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INVITED REVIEW

# Mechanisms of Acupuncture Therapy for Cerebral Ischemia: an Evidence-Based Review of Clinical and Animal Studies on Cerebral Ischemia

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**Abstract** Ischemic stroke is a major cause of mortality and disability worldwide. As a part of Traditional Chinese Medicine (TCM), acupuncture has been shown to be effective in promoting recovery after stroke. In this article, we review the clinical and experimental studies that demonstrated the mechanisms of acupuncture treatment for cerebral ischemia. Clinical studies indicated that acupuncture activated relevant brain regions, modulated cerebral blood flow and related molecules in stroke patients. Evidence from laboratory indicated that acupuncture regulates cerebral blood flow and metabolism after the interrupt of blood supply. Acupuncture regulates multiple molecules and signaling pathways that lead to excitoxicity, oxidative stress, inflammation, neurons death and survival. Acupuncture also promotes neurogenesis, angiogenesis as well as neuroplasticity after ischemic damage. The evidence provided from clinical and laboratory suggests that acupuncture induces multi-level regulation via complex mechanisms and a single factor may not be enough to explain the beneficial effects against cerebral ischemia.

**Keywords** Cerebral ischemia · Acupuncture · Neuroprotection · Recovery enhancement

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## Introduction

Ischemic stroke is a major cause of mortality and disability worldwide (Strong et al. 2007; Mukherjee and Patil 2011). Although many advances have been made in pharmacotherapy and interventional approaches, most of them failed at making the successful transition into cilinical setting, such as excitatory amino acid antagonists, free radical scavenger and endovascular treatment (Muir and Lees 2003; Shuaib et al. 2007; Kidwell et al. 2013). On the basis of the complexity of biochemical events in cerebral ischemia, most neuroprotectants only focus on a single cascade of pathophysiological events that lead to ischemic injury. And the over-specification may in part explain the failure in the clinical studies (Stroke Therapy Academic Industry 1999). It is suggested that cerebral ischemia may be approached with multiple, multifaceted neuroprotective methods (Doyle et al. 2008).

As a part of Traditional Chinese Medicine (TCM), the practice of acupuncture for the treatment of cerebral ischemia can be traced back to ancient china and it is still prevalent worldwide nowdays. The therapeutic effects of acupuncture for cerebral ischemia have been studied both in the clinic and in laboratory. A large number of reviews provided evidence in support of acupuncture as a candidate in improving rehabilitation and lowering the stroke recurrence rate (Li et al. 2015c; Shih et al. 2015; Xin et al. 2015). A systematic review and meta-analysis also showed that acupuncture indeed protected against experimental stroke with the performances of reducing infarct volume and improving neurological function scores (Wang et al. 2014).

This review mainly focuses on the mechanism of acupuncture on cerebral ischemia, including clinical and animal studies. Firstly, we focus on discussion about the progress in the mechanism of acupuncture clinical studies on cerebral stroke. Then we review findings from animal cerebral ischemic

models that have revealed the therapeutic mechanism of acupuncure. Together, these studies present an overall picture of bioactive chemicals and signal transduction pathways that involve in the beneficial effects of acupuncture.

## Evidence from Clicical Studies

### Acupuncture Activated Related Brain Regions in Stroke Patients

Over the recent years, neuroimaging techniques have become widely recognized as the newly acquired technology in stroke studies. Diffusion tensor imaging (DTI) images indicated that stroke patients with acupuncture treatment showed higher apparent diffusion coefficient (ADC) and fractional anisotropy (FA) (Shen et al. 2012). Functional magnetic resonance imaging (fMRI) studies illustrated the functional substrate of the therapeutic effects of acupuncture in stroke patients, and the features of the neural mechanism of acupuncture (Huang et al. 2013; Li et al. 2015b). Acupuncture noticeably inhibited sensation-associated cortex- Brodmann area (BA) 5 (Qi et al. 2014), activated motor cortex (Schaechter et al. 2007) and the somatosensory cortex in stroke patients with somatosensory deficits (Li et al. 2006). It also regulated the sensorimotor network in the ipsilesional hemisphere, increased the cooperation of bilateral sensorimotor networks and promoted the changes of synchronization between the cerebellum and cerebrum in patients with ischemic stroke in the left basal ganglia (Chen et al. 2014). Besides, stroke patients demonstrated significantly attenuated connectivity between cortical and subcortical areas during passive motor task owing to the inefficient information transmissions caused by ischemic cerebral damage. Acupuncture enhanced the connectivity between the cerebellum and the primary sensorimotor cortex, which could compensate for the inefficient effective connectivity (Xie et al. 2014). Moreover, acupuncture enhanced interregional interactions between the anterior cingulate cortex (ACC) and the posterior cingulate cortex (PCC), the regions that involved in memory and cognitive processing. In post-stroke aphasia patients, acupuncture treatment activated the language-related brain areas, including word generation (WG), frontal, temporal, parietal, occipital lobes and insula, which confer the therapeutic benefits of acupuncture (Chau et al. 2010; Li and Yang 2011; Chang et al. 2017). PET-CT images indicated patients with acupuncture stimulation showed more activation in BA13, 19 and 47, where are the cortical association-, motor execution- and vision-related cerebral regions in the healthy hemisphere. Acupuncture also activated the limbic system in the stroke-affected hemisphere (Huang et al. 2012).

### Acupuncture Regulated Blood Flow in Stroke Patients

Restoration of blood flow to the damaged brain region after stroke has a vital impact on better rehabilitation outcome. Stroke patients treated with acupuncture showed increased mean flow velocity (MFV), which indicated acupuncture may be a useful therapy for enhancing cerebral blood flow during post-stroke rehabilitation (Ratmansky et al. 2016). After acupuncture treatment, the pulsatile component of the microcirculatory blood flow (MBF) increased in stroke patients, while MBF variability as well as blood-flow resistance dreased at local vascular beds on the effected side. The local regulation mechanism may attribute to the suppressed sympathetic neural activity (SNA) (Hsiu et al. 2011). Moreover, acupuncture induced the blood-pressure-harmonic-variability responses, including increases in C5, C6 and decreases in CV5, CV7 on the stroke-affected side, while no significant changes were found on the contralateral side (Hsiu et al. 2013). Acupuncture also decreased plasma Endothelin (ET), the potent vasoconstrictor peptide which could lead to cerebrovascular dysfunction via modulating endothelial nitric oxide synthase (eNOS) phosphorylation and NO production (Yang et al. 2007; Faraco et al. 2013). Furthermore, acupuncture reduced hemorheological parameters, such as the index of erythrocyte aggregation, blood sedimentation, the index of erythrocyte rigidity. And acupuncture also accelerated blood flow in the nail fold microcirculation, decreased erythrocyte aggregation and blood lipid (Liu 2004).

