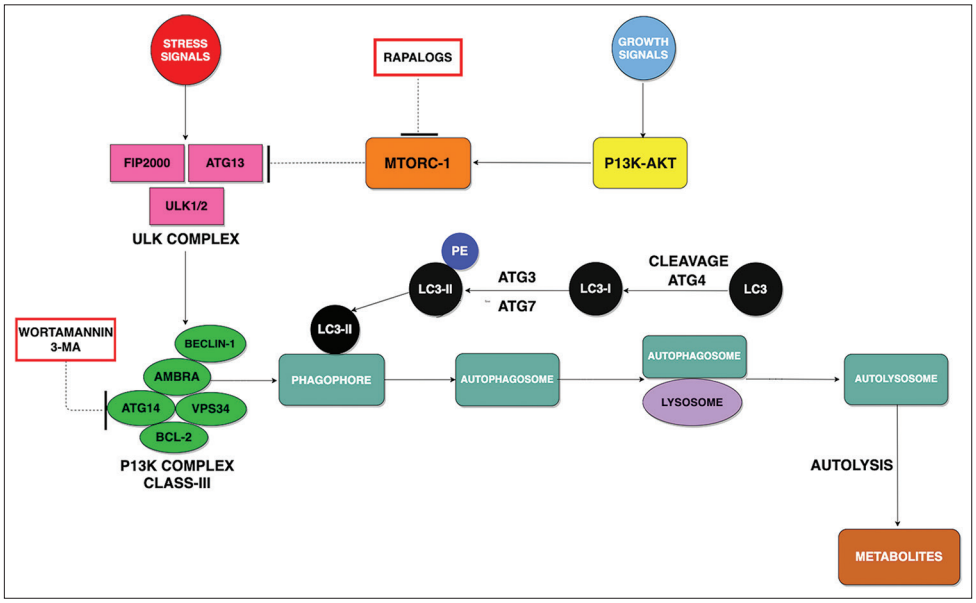

Figure 2: Activation of autophagy signaling pathway. In response to stress signal, autophagy mechanism will be activated. Dotted lines indicate the inhibition of specific autophagy-related pathways, which then activates the AMPK pathway. Beclin-1 and LC3 are the two autophagy-related proteins that can be examined to track the autophagy-related processes.

damage caused by oxygen-glucose deprivation (OGD).[3,28,32] IPC is a brief period of vascular occlusion that has a protection function against I/R injury. It has been an autophagy mediator of I/R injury to initiate protective function. The reperfusion stage begins after 48–72 h of cerebral ischemic assault, which is when the cell death program is activated and occurred at a high rate.[28] AMPK signaling is able to induce the neuroprotective autophagic pathway, particularly in the IPC stage, within the certain window period.[39] Autophagy activity is inversely proportional to the expression of LC3-II to LC3-I ratio and beclin-1.[3,39-41]

During the OGD stage, the progressive autophagy has triggered autophagy-dependent apoptosis of astrocytes, and the presence of apoptotic astrocytes indicates a low chance of recovery.[40,42] The increase in LC3-II, beclin-1, and PI3K III activities decreases neuronal cell survival.[28,32] With increased production of LC3-II protein, the level of p62 protein (an autophagy-specific substrate) is reduced.[42] During autophagy activity, p62 protein recognizes toxic cellular waste which localizes to the autophagosomes formation site, thus decreasing the free form of p62.[3,28] Recently, studies have shown melatonin can block the P13K pathway or alter to another pathway.[43] Beclin-1 converts LC3 into autophagosomes formation and activates PI3K/Akt prosurvival pathway. The activated PI3K survival pathway then reduces p62 expression, resulting in a decrease in infarction size, and saves the remaining brain cells.[37,43]

Acupuncture Regulates Pi3k-Akt-mTOR Signaling Pathway in Treating Ischemic Stroke

The central nervous system (CNS) is a sensitive part of the human body that requires a consistent supply of nutrients and energy. Researchers have corroborated the potentiality of neuroprotective autophagy in treating ischemic stroke. Treatment initiated at an early stage of the disease will give a greater effectiveness.[40] This prospective treatment promotes the survival of neuron cells, particularly the recovering neuron cells. The foundation of acupuncture practice is based on the theory of acupoints and meridians. From the perspective of traditional Chinese medicine, acupuncture applies point stimulations that promote the flow of Qi and treat disease, whereas in the EA technique, the required dosage of manipulations is provided through electrical stimulations to the acupoints. EA is advantageous as the frequency and intensity of the electrical stimulations are adjustable, allowing the required dosage to be administered. Although the mechanism of acupuncture or EA is still unclear, increasing evidence has indicated their remarkable therapeutic effects.[17] Comparatively, EA provides constant manipulations to the acupoints, which makes it more effective than the conventional acupuncture treatment. The positive therapeutic effect of acupuncture is evidenced in various diseases, such as neurological diseases, psychiatric disorders, infections, and pains.[17,30] During acupuncture treatment, needle insertion at specific acupoint triggers the affected nerve afferent fibers to send a signal to the brain. By manipulating and modulating the signaling pathways in the body, acupuncture facilitates in achieving homeostasis, and even exerts beneficial effect in life-threatening diseases such as ischemia stroke.[17,44]

Nutrient-deprived cells can activate either positive or negative pathway. For example, during an ischemic stroke, lack of nutrient supply causes autophagy to favor negative pathways. The game-changer of the autophagy survival signaling pathway is the chance of cell survival, from an uncontrolled and prolonged autophagy activity that leads to unwanted cell

death, termed as apoptosis.[19,45] The AMPK stage inhibits the activation of mTOR during autophagy initiation stage, which activates the mechanism. The autophagy mechanism is activated, especially within the first 24 h of IPC, and the cells undergo lysosome engulfment leading to a chance of survival.[8] All of the above activities are initiated and controlled by the activation of the autophagy mechanism, and acupuncture has been one of the means to modulate AMPK-PI3K-mTOR signaling pathways.[38,46] Rebalancing the energy metabolism requirements of tissue promotes tissue recovery, and particularly improves brain injury. Functional recoveries involving neurogenesis, angiogenesis, anti-apoptosis, and other recovery support mechanisms are observed following acupuncture treatment.[44]

In a study on Sprague Dawley rats with cerebral ischemia/reperfusion, a 3-day acupuncture treatment on LI-11 (Quchi) and ST-36 (Zusanli) for 30 min/day increased the protein levels of P13K and mTOR, which downsized the cerebral infarction.[13] Another study on the same rat population that applied 30 min/day acupuncture on GV-20 (Baihui) point for 5 days, also reported the reduction of cerebral infarction volume and LC3-II expression. Additionally, suppression of phosphorylated Akt and increase of mTOR expression were also observed.[47] These study findings indicated the activation of the neuroprotective autophagy pathway and protection from further brain injury.

