Ameliorating effects of traditional Chinese medicine preparation, Chinese materia medica and active compounds on ischemia/reperfusion-induced cerebral microcirculatory disturbances and neuron damage

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Abbreviations: AIF, apoptosis inducing factor; AMPA, \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; AP-1, activator protein-1; Asp, aspartate; BBB, brain blood barrier; BFGF, basic fibroblast growth factor; BMEC, brain microvascular endothelial cell; BNDF, brain-derived neurotrophic factor; cAMP, cyclic adenosine monophosphate; Cav-1, caveolin-1; CAT, catalase; CBF, cerebral blood flow; COX-2, cyclooxygenase-2; DHR, dihydrorhodamine 123; DPPH, 1,1-diphenyl-2-picrylhydrazyl radical; 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl; ERK, extracellular signal-regulated kinase; GABA, \(\gamma\)-aminobutyric acid; Glu, glutamate; Gly, glycine; GRK2, G protein-coupled receptor kinase 2; GSH, glutathione; GSH-Px, glutathione peroxidase; GSSG, glutathione disulfide; HE, hematoxylin and eosin; HIF, hypoxia-inducible factor; HPLC, high performance liquid chromatography; hs-CRP, high-sensitivity C-reactive protein; I/R, ischemia-reperfusion; I-\(\kappa\)B, Inhibitory \(\kappa\)B; ICAM-1, intercellular adhesion molecule-1; IL-1\(\beta\), interleukin-1\(\beta\); IL-8, interleukin-8; IL-10, interleukin-10; iNOS, inducible nitric oxide synthase; JAM-1, junctional adhesion molecule-1; JNK, Jun N-terminal kinase; LDH, lactate dehydrogenase; MAPK, mitogen activated protein kinase; MCAO, middle cerebral artery occlusion; MDA, malondialdehyde; MPP, matrix metalloproteinases; MPO, myeloperoxidase; MRI, magnetic resonance imaging; NADPH, nicotinamide adenine dinucleotide phosphate; NO, nerve growth factor; NMDA, \(N\)-methyl-D-aspartic acid; NF-\(\kappa\)B, nuclear factor \(\kappa\)B; NO, nitric oxide; NSC, neural stem cells; OGD, oxygen-glucose deprivation; 8-OHdG, 8-hydroxydeoxyguanosine; PARP, poly-ADP-ribose polymerase; PMN, polymorphonuclear; RANTES, regulated upon activation normal T-cell expressed and secreted; ROS, reactive oxygen species; rtPA, recombinant tissue plasminogen activator; SOD, superoxide dismutase; TIMP-1, tissue inhibitor of metalloproteinase-1; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\); TTC, 2,3,5-triphenyltetrazolium chloride; Tuj-1, class III \(\beta\)-tubulin; TUNEL, terminal-deoxynucleotidyl transferase mediated nick end labeling; VEGF, vascular endothelial growth factor; ZO-1, zonula occludens-1

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1. Introduction

Stroke is the second leading cause of mortality in the world, resulting in 6,671,000 deaths (11.9% of all deaths) in 2012. Pathogenically, ischemic stroke caused by vessel occlusions accounts for 85% of all conditions. Since the core of brain tissue undergoes necrotic cell death within a few minutes of the onset of cerebral ischemia, early restoration of blood flow by thrombolytic therapy decreases morbidity and mortality in these patients. Paradoxically, reperfusion itself evokes additional injury to ischemic penumbra, a region bordering the infarct core, causing the so-called ischemia-reperfusion (I/R) injury. Such injury exacerbates brain damage, leading to increases in severe morbidity in surviving victims. I/R imposes multiple insults to the cerebral microcirculation, including reactive oxygen species (ROS) outburst, inflammatory mediator overproduction, leukocyte infiltration, microvessel hyperpermeability, brain blood barrier (BBB) disruption, capillary hyperperfusion, etc. Many of these factors are thought to play significant roles in the pathogenesis of post-ischemic injury in stroke patients. Much effort has been made to attenuate the microcirculatory disturbances by ablating a single factor in the pathogenesis, including introduction of recombinant tissue plasminogen activator (rtPA), antioxidants, anti-intercellular adhesion molecule-1 (ICAM-1) antibody, calcium-stabilizing agents and anti-excitoxotoxic agents. However, clinical trials have failed to show positive effects in patients with ischemic stroke. Other therapeutic approaches, such as anti-inflammatory and anti-apoptotic agents, are being evaluated, but no successful clinical trials have thus far been reported. These results suggest that such microcirculatory disturbances are part of a complicated pathological process involving multiple, coordinated events. Once initiated, the process may only be interrupted by a remedy consisting of multiple compositions that target the underlying insults.