### Acupuncture Modulated Relevant Molecules

In acute stroke patients, serum proteins such as matrix metalloproteinase 9 (MMP9), D-dimer are significantly higher, indicating that the serum proteins could be the biomarkers for the diagnosis of strokes or serve as surrogate markers. A proteomic analysis showed electroacupuncture (EA) modulated the expression of multiple serum proteins in acute ischemic stroke patients. Acupuncture down-regulated serum SerpinG1 and up-regulated the expression of serum complement component I, C3, C4B and Beta-2-glycoprotein I (APOH), which could contribute to the removal of apoptosis cells. In addition, EA also enhanced gelsolin expression, a secreted protein function as controlling cytoskeletal rearrangement. That may contribute to the beneficial effects of EA on motor rehabilitation in acute stroke patients (Pan et al. 2011). Circulating endothelial progenitor cells (EPCs) are involved in the restore of ischemic cerebral tissue. The increase of EPCs is associated with better neurological improvement in patients after acute ischemic stroke (Pias-Peleteiro et al. 2016). EA increased the number of EPCs and the level of plasma vascular endothelial growth factor (VEGF) in patients with stable ischemic stroke (Lee et al. 2015).The contents of thromboxane (TXB2) and prostaglandin 6-Keto-PGF1a in the blood are both the pair of

physiological antagonists. The increase of TXB2 and the decrease of 6-K-P are associated with the aggregation of blood platelets and the formation of thrombus. Acupuncture significantly decreased the plasma levels TXB2 and increased the 6-K-P, indicating that acupuncture could regulate blood circulation in stroke patients (Zhang 2010). Acupuncture reduced the levels of serum high-sensitivity C-reactive protein (hs-CRP) and pro-inflammatory cytokines, such as TNF-α, IL-6 and IL-1β in stroke patients (Wang et al. 2016a).

## Evidence from Experimental Studies

### Acupuncture Regulated Blood Flow and Metabolism

The changes of blood flow during cerebral ischemia exhibit a nonlinear decline. After the occlusion, there is an overshoot of blood flow owing to the compensatory expansion or the contraction of small artery and blood capillary smooth muscle, and then followed by a gradual decline. The early application of acupuncture was proved to be useful for ameliorating blood supply, deferring the descent of blood flow in ischemic region (Gao et al. 2002; Hsieh et al. 2006; Zhou et al. 2013b). Besides, acupuncture was also responsible for blunting the hyperaemia and promoting the recovery of blood flow during reperfusion (Pang et al. 2003; Choi et al. 2010; Zhou et al. 2011). Induced blood supply might attribute to the modulation of acupuncture on renin-angiotensin system (RAS). EA restrained the expression of Angiotensin II and AngII type 1 receptor (AT1R), as well as the downstream Gq and CaM in ischemic hemisphere tissue, which resulted in vasodilatation. Meanwhile, EA increased the expression of AngII type 2 receptor (AT2R) which could enhance cerebral blood flow (CBF) and reduce ischemic volume by mediating the angiogenic effect of AngII (Li et al. 2014). Acupuncture also regulated cerebral blood flow via the modulation on the cholinergic system in cerebral ischemia. Central cholinergic system is susceptible to the process of ischemia, during which the synthesis of acetylcholine (ACh) is impaired (Hartig et al. 2002). EA stimulation increased ACh release and mAChR M3 expression in cerebral cortex. Furthermore, the perfusion responses stimulated by acupuncture were abolished in eNOS KO mice, suggesting the effects of EA were eNOS-dependent (Kim et al. 2013a).

The beneficial effects of acupuncture can attribute to the regulation on energy metabolism. A study using microPET imaging technology reported that acupuncture caused the increase of glucose (Liu et al. 2013b). Other studies demonstrated that acupuncture increased the activities of hexokinase, pyruvate kinase, and glucose 6 phosphate dehydrogenase, and the supply of glucose and oxygen to neural cells in multi-infarct rats (Zhao et al. 2011; Zhang et al. 2014). Recently, another study showed the role of acupuncture on monocarboxylate transporters (MCTs) in energy metabolism. MCTs are responsible for the up-taking and releasing of lactate, which is the obligatory energy substrate for neurons during the recovery period after ischemia (Schurr et al. 2001; Halestrap and Meredith 2004). Acupuncture increased the expression of MCT1 in astrocytes, which could facilitate the transfer and utilization of intracellular lactate (Lu et al. 2015).

### Acupuncture Attenuated Excitotoxicity

Excitotoxicity mediated by glutamate is an important trigger and executioner of tissue damage in cerebral ischemia. It can cause necrosis, apoptosis and inflammation through initiating molecular events as well as the intracellular signaling pathways. Acupuncture could attenuate extracellular glutamate level in experimental cerebral ischemia models (Pang et al. 2003; Lee et al. 2010). The neuroprotective effects were related to the upregulation of acupuncture on glutamate transporter type 1 (GLT-1) and glutamate transporter type 2 (GLT-2), which are responsible for the uptake of extracellular glutamate (Furness et al. 2008; Zhu et al. 2013; Guo et al. 2015). Excessive exposure to glutamate activates two major subfamilies of ligand-gated postsynaptic ion receptors: N-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-Methylisoxazole-4-propionic acid subtype glutamate receptors (AMPARs) (Kwak and Weiss 2006). Both the NMDAR and AMPARs are $Ca^{2+}$ permeable except the glutamate receptor subunit2 (GluR2), a receptor belonging to AMPARs which is uniquely impermeable to $Ca^{2+}$ (Zhao et al. 2012). EA pretreatment (electroacupuncture applied before the onset of cerebral ischemia) increased the expression of GluR2 and attenuated the cerebral ischemic damage in hippocampus after reperfusion (Liu et al. 2015b). Besides, acupuncture also reversed the induced expression of NMDAR1 in middle cerebral artery occlusion (MCAO) model (Sun et al. 2005; Lin and Hsieh 2010).

GABAergic neurons dysfunction is another mechanism for neuronal excitotoxicity (Huang et al. 2010). Acupuncture enhanced the resistance of GABAergic neurons to ischemic damage in cerebral cortex through up-regulating the expression of GABA levels and preventing the impairments of spike encoding as well as synaptic transmission in GABAergic neurons (Gan et al. 2005; Zhang et al. 2011c). Besides, acupuncture promoted motor function recovery by activating GABAB receptor and by increasing the formation of cyclic adenosine monophosphate (cAMP), which can activate protein kinase A (PKA) and cause the phosphorylation of cAMP responsive element-binding protein (CREB) (Jiang et al. 2016).

Accumulation of glutamate and depolarization of neurons that result from energy failure could lead to the disruption of ionic homeostasis (dramatically increases intracellular $Ca^{2+}$ and $Na^{+}$) and it can trigger a series of events which damage tissue profoundly in cerebral ischemia (Furukawa et al. 1997).

Na(v)1.1 and Na(v)1.6 are the sodium channel alpha-subunits that express in central nervous system (CNS) at high levels. Acupuncture up-regulated the Na(v)1.1 expression and down-regulated the Na(v)1.6 expression in ischemic brain, and then leaded to the reduction of persistent $Na^+$ (Ren et al. 2010a, b). Moreover, acupuncture down-regulated the excessive expression of large-conductance $Ca^{2+}$ −activated $K^+$ channels, which is responsible for the delayed neuronal damage after cerebral ischemia (Wang et al. 2016b).