The overexpression of the two key regulators of autophagy, beclin-1 and LC3-II, indicates the activation of autophagy mechanism.[44,48] Beclin-1 is vital in the apoptotic signaling in the autophagy mechanism. By assessing beclin-1, not only the autophagosome activity can be monitored, the severity and progression of ischemia stroke can be classified as well.[49] Another theory is to promote the cell survival pathway by maintaining the Bcl-2–beclin-1 complex. Bcl-2 is an anti-apoptotic protein that inhibits autophagic effects.[10,11] Elevated Bcl-2 expression is neuroprotective against apoptosis via the P13K/Akt pathway.[50] Providing 7-day acupuncture stimulation on GV-20 and GV-24 (Shenting) at 2 h after reperfusion, significantly increased the expression of beclin-1. In another case that started to apply stimulation on the same acupoints at 24 h after middle cerebral artery occlusion (MCAO), the mRNA expression of Bcl-2 mRNA was increased and beclin-1 was suppressed following 6 days of treatment.[51] These results demonstrated that administering acupuncture stimulation can prevent apoptotic activity, switch autophagy mechanism to neuroprotective mode, and stop further progression of ischemia stroke; with conditions, suitable acupoints are selected and stimulations are performed at right timing.

Overall, acupuncture therapy is recommended for the treatment of ischemic stroke, however, further studies are required to provide scientific evidence of the mechanism. In terms of ischemic stroke, EA therapy is effective in modulating autophagy mechanism and promoting neuroprotective function.[17] Primary acupoints are selected and stimulated, which eventually stop the progression of ischemic stroke as evidenced by the reduced cerebral infarction.[3,30] Understanding the acupoints and the right timing to provide acupuncture during the course of ischemic stroke will produce the desired therapeutic effects.[13,44,52] Table 1 summarizes the studies on acupuncture that support its potential in treating ischemic stroke. EA in particular has advantages in improving brain activity and disability movement.[53] This process increases cell survival during stress, and studies have shown that pharmacological targeting therapy is not the only option.[13,54]

The effects of acupuncture meridians and points on autophagy

The different locations of points in the meridians affect the pathway and release of growth factors differently. The mTOR/AMPK pathway is the main pathway that involves many apoptotic factors, such as Bcl-2, p53, NK-kB, P13K-Akt, and AMPK.[52-55] In addition, netrin-1 has been reported to inhibit autophagy via P13K/mTOR pathway. As angiogenic factor enhances the development of nervous and vascular system, the expression of netrin-1 is promoted, resulting in a reduction of infarct size and improvement of motor function.[56-58]

It is found that most acupoints for ischemic stroke therapy are mainly located in governor vessel, large intestine, and stomach meridian. The effective acupoints located on these channels [Tables 1 and 2] are commonly used in treating neurodegenerative diseases. In some cases, stimulation on these acupoints supports the inhibition of apoptosis and promotion of neurogenesis.[59] The brain cell injury benefits from acupoint stimulation by improving the size of the infarct, ameliorating brain function, and preventing further damage.[60] The recommended dosage of EA, 2 Hz electric stimulation for 20–30 min of treatment, has shown an effective outcome in the improvement of ischemic stroke symptoms. The combination of acupoints is selected and applied based on the patient's symptoms.[17]

Governor vessel meridian

GV-20 (Baihui) promotes neurogenesis as well as triggers the proliferation and differentiation of vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF).[50,61] VEGF and BDNF are the vital neurotrophic factors in the survival signaling of neural stem cells (termed as astrocytes). Astrocytes not only function to initiate proliferation but also to promote tissue repair.

Stimulation of BDNF causes neurogenesis in adults in concert with VEGF that acts as an angiogenic factor in angiogenesis.[58,59] GV-26 (Shuigou) is a common acupoint used to promote neurogenesis and modulate angiogenesis via angiotensin.[58,62] GV-14 (Dazhui) is another common acupoint to promote neurogenesis and promote anti-apoptotic mechanisms. Overall, stimulation on these acupoints improves the neurological and motor functions of the brain.[58,62]

A combination of two or more acupoints is usually applied to obtain more effective treatment. The common acupoint combinations GV-20 and GV-14 promote neurogenesis by

increasing the secretion of VEGF and BDNF.[58] The acupoint combination also raises the Bcl-2 expression, indicating that acupuncture can alter the apoptosis and autophagy activity. Promoting Bcl-2 expression and suppressing the Bcl-2/Bax protein has reversed the cell death apoptosis mechanism to favor the neuroprotection effect.[49,51]

Large intestine meridian

Recent studies suggest the vital role of acupoint LI-4 (Hegu) in cell proliferation and angiogenesis during the ischemic condition. Stimulation on LI-4 promotes the stem cell factor in peri-infarct tissue, followed by the expression of the

Table 1: Animal studies of electroacupuncture-mediated autophagy and applied acupoints in ischemic stroke

References	Model type	Treatment effects	Key findings	Country
Liu *et al*.[64]	MCAO/R, rats	LC3-II/LC3-I↓ Beclin-1 ↓ mTOR ↑ ULK1↓ ATG13↓, autophagosomes ↓ autophagolysosomes↓	EA neuroprotective effects of Quchi and Zusanli exert through (PI3K)/Akt signaling pathway by inhibiting autophagosome formation	China
Wu *et al*.[47]	MCAO, rats	mTOR ↑ p-mTOR↓	The EA pretreatment gives a neuroprotective effect of Baihui by regulation of mTOR-mediated signaling pathways	China
Ma *et al*.[52]	MCAO, rats	Bcl-2 ↑ Bax↓	Stimulation on Baihui and Siguan upregulates Bcl-2 and Bax gene which exerts a neuroprotective role	China
Shu *et al*.[49]	MCAO/R, rats	LC3-II/LC3-I↓ Beclin-1↓	Stimulation on Shuiguo acupoint affects the expression of beclin-1, and LC3-II	China
Ting *et al*.[13]	4-VO, rats	LC3↓ Beclin-1 ↓ mTOR↑	Neuroprotective effect through inhibition of autophagy overexpression using stimulation of Baihui, Mingmen, and Zusanli, analyzed by LC3-II, mTOR, and Beclin-1	China
Liu *et al*.[51]	MCAO, rats	Bcl-2 ↑ Bax↓ p38↓	Stimulation effects of Shenting and Baihui in inhibition of apoptosis, through measuring Bcl-2, Bax, p38, ERK1/2, and c-JNK	China
Huang *et al*.[55]	I/R group, rats	Bcl-2 ↑ Bax↓	The protective effect and decrease of apoptosis through Zusanli acupoint stimulation by affecting the expressions of Bcl-2 and Bax	China
Wu *et al*.[71]	I/R group, rats	LC3-II ↓ LC3-II/LC3-I↓ Autophagosomes ↓ p-AKT↑	The effect of Baihui stimulation on mTOR/AMPK/Akt signaling pathway leading to inhibition of autophagy and protective effect	China
Wang *et al*.[72]	MCAO/R, rats	p-mTOR ↑ LC3-II/LC3-I↓ p62 ↑ p-AKT↑ p-mTOR↑	Using Zusanli and Quchi to initiate a neuroprotective effect via P13K/Akt/mTOR signaling pathway. Finding shows a reduced in neuronal apoptosis and autophagy	China

