Journal of Integrative Neuroscience

J. Integr. Neurosci. **2024**; 23(2): 31
https://doi.org/10.31083/j.jin2302031

Review

Mechanisms by Which Electroacupuncture Alleviates Neurovascular Unit Injury after Ischemic Stroke: A Potential Therapeutic Strategy for Ischemic Brain Injury after Stroke

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Academic Editor: Gernot Riedel
Submitted: 15 July 2023 Revised: 24 August 2023 Accepted: 31 August 2023 Published: 18 February 2024

Abstract

Stroke is the most common cerebrovascular disease and one of the leading causes of death and disability worldwide. The current conventional treatment for stroke involves increasing cerebral blood flow and reducing neuronal damage; however, there are no particularly effective therapeutic strategies for rehabilitation after neuronal damage. Therefore, there is an urgent need to identify a novel alternative therapy for stroke. Acupuncture has been applied in China for 3000 years and has been widely utilized in the treatment of cerebrovascular diseases. Accumulating evidence has revealed that acupuncture holds promise as a potential therapeutic strategy for stroke. In our present review, we focused on elucidating the possible mechanisms of acupuncture in the treatment of ischemic stroke, including nerve regeneration after brain injury, inhibition of inflammation, increased cerebral blood flow, and subsequent rehabilitation.

Keywords: ischemic stroke; electroacupuncture; inflammation; nervous system; rehabilitation

1. Introduction

According to previous studies, approximately 13.7 million people are affected and afflicted by stroke each year [1,2]. Stroke is currently the second leading cause of death worldwide [3], accounting for 10% of all deaths [4,5]. Its high mortality, disability, and recurrence rates impose a heavy economic burden on global health care systems. Stroke is broadly categorized as ischemic or hemorrhagic; this review focuses on the mechanisms associated with ischemic stroke. This is the most common type of stroke and is caused by occlusion of cerebral blood vessels or cerebral thrombosis, which results in blockage of cerebral blood flow (CBF) and thereby causes ischemia, hypoxia, and softening or even necrosis of brain tissue.

After ischemic stroke onset, restoring cerebrovascular function to reinitiate the blood supply to the brain is the first priority. Current conventional treatment strategies include angioplasty, stenting, intravenous thrombolytic therapy, and thrombectomy. However, there are obvious limitations to these therapies, including stringent treatment time windows, strict indications for administration, and many contraindications [6,7]. The safety and efficacy of these treatment strategies are also controversial according to previous clinical research [8]. Therefore, there is an urgent need to identify novel effective therapeutic strategies for clinical use.

Acupuncture originated in China, has a history of more than 3000 years, and is an important component of Chinese medicine [9]. It involves the stimulation of specific acupuncture points on the body surface with specially designed metal needles within the theoretical framework of traditional Chinese medicine and utilizes twisting and lifting techniques or electrical impulses to treat diseases [10–12]. Acupuncture is rapidly developing; its use has spread to many countries and its efficacy is widely recognized. Scientists have studied the efficacy and underlying mechanisms of acupuncture, and the effectiveness of acupuncture as an alternative therapy for stroke has been described in the literature [13]. However, the potential mechanisms through which acupuncture may aid the treatment of stroke are not yet fully understood. Therefore, the purpose of this study is to investigate the possible mechanisms of acupuncture in the treatment of stroke. Various studies have demonstrated that acupuncture pretreatment can treat ischemic stroke by

inducing cerebral ischemic tolerance, regulating oxidative stress, increasing CBF, inhibiting apoptosis, and promoting neural regeneration; thus, it is a promising prevention strategy and alternative therapy for stroke [14].

2. Ischemic Stroke and Electroacupuncture

Ischemic stroke is caused by a reduction in or interruption of blood flow in the cerebral arteries due to various causes, including atherosclerosis, cardiogenic embolism, vasculitis, hereditary diseases, and hematologic disorders [15,16]. Ischemic stroke directly impairs neurological function in three main ways. First, ischemia and infarction during ischemic stroke cause direct damage to brain tissue. Second, ischemia induces the production of excess reactive oxygen species (ROS), causing oxidative stress, which exacerbates neuronal dysfunction. Finally, the inflammatory cascade caused by ischemic stroke is thought to result in further neuronal damage.

Acupuncture, which originated in China, has been developed as a unique treatment modality during its long history and is an important part of Chinese medicine [17,18]. Several clinical studies have shown that acupuncture improves postural balance, reduces muscle spasms, and increases muscle strength [19–21]. In electroacupuncture (EA) therapy, a fine needle is inserted into the selected acupoint. After tactile confirmation that the needle is correctly placed, an EA machine is connected to the acupuncture needle, and a low-frequency pulse current similar to that of human bioelectric currents is delivered with different stimulation parameters to treat different diseases [22]. A systematic review and meta-analysis published in 2015 evaluated the clinical efficacy and safety of EA in the treatment of ischemic stroke [23]. The mechanism of EA in the treatment of ischemic stroke has also received much attention recently; scientists conducted a systematic review and analysis of recent clinical studies and found that EA can play a role in the treatment of ischemic stroke through the following five mechanisms: (1) EA can promote neuronal proliferation and differentiation and induce poststroke neurogenesis and neuroprotection, (2) EA can effectively ameliorate the damage caused by cerebral ischemia–reperfusion, (3) EA can increase CBF and alleviate blood–brain barrier dysfunction after stroke, (4) EA can inhibit apoptosis, and (5) EA pretreatment can induce cerebral ischemic tolerance [24–28]. Herein, we review the main potential mechanisms of acupuncture in the treatment of stroke.