### Acupuncture Attenuated Oxidative Stress

Oxidative stress, triggered by the imbalance between the generation and the clearance of ROS, is a major mechanism underlying cerebral ischemia (Chan 2001). The overproduction of ROS causes damage to lipids, proteins, and nucleic acids (Nathan and Ding 2010). Besides, excessive ROS also serve as the signalling molecules, which could trigger apoptosis and inflammation. EA treatment reduced the extent of lipid peroxidation- malondialdehye (MDA) and 4-hydroxynonenal (4-HNE), possibly by increasing the activity and expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Siu et al. 2004a, b; Liu et al. 2006; Sun et al. 2016). In cerebral multi-infarction rats, acupuncture treatment increased Ref-1 expression, the multifunctional protein controlling cellular response to oxidative stress by possessing both DNA repair and transcriptional regulatory activities (Liu et al. 2013a). NADPH oxidase is a major source of ROS during cerebral ischemic injury, which consists of membrane subunits (gp91phox and p22phox), cytosolic subunits (p40phox, p47phox, and p67phox) subunits and Rac1 or 2 (Kahles et al. 2007; Chrissobolis and Faraci 2008; Paravicini and Touyz 2008). Acupuncture suppressed the generation of NADPH oxidase-derived $O_2^-$ and reduced the activity of NADPH oxidase (Guo et al. 2014; Shi et al. 2015).

Under oxidative stress circumstances, the overload of ROS breaks the balanced thiol-redox environment and results in the loss of biological activities in protein. The thioredoxin system comprised of thioredoxin (Trx), thioredoxin reductase (TrxR) and NADPH, is a major cellular thiol-reducing and antioxidant system (Nakamura 2004). Acupuncture could induce the expression of Trx in ischemia-reperfused rat brains (Siu et al. 2005). Since Trx replaces glutathione (GSH) as the substrate for GPx and promotes the expression of manganese superoxide dismutase (Mn-SOD) (Das et al. 1997), it is necessary to study whether there is a cross-talk among different antioxidants regulated by acupuncture.

### Acupuncture Modulated Inflammatory Responses

Inflammatory responses play a critical role in the pathophysiological processes of cerebral ischemia. During cerebral ischemia, the immune cells in CNS such as microglia and astrocytes are activated to produce massive pro-inflammatory cytokines, protease and ROS. These molecules make the neurons more vulnerable, and then contribute to the later ischemic injury (Ceulemans et al. 2010). Acupuncture reduced the content of intercellular adhesion molecule-1 (ICAM-1) (Zhang et al. 2011b), TNF-α, HPS70 (Xu et al. 2014b), IL-1β and enhanced the expression anti-inflammatory cytokines, such as IL-10 in experimental stroke studies (Zhang et al. 2007; Liu et al. 2016b).

Acupucnture also suppressed the activation of nuclear transcription factor kappa B (NF-kB), a major transcription factor that can activate many inflammation-related genes. Acupuncture treatment reduced the expression of IkB kinase beta alpha (IKKα) and IkB kinase beta (IKKβ), the critical kinases that are essential for the phosphorylation and degradation of NF-κB inhibitor (IκB) and the translation of NF-κB to the nucleus. And acupuncture also altered the expression of NF-κB and IκB in the cytoplasm and nucleus in the ischemic cortex (Qin et al. 2013). Besides, acupuncture induced the expression of zinc finger protein A20 and miR-9, which are the upstream regulators of NF-kB signaling pathway (Liu et al. 2016a; Zhan et al. 2016).

The cholinergic anti-inflammatory pathway controls inflammation via neural circuits and suppress the production of pro-inflammatory cytokines (Neumann et al. 2015). EA pretreatment inhibited high mobility group box 1 (HMGB1) release through upregulating a7nAChR to protect the brain from transient cerebral ischemic injury (Wang et al. 2012). Toll-like receptors (TLRs), which enable the immune systems to recognize pathogen-associated molecular patterns and mediate the inflammatory reactions, are involved in the pathophysiological processes of cerebral ischemic injury (Cao et al. 2007). TLR4 activated a common signaling pathway to activate NF-κB, thereby inducing the expression of pro-inflammatory mediators (Barton and Medzhitov 2003). EA suppressed the activation of the Toll-like receptor 4 (TLR4)/NF-κB pathway, accompanied with the decreased levels of inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Lan et al. 2013; Han et al. 2015).

Acupuncture also up-regulates other anti-inflammatory mediators. Monocyte chemotactic protein-induced protein 1 (MCPIP1) was originally identified in human monocytes treated with MCP-1 and is responsible for the negative activation of macrophages (Liang et al. 2008; Zhu et al. 2015). MCPIP1 could reduce pro-inflammatory cytokines through inhibiting the activation of NF-κB (Liang et al. 2008). EA pretreatment-induced neuroprotective effects were depressed in MCPIP1 deficiency condition, followed with the high level of pro-inflammatory cytokines and greater infiltration of leukocytes (Jin et al. 2013).

### Acupuncture Modulated Cell Apoptosis and Autophagy

Cerebral ischemia activates a series of cascades that ultimately lead to neuron death (Sahota and Savitz 2011). It is generally recognised that necrosis occurs within minutes in the core of the ischemic area. And neurons in the penumbra undergo apoptosis after several hours or days owing to multiple pathophysiological processes induced by cerebral ischemia (Doyle et al. 2008). A large number of studies have revealed the role of acupuncture in apoptosis cascades (Fig.1).

There are two classic signaling pathways in cell death: the extrinsic death receptor (DR) and intrinsic mitochondrial apoptotic pathways (Broughton et al. 2009). DR activates the caspases by binding to the specific ligands. And the intrinsic pathway activated by pro-apoptosis proteins such as Bid and Bax, also results in the downstream activation of caspases (Igloffstein and Vogel 1991). EA affected both pathways via decreasing the expression of DR5 and increasing the expression of anti-apoptotic Bcl-2, together with increasing the inhibitor of apoptosis protein (IAP) family- cIAP-1 and cIAP-2 (Wang et al. 2009b; Li et al. 2012; Kim et al. 2013c; Liu et al. 2015a; Lin et al. 2016). In addition, acupuncture reduced the expression of pro-apoptotic Bax by enhancing the expression of HIF-1α as well as suppressing the NF-κB activation (Feng et al. 2013; Zhao et al. 2015). Acupuncture could potentiate Akt activity and suppressed the expression of cleavage caspase 9 (Wang et al. 2002).