MCAO/R: Middle cerebral artery occlusion/reperfusion, 4-VO: Four-vessel occlusion, JNK: Jun N-terminal kinases, ERK: Extracellular signal-regulated kinase, I/R group: Intestinal ischemia/reperfusion group, mTOR: Malian target of rapamycin, AMPK: 5'AMP-activated protein kinase, ATG: Autophagy-related genes, EA: Electroacupuncture, LC3: Light chain 3, PI3K: Phosphatidylinositol-3-kinase, ULK: Unc-51-like-kinase

Table 2: Biological responses induced by the acupoints and the involved signaling pathways

Reference	Acupoints	Results
Xing *et al*., 2018[59]	Hegu (LI-4)	Upregulate angiogenic factors (VEGF and angiogenin-1, LV, and DG)
	Quchi (LI-11)	Promotes neuronal cell proliferation
		Prevent apoptosis via PI3K/Akt pathways. Reduce ischemic brain damage, and improve metabolism
	Zusanli (ST-36)	Improves cognitive hippocampus function
		Stimulates neurogenesis, and exerts neuroprotective roles
	Baihui (GV-20)	Modulation of neurotransmission by increasing dopamine levels
		Improving memory via long-term potentiation recovery in the hippocampus
	Shuigou (GV-26)	Induce neurogenesis, and regulates angiotensin
		Exert an anti-apoptotic effect by activating Akt-mediated pathways
	Quchi (LI-11) Zusanli (ST-36)	Improves in motor function
		Upregulate PI3K/Akt pathway to promote neuronal cell proliferation
	Dazhui (GV-16) Baihui (GV-20)	Neurogenesis, by increased levels of neurotrophic factors (BDNF and VEGF)
		Release of vasodilative mediators
	Baihui (GV-20) Shenting (GV-24)	Improves the impaired function of motor control, and cognition via inhibition of NF-kB-mediated
	Shuigou (GV-26) Chengjiang (CV-24)	Produce anti-apoptotic effects, and modulate neurotransmission by reducing the expression of the NMDA receptor

VEGF: Vascular endothelial growth factor, BDNF: Brain-derived neurotrophic factor, PI3K: Phosphatidylinositol-3-kinase, LV: subventricular zone of the lateral ventricle, DG:dentate gyrus, NF-kB:nuclear factor kappa-light-chain-enhancer of activated B cells, NMDA:N-methyl-D-aspartate

angiogenic factors.[58] Another acupoint that is generally applied in ischemic stroke is LI-11 (Quchi), which has a role in neuronal cell proliferation. Both acupoints block the apoptosis and activate neuroprotective activity through PI3K/Akt pathways, which then improve cerebral function and the recovery of brain damage.[58,59]

Combining acupoint LI-11 and LI-4 is advantageous in promoting the activation of anti-apoptosis and angiogenesis. Similarly, the acupoint combination of LI-11 and ST-36 also induces anti-apoptotic effects via the P13K/Akt signaling pathway.[63,64] In addition, the activated PI3K/Akt pathway promotes neuroprotective effects through mediation of mTORC1, ULK complex, and beclin-1.[62,64] These proteins assist in regulating neurogenesis and angiogenesis, resulting in improvement of motor function. The inhibition of autophagy activity suppresses the expression of the ULK complex and beclin-1 from upregulating protective proteins,[58,64] which then block the apoptosis activity and reduce the apoptotic cells.[64] Suppression of autophagy activity reduces the number of autophagosomes accumulated, causing an inefficient fusion and degradation.[54] Noteworthy, the secretion of BDNF and VEGF is also affected, resulting in increase of cell proliferation, angiogenesis, and cell survival of the brain cells.[58,59] These growth factors upregulate endogenous neurogenesis and support the recovery of brain injury during ischemic stroke.[44,62]

Stomach meridian

ST-36 is another acupoint generally applied to treat various diseases and distinctly improves cognitive hippocampus function. Regulating autophagy pathways not only promotes neurogenesis but also initiates neuroprotection with an anti-apoptotic effect.[58,59] To optimize treatment outcome, it is necessary to combine ST-36 with another acupoint, such as LI-11. In treating ischemic stroke, the combination ST-36 and LI-11 promotes proliferation in the ischemic tissue, impairs apoptosis, promotes neurogenesis, and modulates autophagy via PI3K/Akt and ERK1/2.[64] A significant increased level of mTORC-1 and ULK complex provides neuroprotection effect and affects the autophagosome formation[54] due to the decrease of Atg4 expression that prevents the conversion of LC3B-I to LC3B-II.[44] In addition, stimulation on ST-36 upregulates Bcl-2 and minimizes the brain damage during ischemic injury.[59] A recent study revealed the impact of neuroprotection on proliferation of astrocyte; the presence of astrocytes supports the recovery of neurological function.[41]

Electroacupuncture modulates autophagy and provides neuroprotection

Previous studies have provided evidence that acupuncture effectively treats ischemic stroke by preventing further cell destruction and switching autophagy action to neuroprotective. [65,66] However, the clear mechanism of acupuncture affecting the autophagy mechanism in ischemic stroke is yet to be explored. Table 1 shows that the evidence of EA modulates autophagy via the PI3K-mTOR signaling pathway and AMPK, with improvement in ischemic stroke.[13,23,54] Table 2 summarizes the selections and combinations of acupoints and their effects on ischemic stroke. Preclinical studies are generally performed in Sprague Dawley rats, with a chosen acupoints showing significantly improved cerebral function.[58,59]