3. Method

Studies on the mechanism of acupuncture in treating ischemic stroke models were obtained by a PubMed literature search, which was limited to full-text studies published in English between January 1, 2000, and December 31, 2022. The following search string was used for the literature search: ("electroacupuncture" OR "acupoint") AND ("ischemic stroke" OR "neurogenesis" OR "cerebral ischemia" OR "cerebral reperfusion" OR "cerebral blood flow" OR "apoptosis autophagy" OR "preconditioning" OR "rehabilitation"). We identified a total of 120 published articles. Papers that did not describe the specific mechanisms by which EA treated ischemic stroke injury, papers that had information on stroke and EA but were unrelated, and papers that used EA as a secondary treatment were excluded. Of these, a total of 40 published articles met the inclusion criteria. All searches were limited to animal studies, most of which used a similar animal model of ischemic stroke, the unilateral transient middle cerebral artery occlusion (MCAO) model.

4. Mechanism of Action Underlying the Effect of EA on Neurogenesis and Cell Proliferation

The beneficial effect of EA on neurogenesis after brain injury may be related to neurotrophic factors. Neurogenesis in adult mammals is accomplished primarily through the division of stem and progenitor cells [29]. Adult neurogenesis, i.e., neuronal growth and development, mainly occurs in two brain regions: the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus [30,31]. Reports indicate the presence of neural stem cells (NSCs) in the neocortex, amygdala, striatum, and substantial nigra [32]. These NSCs have the ability to differentiate into different types of neurons, astrocytes, and oligodendrocytes at the time of brain injury [33,34]. Neural progenitor cells (NPCs) are present in the SVZ and migrate to the site of the injury, where they form new functional synapses with the remaining neurons and connect new neural circuits [35,36]. Brain injury activates endogenous neural repair systems, and the proliferation and survival of these neural cells are thought to play a role in neural repair in the brain, which is a potential therapeutic target [37–39]. Neurotrophic factors can prevent ischemic injury and exert neuroprotective effects. Glial cell-derived neurotrophic factor (GDNF) belongs to the transforming growth factor beta (TGF-β) family and was originally found to enhance the function of midbrain dopaminergic neurons by promoting survival and differentiation during the development of the central and peripheral nervous systems [40,41]. Moreover, it was shown that GDNF ligands can promote the neurogenic differentiation of NPCs [42]. Brain-derived neurotrophic factor (BDNF) is a protein that regulates neuronal survival and synaptic plasticity by binding to tyrosine kinase beta (Trkβ) and p75 neurotrophin receptor (p75 NTR) and activates intracellular signaling pathways [43,44]. Lu *et al.* [45] reported in a meta-analysis of 34 studies (1617 animals) that acupuncture can promote the proliferation, differentiation, and migration of nerve cells after stroke. Brain injury can activate the neural repair system, but spontaneous regeneration cannot meet the requirements of brain function recovery [46]. Nerve growth factor (NGF) can promote the growth and dif-

ferentiation of central and peripheral neurons and accelerate the repair of the nervous system after injury, and EA can induce the expression of NGF; the number of BrdU-positive cells was found to be significantly increased by the combination of NGF and EA, indicating increased proliferation and survival of neuronal cells [47,48]. In addition, EA pretreatment elevates stroke-induced striatal neurogenesis and improves neurological recovery through modulation of the resinous acid (RA) pathway [49,50]. Acupuncture alters the expression level of growth-associated protein-43 (GAP-43), a protein specific to the nervous system, and promotes nerve regeneration in the marginal zone of the ischemic lesion [51]. In some MCAO animal models, the expression of BDNF, GDNF, and vascular endothelial growth factor (VEGF) was found to be increased significantly after EA stimulation of Baihui GV20, Dazhui GV14, and Quchi LI11; this resulted in increased proliferation and differentiation of NSCs in the DG and SVZ and promoted the differentiation of proliferating NSCs into neurons and glial cells [24,52–55].

Notch receptors are highly conserved, single-channel transmembrane proteins that are involved in various cytogenesis-related processes, including cell differentiation, apoptosis, and proliferation, and play an important role in the regulation of self-renewal and differentiation of NSCs after ischemic injury. In the MCAO animal model, EA at LI11 and Zusanli ST36 was found to activate the Notch signaling pathway, significantly decreasing cerebral infarct size, improving cerebral nerve function, and promoting the proliferation of NSCs in rats [56–59]. Basic fibroblast growth factor (bFGF) promotes the regeneration and repair of mesodermal ectodermal cells, which is essential for the development and differentiation of the central nervous system (CNS). In both cerebral ischemia/reperfusion (CI/R) and MCAO models, EA treatment was found to significantly increase the expression level of bFGF in the striatum and cortex and thus exert neurogenic and protective effects [60–62]. One of the most abundant neuropeptides in the nervous system, neuropeptide Y (NPY), is a key regulator of homeostasis inside and outside the CNS, while NPY can also promote neurogenesis in the SVZ and DG regions. Furthermore, acupuncture can upregulate the expression of NPY in the CNS [63–66]. In addition to affecting neurogenesis and proliferation, ischemic stroke also severely impairs brain function due to cerebral ischemia/reperfusion.

