INVITED REVIEW

Acupuncture and neuroregeneration in ischemic stroke

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Abstract
Acupuncture is potentially beneficial for post-stroke rehabilitation and is considered a promising preventive strategy for stroke. Electroacupuncture pretreatment or treatment after ischemic stroke by using appropriate electroacupuncture parameters generates neuroprotective and neuroregenerative effects that increase cerebral blood flow, regulate oxidative stress, attenuate glutamate excitotoxicity, maintain blood-brain barrier integrity, inhibit apoptosis, increase growth factor production, and induce cerebral ischemic tolerance.

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Introduction
Ischemic stroke is a major cause of mortality and disability worldwide. Acupuncture, one of the most important components of traditional Chinese medicine, has been shown to activate relevant brain regions, modulate cerebral blood flow (CBF), and regulate multiple molecules and signaling pathways that lead to excitotoxicity, oxidative stress, inflammation, neuronal death, and survival after interruption of blood supply. A large number of animal experiments revealed the neuroprotective effects of acupuncture on ischemic stroke (Feng and Zhang, 2014). Furthermore, acupuncture promotes neurogenesis, angiogenesis, and neural plasticity, in addition to inhibiting apoptosis after ischemic damage. Clinical and laboratory evidence suggests that acupuncture induces multilevel regulation through complex mechanisms against cerebral ischemia (Zhu et al., 2017).

Acupuncture serves not only as a complementary and alternative therapy for poststroke rehabilitation but also as a promising preventive strategy in stroke, which could induce cerebral ischemic tolerance, especially when combined with modern electrotherapy (Li and Wang, 2013). Electroacupuncture (EA) possesses additional characteristics such as acupoint specificity and stimulation parameters, which produce different effects against cerebral ischemia.

EA Pretreatment Induces Tolerance to Cerebral Ischemia
EA pretreatment has been shown to induce ischemic tolerance and may be a promising preventive strategy for patients with high risk of acute ischemic stroke. Many studies have shown that the protective mechanisms of EA pretreatment may involve a series of regulatory molecular pathways including enhancement of antioxidants, regulation of the endocannabinoid system, and inhibition of apoptosis (Li et al., 2012).

Several studies have demonstrated the involvement of different cannabinoid receptors in different types of ischemic tolerance. Pretreatment with EA induces rapid (2 hours after EA) and delayed (24 hours after EA) tolerance to focal cerebral ischemia induced by middle cerebral artery occlusion (MCAo) in rats (Zhang et al., 2003). Cannabinoid receptor type 1 (CB1) was thought to be involved in rapid ischemic tolerance, whereas cannabinoid receptor type 2 (CB2) was considered to contribute to the delayed neuroprotective effect (Ma et al., 2011). Pretreatment with EA in an animal model of focal cerebral ischemia reduced infarct size, improved neurological outcome, and inhibited neuronal apoptosis. EA pretreatment upregulated the neuronal expression of CB1 and increased the production of endocannabinoid 2-arachidonylglycerol and N-arach-idonoylethanolamine-anandamide in rat brains, thereby inducing rapid tolerance to focal cerebral ischemia (Wang et al., 2009). EA pretreatment protects against cerebral ischemia/reperfusion (I/R) injury induced by MCAo in rats through CB1 receptor (CB1R)-mediated phosphorylation of glycogen synthase kinase 3 (Wei et al., 2014). In addition, pretreatment with EA may activate endogenous epsilon protein kinase C-mediated antiapoptosis to protect against ischemic damage after focal cerebral ischemia through CB1-induced rapid tolerance to focal cerebral ischemia in rats (Wang et al., 2011). EA pretreatment (15 Hz) at the *Baihui* (GV20) acupoint could induce rapid tolerance to focal cerebral ischemia (Wang et al., 2005). Additionally, repeated EA pretreatment at GV20 stimulates the release of enkephalins, which may bind to delta- and micro-opioid receptors and induce delayed cerebral ischemic tolerance (Xiong et al., 2007).

EA Pretreatment Regulates Oxidative Stress, Maintains the Integrity of the Blood-Brain Barrier (BBB), and Inhibits Apoptosis
Nitric oxide biosynthesis is a key factor in the pathophysiological response of the brain to hypoxia-ischemia. Brain ischemia activates Ca^{2+}-dependent nitric oxide synthase (NOS) isoforms, namely neuronal NOS (nNOS) and en-

dothelial NOS (eNOS). Although eNOS appears to have neuroprotective properties, nNOS may have neurotoxic effects (Bolaños and Almeida, 1999). Furthermore, delayed ischemia or reperfusion after an ischemic episode induces the expression of Ca^{2+}-independent inducible NOS (iNOS), which may have neurotoxic effects, mainly in glial cells (Garry et al., 2015).

Abrupt reperfusion after ischemia results in overproduction of reactive oxygen species (ROS), which leads to brain injury. Preischemia EA therapy at either *Fengchi* (GB20) or *Zusanli* (ST36), similarly, attenuates lipid peroxidation and reduces ROS production, consequently improving the function of the respiratory chain and the antioxidant capacity in the ischemic penumbra (Siu et al., 2004b; Zhong et al., 2009).

EA pretreatment at GV20 in diabetic mice with cerebral I/R injury reduced infarct size and improved neurological outcomes. EA attenuated cerebral ischemic injury by inhibiting nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-mediated oxidative damage (Guo et al., 2014).

EA preconditioning (2 Hz/15 Hz) at GV20 and *Dazhui* (GV14) can protect the ischemic cerebral cortex tissue from injury in cerebral I/R rats; this may be related to its effects in downregulating the expression of nNOS and iNOS and upregulating the expression of glial fibrillary acidic protein (GFAP) (Zhang et al., 2015c).

EA pretreatment at GV20 significantly reduced BBB permeability and brain edema (Zou et al., 2015). Furthermore, EA pretreatment could alleviate brain edema and BBB dysfunction caused by cerebral ischemia by reducing matrix metalloproteinase-9 (MMP-9) levels (Dong et al., 2009). EA pretreatment significantly attenuated neuronal apoptosis, inhibited caspase-3 activity in the hippocampal CA (Cornu Ammonis) 1 region, and ameliorated learning and memory function in rats exposed to high-sustained positive acceleration (Feng et al., 2010). Moreover, excessive activation of N-methyl-D-aspartate (NMDA) glutamate receptors contributes to neuronal death after stroke. EA pretreatment (1.7 Hz, 1 mA) at GV20, Shenshu (BL23), and ST36 can suppress the increase in hippocampal glutamate content and downregulate NMDA receptor subunit 1 (NR1) mRNA expression in rats with vascular dementia established by MCAo (Meng et al., 2008). EA treatment also reversed the high expression of NR1 and upregulated the level of tropomyosin receptor kinase A (TrkA) in a MCAo rat model; this effect was mediated by the stimulation of the phosphatidylinositol 3-kinase (PI3K) pathway but not the extracellular signal-regulated kinases (ERKs)/mitogen-activated protein kinase (MAPK) pathway. Therefore, EA pretreatment attenuates glutamate excitotoxicity by modulating PI3K pathway (Sun et al., 2005).

EA Pretreatment Influences Growth Factors Following Cerebral Injury

Stem cells from the subventricular and subgranular zones or those recruited from the bone marrow (BM) through peripheral circulation possess a definitive role in neuronal regeneration following cerebral injury. Some of the most commonly explored growth factors include vascular endothelial growth factor (VEGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and insulin growth factor-1 (IGF-1), each of which may activate ischemic brain endogenous repair through specific mechanisms (Dailey et al., 2013).

Among the neurotrophic factors, BDNF and stromal cell-derived factor-1α (SDF-1α) are considered to be potent candidates for the recovery from cerebral ischemia. SDF-1α resulted in neuroprotection against neurotoxic insult, and it induced BM-derived cell targeting in the ischemic brain, thereby reducing the volume of cerebral infarction and improving neural plasticity (Shyu et al., 2008).

EA preconditioning (2 Hz, at GV20 and GV14, for 20 minutes) reduced infarct volume in mice with cortical ischemia, leading to prominent improvement of neurological function. Pretreatment with EA also increased the production of BDNF and SDF-1α, which elicited protective effects against focal cerebral ischemia (Kim et al., 2013a).

Acupuncture therapy was reported to produce alterations in the levels of growth factors such as glial cell line derived neurotrophic factor (GDNF), BDNF, and VEGF (Peplow and Martinez, 2016).

Neuroglobin (NgB) is involved in cellular oxygen homeostasis. It increases oxygen availability to brain tissue and provides protection under hypoxic or ischemic conditions, potentially limiting brain damage. In other words, NgB enhances cell viability under hypoxia and under various types of oxidative stress, and it is beneficial for neurons (Burmester and Hankeln, 2009). Applying EA preconditioning to GV20 has a significant neuroprotective effect on cerebral ischemia-reperfusion, which is closely related to the upregulation of cerebral NgB expression (Xie et al., 2012).

EA pretreatment at GV20 enhanced the neuronal expression of hypoxia-inducible factor-1α (HIF-1α), reduced infarct volume, improved neurological outcome, inhibited neuronal apoptosis, upregulated the expression of B-cell lymphoma 2 (Bcl-2), and downregulated the expression of Bcl-2-associated X protein (Bax) after reperfusion in the penumbra (Zhao et al., 2015).