Initiation stage

A drastic change in AMP/ATP ratio leads to the activation of AMPK, which acts as an energy monitor.[23,33] The activated AMPK then promotes autophagy by inhibiting of mTOR mechanism, resulting in dephosphorylation of Atg13 and ULK1 and formation of ULK1 complex to initiate autophagy.[13,18,67] Recent findings have shown that EA has an effect on autophagy mechanism and maintaining homeostasis. Following 24 h after reperfusion, treatment consisting of 30 min/day of EA on MCAO for 3 days significantly improves the recovery of neurological deficits and cerebral infarct volume.[13,59] To treat ischemic stroke, EA treatment was provided on acupoints LI-11 and ST-36 at a frequency of 1–20 Hz.[13] The expression of Atg13 and ULK1 was reduced while the levels of mTOR were increased, indicating that these acupoints affect the autophagy mechanism.[13,59] In conclusion, EA impairs autophagosome formation that leads to the stimulation of neuroprotective effect.[68]

Another study performed conventional acupuncture and EA on I/R-injured rats using the GV-20 acupoint for 5 days, with each session lasting for 30 min. GV-20 acupoint has been the main acupoint when treating most neurological disease, including ischemic stroke.[47,61] Posttreatment symptoms were reduced in severity, especially in EA-treated mice, and a significant increase of p-mTOR expression inhibited the autophagy. Moreover, the expressions of p53 and LC3-II were also affected.[47]

Nucleation stage

This stage involves many proteins and mechanisms, such as beclin-1, PI3K Class III, and autophagy-related gene (Atg) proteins. Beclin-1 is the main protein in signaling and promoting the nucleation of autophagy. The expressions of beclin-1 and LC3 spike during brain injury and decrease significantly posttreatment, which also affect the Bcl2 family protein.[13,51,55] Positive outcomes of using acupuncture to treat ischemia stroke are resulted from high expressions of mTOR and beclin-1, and low level of LC3 that inhibited the cell death pathway.[62,65]

An EA study on Sprague Dawley rats with cerebral infarct/reperfusion observed the upregulation of mTOR expression and suppression of beclin-1 and LC3 at the end of the study.[13] EA was applied on GV-20, GV-4 (Mingmen), and ST-36 acupoints for 20 min at three different time-points, i.e., 12, 24, and 48 h postreperfusion.[13] The study findings indicated cerebral infarct size decreased with activation of autophagy protective effect.[13,69] Another study on MCAO-induced model rat applied EA on GV-26 at a frequency of 2-20 Hz for 30 min. Rats that were treated at 24 and 48 h postreperfusion showed a significant reduction of cerebral infarct size at

the end of treatment. Compared to the cerebral ischemia/reperfusion (CI/R) control group, the expression of beclin-1 was suppressed in the EA-treated rats.[49] Other EA treatment regimens that had been tested on MCAO rats were applying 30 min of EA on GV-20 and GV-24 (Shenting) at 1–20 Hz for 14 days. At day 6 of treatment, the expression of Bcl-2 was evidently increased and beclin-1 complex was impaired, which then suppressed the formation of autophagy vesicle.[51,70]

Expansion and maturation stage

The most important protein in this phase is LC3 because of the support functions in promoting and cleaving of the protein interaction. In order to extend the double membrane and prepare for autophagosome maturation, the transformed LC3-II attaches to the vesicles.[9,10] Some studies have shown that EA can inhibit the cleavage of LC3 and block the autophagosome formation.

A number of EA preclinical ischemic stroke studies were performed on MCAO rats and provided EA at different acupoints. Stimulation of GV-20 for 30 min/day for 5 continuous days had decreased the LC3-II/LC3-I ratio, promoted protective effect, and influenced p-mTOR.[49,71] Another study applied 30 min/day EA on LI-11 and ST-36 acupoints at 1–20 Hz for 3 days, with emphasis on initiating EA at 24 h postreperfusion injury. The LC3-II/LC3-I ratio was significantly improved after 3 days of EA treatment.[64] Compared to administering EA at 24 and 72 h postreperfusion injury, applying 30 min of EA on GV-26 acupoint at 6 h postreperfusion injury clearly reduced the LC3-II/LC3-I ratio.[49] Overall, these findings suggest that EA impairs autophagosome maturation and formation, which significantly decreases the ratio and cerebral infarct volume.

Fusion of autophagosomes stage

In this stage, mature autophagosomes fuse with lysosomes. When autophagolysosomes are formed, their double-layer membranes are distinctly visible, making them an ideal biomarker.[21,44] The inhibited fusion activity increases the number of autophagosome, leading to the degradation of lysosome. Studies on EA show that certain acupoints inhibited autophagosome fusion. A model of MCAO and reperfusion rats applied 1–20 Hz stimulation on LI-11 and ST-36 for 30 min/day. Autophagolysosomes and lysosomes significantly decreased after 3 days of treatment.[13]

Degradation stage

The last stage of autophagy depends on p62, a protein that helps binding of ubiquitinated proteins to the C-terminal that interacts with LC3-II. The whole process leads to a complex formation until ready for degradation within the autophagolysosomes.[21,44]

A study on cerebral ischemic-induced MCAO rats showed that EA disrupted the degradation in autophagy by needling GV-26 for 30 min/day. The tested time to perform EA was at 6, 24, or 72 h after reperfusion, and p62 expression was significantly reduced in 6-h and 24-h groups.[49] Compared to the 72-h group, the p62 protein expressions were similar between the baseline and posttreatment. Based on the posttreatment effect, the study concluded that EA contributes to the improvement of neurological deficits.[49,65] In addition, a study on ST-36 and LI-11 exerts a neuroprotective function with inhibited expression of p62. This study was conducted on a MCAO/R rat applied for 30-min EA treatment for 3 days.[72] These findings show the ability of p62 suppression and its protective effect.