Recent studies showed that other molecules which participating in apoptosis were regulated by acupuncture as well. The endocannabinoid system (ECS) is a widespread modulatory system that plays a critical role in the response to endogenous and environmental insults (Lu and Mackie 2016). Acupuncture up-regulated the expression of the endocannabinoid and increased the expression of cannabinoid receptor type 1 (CB1) and cannabinoid receptor type 2 (CB2) receptor (Wang et al. 2009a; Ma et al. 2011). Moreover, acupuncture triggered multiple signal transduction events via the activation of the CB1 receptor. EA pretreatment activated the signal transducer and activator of transcription 3 (STAT3), enhanced epsilon protein kinase C (εPKC) and increased phosphorylation of glycogen

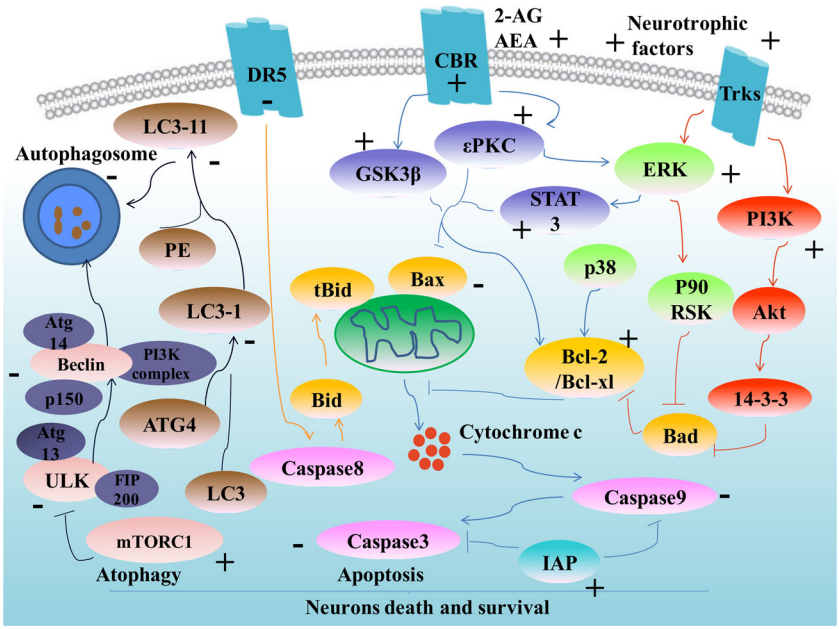


**Fig. 1** Neurons death and survival pathways that involve in the protective effect of acupuncture in experimental stroke models. Symbols + and − respectively indicate the upregulation and downregulation of molecules caused by acupuncture treatment. Symbols → represent stimulatory regulation and ⊣ represent inhibitory regulation. Acupuncture regulates the neurons death and survival through multiple pathways: (i) the extrinsic and intrinsic apoptosis pathways; (ii) autophagy; (iii) PI3k/Akt pathway and ERK pathway. Acupuncture decreases the expression of DR5 and increase the expression of anti-apoptotic Bcl-2, Bcl-xL, cIAP-1 and cIAP-2. Acupuncture also regulates the endocannabinoid system as well as STAT3, εPKC and GSK-3β, which ultimately triggerred the intrinsic apoptotic pathway. Acupuncture activates the p38 MAPK/CREB signaling pathway and regulated Bax-mediated apoptosis. Acupuncture increases the expression of the mTORC1 in the peri-infarct cortex, and decreases the levels of ULK1, Atg13 and Beclin1. Besides, acupuncture reduces the expression of LC3-II, Beclin 1 and the number of autophagosomes. In addition, acupuncture mediates neurotrophic factor and the downstream signaling pathways- both ERK pathway and PI3k pathway, which can phosphorylate Bad and attenuate caspase 3-dependent apoptosis. DR, death receptor; CBR, cannabinoid receptor; TrkR, tropomyosin-related tyrosine kinase receptor; IAP, inhibitor of apoptosis protein; STAT3, signal transducer and activator of transcription 3; 2-AG, 2-arachidonoylglycerol; AEA, N-arach-idonoylethanolamine-anandamide;εPKC, epsilon protein kinase C; GSK-3β, glycogen synthasekinase-3β; PE, phosphatidylethanolamine; LC3, microtubule-associated proteinlight chain 3; ATG4, autophagy-related gene 4; mTORC1, mammalian target of rapamycin complex1; ULK, Atg1/Unc-51-like kinase; Atg13, autophagy related gene 13

synthase kinase-3β (GSK-3β), which ultimately triggered the intrinsic apoptotic pathway (Wang et al. 2011b; Zhou et al. 2013b; Wei et al. 2014).

p38 is the mediator of various cellular signaling pathways in response to stimuli after transient cerebral ischemia (Nozaki et al. 2001). Acupuncture activated the p38 and the cAMP/CREB signaling pathway, and regulated Bax-mediated apoptosis (Cheng et al. 2015; Lin et al. 2015). It is noted that the frequency of EA seemed to act on different Bcl-2 family members. EA-5 Hz activated the p38 MAPK/CREB/Bcl-xL, while EA-25 Hz activated the p38 MAPK/CREB/Bcl-2 signaling pathway. However, the underlying mechanism still needs to be further explored.

Autophagy is responsible for the degradations of long-lived proteins and damaged organelles and plays an important role in maintaining cellular homeostasis (Pan et al. 2008). It has been recognized as the third type of cell death except for necrosis and apoptosis (Adhami et al. 2006). Physiological levels of autophagy are essential for neuronal survival, while excessive activations of autophagy are involved in the pathphysiological processes of cerebral ischemia (Wang et al. 2011a). Acupuncture pretreatment markedly reduced the expression of autophagy markers- microtubule-associated protein 1A light chain 3 (LC3) and Beclin 1 as well as the number of autophagosomes (Wu et al. 2015; Shu et al. 2016). Mammalian target of rapamycin complex1 (mTORC1) plays an important role in autophagosome formation and autophagy. Inactivated mTORC1 can phosphorylate the Atg1/Unc-51-like kinase (ULK) complex (Szymanska et al. 2015), which could trigger autophagy by targeting the autophagosome formation-specific classIIIPI3K complex. Acupuncture could increase the expression of the mTORC1 in the peri-infarct cortex, and decrease the levels of ULK1, autophagy related gene 13(Atg13) and Beclin1 (Liu et al. 2016c).

## Acupuncture Promoted Cell Survival Through Regulating Neurotrophic Factors and the Downstream Signaling Pathway

Neurotrophic factors are known to be involved in neuronal development, proliferation, maturation and outgrowth (Otsuka et al. 2016). The neurotrophins which comprised of brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin (NT) 3 and NT4/5, mediate their functions by binding to tropomyosin-related tyrosine kinase (Trk) receptor family (Roux and Barker 2002). Trk receptors are highaffinity receptors with much greater ligand specificity which include TrkA, TrkB and TrkC (Chopin et al. 2016). TrkA preferentially binds to NGF. TrkB is primarily a receptor to BDNF and NT-4/5 and TrkC is the receptor for NT-3. By binding to Trks, neurotrophins activates their downstream pathways, including mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and PI3K-Akt (Chao 2003). The neurotrophins have been reported to provide neuroprotective effects in cerebral ischemia (Cheng et al. 2004). Acupuncture increased the level of BDNF, glial cell linederived neurotrophic factor (GDNF), tromal cell derivedfactor-1α (SDF-1α) and basic broblast growth factor (bFGF) (Ou et al. 2001; Chen et al. 2012; Kim et al. 2012; Kim et al. 2013b). However, its regulations on vascular endothelial growth factor (VEGF) are somewhat conflicting. One study reported that VEGF remained unchanged after acupuncture pretreatment while another study indicated that acupuncture up-regulated the VEGF expression (Ma and Luo 2008; Kim et al. 2013b; Yu et al. 2014). The contradiction may due to the difference in severity of cerebral ischemia and acupoints that used in these two studies.