Neuroprotective Effect of EA after Ischemic Stroke

EA was applied to *Renzhong* (GV26) and *Neiguan* (PC6) acupoints for 30 minutes, starting immediately after the onset of reperfusion in a MCAo rat model, leading to a significant reduction in ischemic infarction and neurological deficits, upregulation of delta-opioid receptor expression, and protection of the brain from I/R injury (Tian et al., 2008).

EA at GV20 and GV26 reduced neurological deficit, brain swelling, and infarct area; it also increased the percentages of residual cells in the ipsilateral striatum and cortex, and facilitated electroencephalogram recovery following MCAo in monkeys (Gao et al., 2002). EA was found to promote the recovery of neurological function in patients with acute

ischemic stroke and somatosensory evoked potential in rats with MCAo (Si et al., 1998).

A study assessed the hypothesis that EA can enhance cerebral glucose metabolism by using 18F fluorodeoxyglucose/positron emission tomography (PET) imaging in rats subjected to I/R injury. After EA treatment at the *Quchi* (LI11) and ST36 acupoints, T2 weighted imaging revealed a significant reduction in infarct volume, PET imaging of glucose metabolism in the caudate putamen, motor cortex , and somatosensory cortex regions was promoted by EA, accompanied by functional recovery in Catwalk and Rota-rod performance; moreover, EA could promote adenosine monophosphate (AMP) activated protein kinase α (AMPKα) phosphorylation of these regions to enhance neural activity and motor functional recovery after ischemic stroke (Wu et al., 2017).

EA stimulation applied to GV20 and GV14 significantly reduced infarct volume, brain water content, and neuronal injury in rats with MCAo. EA exerts neuroprotective effects against I/R injury by attenuating inflammatory cytokines, upregulating antioxidant systems, and reducing excitotoxicity (Shen et al., 2016).

EA Increases Cerebral Blood Flow in Cerebral Ischemia

In our previous study, we found that 2 or 15 Hz EA at ST36 (bilaterally) could increase CBF in rats with and without cerebral ischemia (Hsieh et al., 2006). In addition, we observed that neither 2 nor 15 Hz EA influenced the expression levels of nitric oxide (NO) in peripheral blood and calcitonin gene-related peptide (CGRP) in the cerebral cortex and thalamus.

EA stimulation at GV26 could promote the proliferation of vascular endothelial cells and increase regional CBF, indicating that EA might promote angiogenesis under cerebral ischemic conditions (Du et al., 2011).

Influence of EA on ROS after Ischemic Stroke

EA treatment at GV26 and GV20 might markedly reduce the neurological deficit score, promote respiratory enzyme activity, and reduce ROS generation, consequently improving respiratory chain function and the antioxidative capability of brain tissues in the infarct penumbra zone (Zhong et al., 2009). These mechanisms may account for the anti-injury effect of EA on brain function in rats with MCAo.

EA treatment at GB20 was suggested to alleviate lipid peroxidation after cerebral I/R injury by promoting the activities of superoxide dismutase and glutathione peroxidase (Siu et al., 2004a). In a previous study, the effect of EA at GV26 and *Chengjiang* (CV24) on NOS in rats with cerebral I/R injury was explored. The study revealed that the abnormally increased expression levels of nNOS and iNOS were reversed partly through the TrkA/PI3K-mediated signal transduction pathway (Chen et al., 2011).

EA stimulation at GV20 and GV14 in mice after MCAo significantly reduced infarct volume and increased cerebral perfusion in the cerebral cortex, consistent with a prominent improvement in neurological function and vestibule-motor function. EA stimulation after moderate focal cerebral ischemia, but not severe ischemia, improves tissue and functional recovery. Acetylcholine (Ach)/eNOS-mediated perfusion augmentation might be related to these beneficial effects of EA by interventions in acute ischemic injury (Kim et al., 2013b).

Cholinergic Anti-Inflammatory Pathway in Attenuation of Neuroinflammation Suggestions and Advises for the Most Promising Further Direction of the Study

EA stimulation in an experimental stroke model improved cerebral perfusion, thus reducing infarct volume and hindering apoptosis, neuronal and peripheral inflammation, and oxidative stress. Furthermore, a dramatically lower reduction in mRNA levels of choline acetyltransferase and α7 nicotinic Ach receptors (α7nAChR) was detected, suggesting the inhibition of central cholinergic system impairment. EA also activated the dorsal motor nucleus of the vagus, thereby implying its role as an alternative modality of parasympathetic nervous system activation for stroke therapy (Chi et al., 2017). A similar result was reported wherein EA at the GV20 and *Shenting* (GV24) acupoints activated the expression of α7nAChR in the hippocampus; EA treatment also led to decreased production of the inflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β), leading to a reduction in neuroinflammatory response (Liu et al., 2017). Our previous study investigated the effect of electric stimulation (ES) of the ears; 2 Hz ES of the ears ameliorated learning and memory impairment and increased the number of α4nAChR in the hippocampus in rats with I/R injury (Kuo et al., 2016). These findings suggest that ES (2 Hz) of the ears exerts neuroprotective effects that are related to acetylcholine release.

Stimulation of acupuncture points on the extremities has been demonstrated to result in stimulation of the vagus nerve. In our previous study, 2 Hz EA stimulation at bilateral ST36 and *Shangjuxu* (ST37) acupoints reduced pulse rate, which indicated that such an application might enhance parasympathetic activity (Hsieh et al., 1999). The auricular branch of the vagus nerve is the only peripheral branch of the vagus nerve. Auricular electrical stimulation on the concha of the ear induced an increase in vagal activity (La Marca et al., 2010). Cholinergic anti-inflammatory pathway is a physiological mechanism by which central nervous system regulates immune response and controls inflammation (Duris et al., 2017). Stimulation of the vagus nerve may represent a new way to prevent pathological inflammation, which has shown potential as a strategy to lessen the inflammatory response and facilitate functional recovery in stroke patients (Neumann et al., 2015). Vagus nerve stimulation is neuroprotective in acute cerebral I/R injury by suppressing inflammation and apoptosis *via* activation of cholinergic and α7 nAChR/Akt pathways (Mravec, 2010; Jiang et al.,

2014). A short period of transcutaneous stimulation in the external ear initiated 30 minutes after contralateral transient MCAo reduces infarct volume by 28% in rats and leads to an improvement in neurological outcome that is sustained at 24 hours (Ay et al., 2015). This convenient technological development deserves further study on the role of vagus nerve stimulation as a neuromodulator in acute and chronic stroke and as a potential secondary preventive option (Cai et al., 2014). In addition, Acupuncture stimulation apply to forepaw in rat can increase the release of acetylcholine in cerebral cortex and increase cortical cerebral blood flow. The afferent pathway involves to group III and IV afferent nerves, whereas intrinsic cholinergic vasodilators originating in the nucleus basalis of Meynert participates in the efferent pathway (Uchida et al., 2000). The brain mediates *via* vagal secretion of ACh to suppress peripheral inflammation and the acetylcholinesterase mRNA-targeting microRNA-132 may play a functional regulator role in brain to body resolution of inflammation (Shaked et al., 2009). Taken together, acupuncture stimulation induces vagal activity and cholinergic anti-inflammatory pathway in attenuation of neuroinflammation possibly are a further direction of the study.

Influence of EA on the BBB after Ischemic Stroke

EA was presumed to improve the function of the BBB, which is generally disrupted after cerebral ischemia (Wu et al., 2001). Applying EA (20 Hz/80 Hz, 1–3 mA) to GV20 and GV14 can reduce ischemic injury of the cerebral cortical neurons and BBB in rats with cerebral I/R injury (Shen et al., 2009). Matrix metalloproteinase (MMPs) are neutral proteases that disrupt the BBB and degrade myelin basic proteins under conditions of neuroinflammation, resulting in brain edema (Candelario-Jalil et al., 2011).

Our previous study indicated that EA at GV20 and GV14 during the subacute phase of cerebral I/R injury significantly reduced cerebral infarct and neurological deficit scores. EA also downregulated the expression of nuclear factor kappa B (NF-κB) p50, and TNF-α, in addition to reducing iNOS and apoptosis levels in the ischemic cortical penumbra to provide neuroprotection (Cheng et al., 2014a). Additionally, EA at GV26 and GV20 in a rat model of cerebral I/R injury markedly reduced neurological deficits and the aquaporin-4 protein and mRNA expression levels of corpus striatum, thereby relieving damage to the BBB (Peng et al., 2012).

EA at GV20 and ST36 can improve neurological outcomes in a cerebral I/R injury rat model and reduce inflammation as well as MMP-9 expression in the brain (Xu et al., 2010; Chen et al., 2012b). Acupuncture and EA administered at the GV20 and ST36 acupoints significantly reduced infarct size and improved neurological function. Furthermore, inflammatory cell infiltration and MMP2, aquaporin 4, and aquaporin 9 expression levels were significantly reduced. Acupuncture and EA can exert similar neuroprotective actions in a MCAo rat model (Xu et al., 2014). EA stimulation (2 Hz, 1 mA) at the GV20 and *Siguan* acupoints (bilateral *Hegu* (LI4) and *Taichong* (LR3)) significantly reduced cerebral infarct area and neurological deficit scores, reduced the number of apoptotic cells, upregulated Bcl-2 protein expression, and downregulated Bax protein expression in rats with MCAo. Additionally, EA stimulation significantly downregulated the expression levels of MMP-9 and simultaneously upregulated the mRNA and protein expression levels of tissue inhibitor of metalloproteinases-1 (TIMP-1) (Ma et al., 2016).