Discussion

In brief, autophagy involves clearing the dysfunctional or aged organelles to maintain cellular homeostasis. The role of autophagy in the human body is to sustain the stability of chromosomes, counteract apoptosis, regulate the immune system and host defenses. When autophagy mechanism is impaired, diseases such as neurodegenerative diseases, tumors, chronic inflammation, steatohepatitis, and muscle damage may develop.[24] In recent years, stroke has been the major global disability, with approximately 87% of all strokes caused by cerebral vessels' blockage.[2] Hypoxic condition and progressive autophagy that occur in stroke, cause neurons to degrade.[1,2,10] The concept of acupuncture is to promote neuroprotection by modulating autophagy mechanisms, but further investigation on the underlying mechanisms is required. EA therapy has been shown to promote the recovery of ischemic cerebrovascular disease effectively.[8,41,73] Applying constant electrical stimulation through the needles increases the efficacy of therapy. PI3K/Akt/mTOR pathway not only regulates cellular apoptosis but also modulates growth and proliferation. Through this pathway, EA blocks the autophagy mechanism, which then improves CNS function and reduces brain injury.[59] Markers such as ULK1 complex, LC3, beclin-1, and other autophagy markers facilitate the evaluation of autophagy activity levels and indicate the efficacy of acupuncture treatment.[28,32] As a result, these markers support the effectiveness of acupuncture therapy in modulating the autophagy mechanism.[41,74,75]

Previous studies have shown that EA treatments are able to modulate the autophagy pathway. However, to optimize EA treatment outcome, certain factors are required to be considered when providing EA treatment. Factors such as the acupoint selection, treatment duration and frequency, wave type, and intensity of electrical stimulation can also affect the treatment result. The above-discussed studies showed that among various factors and groups of ischemic injury, a pretreatment and reperfusion at the first 24-h group resulted in significant recovery from cerebral infarction [Table 1]. The upregulation of P13K/Akt-mTOR and beclin-1 decreases the level of LC3 in the brain. Acupoint selection has a significant role as different points have different benefits [Table 2]. These biomarkers help in monitoring the progress of the therapy and functions of the acupoints. Although animal studies have shown the benefits of EA treatment, EA may exert similar or different effects on human. The studies included in this review highlighted the time of ischemic injury or reperfusion as the essential key to

have high recovery from an injury, particularly in obtaining the neuroprotection activity and neurogenesis.

Table 2 summarizes the selections of common acupoints to treat ischemic stroke. GV-20, GV-24, GV-26, LI-11, and ST-36 are the commonly used acupoints as they can modulate the autophagy mechanism, while many other acupoints boost the treatment effect. Additionally, researches have shown that acupuncture effectively treats ischemic stroke. A traditional Chinese medicine classical book recorded the Shuigou (GV-26) as a popular acupoint to treat strokes since ancient times.[49] The history record showed that this acupoint has unique functions and benefits to treat stroke. The historical evidence demonstrated the credibility of the acupoint's function as the intervention method. The combinations of acupoints are also essential in significantly improving the recovery of brain injury, as the suitable combinations regulate the neurogenesis, blood flow, and neuroprotection from cell deaths. Therefore, the selection of acupoints is crucial in determining the efficacy of the treatment. Acupuncture is one of the good mediators in altering autophagy mechanisms to allowing neuroprotection and prevention of apoptotic mechanisms.

Conclusions

Acupuncture is effective in treating ischemic stroke, by inhibiting autophagy and producing neuroprotective effects. Compared to Western medicine, acupuncture requires more time to produce significant treatment outcome and improvement.[76] Acupuncture is a safer option with fewer side effects, especially for long-term treatment duration. However, acupuncture requires further research and development due to the lack of evidence on detailed mechanism. The ideal treatment window and other factors of acupuncture treatment may result in better treatment outcome and higher survival of neuron cells. The selection of acupoints is crucial to determine the results and effects of autophagy and neuroprotection.

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Conflicts of interest

There are no conflicts of interest.

References

1. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, *et al*. Heart Disease and Stroke Statistics—2021 Update: A Report From the American Heart Association. Circulation. 2021 Jan 27;143(8). https://doi.org/10.1161/CIR.0000000000000950.
2. World Health Organization. Cardiovascular Diseases (CVDs) [Internet]. who.int. World Health Organization: WHO; 2019 [cited 2020 Oct 22]. Available from: https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds).
3. Hou K, Xu D, Li F, Chen S, Li Y. The progress of neuronal autophagy in cerebral ischemia stroke: Mechanisms, roles and research methods. Journal of the Neurological Sciences. 2019;400:72-82.https://doi.org/10.1016/j.jns.2019.03.015.
4. Gattringer T, Posekany A, Niederkorn K, Knoflach M, Poltrum B, Mutzenbach S, *et al*. Predicting Early Mortality of Acute Ischemic Stroke. 2019;50(2):349-56.https://doi.org/10.1161/STROKEAHA.118.022863
5. Adams RJ, Chimowitz MI, Alpert JS, Awad IA, Cerqueria MD, Fayad P, *et al*. Coronary Risk Evaluation in Patients With Transient Ischemic Attack and Ischemic Stroke. Circulation. 2003 Sep 9;108(10):1278–90. https://doi.org/10.1161/01.CIR.0000090444.87006.CF.
6. Yang Z, Zhong L, Zhong S, Xian R, Yuan B. Hypoxia induces microglia autophagy and neural inflammation injury in focal cerebral ischemia model. Experimental and Molecular Pathology. 2015;98(2):219-24. https://doi.org/10.1016/j.yexmp.2015.02.003.
7. Khoshnam SE, Winlow W, Farzaneh M, Farbood Y, Fathi Moghaddam H. Pathogenic mechanisms following ischemic stroke. Neurological Sciences. 2017;38.https://doi.org/10.1007/s10072-017-2938-1.
8. Wang Y, Reis C, Applegate R, Stier G, Martin R, Zhang JH. Ischemic conditioning-induced endogenous brain protection: Applications pre-, per- or post-stroke. Experimental Neurology. 2015;272:26-40.https://dx.doi.org/10.1016%2Fj.expneurol.2015.04.009.
9. Advani R, Naess H, Kurz MW. The golden hour of acute ischemic stroke. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine. 2017;25.https://doi.org/10.1186/s13049-017-0398-5.
10. Wang P, Shao B-Z, Deng Z, Chen S, Yue Z, Miao C-Y. Autophagy in ischemic stroke. Progress in Neurobiology. 2018;163-164:98-117. https://doi.org/10.1016/j.pneurobio.2018.01.
11. Luo Y, Tang H, Li H, Zhao R, Huang Q, Liu J. Recent advances in the development of neuroprotective agents and therapeutic targets in the treatment of cerebral ischemia. European Journal of Medicinal Chemistry. 2019;162:132-46.https://doi.org/10.1016/j.ejmech.2018.11.014.
12. Ayuso MI, Gonzalo-Gobernado R, Montaner J. Neuroprotective diets for stroke. Neurochemistry International. 2017;107:4-10.https://doi.org/10.1016/j.neuint.2017.01.013.
13. Ting Z, Jianbin Z, Luqi H. Protective effect of electroacupuncture on neurons autophagy in perfusion period of cerebral ischemia. Neurosci Lett. 2017;661:415.https://doi.org/10.1016/j.neulet.2017.06.043.
14. Dong Q, Lin X, Shen L, Feng Y. The protective effect of herbal polysaccharides on ischemia-reperfusion injury. International Journal of Biological Macromolecules. 2016;92:43140.https://doi.org/10.1016/j.ijbiomac.2016.07.052.
15. Xiaoxi L, Xuezhu Z, Kun N, Yujie J, Jing L, Zhenzhen L, et al. Effect of electro-scalp acupuncture on acute ischemic stroke: a randomized, single blind, trial. Journal of Traditional Chinese Medicine. 2018;38(1):95-100.https://doi.org/10.1016/j.jtcm.2018.02.012.
16. Bibak B, Teymouri M, Mohammadi A, Haghbin A, Pan Y, Kasaian J, *et al*. Tongluojiunao, a traditional Chinese medication with neuroprotective ability: A review of the cellular, molecular and physiological mediators of TLJN's effectiveness. Biomedicine & Pharmacotherapy. 2019;111:485-95.https://doi.org/10.1016/j.biopha.2018.12.082.
17. Organization WH. Acupuncture: Review and Analysis of Reports on Controlled Clinical Trials [Internet]. Google Books. World Health Organization; 2002. Available from: https://books.google.com/books?id=vHU0DgAAQBAJ&printsec=frontcover#v=onepage&q&f=fal. [cited 2020 Oct 24].
18. Chen F, Qi Z, Luo Y, Hinchliffe T, Ding G, Xia Y, *et al*. Non-pharmaceutical therapies for stroke: Mechanisms and clinical implications. Progress in Neurobiology. 2014;115:24669.https://doi.org/10.1016/j.pneurobio.2013.12.007.
19. Hayat MA. Chapter 1 - Introduction to Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection and Aging, Volumes 1–4. In: Hayat MA, editor. Autophagy: Cancer, Other Pathologies, Inflammation, Immunity, Infection, and Aging. Amsterdam: Academic Press; 2014. p. 1-32.https://doi.org/10.1016/B978-0-12-405530-8.00001-7.
20. Ma X, Li L, He T, Brown MA, Zhong Q. Chapter 2 - Overview of Autophagy and Its Molecular Regulation. In: Ren J, Sowers JR,