Furthermore, the downstream signaling pathway of neurotrophic factors- PI3K/Akt pathway was involved in the regulation of acupuncture. EA significantly up-regulated the expression of PI3K and promoted the phosphorylation of Akt in ischemic cerebral tissues through regulating the Bcl-2/Bax ratio (Chen et al. 2012; Xue et al. 2014). By regulating the trkA–PI3K pathway, acupuncture also down-regulated the expression of transient receptor potential melastatin 7 (TRPM7), the $Ca^{2+}$ - permeable nonselective cation channel (Zhao et al. 2007).

ERK, a member of MAPK family, was also involved in the protective progress (Cheng et al. 2014). However, another study reported that acupuncture up-regulated the level of TrkA, and acupuncture appeared to activate the PI3K pathway instead of ERK pathway (Sun et al. 2005). As the Trks are receptors with greater ligand specificity, the inconsistent phenomenon may result from the diversity of neurotrophins stimulated by different acupuncture manipulation.

## Acupuncture Restored Blood–Brain Barrier (BBB) Integrity

The BBB formed by endothelial tight junctions, pericytes, perivascular astrocytes and basement membrane, is responsible for maintaining the homeostasis of CNS and the optimal brain function (Bradbury 1985). The disruption of BBB resulting from the cerebral ischemia pathological processes leads to the secondary injury such as oedema during cerebral ischemia (Yang and Rosenberg 2011). Acupuncture alleviated the insult caused by cerebral ischemia via preventing the breakdown of BBB (Wu et al. 2001). EA pretreatment reversed the degradation of tight junctions, including claudin-5 and occludin in the endothelial cells. Moreover, EA pretreatment reduced the increased expression of p-caveolin-1, which is able to increase the density of caveolae and cause transcytosis of roteins (Zou et al. 2015). In addition, EA could improve neurological dysfunction by enhancing cells proliferation and differentiation into mature neurons (Tao et al. 2010).

During the processes of ischemic stroke, MMPs are activated, which can disrupt the BBB, and provoke oedema formation and brain haemorrhage (Lee et al. 2014). Acupuncture attenuated the expression and activity of MMP9 and MMP2 (Dong et al. 2009; Xu et al. 2014a). Besides, acupuncture also increased the expression of tissue inhibitor of metalloproteinases-1 (TIMP-1) and modulated the balance of MMP-9/TIMP-1 (Ma et al. 2016).

Aquaporins (AQPs) are a family of water channel proteins that control the movement of water across cell membranes. AQP4 and AQP9 are highly expressed in astrocytes and vascular endothelial cells after cerebral ischemia, and are responsible for oedema via accelerating water transport. Acupuncture ameliorated oedema and the underlying mechanisms were associated with attenuating the expression of AQP4 and AQP9 (Zhang et al. 2011a; Peng et al. 2012; Xu et al. 2014a; Jung et al. 2016).

## Acupuncture Promoted Neurogenesis, Reactive Astrocytes and Angiogenesis

Adult neurogenesis covers a series of progresses, including proliferation, fate specification, neuronal maturation, targeting, and synaptic integration of the new born neurons (Ming and Song 2005). Studies have reported that neurogenesis may be a therapeutic target to alleviate the insult following cerebral ischemia (Kokaia and Lindvall 2003; Liu et al. 2009). Although neurogenesis can be triggered after brain ischemia, the endogenous neurogenesis is still limited and the beneficial effects to promote the recovery are inadequate with the fact that the new neurons did not exert long-term survival (Arvidsson et al. 2002; Zhang et al. 2008). Hence, improving the poor survival of newborn neurons derived from neurogenesis is necessary for the recovery of cerebral ischemia. Recent studies suggested that acupuncture is effective in targeting neurogenesis in cerebral ischemia (Cheng et al. 2009; Luo et al. 2014; Lu et al. 2016). Acupuncture enhanced the proliferation in hippocampal dentate gyrus and hippocampal CA1 area, and promoted the neuroblast differentiation and increased the number of tertiary dendrites in the subgranular zone of the DG (Kim et al. 2001; Kim et al. 2002; Hwang et al. 2010; Li et al. 2015a). Besides, acupuncture increased the total numbers of BrdU/Dcx, which indicated acupuncture promoted the proliferation and differentiation of neural stem cells (NSCs) (Tao et al. 2010). The activations of BDNF/VEGF signaling pathway together with ERK signaling pathway, retinoic acid (RA) signaling pathway and canonical Notch pathway were responsible for the proliferation-promoting effects of acupuncture (Zhao et al. 2012; Hong et al. 2013; Xie et al. 2013; Huang et al. 2014; Kim et al. 2014). Moreover, acupuncture promoted the proliferation of neural progenitor cells (NPCs) by increasing the levels of Wnt1 and β-catenin and suppressing GSK3 transcription (Chen et al. 2015).

Astrocytes represent the most numerous glial cell type in CNS and play a fundamental role in providing structural, trophic, and metabolic support to neurons and modulating synaptic activity (Chen and Swanson 2003). It is considered that the failure of astrocytes to support the essential metabolic necessary for neurons resulted in cerebral ischemia insult (Takano et al. 2009). The ultimate rehabilitation and recovery are affected by the status of astrocytes, which can activate through activating neurogenesis and synaptic reorganization (Song et al. 2002). Acupuncture activated astrocytes in the peri-infarct region of the ischemic cerebral hemisphere and reduced excess reactive gliosis (Han et al. 2010). In addition, acupuncture contributed to the proliferation of GFAP/vimentin/nestin-positive reactive astrocytes and the secretion of BDNF derived from reactive astrocytes (Tao et al. 2016).

Angiogenesis might be another mechanism underlie the beneficial effects of acupuncture for cerebral ischemia. EA promoted angiogenesis via enhancing vascular endothelial cell proliferation (Du et al. 2011), upregulating the expressions of VEGF and angiogenin-1 (Ang-1) and downregulating the expression of anti-angiogenesis factors-endostatin (Wang et al. 2003; Ma and Luo 2008). Stem cell factor (SCF) /c-kit pathway is involved in neurogenesis after cerebral ischemia. Study indicated that acupuncture could facilitate the vasculogenesis and recovery by increasing the expression of MMP-9 and the binding of SCF to c-kit, which could result in the promotion of the EPCs mobilization, migration and homing (Lu et al. 2013). However, the neovascularization may be detrimental in patients with co-morbidities. For instance, the break and leak of immature vessels aggravated vascular and neuronal damage after stroke in diabetes patients (Caldwell et al. 2003; Caldwell et al. 2005; Navaratna et al. 2009). Further studies are needed to make sure the significance of acupuncture on angiogenesis in models with co-morbidities.