5. Mechanism Underlying the Effect of EA in Cerebral Ischemia/Reperfusion Injury

5.1 Oxidative Stress and EA

CI/R leads to the generation of large amounts of ROS, resulting in an imbalance between oxidative and antioxidative status in the body and thus oxidative stress, which in turn causes cellular damage and necrosis, which is a key factor in ischemic brain injury [67,68]. Mitochondria are the main site of aerobic cellular respiration, supplying energy to cells and participating in cell differentiation, apoptosis, and information transfer [69]. Under normal physiological conditions, the respiratory chain of mitochondria is the main source of ROS, but CI/R increases the leakage of electrons generated in the respiratory chain, resulting in the production of large amounts of ROS [70]. Furthermore, CI/R causes a large decrease in the level of superoxide dismutase (SOD), a key factor in maintaining oxidative and antioxidative balance, impairing its function as a free radical scavenger and the redox balance of mitochondria, and causing the excessive production of ROS and free radicals with impaired antioxidant capacity, ultimately leading to mitochondrial dysfunction [71,72]. Experiments have shown that EA of Fengchi GB 20 can activate the antioxidant enzyme system and increase the ability of SOD and glutathione peroxidase (GSH-Px) to scavenge excessive ROS; this increase in SOD activity involves the nuclear factor erythroid-2 related factor 2 (Nrf2)/heme oxygenase (HO-1) signaling pathway, and the increase in GSH-Px activity may be associated with increased antioxidant activity by glutathione (GSH) [73,74]. CI/R causes lipid peroxidation to produce malondialdehyde (MDA) and 4-hydroxynonenoic acid (4-HNE), and EA at GB20 and ST36 can reduce the degree of lipid peroxidation and MDA production during MCAO [75]. A systematic review and meta-analysis published in 2014 showed that EA reduced the infarct size and improved neural function in animal models of cerebral ischemia [76]. The respiratory chain function of mitochondria was found to be significantly improved after EA at Shuigou GV26 and GV20, and the activities of succinate dehydrogenase (SDH), cytochrome C oxidoreductase, and NADH dehydrogenase (NADH-Q) reductase were found to be increased, resulting in an elevated antioxidant capacity and inhibition of ROS production [77]. It was also found that CI/R impaired the phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT)-mammalian target of rapamycin (mTOR)-mediated autophagy–lysosome pathway (ALP) to increase the percentage of dysfunctional mitochondria while impairing mitochondrial autophagy, an important change associated with CI/R, and EA restored mitochondrial autophagy through the Pink1/Parkin signaling pathway to ameliorate the impairment of the ALP [78,79].

5.2 Anti-Inflammatory Effect of EA

The inflammatory cascade after ischemic stroke results in the activation of a series of inflammatory cells and the release of inflammatory signals. Acupuncture exerts anti-inflammatory effects by regulating multiple immune cell populations and inflammatory transmission, which involves glial cells, vagal cholinergic anti-inflammatory pathways, and leukocytes (Fig. 1).

5.2.1 Glial Cells

Microglia are the most numerous immune cells in the brain, accounting for 5–10% of all cells in the brain [80].