For more than two thousand years, traditional Chinese medicine (TCM) has been used in China, Korea, Japan and other Asian countries for the clinical treatment of cerebrovascular diseases including stroke, encephalitis, dizziness, insomnia, amnesia, and dementia. In China, the use of compound TCM preparations to treat cerebrovascular diseases dates back to the Han Dynasty. “Treatise on Cold Damage (Shang Han Lun)” and “Synopsis of the Golden Chamber (Jin Gui Yao Lue)” appeared at that time which recorded several classical formulas, including Chaihu Jia Longgu Muli Tang, Guizhi Fuling Wan, Gualou Guizhi Tang, and others, which were devoted to cope with cerebrovascular diseases. In the Tang dynasty, medical formulas were further developed, as shown by “Important Prescriptions Worth a Thousand Gold for Emergency (Quan Jin Fang)” and “Arcane Essentials from the Imperial Library (Wai Tai Mi Yao); these documented the use of Xiaoxuming Tang, Dihuang Yinzi and Huanglian Jiedu Tang for the treatment of cerebrovascular diseases.

In the dynasties that followed, more compound TCM preparations were used in clinic. In the Song dynasty, treatments included Sijunzi Tang and Longdan Xiegan Tang, documented from “Formulary of the Bureau Taiping People’s Welfare Pharmacy (Tai Ping Hui Min He Ji Ju Fang)”. In the Yuan dynasty, medicines included Shengmai San, as reported in “Revelation of Medicine (Yi Xue Qi Yuan)”. In the Jin dynasty, the use of Chaihu Shugan San was mentioned in “Jing-Yue’s Collected Works (Jing Yue Quan Shu)”. Finally, in the Qing dynasty, Buyang Huanwu Tang and Taohong Siwu Tang were used according to “Correction on Errors in Medical Works (Yi Lin Gai Cuo)” and “Golden Mirror of the Medical Ancestors (Yi Zong Jin Jian), respectively.