## Acupuncture Induced Neuroplasticity

Neuroplasticity has ben defined as the ability of neurons and circuits to modify their functional activity and the synaptic organization in conformity to variations in activity. The maintenance of neuroplastic activity is essential for nerve recovery after cerebral ischemia. EA treatment increased the levels of dopamine (Chuang et al. 2007), the growth associated protein 43 (GAP-43) as well as the tyrosine hydroxylase (TH) in dopaminergic (DAergic) neurons. However, these effects were reversed by D2R-selective antagonist. That indicated the oplastic mechanism of EA was related to themodulation of acupuncture on D2 receptors in DAergic neurons (Xu et al. 2013a). Acupuncture also played a role in the synaptic

reconstruction in cerebral ischemia (Xu et al. 2012, 2013b). Acupuncture increased the dendritic spine density through regulating the ephrin-A5 expression, the ligand of Eph that affect the structure of dendritic spine (Ren et al. 2008). In addition, the beneficial effects of acupuncture on synaptic plasticity were related to astrocytes, which are responsible for synaptic plasticity via participating in the processes of transmission, formation and reorganization (Baranano et al. 2001; Mauch et al. 2001; van der Hel et al. 2005; Luo et al. 2011).

## Conclusions and Consideration

As an age-old healing art, acupuncture has been shown to be an effective therapy for cerebral ischemia. In stroke patients, acupuncture activated specific brain regions, modulated cerebral blood flow and relevant molecules. On the other hand, evidence from animal studies indicated the therapeutic mechanisms of acupuncture cover nearly all the molecular and cellular events during the pathological process of cerebral ischemia (Fig.2). It seems that the

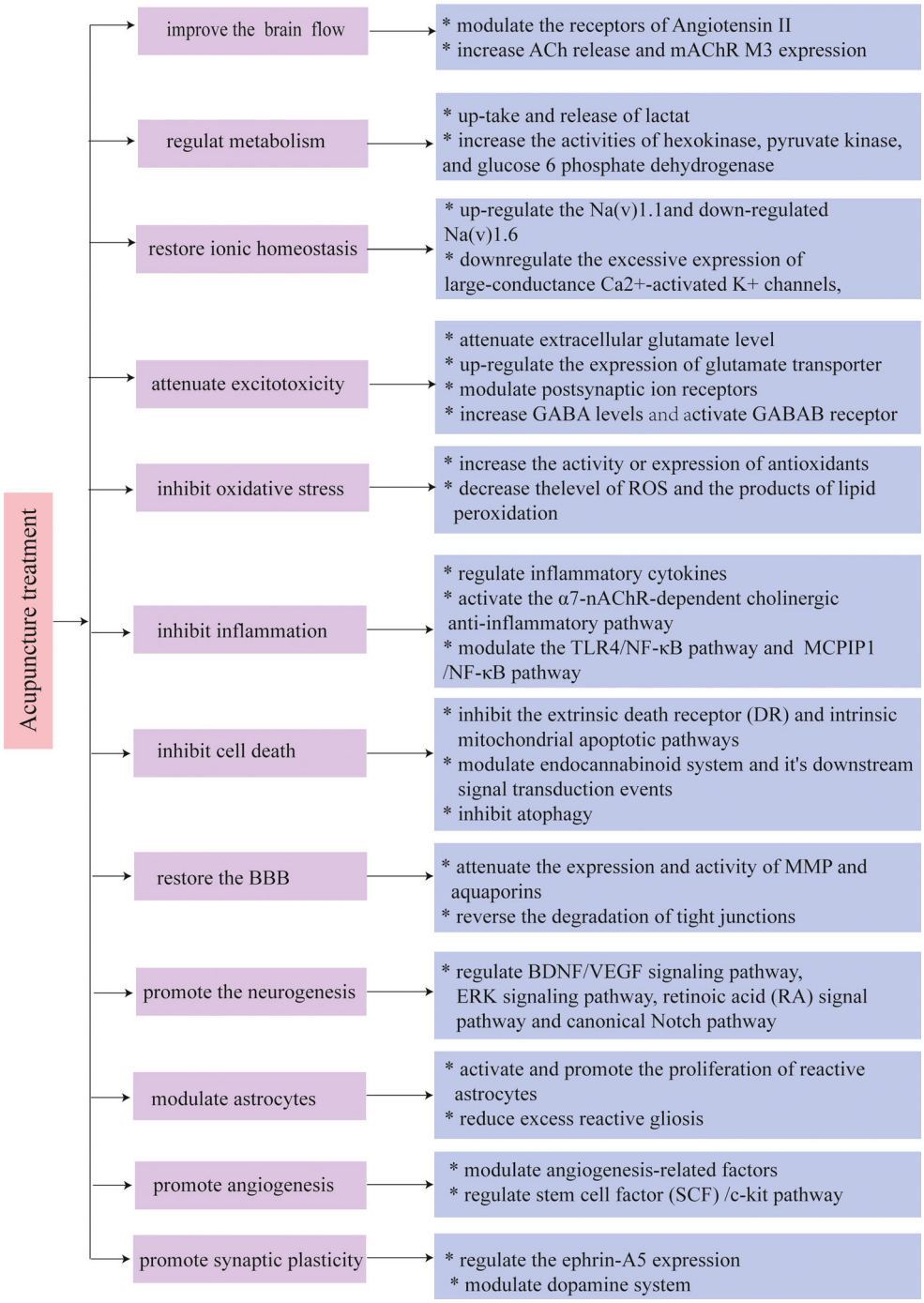


**Fig. 2** Efects of acupuncture on molecular and cellular events during the pathological process of cerebral ischemia in animal models

therapic effects of acupuncture are multi-faceted and the modulation produced by acupuncture on these pathways ultimately paves the way for stroke recovery.

From a research standpoint, acupuncture may be used as a non-pharmaceutical therapy for stroke in which potential therapeutic targets could be identified and investigated. It requires more studies on how acupuncture influences relevant brain network, molecules or signaling pathways. However, problems with small sample size and methodological weaknesses, including unclear methods of randomization and the lack of long-term follow-up exist in the acupuncture clinical studies (Park et al. 2001; Zhang et al. 2005). Larger and more definite clinical trials are necessary for advancing our understanding of the mechanistic basis as well as providing quality evidence for successful clinical application.

In general, acupuncture is mainly used for the rehabilitation and the treatment for complications after stroke. In fact, acupuncture also has been used for the prevention of disease. Evidence from laboratory indicated that acupuncture pretreatment induced significant ischemic tolerance and increased the likelihood of achieving protection against ischemic injury (Wang et al. 2009a; Wang et al. 2011b; Jin et al. 2013; Zhou et al. 2013a). As early initiation of therapeutic modalities is not always available in clinical condition owing to narrow therapeutic treatment window, acupuncture therapy especially acupuncture pretreatment may be a potential method to against cerebral ischemic damage.

Notwithstanding the fact that acupuncture demonstrated neuroprotective effects in the laboratory, there are several obstacles hampering the translation from animal to clinical research. The specificity and accurate location of acupoints are the basis of acupuncture treatment and there are more than three hundred acupionts in human body. As the experimental animals were smaller, the acupoints in animals that mapped by the human acupoints are much less than those in human. More importantly, animal studies that explore the mechanism emphasis on standardization acupuncture manipulation and reproducibility, but clinical studies aiming to validate the effectiveness of acupuncture highlight individual therapy. That makes the therapeutic regimens different between clinical and animal studies, of which single or no more than five acupoints are used in animal studies, while more acupoints or combinations with other medical treatments are selected in clinical studies. These differences make the findings in animal studies hardly or partly to serve the clinical studies of acupuncture.