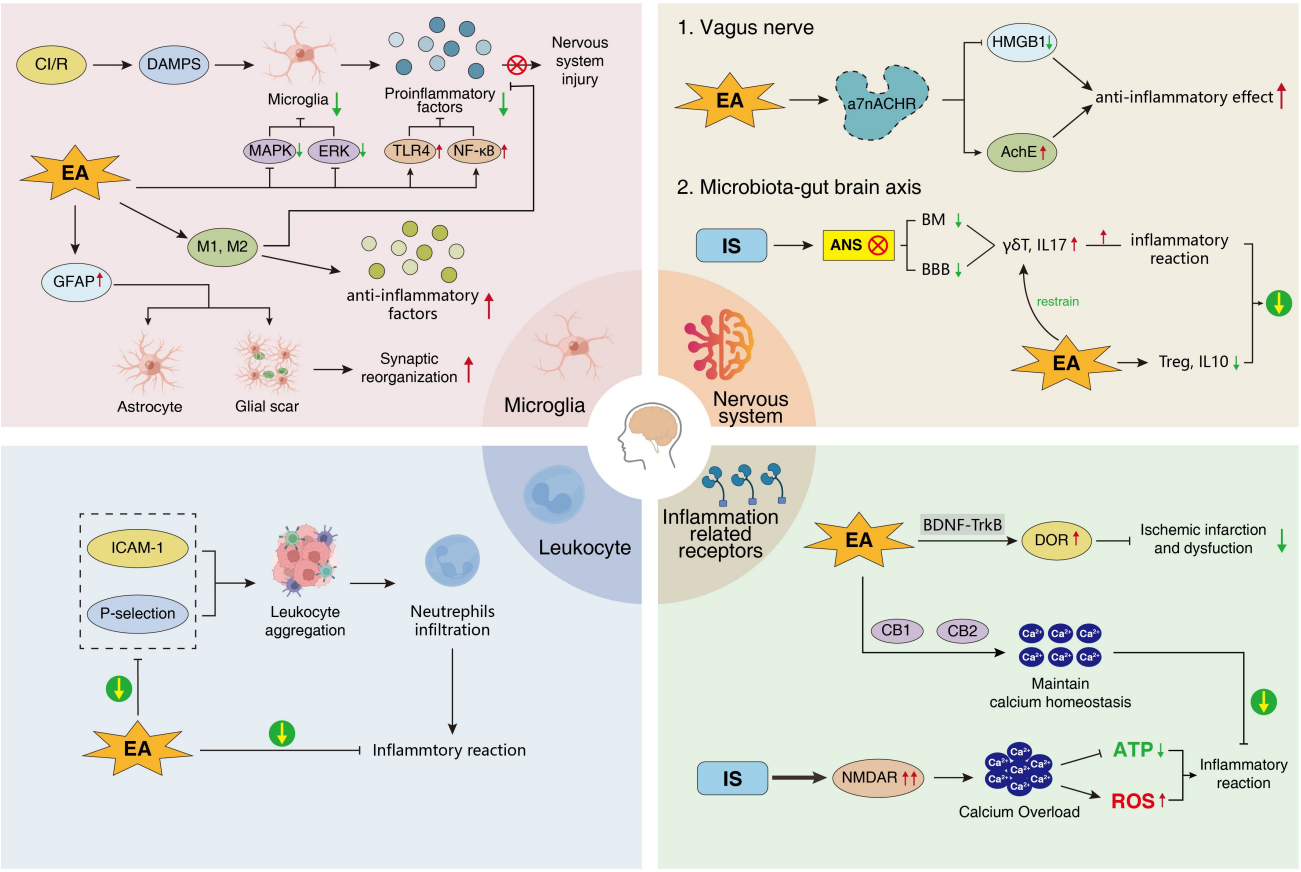

Fig. 1. Anti-inflammatory effects of EA. EA, Electroacupuncture; GFAP, glial fibrillary acidic protein; M1, M1 microglia; M2, M2 microglia; CI/R, cerebral infarction/reperfusion; DAMPs, damage-related molecular patterns; MAPK, mitogen-activated protein kinase; ERK, extracellular regulatory protein kinase; TLR4, Toll-like receptor 4; NF-κB, enhanced κ-light chain in B cells activated by nuclear factor; a7nAChR, a7 nicotinic acetylcholine receptor; HMGB1, high mobility group protein B1; AchE, acetylcholinesterase; IS, ischemic stroke; ANS, autonomic nervous system; BM, bowel movement; BBB, blood brain barrier; $\gamma\delta$T, T cell subpopulation; IL17, interleukin 17; Treg, regulatory T cell; IL10, interleukin 10; ICAM-1, intercellular cell adhesion molecule-1; P-selection, platelet p-selectin; BDNF-TrkG, brain-derived neurotrophic factor (BDNF)-tyrosine kinase receptor B (TrkB); DOR, delta-opioid receptor; CB1, type I cannabinoid receptor; CB2, type II cannabinoid receptor; NMDAR, N-methyl-D-aspartate type glutamate receptor; ATP, adenosine triphosphate; ROS, reactive oxygen species.

Damaged neurons release damage-related molecular patterns (DAMPs) after CI/R, and DAMPs rapidly activate microglia [56]. On the one hand, the anti-inflammatory factors (interleukin, IL-4, IL-10, IL-13, TGF-β) and neuroprotective factors secreted by activated microglia can remove harmful substances from damaged neurons in the CNS and promote the repair of neurological functions [81,82]. On the other hand, excessively activated microglia secrete high levels of proinflammatory factors (e.g., tumor necrosis factor alpha (TNF-α), IL-1β, IL-6, and matrix metalloproteinases), causing neurotoxicity, inhibiting the repair of the nervous system, and aggravating damage [83–85]. EA effectively reduces microglial activation, which is associated with inhibition of the P38 mitogen-activated protein kinase (MAPK) and extracellular regulatory protein kinase (ERK) pathways, reduces the levels of proinflammatory factors, and controls inflammatory responses [86]. In an animal model of MCAO, acupuncture at Neiguan PC6 and LI11 inhibited Toll-like receptor 4/enhanced κ-light chain in B cells activated by nuclear factor (TLR4/NF-κB) pathway activation; decreased the expression of IL-1β, TLR4, TNF-α, inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ), NF-κB, RelA (P65), and tumor necrosis factor-associated factor 6 (TRAF6); and alleviated neuronal injury [87]. Microglia can be polarized toward two phenotypes, the proinflammatory M1 phenotype and the anti-inflammatory M2 phenotype, which contribute to neurological damage and neuroprotection, respectively [87]. Acupuncture at ST36 was shown to induce a shift from M1 polarization to M2 polarization, achieving a balance between the two polarization states by suppressing proinflammatory factor expression and increasing anti-inflammatory and repair factor expression [74]. At the site of CI/R injury, many reactive astrocytes and microglia proliferate and dif-

ferentiate, forming a glial scar [88]. Increased secretion of chondroitin sulfate proteoglycans (CSPGs) by cells forming the glial scar is an important hindrance to regeneration and functional recovery of axons [89,90]. Astrocytes have a nutritional and protective role in neuronal development and are involved in the formation of the blood–brain barrier and synaptic signaling [91]. In an animal model of stroke, the expression of the astrocyte activation marker GFAP was found to increase substantially after EA at GV20 and GV14 but decrease after a period of time, suggesting that EA has the potential to activate astrocytes in the area of injury and prevent excessive glial scar production [92]. In addition, EA increases the synaptic density and thickness and plays an active role in synaptic reorganization [93].

5.2.2 Vagus Nerve

Stimulation of the vagus nerve may be a potential therapeutic strategy to effectively reduce the inflammatory response and promote neurological recovery [94]. The vagal cholinergic anti-inflammatory pathway can inhibit the release of TNF-α and proinflammatory factors such as IL-1β, IL-6, and IL-18 by macrophages through electrical stimulation [95,96]. The a7 nicotinic acetylcholine receptor (a7nACHR) is the key to the function of the cholinergic anti-inflammatory pathway [97]. EA can exert anti-inflammatory effects by suppressing the function of the reflex center of the innate immune system [96]. In an experimental model of stroke, EA activates a7nACHR to inhibit high mobility group protein B1 (HMGB1), a nuclear protein with proinflammatory effects [98]. Acetylcholine secreted by the vagal nerve inhibits peripheral inflammation in the brain, and EA of the rat forepaw increases acetylcholine release, which may be related to the targeting of miR-132, an inflammatory regulator, by acetylcholinesterase (AchE) [99,100]. In addition, EA at GV20 and GV14 exerts neuroprotective effects by reducing the expression of five subtypes of another muscarinic cholinergic receptor to ameliorate damage to the central cholinergic system [101].