Zhang Y, editors. Autophagy and Cardiometabolic Diseases. London: Academic Press; 2018. p. 19-27.https://doi.org/10.1016/B978-0-12-805253-2.00002-X.
21. Parzych KR, Klionsky DJ. An overview of autophagy: morphology, mechanism, and regulation. Antioxid Redox Signal. 2014;20(3):460-73. https://doi.org/10.1089/ars.2013.5371.
22. Yu L, Chen Y, Tooze SA. Autophagy pathway: Cellular and molecular mechanisms. Autophagy. 2018;14(2):207-15.https://doi.org/10.1080/15548627.2017.1378838.
23. Jiang S, Li T, Ji T, Yi W, Yang Z, Wang S, *et al*. AMPK: Potential Therapeutic Target for Ischemic Stroke. Theranostics. 2018;8(16):4535-51.https://dx.doi.org/10.7150%2Fthno.25674.
24. Maday S. Mechanisms of neuronal homeostasis: Autophagy in the axon. Brain Research. 2016;1649:143-50.https://doi.org/10.1016/j.brainres.2016.03.047.
25. Ge Y, Huang M, Yao Y-m. Autophagy and proinflammatory cytokines: Interactions and clinical implications. Cytokine & Growth Factor Reviews. 2018;43:3846.https://doi.org/10.1016/j.cytogfr.2018.07.001.
26. Hawkins PT, Stephens LR. PI3K signalling in inflammation. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. 2015;1851(6):882-97.https://doi.org/10.1016/j.bbalip.2014.12.006.
27. Oh WJ, Jacinto E. mTOR complex 2 signaling and functions. Cell Cycle. 2011;10(14):230516. https://doi.org/10.4161/cc.10.14.16586.
28. Huang X-p, Ding H, Lu J-d, Tang Y-h, Deng B-x, Deng C-q. Autophagy in cerebral ischemia and the effects of traditional Chinese medicine. Journal of Integrative Medicine. 2015;13(5):289-96.https://doi.org/10.1016/S2095-4964(15)60187-X.
29. Aroor AR, Jia G, Sowers JR. Chapter 1 - Overview of Autophagy and Cardiometabolic Syndrome. In: Ren J, Sowers JR, Zhang Y, editors. Autophagy and Cardiometabolic Diseases.London: Academic Press; 2018. p. 3-17.https://doi.org/10.1016/B978-0-12-805253-2.00001-8.
30. Ravanan P, Srikumar IF, Talwar P. Autophagy: The spotlight for cellular stress responses. Life Sciences. 2017;188:53-67.https://doi.org/10.1016/j.lfs.2017.08.029.
31. Tan F, Cao B, Zhan J, Lai P. Mechanism underlying treatment of ischemic stroke using acupuncture: transmission and regulation. Neural Regeneration Research. 2021;16(5):944.https://doi.org/10.4103/1673-5374.297061.
32. Wang P, Miao CY. Chapter 12 - Autophagy and Stroke. In: Ren J, Sowers JR, Zhang Y, editors. Autophagy and Cardiometabolic Diseases. London: Academic Press; 2018. p. 13748.https://doi.org/10.1016/B978-0-12-805253-2.00012-2.
33. Sun Y, Zhu Y, Zhong X, Chen X, Wang J, Ying G. Crosstalk Between Autophagy and Cerebral Ischemia. 2019;12(1022).https://doi.org/10.3389/fnins.2018.01022.
34. Huang L, Chen C, Zhang X, Li X, Chen Z, Yang C, *et al*. Neuroprotective Effect of Curcumin Against Cerebral Ischemia-Reperfusion Via Mediating Autophagy and Inflammation. Journal of molecular neuroscience: MN. 2018;64(1):129-39.https://doi.org/10.1007/s12031-0171006-x.
35. Klionsky D, Abdel-Aziz A, Abdelfatah S, Abdellatif M, Abdoli A, Abel S et al. Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)1. Autophagy. 2021;17(1):1-382.https://doi.org/10.1080/15548627.2020.1797280.
36. Li Y, Zhong W, Jiang Z, Tang X. New progress in the approaches for blood–brain barrier protection in acute ischemic stroke. Brain Research Bulletin. 2019;144:4657.https://doi.org/10.1016/j.brainresbull.2018.11.006.
37. Bu Q, Liu X, Zhu Y, Liu Y, Wang Y. w007B protects brain against ischemia–reperfusion injury in rats through inhibiting inflammation, apoptosis and autophagy. Brain Research. 2014;1558:100-8.https://doi.org/10.1016/j.brainres.2014.02.034\.
38. Xing Y, Zhang M, Wang M, Feng Y, Dong F, Zhang F. The Anti-apoptosis Effect of Single Electroacupuncture Treatment via Suppressing Neuronal Autophagy in the Acute Stage of Ischemic Stroke Without Infarct Alleviation. Frontiers in Cellular Neuroscience. 2021;15.https://doi.org/10.3389/fncel.2021.633280\.
39. Gustafsson ÅB, Mentzer RM. Chapter 9 - Autophagy: An Endogenous Survival Mechanism and Cardioprotective Response to Ischemic Stress. In: Gottlieb RA, editor. Autophagy in Health and Disease. Saint Louis: Academic Press; 2013. p. 141-57.https://doi.org/10.1016/B978-0-12-3851017.00009-7.