Appropriate acupuncture manipulation is the important factor that affects the efficacy of acupuncture. The variations in acupuncture manipulation, for example, timing, duration, acupuncture models (EA or MA), acupoints (Fig.3), frequency may influence the outcomes of acupuncture studies (Zhang et al. 2015). Acupuncture application during MCAO reduced larger infarct volume than acupuncture performed after MCAO (Zhou et al. 2013a). Furthermore, the beneficial effects of acupuncture against cerebral ischemia are dependent on the appropriate duration of acupuncture. EA given for 5–30 min demonstrated better outcomes in reducing ischemic infarct volume, neurological deficits and mortality rate, while long duration of acupuncture stimulation could aggravate ischemic injury to brain (Zhou et al. 2013a). However, acupuncture manipulation performed in laboratory varies with different acupuncture studies (Table 1). For the sake of research, establishing the optimizing acupuncture manipulation by scientific testing is a critical issue that should not be overlooked in studies.

Although advances have been made in the studies of acupuncture parameters in studies, how would these kinds of studies be translated into clinical studies remains a major question. To overcome this flaw, we believe that the following points need to be paid attention in acupuncture studies. Firstly, we should justify the changes of molecules or signaling pathways in animal studies to understand the mechanism of acupuncture when we use specific

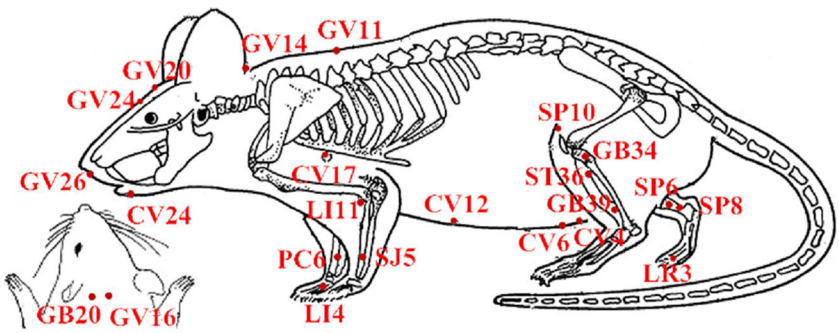


**Fig. 3** Acupoints commonly used in experimental stroke studies. GV20, baihui; GV26, shuigou (renzhong); GV14, dazhui; CV24, chengjiang; CV16, fengfu; GB20, fengchi; GB34, yanglingquan; LI11, quchi; LI4, hegu;GB39, xuanzhong; LR3,taichong; SP6, sanyinjiao; SP8, diji; ST36, zusanli; PC6, neiguan; SJ5, waiguan; CV6, qihai; CV17, danzhong; CV12, zhongwan; GV11, shendao; SP10,xuehai

**Table 1** Acupoints and manipulation parameters used in animal studies for cerebral ischemia

| Study | Acupoint | Pre/post- treatment | Method | Frequency | Intensity | Depth | Duration |
|---|---|---|---|---|---|---|---|
| Hsieh et al. 2006 | ST36<br>SP6 | post-treatment | EA | 2Hz/15Hz | 5 mA | 2 mm | 20 min |
| Zhou et al. 2011 | GV26<br>GV20 | post-treatment | EA | different frequencies | different intensities | GV20:2mm<br>GV26:1mm | 30min |
| Kim et al. 2013a | GV20<br>GV14 | post-treatment | EA | 2 Hz | 1 mA | | 20 min |
| Choi et al. 2010 | GB34<br>GB39 | post-treatment | MA | | | 5 mm | |
| Li et al. 2014 | GV26 | post-treatment | EA | 15 Hz | 1 mA | 2 mm | 20min |
| Liu et al. 2013b | GV20<br>GV26 | post-treatment | EA | 2 Hz | | GV20:5.0-10.0 mm<br>GV26:2.0-3.0 mm | 30min |
| Wang et al. 2002 | GV20<br>GV26 | post-treatment | EA | 20/3 Hz | 3 mA | | 1 h |
| Cheng et al. 2015 | GV20<br>GV16 | post-treatment | EA | 5Hz or 25Hz | 2.7-3.0 mA | GV20: 4 mm<br>GV16: 7.5 mm | 25min |
| Chen et al. 2012 | LI11<br>ST36 | post-treatment | EA | 1/20 Hz | | 2-3 mm | 30min |
| Kim et al. 2013c | GV20<br>CV6 | post-treatment | EA | 2 Hz | 1 mA | 3 mm | 30min |
| Zhao et al. 2007 | GV26<br>CV24 | post-treatment | EA | 4/16 Hz | | | 30min |
| Wu et al. 2015 | GV20 | pre-treatmnet | EA | 2/15 Hz | 1 mA | | 30 min |
| Kim et al. 2013b | GV20<br>GV14 | pre-treatmnet | EA | 2 Hz | 1 mA | 3 mm | 20min |
| Ma and Lou 2008 | LI4 | post-treatment | EA | 40/60 Hz | 1.5V | | 15min |
| Ou et al. 2001 | GV20<br>GV26 | post-treatment | EA | 18/3.85 Hz | 1.8–2.5/ 1.4–2.5 mA | | 1h |
| Sun et al. 2005 | GV26<br>CV24 | post-treatment | EA | 16/4 Hz | | | 30 min |
| Cheng et al. 2014 | GV20<br>GV14 | post-treatment | EA | 5 Hz | 2.7–3.0 mA | GV20:4mm<br>GV14:5mm | 25 min |
| Kim et al. 2001 | ST36 | post-treatment | MA | | | | 20 min |
| Luo et al. 2011 | GV20<br>GV14 | post-treatment | EA | 4/20 Hz | 1–3 mA | GV20:0.5 cun<br>GV14:0.25cun | 30 min |
| Hong et al. 2013 | ST36<br>LI11 | post-treatment | EA | 5/20 Hz | 2–4 mA | 5mm | 20min |
| Zhao et al. 2012 | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Tao et al. 2016 | ST36<br>LI11 | post-treatment | EA | 1/20Hz | | 2-3 mm | 30 min |
| Han et al. 2010 | GV20<br>GV14 | post-treatment | EA | 20 Hz | 1-2 mA | | 30 min |
| Huang et al. 2014 | ST36<br>LI11 | post-treatment | EA | 1-20 Hz | | | 30 min |
| Kim et al. 2014 | GV20<br>GV14 | post-treatment | EA | 2 Hz | | 2mm | 20min |
| Ren et al. 2008 | PC6<br>SP6<br>SJ5<br>ST36 | post-treatment | EA | 10 Hz | 1 mA | | 30 min |
| Xie et al. 2013 | ST36<br>LI11 | post-treatment | EA | 1/20 Hz | | 2-3 mm | 30 min |
| Jin et al. 2013 | GV20 | pre-treatment | EA | 2/15 Hz | 1mA | | 30 min |