5.2.3 Leukocytes

After ischemic injury, the production of adhesion molecules and chemotactic mediators of leukocytes increases, resulting in the recruitment of large numbers of leukocytes to the injured area and their adhesion to endothelial cells, as well as an increase in neutrophil infiltration, which exacerbates the release of proinflammatory factors and the inflammatory response [102,103]. Intercellular cell adhesion molecule-1 (ICAM-1) is an important receptor that mediates the leukocyte adhesion response, and its expression level is increased in response to inflammatory stimuli in ischemic injury, thereby exacerbating leukocyte adhesion and infiltration [104,105]. The expression of platelet p-selectin (P-selectin), an adhesion molecule, is upregulated after ischemic injury, causing leukocytes to roll on stimulated endothelial cells, resulting in leukocyte extravasation and aggravating the inflammatory response while causing platelets to adhere and aggregate and lose their stability, thus forming a thrombus [106]. EA was found to inhibit the expression of ICAM-1 and P-selectin, reduce the adhesion and infiltration of leukocytes, and exert an anti-inflammatory effect [107].

5.2.4 Other Transmitters and Receptors Involved in Inflammation

In addition to its analgesic and sedative effects, activation of the delta-opioid receptor (DOR) has been shown to effectively protect against ischemic-hypoxic injury during CI/R by reducing excitatory neurotransmitter expression through ion homeostasis and reducing impaired neurotransmission, which may be related to the BDNF-tyrosine kinase receptor B (TrkB) signaling pathway. EA at GV26 and PC6 was shown to upregulate DOR expression by mediating the BDNF-TrkB signaling pathway and significantly reduce ischemic infarction and functional impairment [108,109]. Glutamate is an important excitatory neurotransmitter in the CNS. Ischemic transporter dysfunction in the brain after stroke leads to excessive release of glutamate and overactivation of *N*-methyl-*D*-aspartate (NMDA) glutamate receptor (NMDAR), which induces excitotoxicity leading to neuronal cell injury and death [110]. In an animal model of ischemia induced by vascular occlusion, glutamate levels were found to be significantly higher in the control group (135.19 ± 23.76 µm) than in the needle stimulation group (72.20 ± 27.15 µm) after acupuncture at Yanglingquan GB34 versus Xuanzhong GB39, which may be related to the reversal of high expression of the NMDAR1 subunit by EA [111,112]. Calcium overload due to abnormal release of glutamate causes an imbalance in calcium ion homeostasis [113]. Calcium overload blocks ATP synthesis and contributes to excessive ROS production, leading to mitochondrial dysfunction while inducing NLRP3 inflammasome activation and exacerbating the inflammatory cascade [114,115]. Calmodulin-dependent protein kinase (CaMKII), an important receptor for calcium signaling, inhibits CaMKII-AMPA receptor 1 (GluA1) phosphorylation to exert anti-inflammatory effects in a complete Freund's adjuvant (CFA)-induced mouse model of inflammation [116]. The analgesic and anti-inflammatory effects of EA in a CFA-induced inflammation model are closely related to the cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2) receptors [117]. Studies have shown that endogenous cannabinoids can regulate various ion channels, including T-type calcium channels, and that CB1 and CB2 receptors are key regulators that can help maintain calcium homeostasis and reduce subsequent inflammatory damage [118,119]. NLRP3 is involved in the inflammatory response by inducing the secretion and maturation of IL-18 and IL-1β. As a conserved anti-inflammatory miRNA, miR-223 can negatively regulate NLRP3 expression to inhibit the inflammatory response [120,121]. In an MCAO rat

model, EA at Waiguan TE5 and ST36 was shown to upregulate the expression of miR-223 in the peri-infarct cortex, by inhibiting the miR-223/NLRP3 pathway, and to reduce the expression of NLRP3, caspase-1, IL-1β, and IL-18 to alleviate neuroinflammation [122].

5.2.5 Microbiota–Gut–Brain Axis

The gut is the most important immune organ of the human body, accounting for approximately 70% of the immune function of the whole body. The microbiota–gut–brain axis (MGBA) is a bidirectional regulatory network between the brain and the gut [123]. After stroke, homeostasis of the intestinal microbiota is disrupted, dysregulation of the autonomic nervous system (ANS) leads to the weakening of intestinal movement and barrier function, and proinflammatory factors in the intestine enter the brain through the damaged blood–brain barrier to aggravate injury [124]. Studies have shown that T cells play a key role in tissue damage secondary to ischemic stroke [125]. The initial inflammatory cascade causes T cells to migrate to, and T cell subpopulation ($\gamma\delta$T) cells to be transported to, the pia mater of the brain where they secrete the proinflammatory factor IL-17 to aggravate the inflammatory response [123,126]. CD4+CD25+ regulatory T (Treg) cells play an important role in peripheral immunity. After stroke, Treg cells migrate to the intestine with the help of mesenteric lymph node dendritic cells, and the expression of the anti-inflammatory factor IL-10 is upregulated to inhibit IL-17-mediated inflammation and the proliferation and differentiation of $\gamma\delta$T cells [123,127]. Studies have shown that EA at GV20, GV14, Shenshu BL23, and ST36 can alleviate the disruption of the intestinal microbiota, inhibit the inflammatory response, and promote the recovery of neurological function [128]. In an MCAO model, the proportion of CD3+TCR$\gamma\delta$+carboxyfluorescein diacetate succinimidyl ester (CFSE)+ cells was found to decrease from 12.06% to 6.52% after EA at GV20, and this change was related to an increase in the number of Treg cells in the brain and small intestine and the inhibition of $\gamma\delta$T cell function [129].