Nowadays in China, several new compound TCM preparations have been formulated for the treatment of cerebrovascular diseases. Based on classical formulas and approved by the State Food and Drug Administration (SFDA), these include Cerebralcare Granule (Yangxue Qingnao granule), Tongxinluo capsule, Shenfu injection, Danhong injection, Huatuo Zaizao extractum. The name, composition and origin of the compound TCM preparations that are derived from TCM literatures or approved by the SFDA and cited in the present review are listed in Table 1. In addition, Chinese materia medica as well as active ingredients and components included in compound TCM preparations are listed in Table 2. These represent substances of recent research interest as related to their possible roles in the pathogenesis of I/R-induced brain injury, neuron damage and the underlying mechanisms. The present review is based on 139 references published from 1995 to 2014, mainly focusing on the ameliorating effects and underlying mechanisms of TCM preparations, Chinese materia medica, and active compounds on I/R-induced cerebral microcirculatory disturbances, brain injury and neuron damage.
| Compound TCM preparation | Composition | Origin |
|--------------------------|-------------|--------|
| Buyang Huanwu decoction | Huangqi (Radix Astragali seu Hedysari), Fangdeng (Radix Angelicae Sinensis), Chishao (Radix Paeoniae Rubra), Douchi (Bassia scoparia), Dazao (Fructus Jujubae), Maqianzi (Semein Strychni), Honghua (Flos Carthami), Maqianzi (Semein Strychni) | “Correction on Errors in Medical Works” (Qing dynasty) |
| Chaihu Jia Longgu Muli Tang | Chaihu (Radix Bupleuri), Longgu (Os Draconis), Huanggao (Radix Scutellariae), Shengjiang (Rhizoma Zingiberis), Qiandan (Minium), Shanzhuyu (Fructus Corni), Shanyao (Geum urbanum), Taoren (Semen Persicae), Fuling (Poria), Banxia (Rhizoma Pinelliae), Dahuang (Radix et Rhizoma Rhei), Muli (Concha Ostreae), Dazao (Fructus Jujubae) | (Han dynasty) |
| Danhong injection | Danshen (Radix Salviae Miltiorrhizae), Honghua (Flos Carthami) | Approved by SFDA (Z20026866) |
| Dihuang Yinzi | Shengdihuang (Radix Rehmanniae Recens), Eupolyphaga (Eupolyphaga Chinensis), Mianping (Eupolyphaga Chinensis), Shanzhuyu (Fructus Corni), Dihuang (Radix et Rhizoma Rhei), Xiaohuixiang (Fructus Foeniculi), Dazao (Fructus Jujubae) | “Arcane Essentials from the Imperial Library” (Tang dynasty) |
| Fufang Danggu injection | Danggui (Radix Angelicae Sinensis), Chuanxiong (Rhizoma Ligustici Chaixxiang), Honghua (Flos Carthami) | Approved by SFDA (Z42021410) |
| Gualou Guizhi Tang | Gualou (Fruitus Trichosanthis), Guizhi (Ramulus Cinnamomi), Baishao (Radix Paeoniae Alba), Gouqizi (Fructus Lycii), Huangqi (Radix Astragali seu Hedysari), Shuizhi (Hirudo), Quanxie (Scorpion) | “Synopsis of the Golden Chamber” (Han dynasty) |
| Guizhi Fuling Wan | Guizhi (Ramulus Cinnamomi), Fuling (Poria), Gancao (Radix Glycyrrhizae), Mudanpi (Cortex Moutan Radicis), Chishao (Radix Paeoniae Rubra), Taoren (Semen Persicae) | “Synopsis of the Golden Chamber” (Han dynasty) |
| Huatuo Zaizao extractum | Danggui (Radix Angelicae Sinensis), Chuanxiong (Rhizoma Ligustici Chaixxiang), Bingpian (Bomeolum Syntheticum), Baishao (Radix Paeoniae Alba), Renshen (Radix Ginseng), Wuweizi (Fructus Schisandrae Chinensis), Majianzi (Semen Styrchi), Honghua (Flos Carthami), Tiannanxing (Rhizoma Arisaematis) | Approved by SFDA (Z44020748) |
| Huanshaodan decoction | Shuidihuang (Radix Rehmanniae Preparata), Shanzhuyu (Fructus Corni), Shanyao (Geum urbanum), Maqianzi (Semein Strychni), Honghua (Flos Carthami), Chishao (Radix Paeoniae Rubra), Taoren (Semen Persicae), Fuling (Poria) | Approved by SFDA (Z50020189) |
| Huanglian Jiedu Tang | Huangqin (Radix Scutellariae), Huanglian (Rhizoma Coptidis), Huangqin (Radix Scutellariae), Baishao (Radix Paeoniae Alba), Renshen (Radix Ginseng), Shuizhi (Hirudo), Quanxie (Scorpion), Chishao (Radix Paeoniae Rubra), Dilong (Lumbricus), Chuanxiong (Rhizoma Ligustici Chaixxiang), Tianma (Rhizoma Gastrodiae), Loulu (Radix Rhapontici) | “Arcane Essentials from the Imperial Library” (Tang dynasty) |
| Naoshuang capsule | Puhuang (Pollen Typhae), Chishao (Radix Paeoniae Rubra), Yujin (Radix Curcumae), Tianma (Rhizoma Coptidis), Loulu (Radix Rhapontici), Dazao (Fructus Jujubae), Maqianzi (Semein Strychni), Chishao (Radix Paeoniae Rubra), Dilong (Lumbricus), Chuanxiong (Rhizoma Ligustici Chaixxiang), Tianma (Rhizoma Gastrodiae), Loulu (Radix Rhapontici) | Approved by SFDA (Z20040093) |
| Shenfu injection | Renshen (Radix Ginseng), Fuzi (Radix Aconiti Lateralis Preparata), Chishao (Radix Paeoniae Rubra), Dazao (Fructus Jujubae), Maqianzi (Semein Strychni), Chishao (Radix Paeoniae Rubra), Dilong (Lumbricus), Chuanxiong (Rhizoma Ligustici Chaixxiang), Tianma (Rhizoma Gastrodiae), Loulu (Radix Rhapontici) | Approved by SFDA (Z51020664) |
| Shenqi Fuzheng injection | Danshen (Radix Salviae Miltiorrhizae), Huangqi (Radix Astragali seu Hedysari), Maqianzi (Semein Strychni), Honghua (Flos Carthami) | Approved by SFDA (Z19990065) |
| Shengmai San | Renshen (Radix Ginseng), Maozhu (Radix Ophiopogonis), Wuweiizi (Fructus Schisandrae Chinensis), Majianzi (Semen Styrchi), Honghua (Flos Carthami), Chishao (Radix Paeoniae Rubra), Taoren (Semen Persicae), Fuling (Poria) | “Revelation of Medicine” (Jin dynasty) |
| Taohong Siwu Tang | Shuidihuang (Radix Rehmanniae Preparata), Danggui (Radix Angelicae Sinensis), Baishao (Radix Paeoniae Alba), Chuanxiong (Rhizoma Ligustici Chaixxiang), Taoren (Semen Persicae), Honghua (Flos Carthami), Xingren (Semen Ziziphi Spinosae), Bingpian (Bomeolum Syntheticum), Wugong (Scolopendra), Tanxiang (Lignum Santali Albi) | “Golden Mirror of the Medical Ancestors” (Qing dynasty) |
| Tianma Gouteng granule | Tianma (Rhizoma Gastrodiae), Gouteng (Ramulus Uncariae cum Uncis), Shengjiang (Rhizoma Zingiberis), Qiandan (Minium), Shanzhuyu (Fructus Corni), Dihuang (Radix et Rhizoma Rhei), Baishao (Radix Paeoniae Alba), Fuzi (Radix Aconiti Lateralis Preparata), Shengjiang (Rhizoma Zingiberis) | Approved by SFDA (Z51021084) |
| Tongxinluo capsule | Renshen (Radix Ginseng), Shuizhi (Hirudo), Quanxie (Scorpio), Chishao (Radix Paeoniae Rubra), Chuanxiong (Rhizoma Ligustici Chaixxiang), Tianma (Rhizoma Gastrodiae), Loulu (Radix Rhapontici), Dazao (Fructus Jujubae), Maqianzi (Semein Strychni), Chishao (Radix Paeoniae Rubra), Dilong (Lumbricus), Chuanxiong (Rhizoma Ligustici Chaixxiang), Tianma (Rhizoma Gastrodiae), Loulu (Radix Rhapontici) | Approved by SFDA (Z19980015) |
| Xiaoxing decoction | Mahuang (Herba Ephedrae), Guizhi (Ramulus Cinnamomi), Fangfeng (Radix Saposhnikoviae), Fangji (Radix Stephaniae Tetrandrae), Xingren (Semen Armeniacae Amarum), Huangqin (Radix Scutellariae), Renshen (Radix Ginseng), Gancao (Radix Glycyrrhizae), Dazao (Fructus Jujubae), Chuanxiong (Rhizoma Ligustici Chaixxiang), Baishao (Radix Paeoniae Alba), Fuzi (Radix Aconiti Lateralis Preparata), Shengjiang (Rhizoma Zingiberis) | “Important Prescriptions Worth a Thousand Gold for Emergency” (Tang dynasty) |
### Table 1 (continued)