40. Liu Z, Chopp M. Astrocytes, therapeutic targets for neuroprotection and neurorestoration in ischemic stroke. Progress in Neurobiology. 2016;144:10320.https://doi.org/10.1016/j.pneurobio.2015.09.008.
41. Tao J, Zheng Y, Liu W, Yang S, Huang J, Xue X, *et al*. Electro-acupuncture at LI11 and ST36 acupoints exerts neuroprotective effects via reactive astrocyte proliferation after ischemia and reperfusion injury in rats. Brain Research Bulletin. 2016;120:1424.https://doi.org/10.1016/j.brainresbull.2015.10.011.
42. Zhou S, Qiao B, Chu X, Kong Q. Oxymatrine attenuates cognitive deficits through SIRT1mediated autophagy in ischemic stroke. Journal of Neuroimmunology. 2018;323:136-42. https://doi.org/10.1016/j.jneuroim.2018.06.018.
43. Ramos E, Patiño P, Reiter RJ, Gil-Martín E, Marco-Contelles J, Parada E, *et al*. Ischemic brain injury: New insights on the protective role of melatonin. Free Radical Biology and Medicine. 2017;104:32-53.https://doi.org/10.1016/j.freeradbiomed.2017.01.005.
44. Huang Y-G, Tao W, Yang S-B, Wang J-F, Zhigang M, Feng Z-T. Autophagy: Novel insights into therapeutic target of electroacupuncture against cerebral ischemia/ reperfusion injury. Neural Regeneration Research. 2019;14:954.https://dx.doi.org/10.4103%2F1673-5374.250569.
45. Napoletano F, Baron O, Vandenabeele P, Mollereau B, Fanto M. Intersections between Regulated Cell Death and Autophagy. Trends in Cell Biology. 2019;29(4):32338.https://doi.org/10.1016/j.tcb.2018.12.007.
46. Xiao Y, Chen W, Zhong Z, Ding L, Bai H, Chen H et al. Electroacupuncture preconditioning attenuates myocardial ischemia-reperfusion injury by inhibiting mitophagy mediated by the mTORC1-ULK1-FUNDC1 pathway. Biomedicine & Pharmacotherapy. 2020;127:110148.https://doi.org/10.1016/j.biopha.2020.110148.
47. Wu ZQ, Cui SY, Zhu L, Zou ZQ. Study on the Mechanism of mTOR-Mediated Autophagy during Electroacupuncture Pretreatment against Cerebral Ischemic Injury. Evid Based Complement Alternat Med. 2016;2016:9121597. doi: 10.1155/2016/9121597. Epub 2016 Jul 28. PMID: 27547233; PMCID: PMC4980529..
48. Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, *et al*. Distinct Roles of Autophagy in the Heart During Ischemia and Reperfusion. Circulation Research. 2007;100(6):91422. https://doi.org/10.1161/01.RES.0000261924.76669.36.
49. Shu S, Li C-M, You Y-L, Qian X-L, Zhou S, Ling C-q. Electroacupuncture Ameliorates Cerebral Ischemia-Reperfusion Injury by Regulation of Autophagy and Apoptosis. Evidence-Based Complementary and Alternative Medicine. 2016;2016:1-8.http://dx.doi.org/10.1155/2016/7297425.
50. Li W, Ye A, Ao L, Zhou L, Yan Y, Hu Y, *et al*. Protective Mechanism and Treatment of Neurogenesis in Cerebral Ischemia. Neurochemical Research. 2020;45(10):2258-2277.https://doi.org/10.1007/s11064-020-03092-1.
51. Liu J, Wang Q, Yang S, Huang J, Feng X, Peng J, *et al*. Electroacupuncture Inhibits Apoptosis of Peri-Ischemic Regions via Modulating p38, Extracellular Signal-Regulated Kinase (ERK1/2), and c-Jun N Terminal Kinases (JNK) in Cerebral Ischemia-Reperfusion-Injured Rats. Med Sci Monit. 2018;24:4395-404.https://doi.org/10.12659/MSM.908473.
52. Ma R, Yuan B, Du J, Wang L, Ma L, Liu S, *et al*. Electroacupuncture alleviates nerve injury after cerebra ischemia in rats through inhibiting cell apoptosis and changing the balance of MMP9/TIMP-1 expression. Neurosci Lett. 2016;633:158-64.https://doi.org/10.1016/j.neulet.2016.09.033.
53. Xu Q, Yang J-W, Cao Y, Zhang L-W, Zeng X-H, Li F, *et al.* Acupuncture improves locomotor function by enhancing GABA receptor expression in transient focal cerebral ischemia rats. Neurosci Lett. 2015;588:88-94. https://doi.org/10.1016/j.neulet.2014.12.057.
54. Xue X, You Y, Tao J, Ye X, Huang J, Yang S,*et al*. Electro-acupuncture at points of Zusanli and Quchi exerts anti-apoptotic effect through the modulation of PI3K/Akt signaling pathway. Neurosci Lett. 2014;558:14-9.https://doi.org/10.1016/j.neulet.2013.10.029
55. Huang Z, Han Y-m, Hong X-p, Duan Y-j, Chen T, Chen J-r. Protective effect of electroacupuncture preconditioning at zúsānlǐ (足三里 ST36) on mitochondria in the intestinal ischemia/reperfusion injury. World