6. Mechanism Underlying the Effect of EA on Pathological Changes Related to the Blood–Brain Barrier, Cerebrovascular Function, and Cerebral Blood Flow after Ischemic Stroke

6.1 Blood–Brain Barrier

The blood–brain barrier (BBB) consists of the brain capillary wall and glial cells and is a barrier between the blood circulation and brain tissue. Due to its poor permeability, the BBB can limit the exchange of free substances between blood and brain tissue, and it has a role in maintaining the homeostasis of the brain's internal environment and protecting neural tissue from damage by toxins and pathogens [130–132]. After ischemic injury, the integrity of the BBB is disrupted and its permeability increases, resulting in vasogenic edema and hemorrhagic transformation [133]. In a CI/R rat model, EA at GV20 and GV14 can reduce ischemic damage to brain cortical neurons and the BBB [134]. In addition, EA downregulates the expression of Nogo-A, an inhibitory neuroregenerative factor, to alleviate BBB damage [135]. EA at GV20, GV26, and ST36 improved the vascular ultrastructure of brain tissue in CI/R rats, promoting capillary generation and restoration of vascular function, which was closely related to the upregulation of *VEGF* mRNA expression [136]. The expression of metalloproteinase inhibitor-1 (*TIMP-1*) mRNA and protein tissue inhibitor was found to be dysregulated after EA at GV20, Hegu LI4, and Taichong LR3 in an MCAO animal model [137]. The protein and mRNA expression levels of matrix metalloproteinase 9 (MMP-9) were shown to significantly reduced in the BBB of CI/R rats after EA at GV20 and GV26 [138]. Reduced inflammatory cell infiltration and upregulation of matrix metalloproteinase 2/water channel protein (MMP2/AQP) expression occurred after EA at GV20 and ST36 [139]. The above experimental results all indicate that acupuncture can ameliorate BBB injury and exert neuroprotective effects on ischemic brain tissue.

6.2 Angiogenesis

VEGF promotes the proliferation and division of endothelial cells, increases vascular permeability, and promotes neoangiogenesis [132,140]. EA was shown to activate the HIF-1α/VEGF/Notch1 signaling pathway and promote angiogenesis after ischemic injury via exosomal miR-210 [141]. Furthermore, in an MCAO animal model, EA can effectively promote angiogenesis and neurological recovery through the EphB4/EphB2-mediated Src/PI3K signaling pathway [142]. The PI3K-AKT pathway increases the secretion of VEGF through hypoxia-inducible factor 1 (HIF-1) and regulates the expression of angiogenesis factors such as nitric oxide and angioplasty, which play a major role in the process of angiogenesis, while the activation of the PI3K-AKT pathway promotes the neuroprotective effect of the opiate receptor agonist (D-Ala2, D-Leu5)-Enkephalin (DADLE) [143,144]. In a CI/R animal model, the relative protein expression of PI3K p85, PI3K p110 and p-AKT was found to be upregulated in the acupuncture group, and the expression of VEGF, GAP-43, and synaptophysin (SYN) was shown to be significantly increased, which indicates that acupuncture exerts its angiogenic and protective effects through the PI3K-AKT signaling pathway [145].

6.3 Cerebral Blood Flow

A $>$100% increase in blood flow at the ischemic foci and significant alleviation of ischemic infarction and nerve injury were observed in MCAO model animals treated with EA at GV26 (1.0–1.2 mA and 5–20 Hz) compared with those treated with EA at GV20 [146]. Data from another ex-

Table 1. Summary of the acupuncture frequency and effects for the selected acupoints mentioned in this review.

Acupoint	Acupuncture Parameters	Outcome	Model	Year	Reference
Fengchi GB20	2 Hz, 30 min	Affected the degree of lipid peroxidation and MDA production after MCAO	MCAO	2004	Siu *et al.* [75]
Shuigou GV26	5/20 Hz, 2–4 mA, 30 min, 1 mm	Significantly increased respiratory enzyme activity and reduced ROS production	MCAO	2009	Zhong *et al.* [77]
Yanglingquan GB34 Xuanzhong GB39	2 Hz, 10 min, 5 mm	Decreased glutamate levels	MCAO	2010	Lee *et al.* [111]
Quchi LI11	1/20 Hz, 30 min, 2–3 mm	Promoted the activation of the PI3K/Akt pathway and inhibited apoptosis in the brain	MCAO	2012	Chen *et al.* [152]
Sanyinjiao SP6	2 Hz, 20 min	Increased LTP	AD	2012	He *et al.* [174]
Baihui GV20	2 Hz, 20 min, 10 days, 2 mm	Significantly increased the mRNA expression of *BDNF* and *VEGF*	MCAO	2014	Kim *et al.* [52]
Neiguan PC6	2/15 Hz, 1 mA, 30 min, 5 days	Decreased IL-1β, TLR4, TNF-α, IKKβ, NF-κB, P65, and TRAF6 levels	MCAO	2015	Han *et al.* [87]
Zusanli ST36	1–20 Hz, 0.2 mA, 30 min, 3 days	Decreased NF-κB p65, p38MAPK, MyD88, TNF-α, IL-1β, and IL-6 levels	MCAO	2016	Liu *et al.* [176]
Hegu LI4 Taichong LR3	2 Hz, 1 mA, 7 days	Increased Bcl-2 protein and TIMP-1 levels and decreased Bax protein and MMP-9 levels	MCAO	2016	Ma *et al.* [137]
Dazhui GV14	2/15 Hz, 1 mA, 30 min, 5 mm	Significantly reduced the mRNA levels of choline acetyltransferase, five subtypes of muscarinic receptors, and a7NACHR	MCAO/R	2018	Chi *et al.* [101]
Shenshu BL23	2/100 Hz, 1 mA, 40 min	Decreased the Bax/Bcl 2 ratio and cleaved cystatin-3 levels	MCAO	2022	Long *et al.* [153]
Shenting GV24	2/20 Hz, 1–3 mA, 30 min, 3 mm, 20 days	Increased synaptic transmission efficiency and plasticity in the CA3-CA1 region of the hippocampus	VCI	2022	Dai *et al.* [170]