Bloodletting puncture at *Jing*-well points has been demonstrated to alleviate cerebral edema, which mainly results from the disruption of the BBB. A study reported that bloodletting puncture at 12 *Jing*-well points of the hand once a day could reduce water content in the brain and the permeability of the BBB in a MCAo rat model, in addition to ameliorating tight junctions, as observed under electron microscopy; the expression levels of occludin and claudin-5 were also upregulated, whereas intercellular adhesion molecule-1 (ICAM-1) and VEGF were downregulated (Yu et al., 2017). Another study observed the effects of bloodletting puncture at *Jing*-well points at the distal ends of the finger and toe on survival rate, survival time, and brain edema in rats with cerebral ischemia; the study revealed that bloodletting puncture prolonged the survival time of the rats and improved ischemic brain edema (Gao et al., 2012).

Antiapoptotic Effect of EA after Ischemic Stroke

EA (2 Hz, 1 mA) applied to a MCAo rat model resulted in a marked reduction of infarct area after stroke and a reduction in the number of apoptotic cells. Moreover, EA enhanced the expression of the antiapoptotic markers Bcl-2, Bcl-xL, and both cIAP-1 and -2. The activities of caspase-3, -8, and -9 were also markedly inhibited by the antiapoptotic effects of EA treatment (Kim et al., 2013c).

Neuroregenerative Effect of EA after Ischemic Stroke

EA could upregulate GDNF expression and extend the duration of this upregulated expression after ischemic insult (Wei et al., 2000). The results of our previous study indicated that 5 Hz EA at GV20 and GV14 upregulated BDNF expression and thus provided BDNF-mediated neuroprotection against caspase-3-dependent neuronal apoptosis by activating the Raf-1/MEK1/2/ERK1/2/p90RSK/Bad signaling cascade in mild MCAo rats (Cheng et al., 2014b).

A rat model of cerebral I/R was established by suture occlusion of the left middle cerebral artery. Low-frequency continuous-wave EA (frequency, 2–6 Hz; current intensity, 2 mA) stimulation of the brachial plexus trunk on the right side increased the amount of BDNF and alleviated neurological function deficits (Guo and Wang, 2012). EA at GV20 improved motor recovery and stimulated BDNF/TrkB expression in rats with cerebral ischemia (Kim et al., 2012). Applying EA stimulation (2 Hz) to GV20 and GV14 after ischemic stroke in a MCAo mouse model may promote

poststroke functional recovery by enhancing the proliferation and differentiation of neural stem cells *via* the BDNF and VEGF signaling pathways (Kim et al., 2014). SDF-1α plays a crucial role in regulating the mobilization, migration, and homing of endothelial progenitor cells (EPCs). The administration of EA at the GV20 and *Siguan* acupoints in a rat model after focal cerebral I/R could accelerate and increase the formation of an SDF-1α concentration gradient to further induce the mobilization of EPCs and angiogenesis in the ischemic brain and improve neurological function recovery (Xie et al., 2016). EA at GV20 and GV26 with dense-sparse waveforms was effective in attenuating cerebral ischemic injuries and upregulating endogenous IGF-1 expression following MCAo in monkeys; this might be an important mechanism by which EA exerts its neuroprotective effects against cerebral ischemia (Gao et al., 2006). EA at GV20 and GV26 (with a "disperse-dense" wave at 2 and 150 Hz, alternately, and a constant intensity of 3 mA) increased the serum levels of transforming growth factor beta (TGF-β1) in rats with acute cerebral I/R injury, thereby exerting its neuroprotective effects (Wang et al., 2016).

Inflammation after stroke is the main cause of cerebral I/R injury. EA ("disperse-dense" wave at 2 to 100 Hz, alternately; 2 mA) at GV20 and *Qubin* (GB7) could attenuate cerebral I/R injury and suppress leukocyte infiltration by reducing cyclooxygenas-2 (COX-2) and NF-κB levels and enhancing TGF-β1 expression in brain tissues of rats with MCAo (Zhang et al., 2009). EA treatment (60 Hz, 1 second and 2 Hz, 3 seconds alternately at an intensity of 10 mA) at *Fengfu* (GV16) and *Jinsuo* (GV8) activates cell proliferation and facilitates neurogenesis as well as maturation of newly generated neurons; thus, in addition to neuronal regeneration, EA can improve newborn neuron migration and its maturation in the striatum of adult rat brains after stroke (Yang et al., 2005). Blood-derived leukocytes and resident microglia are the most activated inflammatory cells that accumulate in brain tissue after cerebral ischemia, leading to inflammatory injury. Microglia, the major source of cytokines and other immune molecules of the central nervous system (CNS), are the first nonneuronal cells that respond to CNS injury, and they become phagocytic when fully activated by neuronal death.

Cerebral ischemia might promote the proliferation of neural stem cells, and some of them could differentiate into astroglia or neurons. EA could enhance the proliferation and differentiation of neural cells into mature neurons, which might be one of the key reasons why EA could improve neurological dysfunction (Tao et al., 2010). EA was stated to maintain the structural integrity of astrocyte (Xiao et al., 2013). Applying EA at GV20 and GV14 has the potential to activate astrocytes in the peri-infarct region and to avoid excess reactive gliosis in a MCAo rat model; moreover, it can facilitate the recovery of postischemic behavioral dysfunction (Han et al., 2010). EA treatment significantly promoted the recovery of neurological deficits in MCAo rats, which correlated with enhanced lactate energy metabolism in the resident astrocytes around the ischemic area and upregulated the expression of the lactate transporter monocarboxylate transporter 1 (MCT1) in these astrocytes (Lu et al., 2015). Applying EA (4/20 Hz, 1–3 mA) at GV20 and GV14 in MCAo rats is useful for synaptic reorganization, which may be associated with its effect on intervening the activation state of astrocytes; thus, acupuncture could initiate the adjustment of neuron–glial networks and thus improve synaptic reorganization, which may be key for the treatment of cerebral ischemia (Luo et al., 2011). Reactive astrogliosis is a common phenomenon in CNS injury such as ischemic stroke. EA treatment at the LI11 and ST36 acupoints attenuated neurological deficits and cerebral infarct volume in I/R injured rats. This treatment also exerted neuroprotection through the proliferation of GFAP/vimentin/nestin-positive reactive astrocytes and the potential secretion of reactive astrocytes-derived BDNF (Tao et al., 2016).

EA at GV20 and GV14 in mice using bilateral common carotid artery stenosis attenuated spatial and short-term memory impairments and enhanced oligodendrocyte differentiation from oligodendrocyte precursor cells. EA stimulation promotes the recovery of memory function following white matter injury through a mechanism that promotes oligodendrocyte regeneration and involves neurotrophin (NT) 4/5-TrkB signaling (Ahn et al., 2016). A study compared the effects of EA (15 Hz, 1–3 mA) applied bilaterally at *Sanyinjiao* (SP6) and *Fenglong* (ST40) with those of manual acupuncture at GV20 and GV26 (punctured and stimulated manually for 1 min) in rats with hyperlipidemia plus cerebral ischemia (Ren et al., 2010). Manual acupuncture and EA significantly upregulated the expression levels of vimentin and beta-Tubulin (Tju-1) in the subependymal ventricular zone of the ischemic side, suppressing the reduction of proliferation and differentiation of neural stem cells (Ren et al., 2010). Our previous study investigated whether acupuncture enhances neuronal regeneration in rats with ischemic stroke. We observed that EA (2 Hz) at both ST36 and ST37 acupoints reduced the infarction/hemisphere ratio, reduced the modified neurological severity score, and increased the rotarod test time. Additionally, EA (2 Hz) reduced nestin immunoreactive cells in the penumbra and ischemic core areas; it also reduced Ki67 and increased GFAP immunoreactive cells in the penumbra area. Our findings suggest that EA (2 Hz) at the ST36 and ST37 acupoints exerts a neuroprotective role (Liao et al., 2017).