**Table 1** (continued)

| Study | Acupoint | Pre/post- treatment | Method | Frequency | Intensity | Depth | Duration |
|---|---|---|---|---|---|---|---|
| Lan et al. 2013 | ST36<br>LI11 | post-treatment | EA | 1/20Hz | 0.01 mA | 2-3 mm | |
| Wang et al. 2012 | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Guo et al. 2014 | GV20 | pre-treatment | EA | 2/15 Hz | lmA | | 30min |
| Siu et al. 2005 | ST36<br>GB20 | post-treatment | EA | 2 Hz | 0.7V | | 30min |
| Siu et al. 2004b | GB20 | post-treatment | EA | 2 Hz | 0.7V | | 30min |
| Shi et al. 2015 | GV20<br>ST36 | post-treatment | MA | | | 5mm for rat<br>3mm for mice | 30s |
| Siu et al. 2004a | ST36<br>GB20 | pre-treatment | EA | 2Hz | 0.7mV | | 30min |
| Ma et al. 2011 | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Wei et al. 2014 | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Wang et al. 2011b | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Wang et al. 2009a | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Dong et al. 2009 | GV20 | pre-treatment | EA | 15 Hz | 1 mA | | 30 min |
| Jung et at. 2016 | GV20<br>GV14 | pre-treatment | EA | 2 Hz | 1 mA | 3 mm | 20min |
| Xu et al. 2014a | GV20<br>ST36 | pre-treatment | EA and MA | 2 Hz | 1 mA | | 20 min |
| Zhang et al. 2011a | GV20 | post-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Liu et al. 2015b | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Guo et al. 2015 | GV20<br>GVI4 | pre-treatment | EA | 2/15 Hz | | GV20: 3mm<br>GV14:10ram | 30min |
| Zhu et al. 2013 | GV20 | pre-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Lin et al. 2010 | GV20 | post-treatment | EA | 2Hz | 2 mA | | 20min |
| Gao et al. 2002 | GV20<br>GV26 | post-treatment | EA | 18/3.85Hz | 7-8.8/6-8.8 mA | | 1h |
| Lu et al. 2015 | PC6<br>LI11 | post-treatment | EA | 2/15 Hz | 1 mA | PC6:2 mm<br>LI11:4 ram | 20 min |
| Tao et al. 2010 | ST36<br>LI11 | post-treatment | EA | 120 Hz | | 2-3 mm | 30min |
| Han et al. 2015 | PC6<br>LI11<br>SP8 | post-treatment | EA | 2/15 Hz | 1 mA | | 30 min |
| Gan et al. 2005 | GV20<br>GV26 | post-treatment | EA | 3.58-6.25 Hz | 1.4-2.0 mA | | 1h |
| Pang et al. 2003 | GVI6<br>GV11 | post-treatment and pre-treatment | EA | 7 Hz | 6 mA | | 30min |
| Zhang et al. 2014 | CV17<br>CV12 | post-treatment | MA | | | CVI7: 2-3 mm<br>CVI2: 3-4mm | 30 s |
| | CV6 | | | | | CV6: 3-4mm | |
| | ST36 | | | | | ST36: 3-4 mm | |
| | SP10 | | | | | SP10: 2-3 mm | |
| Ma et al. 2016 | GV20<br>LI4<br>LR3 | post-treatment | EA | 2Hz | 1mA | | 30 min |
| Zou et al. 2015 | GV20 | pre-treatment | EA | 2/15 Hz | 1mA | | 30 min |
| Jiang et al. 2016 | LI4<br>ST36<br>GB34<br>SJ5 | post-treatment | EA | 1-20 Hz | | 2-3 mm | 30min |

**Table 1** (continued)

| Study | Acupoint | Pre/post- treatment | Method | Frequency | Intensity | Depth | Duration |
|---|---|---|---|---|---|---|---|
| Xue et al. 2014 | ST36 LI11 | post-treatment | EA | 4/20Hz | | 2-3 mm. | 30min |
| Liu et al. 2016a, b, c | ST36 | post-treatment | EA | 1-20 Hz | | 2-3 mm | 30min |
| Feng et al. 2013 | GV20 GV24 | post-treatment | EA | 1-20Hz | | 2-3mm | 30min |
| Lin et al. 2016 | SJ5 | post-treatment | MA | | | 2-3 mm | 20min |
| Zhao et al. 2015 | GV20 | pre-treatment | EA | 2/15Hz | 1mA | | 30min |
| Hwang et al. 2010 | ST36 | post-treatment | MA and EA | 5/20 Hz | 2-4 mA | 5mm | 20min |
| | GV20 | | | | | | 20min |
| Xu et al. 2013a | GB 20 | post-treatment | EA | 2 Hz | 3.0 mA | 8 mm | 50 min |
| Luo et al. 2014 | GV26 | post-treatment | MA | | | 2 mm | 1min |
| Lu et al. 2013 | LI4 | post-treatment | EA | 40/60 Hz | 1mA | 4-6mm | 15min |
| Yu et al. 2014 | GV20 GVI4 | post-treatment | EA | 30/100Hz | 5V | GV20:4mm Gv14:5mm | 20min |
| | CV24 | | | | | CV24:1mm | |
| | CV4 | | | | | CV4: 5mm | |
| | CV6 | | | | | CV6:5ram | |
| Cheng et al. 2009 | GV20 | post-treatment | EA | 4Hz | 2mA | | 20min |
| Pang et al. 2003 | GVI6 GV11 | post-treatment | EA | 7Hz | 6mA | | 30mm |
| Lin and Hsieh 2010 | GV20 | post-treatment | EA | 2Hz | 2mA | 0.5mm | 20min |
| Wang et al. 2016b | GV26 | post-treatment | EA | 2Hz | 1mA | 2mm | 10min |
| Sun et al. 2016 | GV20 | pre-treatment | EA | 2/15 Hz | 1mA | | 30min |
| Lin et al. 2015 | GV20 GV24 | post-treatment | EA | 20Hz | 1-3mA | 2-3mm | 30min |
| Zhan et al. 2016 | GV20 LI4 | post-treatment | EA | 20/2Hz | 1mA | | 35min |
| | LR3 | | | | | | |
| Qin et al. 2013 | GV20 LI4 | post-treatment | EA | 20/2Hz | 1mA | | 30min |
| | LR3 | | | | | | |

acupuncture parameter. Secondly, make comparison of the acupuncture therapeutic effects to determine the optimal acupuncture regimen when we use different acupuncture parameters. Thirdly, we should testify if the optimal acupuncture regimen acquired in animal studies can be applied to clinical studies. In other words, the results of animal studies should be used to provide biological evidence for acupuncture and to assist in screening the best acupuncture therapeutic regimen for clinical.

In conclusion, acupuncture exerts beneficial effects through modulating a series of biological events in cerebral ischemia. More studies are still needed to further explore its biological significance and the deeper mechanisms. In particular, studies should focus on the mechanism of acupuncture on stroke-related complication such as motor dysfunction, which is accociated with neuroplasticity. Besides, more animal acupuncure translational studies are encouraged to translating into clinical studies.

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**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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