MDA, malondialdehyde; MCAO, middle cerebral artery occlusion; VCI, vascular cognitive impairment; LTP, long-term potentiation; AD, Alzheimer's disease; VEGF, vascular endothelial growth factor; MMP-9, matrix metalloproteinase 9; MCAO/R, Middle cerebral artery occlusion/reperfusion; TNF-α, tumor necrosis factor alpha; IKKβ, inhibitor of nuclear factor kappa-B kinase subunit beta; P65, RelA; TRAF6, tumor necrosis factor-associated factor 6; p38MAPK, p38 mitogen-activated protein kinase; MyD88, Myeloid differentiation factor-88; TIMP-1, tissue inhibitor of metalloproteinases-1.

periment showed that CBF was elevated in all brain regions in CI rats after two applications of EA at bilateral ST36 or 15 Hz EA [147]. After EA at ST36 and LI11, cerebrovascular resistance (CVR) was reduced, cerebral blood flow was increased, and meningeal microcirculation was improved [148].

7. The Mechanism by Which EA Inhibits Apoptosis and Autophagy

Apoptosis is a genetically controlled, programmed process of autonomous cell death, and can be induced by excess free radicals generated in acute cerebral ischemia, by a Ca^{2+} concentration surge, or by excitotoxicity; however, apoptosis in the ischemic penumbra seems to be reversible [149,150]. NGF, a nerve growth factor involved in neuroprotection and functional repair, acts through the ERK pathway and the PI3K pathway mediated by TrkA, a high-affinity receptor for NGF. EA was found to decrease NR1 subunit expression while upregulating TrkA expression and to mediate the TrkA-PI3K pathway to inhibit the increase in transient receptor potential melastatin-subfamily member 7 (TRPM7) expression induced by ischemia [110,112,151]. In a rat model of I/R injury, EA at LI11 and ST36 was found to activate the PI3K-AKT signaling pathway, increase the expression of the PI3K activators BDNF and GDNF, upregulate the expression of the antiapoptotic protein Bcl-2, and decrease the expression of the proapoptotic protein Bax, inducing the formation of a stable heterogeneous structure and thus exerting an inhibitory and neuroprotective effect against cell apoptosis [152]. Pretreatment by EA at GV20, BL23, and Sanyinjiao SP6 was found to reduce the Bax/Bcl 2 ratio and inhibit the expression of cleaved cystatin-3, which attenuated neuronal apoptosis and was associated with EA-mediated inhibition of transient receptor vanilloid (TRPV1) [153,154].

Autophagy is a process by which cells degrade their own components using lysosomes; autophagy and apoptosis jointly maintain cellular homeostasis under normal

physiological conditions [155,156]. Cellular autophagy is regulated by Unc-51-like autophagy-activated kinase 1 (UIK1) and FUN14-containing structural domain protein 1 (FUNDC1) [157]. Pretreatment by EA at GV20 and GV26 was shown to suppress p-ULK1 and FUNDC1 expression and upregulate p-mTORC1 and microtubule-associated protein light chain 3 (LC3-I) expression, thereby improving neurological function and reducing the infarct volume [158]. The silent information regulator 1 (SIRT1)-forkhead box proteins O1 (FOXO1) signaling pathway is an important factor in autophagy regulation. After EA pretreatment, the ratio of IC3-II/LC3-I is decreased; the complex effect of acetylated (AC)-FOXO1 and Atg7 is reduced; the levels of p62, SIRT1, and FOXO1 are elevated; and the number of autophagosomes in CI/R rats is significantly reduced. The neuroprotective effect of EA pretreatment may be related to the activation of the SIRT1-FOXO1 signaling pathway [159].