The PI3K/Akt pathway, a critical mediator of cell survival, is suppressed during cerebral I/R injury. Administering EA at the LI11 and ST36 acupoints on the contralateral paralyzed limb significantly improved neurological deficits and cerebral infarction and activated PI3K/Akt signaling in ischemic cerebral tissues, resulting in the inhibition of cerebral cell apoptosis; this treatment also increased serum secretion levels of the PI3K activators BDNF and GDNF, in addition to upregulating the antiapoptotic Bcl-2/Bax ratio in the ischemic cerebrum (Chen et al., 2012a). Furthermore, EA treatment at ST36 and LI11 in cerebral I/R injury rats improved neurological deficit and cerebral infarction and profoundly activated PI3K/Akt signaling, resulting in the

inhibition of cerebral cell apoptosis in the ischemic penumbra; this treatment increased PI3K, p-Akt, p-Bad, and Bcl-2 expression at the protein level but inhibited Bax and cleaved Caspase-3-positive expression (Xue et al., 2014). The ERK pathway, a critical mediator of cell proliferation, is activated in cerebral I/R injury. A study revealed that EA at the LI11 and ST36 acupoints significantly ameliorated neurological deficits and cerebral infarction in cerebral I/R-injured rats, increased the phosphorylation levels of ERK, and increased the protein expression levels of Ras, cyclin D1, and cyclin-dependent kinase (CDK)4; EA-mediated activation of the ERK pathway resulted in the stimulation of cerebral cell proliferation (Xie et al., 2013). EA probably protects cerebral I/R injury by alleviating the damage to the ultrastructure of brain cells and downregulating the expression levels of neurite outgrowth inhibitor-A (Nogo-A) (Liang et al., 2012). Administering EA at bilateral PC6, SP6, GV26, and GV20 could effectively improve neurological function in rats with cerebral infarction; this may be attributed to its effects on the upregulation of cerebral VEGF, nerve growth-associated protein-43 (GAP-43), synaptophysin (SYN), and myelin basic protein (MBP) expression and the downregulation of Nogo-A protein, thus indicating its protective effects on the neurovascular unit (Han et al., 2013). EA (50 Hz, 3 mA) could enhance the recovery of neurological function, reduce cerebral infarction volume, and increase HIF-1α expression in ischemic rats (Li et al., 2017). Intracellular protein denaturation is a significant pathological step in acute conditions such as stroke and myocardial infarction. Heat shock proteins (HSPs) are fundamental for intracellular protein repair and work, because they prevent protein aggregation and assist the refolding of denaturated proteins (Cakmak, 2009). HSP70 and other endogenous injury-signaling molecules are released by damaged cells. One of the molecular mechanisms involved in EA treatment was revealed to be the promotion of the expression of inducible HSP70 (Sun et al., 2003). Conversely, another study that administered EA (2–100 Hz, 2 mA) at the GV20 and ST36 acupoints in rat models of cerebral I/R injury lowered the peak levels of adrenocorticotrophic hormone and HSP70, suggesting that EA may inhibit excessive stress, reduce inflammation, and promote neural repair, thus facilitating healing in ischemic stroke (Shi et al., 2017).

EA Attenuates Glutamate Excitotoxicity *via* NMDA Receptors after Ischemic Stroke

Ischemia impairs brain function and networks. The loss of GABAergic neurons disrupts the balance of excitation and inhibition, which causes further neural excitotoxicity and nerve cell death. Acupuncture at GV20 improves ischemic stroke by preventing the impairment of cortical GABAergic neurons (Zhang et al., 2011). Cerebral ischemia induces excessive glutamate release and excitotoxicity. NMDA receptors are responsible for glutamate-induced excitotoxicity in the postischemic brain. Both hyperemia and glutamate overrelease after ischemia were reported to be vital factors for brain damage due to reperfusion injury, and EA treatment (7 Hz, 6 mA) at GV16 and Shendao (GV11) for 30 minutes might inhibit the overrelease of glutamate and thus protect neurons against I/R injury (Pang et al., 2003).

EA could regulate the content of Ca^{2+} in the ischemic area of the brain and inhibit Ca^{2+} overload in order to protect the neurons in rats with focal cerebral ischemia (Xu et al., 2002). Our previous study indicated that 2 Hz EA at GV20 reduced the expression levels of NR1 and transient receptor potential vanilloid subtype 1 (TRPV1) receptors in the hippocampal CA1 areas in a vascular dementia model induced by the MCAo technique. Additionally, our previous study indicated that MCAo-induced behavior and long-term potentiation impairment were improved (Lin and Hsieh, 2010).

Glutamate-NMDA receptor excitotoxicity and oxidative stress are two common mechanisms associated with most neurodegenerative diseases, and neurotoxicity through these two mechanisms is dependent upon cAMP response element-binding protein (CREB) and NF-κB DNA transcription that regulates the vitality of neurons (Zou and Crews, 2006). A study investigated the mechanisms by which EA ameliorates learning and memory in rats following MCAo surgery. EA at GV24 and GV20 significantly ameliorated neurological deficits and reduced cerebral infarct volume. In addition, EA improved learning and memory abilities in the rats and markedly activated the CREB signaling pathway, resulting in the inhibition of cerebral cell apoptosis in the ischemic penumbra. Furthermore, EA increased the activity of superoxide dismutase and glutathione peroxidase, the protein expression levels of phosphorylated CREB and Bcl 2, and the mRNA expression levels of Bcl 2. Conversely, it reduced the levels of malondialdehyde and inhibited the expression levels of Bax (Lin et al., 2015).

EA Parameters for Treating Cerebral Ischemic Injury

Research demonstrated that the appropriate electrical stimulation parameters of EA pretreatment to induce cerebral ischemic tolerance in rats are a density-sparse wave of 2/15 Hz and a current intensity of 1 mA applied for 30 minutes per day for 5 consecutive days. The density-sparse wave had the most obvious neuroprotective effect, followed by the intermittent wave, whereas the neuroprotective effect of the continuous wave was relatively poor (Yang et al., 2004). EA pretreatment at GV20 could induce more robust neuroprotection against cerebral I/R injury than did stimulation 1 cm lateral to GV20 or the nonmeridian points of the distal limbs; this thus indicates the acupoint specificity of EA pretreatment (Lu et al., 2002; Li et al., 2012).

Previously, experiments were performed on rats subjected to MCAo using different intensities and frequencies of EA at GV26 and GV20 to optimize the stimulation parameters. The results showed that EA at 1.0–1.2 mA and 5–20 Hz remarkably increased blood flow and reduced ischemic infarction, neurological deficits, and death rates. The "nonoptimal" parameters of EA (*e.g.*, < 0.6 mA or > 40 Hz) could not im-

prove the blood flow or reduce ischemic injury. In addition, the same EA treatment with optimal parameters could not increase blood flow in naive brains (Zhou et al., 2011).

EA at GV20 and GV26 by using a sparse-dense wave (5 Hz/20 Hz) at 1.0 mA for 30 minutes greatly increased CBF, reduced infarction, significantly improved neurological deficit, and reduced the death rate in a rat model of cerebral ischemia caused by right MCAo. A similar result was observed with EA at the left (contralateral to the ischemic side) forelimb (LI11 and PC6). By contrast, EA at the right LI11 and PC6 and at the acupoints in the hindlimb (GB34 and SP6) had no such effect. These results imply that EA protection against cerebral ischemia is relatively acupoint specific (Zhou et al., 2013).

Acupuncture stimulation of the cheek, forepaw, upper arm, and hindpaw, but not the chest, back, lower leg, or perineum, produced significant increases in CBF in anesthetized rats (Uchida et al., 2000). A study was designed to investigate the effects of different frequencies of EA on the latent period and wave amplitude of motor-evoked potentials in rats with focal cerebral infarction. EA was applied to GV26 at a frequency of 2 Hz, 50 Hz, or 100 Hz (intensity 1 mA) for 10 min twice daily for 3 days. The latency on the affected side in the 2 Hz group was significantly shortened ($P < 0.05$), whereas the amplitude was significantly increased when compared with the model group, indicating that low-frequency EA at GV26 can promote recovery of motor function after focal cerebral ischemic injury in rats (Yao et al., 2012).

Another study investigated optimal EA frequencies for maintaining the structural integrity of ischemic brain tissue. In a rat model of MCAo, EA (15 and 30 Hz) at bilateral LI11 and ST36 reduced neurological deficit, increased GFAP expression, and alleviated ultrastructural damage of astrocytes at the edge of the infarct, compared with the group with no treatment or EA treatment at 100 Hz. EA interventions at frequencies of 15 and 30 Hz can favorably maintain the structural integrity of astrocytes and play a protective role in cerebral ischemic injury (Xiao et al., 2013).

In another study, the effects of different intensities of EA on neuroprotection were observed in rats with cerebral I/R injury. EA stimulations at GV20, Mingmen (GV4), and ST36 with the same stimulation waveform (30 to 50 Hz) and different electric current intensities (5, 3, and 1 mA) were performed. The 3 mA EA was found to be the most effective in strengthening aerobic metabolism, maintaining the ionic equilibrium in the exterior and interior brain cells, and relieving cellular edema by reinforcing the activities of Na^+-K^+-ATPase (Tian et al., 2015).

EA-induced neuroprotection against cerebral I/R injury depends on an optimal EA duration. A study applied EA (5 Hz/20 Hz at 1 mA) at the GV26 and GV20 acupoints for 5, 15, 30, and 45 minutes after 24 hours reperfusion in rats exposed to right MCAo for 60 minutes. The study revealed that 30 minutes of EA, starting at 5 minutes after the onset of MCAo (EA during MCAO) or 5 minutes after reperfusion (EA after MCAO), significantly reduced infarct volume, improved neurological deficit, and reduced the death rate. Moreover, the protective effect was proportional to the increase in the duration of stimulation in the EA group with MCAo, with the maximum increase observed between 5 and 30 minutes of stimulation. EA for 45 minutes did not cause a reduction in infarct volume or neurological deficits; instead, it showed an increase in death rate in this group (Zhou et al., 2013). In another study, the PC6 acupoint in rats with MCAo was needled at a fixed frequency (3 Hz) with different durations (5, 60, and 180 seconds) under a twisting-rotating acupuncture method. Results showed that different durations of acupuncture had different therapeutic effects, with the 60 seconds duration yielding a more favorable therapeutic effect than did the other two durations for ischemic stroke (Zhang et al., 2015a). To observe the effects of different needle-retaining durations on hemorheology in patients with ischemic stroke, EA was applied at several acupoints including the *Jianyu* (LI15), LI11, *Waiguan* (TW5), *Hegu* (LI4), *Futu* (ST32), and ST36 acupoints by using a frequency of 2 Hz at 2–6 mA for 20, 40, and 60 minutes separately; the treatment was performed once daily for 10 times. The therapeutic effects on various parameters of hemorheology in the 60 minutes group were more favorable than those in the 20 and 40 minutes groups (He et al., 2007).