Journal of Acupuncture - Moxibustion. 2018;28(3):191-7.https://doi.org/10.1016/j.wjam.2018.08.006.
56. Dai S-H, Chen T, Li X, Yue K-Y, Luo P, Yang L-K, *et al*. Sirt3 confers protection against neuronal ischemia by inducing autophagy: Involvement of the AMPK-mTOR pathway. Free Radical Biology and Medicine. 2017;108:345-53.https://doi.org/10.1016/j.freeradbiomed.2017.04.005.
57. Tang T, Gao D, Yang X, Hua X, Li S, Sun H. Exogenous Netrin-1 Inhibits Autophagy of Ischemic Brain Tissues and Hypoxic Neurons via PI3K/mTOR Pathway in Ischemic Stroke. Journal of Stroke and Cerebrovascular Diseases. 2019;28(5):133845.https://doi.org/10.1016/j.jstrokecerebrovasdis.2019.01.032.
58. Chavez L, Huang S, MacDonald I, Lin J, Lee Y, Chen Y. Mechanisms of Acupuncture Therapy in Ischemic Stroke Rehabilitation: A Literature Review of Basic Studies. International Journal of Molecular Sciences. 2017;18(11):2270.https://doi.org/10.3390/ijms18112270.
59. Xing Y, Zhang M, Li W, Dong F, Zhang F. Mechanisms Involved in the Neuroprotection of Electroacupuncture Therapy for Ischemic Stroke. Frontiers in Neuroscience. 2018;12.https://dx.doi.org/10.3389%2Ffnins.2018.00929.
60. Li X, Wang Q. Acupuncture Therapy for Stroke Patients. International Review of Neurobiology. 2013;:159-179.https://doi.org/10.1016/B978-0-12-411545-3.00008-0.
61. Jittiwat J. Laser Acupuncture at GV20 Improves Brain Damage and Oxidative Stress in Animal Model of Focal Ischemic Stroke. Journal of Acupuncture and Meridian Studies. 2017;10(5):324-30.https://doi.org/10.1016/j.jams.2017.08.003
62. Lai H-C, Chang Q-Y, Hsieh C-L. Signal Transduction Pathways of Acupuncture for Treating Some Nervous System Diseases. Evidence-Based Complementary and Alternative Medicine. 2019;2019:1-37. https://doi.org/10.1155/2019/2909632.
63. Lu H, Wang Z, Gan Y, Zhang C. Study on timing and dosage of acupuncture in stroke treatment. World Journal of Acupuncture - Moxibustion. 2018;28(2):1214.https://doi.org/10.1016/j.wjam.2018.06.007.
64. Liu W, Shang G, Yang S, Huang J, Xue X, Lin Y, *et al*. Electroacupuncture protects against ischemic stroke by reducing autophagosome formation and inhibiting autophagy through the mTORC1-ULK1 complex-Beclin1 pathway. International Journal of Molecular Medicine. 2015;37(2):309-318.https://doi.org/10.3892/ijmm.2015.2425.
65. Han X, Bai L, Sun C, Niu X, Ning Y, Chen Z, *et al*. Acupuncture Enhances Communication between Cortices with Damaged White Matters in Poststroke Motor Impairment. Evidence-based complementary and alternative medicine: eCAM. 2019;2019:4245753. https://doi.org/10.1155/2019/4245753.
66. Fu C, Zheng Y, Lin K, Wang H, Chen T, Li L, *et al*. Neuroprotective effect of apigenin against hypoxic-ischemic brain injury in neonatal rats via activation of the PI3K/Akt/Nrf2 signaling pathway. Food & Function. 2021;12(5):2270-2281. https://doi.org/10.1039/D0FO02555K.
67. Peña-Oyarzun D, Bravo-Sagua R, Diaz-Vega A, Aleman L, Chiong M, Garcia L, *et al*. Autophagy and oxidative stress in non-communicable diseases: A matter of the inflammatory state? Free Radical Biology and Medicine. 2018;124:6178.https://doi.org/10.1016/j.freeradbiomed.2018.05.084.
68. Xing Y, Zhang M, Wang M, Feng Y, Dong F, Zhang F. The Anti-apoptosis Effect of Single Electroacupuncture Treatment via Suppressing Neuronal Autophagy in the Acute Stage of Ischemic Stroke Without Infarct Alleviation. Frontiers in Cellular Neuroscience. 2021;15.https://doi.org/10.3389/fncel.2021.633280\.
69. Luo D, Chen R, Liang F. Modulation of Acupuncture on Cell Apoptosis and Autophagy. Evidence-Based Complementary and Alternative Medicine. 2017;2017:1-11.https://doi.org/10.1155/2017/8268736
70. Feng X, Wang H, Liu F, Li R, Wan M, Li J, *et al*. Electroacupuncture improves learning and memory functions in a rat cerebral ischemia/reperfusion injury model through PI3K/Akt signaling pathway activation. Neural Regeneration Research. 2021;16(6):1011.https://doi.org/10.4103/1673-5374.300454.
71. Wu ZQ, Cui SY, Zou ZQ. Cerebral protection by electroacupuncture pretreatment: a mechanism via autophagy involving the mTORC1 pathway. Int J Clin Exp Pathol [Internet] 2017. Available from: http://www.ijcep.com/files/ijcep0048006.pdf. [cited 2020 November 28].
72. Wang M, Zhang M, Feng Y, Xing Y, Tan Z, Li W, *et al*. Electroacupuncture Inhibits Neuronal Autophagy and Apoptosis via the PI3K/AKT Pathway Following Ischemic Stroke. Frontiers in Cellular Neuroscience. 2020;14.https://doi.org/10.3389/fncel.2020.00134.
73. Patel RAG, McMullen PW. Neuroprotection in the Treatment of Acute Ischemic Stroke. Progress in cardiovascular diseases. 2017;59(6):542-8. https://doi.org/10.1016/j.pcad.2017.04.005.
74. Kim YR, Kim HN, Jang JY, Park C, Choi YH, Shin HK, *et al*. Electroacupuncture confers beneficial effects through ionotropic glutamate receptors involving phosphatidylinositol-3 kinase/Akt signaling pathway in focal cerebral ischemia in rats. European Journal of Integrative Medicine. 2012;4(4):e413-e20.https://doi.org/10.1016/j.eujim.2012.06.004.
75. Yu B, Xing Y, Zhang F, Moss M. The Therapeutic Effect of Electroacupuncture Therapy for Ischemic Stroke. Evidence-Based Complementary and Alternative Medicine. 2020;2020:1-9.https://doi.org/10.1155/2020/6415083.
76. Farooqui A. Potential Neuroprotective Strategies for Ischemic Injuries. Ischemic and Traumatic Brain and Spinal Cord Injuries. 2018;:89-154. https://doi.org/10.1016/B978-0-12-813596-9.00003-.