8. EA Pretreatment for Cerebral Ischemic Tolerance

As early as the pre-Qin period in ancient China, the concept of prevention before disease has been followed in Chinese medicine [28]. Preventive acupuncture can open the meridians, regulate the organs, balance yin and yang, support the positive, and eliminate the evil [160]. Acupuncture is widely used in the prevention and treatment of ischemic stroke because of its few side effects and high safety and efficacy [28]. EA pretreatment was found to confer tolerance to cerebral ischemic injury in rats; Furthermore, neurological impairment was shown to be significantly increased and the infarct volume (38.3 ± 25.4 mm^3) was found to be significantly reduced in MCAO model animals subjected to repeated pretreatments with EA at GV20 compared with control MCAO model animals (220.5 ± 66.0 mm^3) and animals in the isoflurane anesthesia group (168.6 ± 57.6 mm^3) [161]. In addition, cerebral ischemia tolerance induced by EA preconditioning is closely related to the endocannabinoid system, and the CB1 receptor-mediated PI3K/Akt/GSK-3β signaling pathway plays an important role in cerebral ischemic injury [162]. In MCAO rat models, EA preconditioning enhances the activation of signal transducer and activator of transcription 3 (STAT3) and protein kinase Cϵ (PKCϵ) by upregulating the expression of the CB1 receptor and increases the levels of the endocannabinoids 2-arachidonic glycerol and n-arachidonic ethanolamine, which significantly reduces the infarct volume after reperfusion, improves nerve function, and inhibits neuronal apoptosis [163–165]. In addition, monocyte chemotactic protein-inducible protein 1 (MCPIP1) is also involved in EA preconditioning-induced cerebral ischemic tolerance, and the neuroprotective effects of EA preconditioning were found to be significantly decreased in MCPIP1-deficient MCAO model animals [166].

9. Rehabilitation Phase

The main sequelae of ischemic stroke sequelae are numbness, hemiparesis, motor dysfunction, cognitive dysfunction, and memory loss, which are often responsible for the high disability rate and poor outcomes. Zhan *et al*. [167] reported in a meta-analysis of 14 randomized controlled trials {896 patients with poststroke cognitive impairment (PSCI)} that EA improved cognitive function and motor function in patients with PSCI. In a stroke rehabilitation experiment, the control group was given standard physiotherapy, and the intervention group received acupuncture. Analysis of the experimental data showed that the immediate and long-term outcomes of the intervention group were better than those of the control group, with EA significantly ameliorating spasticity and motor dysfunction of the limbs caused by stroke and restoring the ability of the patients to perform daily life activities [168]. Another study on stroke hemiplegia found that acupuncture significantly improved muscle spasms and mobility in hemiplegic limbs [169]. Ischemic stroke induces vascular cognitive impairment (VCI), and the learning and memory abilities of VCI model rats are improved after EA at GV20 and Shenting GV24, which may be related to the fact that EA increases the postsynaptic current frequency in neurons in the hippocampal CA3-CA1 regions, promoting connectivity and plasticity [170]. Furthermore, acupuncture at ST36 was shown to alleviate cognitive dysfunction and normalize cAMP concentrations, Protein kinase A (PKA) activity, and phosphorylation of cAMP response element binding protein (pCREB) and pERK expression in patients with vascular dementia, while blocking the PKA signaling pathway was found to reverse the beneficial effect of acupuncture, indicating that acupuncture improves hippocampal function through the regulation of the cAMP/PKA/CREB signaling pathway [171]. Long-term potentiation (LTP) in the hippocampus is responsible for memory formation and learning, and induction of LTP is dependent on NMDAR activation [172]. EA is able to reverse LTP impairment in a rat depression model, possibly by upregulating the expression of the NMDAR subunit NMDARs with NR2B subunits (GluN2B) in the hippocampus [173]. EA at ST36 and SP6 was shown to reduce local circuit inhibition and enhance LTP, possibly by promoting synaptic transmission via inhibition of gamma-aminobutyric acid (GABA) release from interneurons to increase the excitability of granule cells [174]. Acupuncture can activate language-related brain areas and rebuild the neural network responsible for language to relieve language impairment [175] (Table 1, Ref. [52,75,77,87,101,111,137,152,153,170,174,176]).

10. Issues Related to Acupuncture

Acupuncture is a promising alternative treatment option for ischemic stroke with high efficacy, safety, and convenience. However, the efficacy of acupuncture has been challenged and questioned, the fundamental reason being

that some of the mechanisms of action are still unclear. At present, there have been few international reports or studies on acupuncture treatment; high-quality acupuncture research studies are lacking and the theoretical basis for the benefits of acupuncture has not yet been adequately described. Furthermore, most of the research on acupuncture has been performed in China; clinical research results from China are not accepted by international mainstream medicine, and the acupuncture methods used in this research are not in line with international standards. Research methods for studying acupuncture are not consistent with modern scientific methods, and no method has yet been established to evaluate the clinical efficacy of acupuncture. It is difficult to perform a randomized controlled trial (RCT) study on acupuncture; furthermore, the use of blinding and placebos in acupuncture research is also controversial and it can be difficult to determine the criteria for a meta-analysis of acupuncture studies. The qualifications of the physician are also an important consideration related to acupuncture, as the teaching modes of major universities and training institutions vary greatly; thus, the theoretical knowledge and operational skills of acupuncturists worldwide are not necessarily consistent, meaning that treatment effects vary from person to person. In addition, due to interindividual variability, the location of acupuncture points differs among individuals, which in turn leads to biased clinical conclusions, and the optimal frequency, timing, and methods of acupuncture have also yet to be determined.

11. Conclusions

This study reviewed the evidence for the beneficial effects of EA on ischemic stroke in animal studies. EA can promote nerve cell regeneration after ischemic stroke and alleviate CI/R injury by reducing oxidative stress and inhibiting the inflammatory response. EA can also improve cerebrovascular function, affect angiogenesis, and reduce apoptosis and autophagy. Furthermore, EA preconditioning can increase ischemic tolerance and contribute substantially to subsequent rehabilitation.

Author Contributions

QX and MG designed the review and wrote the manuscript. AS, CF and ST designed and drew pictures and tables. YD and AZ collected and organized reference materials. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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