To investigate the differential effects between multiple EA and single-time EA stimulation on ischemic injury, a previous study found that both methods significantly reduced MCAo-induced ischemic infarction; however, only multiple EA attenuated sensorimotor dysfunctions. The short-term effect of single-time EA stimulation differs from the cumulative effect of multiple EA, which possibly depends on their differential modulation of the expression of neurotrophic signaling molecules (Wang et al., 2014).

A multicenter, single-blinded, randomized controlled trial was performed in China. In this trial, 862 patients with limb paralysis between 3 and 10 days after ischemic stroke onset were allocated to acupuncture plus standard care or standard care alone. The acupuncture was applied 5 times per week for 3 to 4 weeks. Fewer patients appeared to die or become dependent in the acupuncture group when compared with the control group at 6 months, particularly in the subgroup receiving ≥ 10 sessions of the treatment (Zhang et al., 2015b).

Effectiveness of Acupuncture in Acute Ischemic Stroke Patients

A systematic review showed that scalp acupuncture in patients with acute ischemic stroke appears to improve the neurological deficit score and clinical effective rate when compared with Western conventional medicine (Wang et al., 2012). Studies have examined the additional therapeutic effects of EA in patients with first-ever ischemic stroke. In one study, the study and control groups underwent a conventional rehabilitation program, with the study group receiving an additional eight courses of EA over a period of 1 month. The study results revealed that EA could improve

motor function, especially in the upper limbs (Hsieh et al., 2007). In another study, 290 patients aged 40–75 years with a first onset of acute ischemic stroke (more than 24 hours but within 14 days) were treated using standard treatment methods, and they were randomly allocated to an intervention group (treated with resuscitating acupuncture) or a control group (treated using sham-acupoints). The results of this clinical trial showed a clinically relevant reduction in relapse in patients treated with resuscitating acupuncture intervention by the end of 6 months, compared with those who underwent needling at the sham-acupoints. Resuscitating acupuncture intervention could also improve self-care ability and quality of life in these patients (Shen et al., 2012). In our previous, randomized, single-blinded, controlled study, 30 first-time ischemic stroke patients underwent acupuncture treatment along with the manual twisting of needles at GV20 and four spirit acupoints (1.5 cun anterior, posterior, left, and right from GV20) for 20 minutes. The displacement area from the center of gravity decreased in the experimental group, suggesting that acupuncture stimulation may induce an immediate effect that improves balance function in stroke patients (Liu et al., 2009).

Increased CBF was observed in single-photon emission computed tomography (SPECT) brain perfusion images obtained in six patients with MCAo following acupuncture at acupoints LI4, *Shousanli* (LI10), LI11, LI15, and *Jugu* (LI16), and at TW5, in the affected arm. Acupuncture stimulation after stroke appears to activate the perilesional sites and may aid in brain reorganization (Lee et al., 2003).

PET is used to observe cerebral function. A study applied this method to observe cerebral functioning in six patients suffering from ischemic stroke after receiving EA treatment at GV20 and right GB7. The results revealed that glucose metabolism had changed significantly in the primary motor area, premotor cortex, and superior parietal lobule bilaterally, as well as in the supplementary motor area on the unaffected hemisphere immediately after the first EA treatment. Similarly, significant changes in glucose metabolism were noted in other areas such as the insula, putamen, and cerebellum. This thus demonstrates that EA was very useful for cerebral motor plasticity after the ischemic stroke (Fang et al., 2012).

Conclusion

In recent years, results from numerous animal experiments and clinical research have uncovered some of the resulting molecular and biophysical correlates of acupuncture or EA in alleviating cerebral ischemic injury. Acupuncture intervention can significantly reduce the size of the infarcted area, improve cerebral blood circulation to promote regional energy metabolism, regulate blood lipid metabolism to resist cerebral free radical damage, inhibit cerebral cortical apoptosis, reduce the amount of excitatory amino acids to lower neurogenic toxicity, reduce calcium overloading, ease cerebral vascular immune-inflammatory reactions, and upregulate the expression of antiapoptosis genes and neurotrophic factors, thereby promoting the proliferation and differentiation of neural stem cells in the focal cerebral cortex and hippocampus. Acupuncture stimulation induces vagal activity and cholinergic anti-inflammatory pathway in attenuation of neuroinflammation possibly are a further direction of the study. In addition, the acupoint that is in common use for neuroprotection or neuroregeneration in ischemic stroke in **Figure 1**.

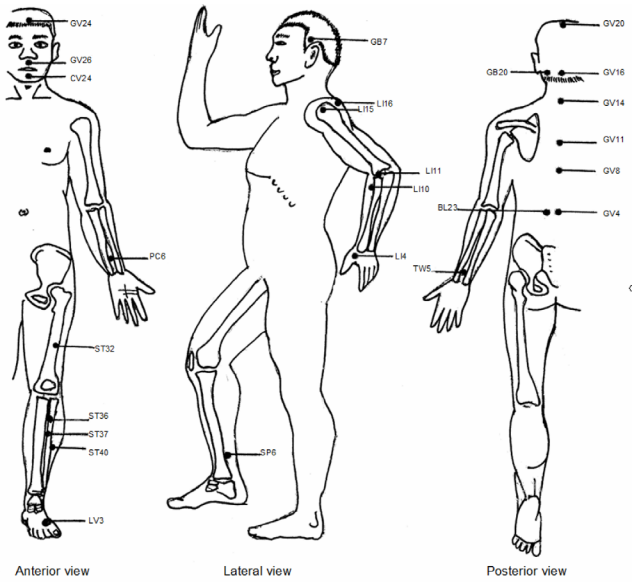

Figure 1 The acupoint that is in common use for neuroprotection or neuroregeneration in ischemic stroke.
LI4: *Hegu*; LI10: *Shousanli*; LI11: *Quchi*; LI15: *Jianyu*; LI16: *Jugu*; ST32: *Futu*; ST36: *Zusanli*; ST37: *Shangjuxu*; ST40: *Fenglung*; SP6: *Sanyinjiao*; BL23: *Shenshu*; PC6: *Neiguan*; TE5: *Waiguan*; GB7: *Qubin*; GB20: *Fengchi*; LV3: *Taichong*; GV24: *Chengjiang*; GV4: *Mingmen*; GV8: *Jinsuo*; GV11: *Shendao*; GV16: *Fengfu*; GV20: *Baihui*; GV24: *Shenting*; GV26: *Renzhong*.

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Open peer review report:
Reviewer: *Krystyna Domanska-Janik, Mossakowski Medical Research Center, Polish Academy of Sciences, Poland.*
Comments to authors: *The authors did an interesting and comprehensive reviewing research of the latest achievements in the science devoted to summarize the biochemical and physio/pathological correlates of beneficial effects of the electro-acupuncture (EA) applied in an early and late phases of ischemic/reperfusion (I/R) brain injury. Due to well known facts that multiple effects observed after EA application to I/R injured brain tissue are extremely complex and involve a variety of interacting biological systems and their underlying pathomechanisms, such comprehensive description of the actual "state of art" is important for further understanding and propagation of all practical benefits which could be achieved by this treatment.*

References

Ahn SM, Kim YR, Kim HN, Shin YI, Shin HK, Choi BT (2016) Electroacupuncture ameliorates memory impairments by enhancing oligodendrocyte regeneration in a mouse model of prolonged cerebral hypoperfusion. Sci Rep 6:28646.

Ay I, Napadow V, Ay H (2015) Electrical stimulation of the vagus nerve dermatome in the external ear is protective in rat cerebral ischemia. Brain Stimul 8:7-12.

Bolaños JP, Almeida A (1999) Roles of nitric oxide in brain hypoxia-ischemia. Biochim Biophys Acta 1411:415-436.

Burmester T, Hankeln T (2009) What is the function of neuroglobin? J Exp Biol 212:1423-1428.

Cai PY, Bodhit A, Derequito R, Ansari S, Abukhalil F, Thenkabail S, Ganji S, Saravanapavan P, Shekar CC, Bidari S, Waters MF, Hedna VS (2014) Vagus nerve stimulation in ischemic stroke: old wine in a new bottle. Front Neurol 5:107.

Cakmak YO (2009) A review of the potential effect of electroacupuncture and moxibustion on cell repair and survival: the role of heat shock proteins. Acupunct Med 27:183-186.

Candelario-Jalil E, Thompson J, Taheri S, Grossetete M, Adair JC, Edmonds E, Prestopnik J, Wills J, Rosenberg GA (2011) Matrix metalloproteinases are associated with increased blood-brain barrier opening in vascular cognitive impairment. Stroke 42:1345-1350.

Chen A, Lin Z, Lan L, Xie G, Huang J, Lin J, Peng J, Tao J, Chen L (2012a) Electroacupuncture at the Quchi and Zusanli acupoints exerts neuroprotective role in cerebral ischemia-reperfusion injured rats via activation of the PI3K/Akt pathway. Int J Mol Med 30:791-796.

Chen SH, Sun H, Xu H, Zhang YM, Gao Y, Li S (2012b) Effects of acupuncture of "Baihui"(GV 20) and "Zusanli"(ST 36) on expression of cerebral IL-1beta and TNF-alpha proteins in cerebral ischemia reperfusion injury rats. Zhen Ci Yan Jiu 37:470-475.

Chen SX, Ding MC, Dai KY (2011) Effect of electroacupuncture on nitric oxide synthase in rats with cerebral ischemia-reperfusion injury. Zhongguo Zhong Xi Yi Jie He Za Zhi 31:784-788.

Cheng CY, Lin JG, Tang NY, Kao ST, Hsieh CL (2014a) Electroacupuncture-like stimulation at the Baihui (GV20) and Dazhui (GV14) acupoints protects rats against subacute-phase cerebral ischemia-reperfusion injuries by reducing S100B-mediated neurotoxicity. PLoS One 9:e91426.

Cheng CY, Lin JG, Su SY, Tang NY, Kao ST, Hsieh CL (2014b) Electroacupuncture-like stimulation at Baihui and Dazhui acupoints exerts neuroprotective effects through activation of the brain-derived neurotrophic factor-mediated MEK1/2/ERK1/2/p90RSK/bad signaling pathway in mild transient focal cerebral ischemia in rats. BMC Complement Altern Med 14:92.

Chi L, Du K, Liu D, Bo Y, Li W (2017) Electroacupuncture brain protection during ischemic stroke: A role for the parasympathetic nervous system. J Cereb Blood Flow Metab doi: 10.1177/0271678X17697988.

Dailey T, Tajiri N, Kaneko Y, Borlongan CV (2013) Regeneration of neuronal cells following cerebral injury. Front Neurol Neurosci 32:54-61.

Dong H, Fan YH, Zhang W, Wang Q, Yang QZ, Xiong LZ (2009) Repeated electroacupuncture preconditioning attenuates matrix metalloproteinase-9 expression and activity after focal cerebral ischemia in rats. Neurol Res 31:853-858.

Du Y, Shi L, Li J, Xiong J, Li B, Fan X (2011) Angiogenesis and improved cerebral blood flow in the ischemic boundary area were detected after electroacupuncture treatment to rats with ischemic stroke. Neurol Res 33:101-107.

Duris K, Lipkova J, Jurajda M (2017) Cholinergic anti-inflammatory pathway and stroke. Curr Drug Deliv 14:449-457.

Fang Z, Ning J, Xiong C, Shulin Y (2012) Effects of electroacupuncture at head points on the function of cerebral motor areas in stroke patients: a PET study. Evid Based Complement Alternat Med 2012:902413.

Feng R, Zhang F (2014) The neuroprotective effect of electro-acupuncture against ischemic stroke in animal model: a review. Afr J Tradit Complement Altern Med 11:25-29.

Feng S, Wang Q, Wang H, Peng Y, Wang L, Lu Y, Shi T, Xiong L (2010) Electroacupuncture pretreatment ameliorates hypergravity-induced impairment of learning and memory and apoptosis of hippocampal neurons in rats. Neurosci Lett 478:150-155.

Gao H, Guo J, Zhao P, Cheng J (2002) The neuroprotective effects of electroacupuncture on focal cerebral ischemia in monkey. Acupunct Electrother Res 27:45-57.

Gao H, Guo J, Zhao P, Cheng J (2006) Influences of electroacupuncture on the expression of insulin-like growth factor-1 following, focal cerebral ischemia in monkeys. Acupunct Electrother Res 31:259-272.

Gao L, Chen Z, Tian L, Li Z, Guo Y (2012) Effects of bloodletting puncture at Jing-Well points in distal ends of finger and toe on survival rate and brain edema in cerebral ischemic rats. J Tradit Chin Med 32:471-476.

Garry PS, Ezra M, Rowland MJ, Westbrook J, Pattinson KT (2015) The role of the nitric oxide pathway in brain injury and its treatment--from bench to bedside. Exp Neurol 263:235-243.

Guo F, Song W, Jiang T, Liu L, Wang F, Zhong H, Yin H, Wang Q, Xiong L (2014) Electroacupuncture pretreatment inhibits NADPH oxidase-mediated oxidative stress in diabetic mice with cerebral ischemia. Brain Res 1573:84-91.

Guo Z, Wang L (2012) Electroacupuncture stimulation of the brachial plexus trunk on the healthy side promotes brain-derived neurotrophic factor mRNA expression in the ischemic cerebral cortex of a rat model of cerebral ischemia/reperfusion injury. Neural Regen Res 7:1618-1623.

Han X, Huang X, Wang Y, Chen H (2010) A study of astrocyte activation in the periinfarct region after cerebral ischemia with electroacupuncture. Brain Inj 24:773-779.

Han YS, Xu Y, Han YZ, Xu L, Liu XG, Liu ZB, Wang P (2013) Protective effect of electroacupuncture intervention on neurovascular unit in rats with focal cerebral ischemia-reperfusion injury. Zhen Ci Yan Jiu 38:173-180.

He YZ, Han B, Zheng SF, Wang LN, Chen ZM, Hu J, Li JM, Peng JX (2007) Effect of different acupuncture needle-retaining time on hemorheology in ischemic stroke patients. Zhen Ci Yan Jiu 32:338-341.

Hsieh CL, Lin JG, Li TC, Chang QY (1999) Changes of pulse rate and skin temperature evoked by electroacupuncture stimulation with different frequency on both Zusanli acupoints in humans. Am J Chin Med 27:11-18.

Hsieh CL, Chang QY, Lin IH, Lin JG, Liu CH, Tang NY, Lane HY (2006) The study of electroacupuncture on cerebral blood flow in rats with and without cerebral ischemia. Am J Chin Med 34:351-361.

Hsieh RL, Wang LY, Lee WC (2007) Additional therapeutic effects of electroacupuncture in conjunction with conventional rehabilitation for patients with first-ever ischaemic stroke. J Rehabil Med 39:205-211.

Jiang Y, Li L, Liu B, Zhang Y, Chen Q, Li C (2014) Vagus nerve stimulation attenuates cerebral ischemia and reperfusion injury via endogenous cholinergic pathway in rat. PLoS One 9:e102342.

Kim JH, Choi KH, Jang YJ, Kim HN, Bae SS, Choi BT, Shin HK (2013a) Electroacupuncture preconditioning reduces cerebral ischemic injury via BDNF and SDF-1alpha in mice. BMC Complement Altern Med 13:22.

Kim JH, Choi KH, Jang YJ, Bae SS, Shin BC, Choi BT, Shin HK (2013b) Electroacupuncture acutely improves cerebral blood flow and attenuates moderate ischemic injury via an endothelial mechanism in mice. PLoS One 8:e56736.

Kim MW, Chung YC, Jung HC, Park MS, Han YM, Chung YA, Maeng LS, Park SI, Lim J, Im WS, Chung JY, Kim M, Mook I, Kim M (2012) Electroacupuncture enhances motor recovery performance with brain-derived neurotrophic factor expression in rats with cerebral infarction. Acupunct Med 30:222-226.

Kim YR, Kim HN, Ahn SM, Choi YH, Shin HK, Choi BT (2014) Electroacupuncture promotes post-stroke functional recovery via enhancing endogenous neurogenesis in mouse focal cerebral ischemia. PLoS One 9:e90000.

Kim YR, Kim HN, Jang JY, Park C, Lee JH, Shin HK, Choi YH, Choi BT (2013c) Effects of electroacupuncture on apoptotic pathways in a rat model of focal cerebral ischemia. Int J Mol Med 32:1303-1310.

Kuo CT, Lin YW, Tang NY, Cheng CY, Hsieh CL (2016) Electric stimulation of the ears ameliorated learning and memory impairment in rats with cerebral ischemia-reperfusion injury. Sci Rep 6:20381.

La Marca R, Nedeljkovic M, Yuan L, Maercker A, Elhert U (2010) Effects of auricular electrical stimulation on vagal activity in healthy men: evidence from a three-armed randomized trial. Clin Sci (Lond) 118:537-546.

Lee JD, Chon JS, Jeong HK, Kim HJ, Yun M, Kim DY, Kim DI, Park CI, Yoo HS (2003) The cerebrovascular response to traditional acupuncture after stroke. Neuroradiology 45:780-784.

Li C, Zhang T, Yu K, Xie H, Bai Y, Zhang L, Wu Y, Wang N (2017) Neuroprotective effect of electroacupuncture and upregulation of hypoxia-inducible factor-1alpha during acute ischaemic stroke in rats. Acupunct Med 35:360-365.

Li X, Wang Q (2013) Acupuncture therapy for stroke patients. Int Rev Neurobiol 111:159-179.

Li X, Luo P, Wang Q, Xiong L (2012) Electroacupuncture pretreatment as a novel avenue to protect brain against ischemia and reperfusion injury. Evid Based Complement Alternat Med 2012:195397.

Liang YQ, Tan F, Chen J (2012) Effects of electroacupuncture on the ultrastructure and the Nogo-A expressions in the cerebral cortex in rats with cerebral ischemia-reperfusion. Zhongguo Zhong Xi Yi Jie He Za Zhi 32:209-213.

Liao SL, Lin YW, Hsieh CL (2017) Neuronal regeneration after electroacupuncture treatment in ischemia-reperfusion-injured cerebral infarction rats. Biomed Res Int 2017:3178014.

Lin R, Lin Y, Tao J, Chen B, Yu K, Chen J, Li X, Chen LD (2015) Electroacupuncture ameliorates learning and memory in rats with cerebral ischemia-reperfusion injury by inhibiting oxidative stress and promoting p-CREB expression in the hippocampus. Mol Med Rep 12:6807-6814.

Lin YW, Hsieh CL (2010) Electroacupuncture at Baihui acupoint (GV20) reverses behavior deficit and long-term potentiation through N-methyl-d-aspartate and transient receptor potential vanilloid subtype 1 receptors in middle cerebral artery occlusion rats. J Integr Neurosci 9:269-282.

Liu J, Li C, Peng H, Yu K, Tao J, Lin R, Chen L (2017) Electroacupuncture attenuates learning and memory impairment via activation of alpha7nAChR-mediated anti-inflammatory activity in focal cerebral ischemia/reperfusion injured rats. Exp Ther Med 14:939-946.

Liu SY, Hsieh CL, Wei TS, Liu PT, Chang YJ, Li TC (2009) Acupuncture stimulation improves balance function in stroke patients: a single-blinded controlled, randomized study. Am J Chin Med 37:483-494.

Lu Y, Zhao H, Wang Y, Han B, Wang T, Zhao H, Cui K, Wang S (2015) Electro-acupuncture up-regulates astrocytic MCT1 expression to improve neurological deficit in middle cerebral artery occlusion rats. Life Sci 134:68-72.

Lu ZH, Xiong LZ, Zhu ZH, Wang Q, Zheng Y, Zheng HX, Hou LZ Chen M (2002) Acupoint specificity of electroacupuncture preconditioning effect on cerebral ischemia injury in rats. Zhongguo Zhenjiu 22:671-673.

Luo Y, Xu NG, Yi W, Yu T, Yang ZH (2011) Study on the correlation between synaptic reconstruction and astrocyte after ischemia and the influence of electroacupuncture on rats. Chin J Integr Med 17:750-757.

Ma L, Zhu Z, Zhao Y, Hou L, Wang Q, Xiong L, Zhu X, Jia J, Chen S (2011) Cannabinoid receptor type 2 activation yields delayed tolerance to focal cerebral ischemia. Curr Neurovasc Res 8:145-152.

Ma R, Yuan B, Du J, Wang L, Ma L, Liu S, Shu Q, Sun H (2016) Electroacupuncture alleviates nerve injury after cerebra ischemia in rats through inhibiting cell apoptosis and changing the balance of MMP-9/TIMP-1 expression. Neurosci Lett 633:158-164.

Meng PY, Sun GJ, Liu SH, Yan HM (2008) Effect of electroacupuncture pretreatment on glutamate-NMDAR signal pathway in hippocampal neurons of vascular dementia rats. Zhen Ci Yan Jiu 33:103-106.

Mravec B (2010) The role of the vagus nerve in stroke. Auton Neurosci 158:8-12.

Neumann S, Shields NJ, Balle T, Chebib M, Clarkson AN (2015) Innate immunity and inflammation post-stroke: an alpha7-nicotinic agonist perspective. Int J Mol Sci 16:29029-29046.

Pang J, Itano T, Sumitani K, Negi T, Miyamoto O (2003) Electroacupuncture attenuates both glutamate release and hyperemia after transient ischemia in gerbils. Am J Chin Med 31:295-303.

Peng Y, Wang H, Sun J, Chen L, Xu M, Chu J (2012) Electroacupuncture reduces injury to the blood-brain barrier following cerebral ischemia/ reperfusion injury. Neural Regen Res 7:2901-2906.

Peplow PV, Martinez B (2016) Prevention and protection against cerebral ischemic injury using acupuncture. Neural Regen Res 11:559-560.

Ren XJ, Ma HF, Tu Y (2010) Effect of electroacupuncture on proliferation and differentiation of neural stem cells in the subependymal zone of the cerebral lateral ventricle in rats with hyperlipemia and cerebral ischemia. Zhen Ci Yan Jiu 35:347-353.

Shaked I, Meerson A, Wolf Y, Avni R, Greenberg D, Gilboa-Geffen A, Soreq H (2009) MicroRNA-132 potentiates cholinergic anti-inflammatory signaling by targeting acetylcholinesterase. Immunity 31:965-973.

Shen MH, Li ZR, Xiang XR, Niu WM (2009) Effect of electroacupuncture on cerebral cortex ultrastructure in rats with cerebral ischemia-reperfusion injury. Zhen Ci Yan Jiu 34:167-170.

Shen MH, Zhang CB, Zhang JH, Li PF (2016) Electroacupuncture attenuates cerebral ischemia and reperfusion injury in middle cerebral artery occlusion of rat via modulation of apoptosis, inflammation, oxidative stress, and excitotoxicity. Evid Based Complement Alternat Med 2016:9438650.

Shen PF, Kong L, Ni LW, Guo HL, Yang S, Zhang LL, Zhang ZL, Guo JK, Xiong J, Zhen Z, Shi XM (2012) Acupuncture intervention in ischemic stroke: a randomized controlled prospective study. Am J Chin Med 40:685-693.

Shi P, Sun LL, Lee YS, Tu Y (2017) Electroacupuncture regulates the stress-injury-repair chain of events after cerebral ischemia/reperfusion injury. Neural Regen Res 12:925-930.

Shyu WC, Lin SZ, Yen PS, Su CY, Chen DC, Wang HJ, Li H (2008) Stromal cell-derived factor-1 alpha promotes neuroprotection, angiogenesis, and mobilization/homing of bone marrow-derived cells in stroke rats. J Pharmacol Exp Ther 324:834-849.

Si QM, Wu GC, Cao XD (1998) Effects of electroacupuncture on acute cerebral infarction. Acupunct Electrother Res 23:117-124.

Siu FK, Lo SC, Leung MC (2004a) Electroacupuncture reduces the extent of lipid peroxidation by increasing superoxide dismutase and glutathione peroxidase activities in ischemic-reperfused rat brains. Neurosci Lett 354:158-162.

Siu FK, Lo SC, Leung MC (2004b) Effectiveness of multiple pre-ischemia electro-acupuncture on attenuating lipid peroxidation induced by cerebral ischemia in adult rats. Life Sci 75:1323-1332.

Sun N, Shi J, Chen L, Liu X, Guan X (2003) Influence of electroacupuncture on the mRNA of heat shock protein 70 and 90 in brain after cerebral ischemia/reperfusion of rats. J Huazhong Univ Sci Technolog Med Sci 23:112-115.

Sun N, Zou X, Shi J, Liu X, Li L, Zhao L (2005) Electroacupuncture regulates NMDA receptor NR1 subunit expression via PI3-K pathway in a rat model of cerebral ischemia-reperfusion. Brain Res 1064:98-107.

Tao J, Xue XH, Chen LD, Yang SL, Jiang M, Gao YL, Wang XB (2010) Electroacupuncture improves neurological deficits and enhances proliferation and differentiation of endogenous nerve stem cells in rats with focal cerebral ischemia. Neurol Res 32:198-204.

Tao J, Zheng Y, Liu W, Yang S, Huang J, Xue X, Shang G, Wang X, Lin R, Chen L (2016) Electro-acupuncture at LI11 and ST36 acupoints exerts neuroprotective effects via reactive astrocyte proliferation after ischemia and reperfusion injury in rats. Brain Res bulletin 120:14-24.

Tian WQ, Peng YG, Cui SY, Yao FZ, Li BG (2015) Effects of electroacupuncture of different intensities on energy metabolism of mitochondria of brain cells in rats with cerebral ischemia-reperfusion injury. Chin J Integr Med 21:618-623.

Tian XS, Zhou F, Yang R, Xia Y, Wu GC, Guo JC (2008) Electroacupuncture protects the brain against acute ischemic injury via up-regulation of delta-opioid receptor in rats. Zhong Xi Yi Jie He Xue Bao 6:632-638.

Uchida S, Kagitani F, Suzuki A, Aikawa Y (2000) Effect of acupuncture-like stimulation on cortical cerebral blood flow in anesthetized rats. Jpn J Physiol 50:495-507.

Wang C, Yang F, Liu X, Liu M, Zheng Y, Guo J (2014) Neurotrophic signaling factors in brain ischemia/reperfusion rats: differential modulation pattern between single-time and multiple electroacupuncture stimulation. Evid Based Complement Alternat Med 2014:625050.

Wang Q, Xiong L, Chen S, Liu Y, Zhu X (2005) Rapid tolerance to focal cerebral ischemia in rats is induced by preconditioning with electroacupuncture: window of protection and the role of adenosine. Neurosci Lett 381:158-162.

Wang Q, Peng Y, Chen S, Gou X, Hu B, Du J, Lu Y, Xiong L (2009) Pretreatment with electroacupuncture induces rapid tolerance to focal cerebral ischemia through regulation of endocannabinoid system. Stroke 40:2157-2164.

Wang Q, Li X, Chen Y, Wang F, Yang Q, Chen S, Min Y, Li X, Xiong L (2011) Activation of epsilon protein kinase C-mediated anti-apoptosis is involved in rapid tolerance induced by electroacupuncture pretreatment through cannabinoid receptor type 1. Stroke 42:389-396.

Wang WB, Yang LF, He QS, Li T, Ma YY, Zhang P, Cao YS (2016) Mechanisms of electroacupuncture effects on acute cerebral ischemia/reperfusion injury: possible association with upregulation of transforming growth factor beta 1. Neural Regen Res 11:1099-1101.

Wang Y, Shen J, Wang XM, Fu DL, Chen CY, Lu LY, Lu L, Xie CL, Fang JQ, Zheng GQ (2012) Scalp acupuncture for acute ischemic stroke: a meta-analysis of randomized controlled trials. Evid Based Complement Alternat Med 2012:480950.

Wei G, Huang Y, Wu G, Cao X (2000) Regulation of glial cell line-derived neurotrophic factor expression by electroacupuncture after transient focal cerebral ischemia. Acupunct Electrother Res 25:81-90.

Wei H, Yao X, Yang L, Wang S, Guo F, Zhou H, Marsicano G, Wang Q, Xiong L (2014) Glycogen synthase kinase-3beta is involved in electroacupuncture pretreatment via the cannabinoid CB1 receptor in ischemic stroke. Mol Neurobiol 49:326-336.

Wu J, Lin B, Liu W, Huang J, Shang G, Lin Y, Wang L, Chen L, Tao J (2017) Roles of electro-acupuncture in glucose metabolism as assessed by 18F-FDG/PET imaging and AMPKalpha phosphorylation in rats with ischemic stroke. Int J Mol Med 40:875-882.

Wu XD, Du LN, Wu GC, Cao XD (2001) Effects of electroacupuncture on blood-brain barrier after cerebral ischemia-reperfusion in rat. Acupunct Electrother Res 26:1-9.

Xiao Y, Wu X, Deng X, Huang L, Zhou Y, Yang X (2013) Optimal electroacupuncture frequency for maintaining astrocyte structural integrity in cerebral ischemia. Neural Regen Res 8:1122-1131.

Xie C, Gao X, Luo Y, Pang Y, Li M (2016) Electroacupuncture modulates stromal cell-derived factor-1alpha expression and mobilization of bone marrow endothelial progenitor cells in focal cerebral ischemia/reperfusion model rats. Brain Res 1648:119-126.

Xie G, Yang S, Chen A, Lan L, Lin Z, Gao Y, Huang J, Lin J, Peng J, Tao J, Chen L (2013) Electroacupuncture at Quchi and Zusanli treats cerebral ischemia-reperfusion injury through activation of ERK signaling. Exp Ther Med 5:1593-1597.

Xie YN, Wang F, Wang Q, Li XY, Zhang QM, Li X, Xiong LZ (2012) Involvement of cerebral neuroglobin in electroacupuncture preconditioning-induced protection effect in cerebral ischemia-reperfusion rats. Zhen Ci Yan Jiu 37:380-384.

Xiong LZ, Yang J, Wang Q, Lu ZH (2007) Involvement of delta-and mu-opioid receptors in the delayed cerebral ischemic tolerance induced by repeated electroacupuncture preconditioning in rats. Chin Med J (Engl) 120:394-399.

Xu H, Hong LC, Huang YQ, Chen SH, Sun H (2010) The expression of MMP-9 and ICAM-1 in rats with cerebral ischemic/reperfusion treated by acupuncture. Jichu Yixue yu Linchuang 30:731-736.

Xu H, Zhang Y, Sun H, Chen S, Wang F (2014) Effects of acupuncture at GV20 and ST36 on the expression of matrix metalloproteinase 2, aquaporin 4, and aquaporin 9 in rats subjected to cerebral ischemia/reperfusion injury. PLoS One 9:e97488.

Xu NG, Yi W, Lai XS (2002) Effect of electro-acupuncture on calcium content in neurocytes of focal cerebral ischemia. Zhongguo Zhong Xi Yi Jie He Za Zhi 22:295-297.

Xue X, You Y, Tao J, Ye X, Huang J, Yang S, Lin Z, Hong Z, Peng J, Chen L (2014) Electro-acupuncture at points of Zusanli and Quchi exerts anti-apoptotic effect through the modulation of PI3K/Akt signaling pathway. Neurosci Lett 558:14-19.

Yang J, Xiong LZ, Wang Q, Liu Y, Chen S, Xu N (2004) Effects of different stimulating parameters and their various combinations on electroacupuncture-induced cerebral ischemic tolerance in rats. Zhongguo Zhenjiu 24:208-212.

Yang ZJ, Shen DH, Guo X, Sun FY (2005) Electroacupuncture enhances striatal neurogenesis in adult rat brains after a transient cerebral middle artery occlusion. Acupunct Electrother Res 30:185-199.

Yao WP, Wang S, Han L, Ma JQ, Shen Y (2012) Effects of different frequencies of electro-acupuncture at shuigou (GV 26) on recovery of motor function in rats with focal cerebral ischemic injury. J Tradit Chin Med 32:99-104.

Yu N, Wang Z, Chen Y, Yang J, Lu X, Guo Y, Chen Z, Xu Z (2017) The ameliorative effect of bloodletting puncture at hand twelve Jing-well points on cerebral edema induced by permanent middle cerebral ischemia via protecting the tight junctions of the blood-brain barrier. BMC Complement Altern Med 17:470.

Zhang C, Wen Y, Fan XN, Tian G, Zhou XY, Deng SZ, Meng ZH (2015a) Therapeutic effects of different durations of acupuncture on rats with middle cerebral artery occlusion. Neural Regen Res 10:159-164.

Zhang HX, Wang Q, Zhou L, Liu LG, Yang X, Yang M, Liu YN, Li X (2009) Effects of scalp acupuncture on acute cerebral ischemia-reperfusion injury in rats. Zhong Xi Yi Jie He Xue Bao 7:769-774.

Zhang M, Liu J, Zhang H (2003) Rapid and delayed tolerance to focal cerebral ischemia induced by pretreatment with single electroacupuncture in rats. Zhonghua Mazui Xue Zazhi 23:355-357.

Zhang S, Li G, Xu X, Chang M, Zhang C, Sun F (2011) Acupuncture to point Baihui prevents ischemia-induced functional impairment of cortical GABAergic neurons. J Neurol Sci 307:139-143.

Zhang S, Wu B, Liu M, Li N, Zeng X, Liu H, Yang Q, Han Z, Rao P, Wang D (2015b) Acupuncture efficacy on ischemic stroke recovery: multicenter randomized controlled trial in China. Stroke 46:1301-1306.

Zhang YG, Gong X, Hou LQ (2015c) Effects of electroacupuncture preconditioning on expression of nitric oxide synthase and glial fibrillary acidic protein in cortex of focal cerebral ischemia-reperfusion rats. Zhen Ci Yan Jiu 40:113-118.

Zhao Y, Deng B, Li Y, Zhou L, Yang L, Gou X, Wang Q, Chen G, Xu H, Xu L (2015) Electroacupuncture pretreatment attenuates cerebral ischemic injury via notch pathway-mediated up-regulation of hypoxia inducible factor-1alpha in rats. Cell Mol Neurobiol 35:1093-1103.

Zhong S, Li Z, Huan L, Chen BY (2009) Neurochemical mechanism of electroacupuncture: anti-injury effect on cerebral function after focal cerebral ischemia in rats. Evid Based Complement Alternat Med 6:51-56.

Zhou F, Guo J, Cheng J, Wu G, Xia Y (2011) Electroacupuncture increased cerebral blood flow and reduced ischemic brain injury: dependence on stimulation intensity and frequency. J Appl Physiol (1985) 111:1877-1887.

Zhou F, Guo J, Cheng J, Wu G, Xia Y (2013) Effect of electroacupuncture on rat ischemic brain injury: importance of stimulation duration. Evid Based Complement Alternat Med 2013:878521.

Zhu W, Ye Y, Liu Y, Wang XR, Shi GX, Zhang S, Liu CZ (2017) Mechanisms of acupuncture therapy for cerebral ischemia: an evidence-based review of clinical and animal studies on cerebral ischemia. J Neuroimmune Pharmacol 12:575-592.

Zou J, Crews F (2006) CREB and NF-kappaB transcription factors regulate sensitivity to excitotoxic and oxidative stress induced neuronal cell death. Cell Mol Neurobiol 26:385-405.

Zou R, Wu Z, Cui S (2015) Electroacupuncture pretreatment attenuates bloodbrain barrier disruption following cerebral ischemia/reperfusion. Mol Med Rep 12:2027-2034.