| Compound TCM preparation | Composition | Origin |
|--------------------------|-------------|--------|
| Xingnaojing injection    | Shexiang (Moschus), Yujin (Radix Curcumae), Bingpian (*Bomeolium Syntheticum*), Zhizi (Fructus Gardeniae) | Approved by SFDA (Z53021638) |
| Cerebralcare Granule™ (Yangxue Qingnao granule) | Danggui (Radix Angelicae Sinensis), Chuanxiong (Rhizoma Ligustici Chuaxiong), Baishao (Radix Paoniae Alba), Shudihuang (Radix Rehmanniae Preparata), Gouteng (Ramulus Uncariae cum Uncis), Jixueteng (Caulis Spatholobi), Xiakucuo (Spica Prunellae), Juemengzi (Semen Cassiae), Zhennhumu (Concha Margaritifera), Yanhusuo (Rhizoma Corydalis), Xixin (Herba Asari) | Approved by SFDA (Z10960082) |

### Table 2

The structures and sources of active components of Chinese materia medica.

| Active component          | Structure | Source                        | Ref. |
|---------------------------|-----------|-------------------------------|------|
| 3’-Methoxy-puerarin       | ![Structure](image1.png) | Gegen (Radix puerariae)       | 12   |
| Astragaloside             | ![Structure](image2.png) | Huangqi (Radix Astragali seu Hedysari) | 13–17|
| Apigenin                  | ![Structure](image3.png) | Celery                        | 18   |
| Baicalin                  | ![Structure](image4.png) | Huangqin (Radix Scutellariae)  | 19–23|
| Calycosin                 | ![Structure](image5.png) | Maoruihua (*Verbascum Thapsus*) | 24   |
| Eupatilin                 | ![Structure](image6.png) | Aiye (*Artemisia Argyi*)       | 25   |
| Ginsenoside Re            | ![Structure](image7.png) | Renshen (Radix Ginseng)       | 26   |
| Ginsenoside Rg3           | ![Structure](image8.png) | Renshen (Radix Ginseng)       | 27   |
| Honokiol                  | ![Structure](image9.png) | Houpo (Cortex Magnoliae Officinalis) | 28   |
| Icariin                   | ![Structure](image10.png) | Yinyanghuo (Herba Epimedii)   | 29   |
| Active component          | Structure | Source                   | Ref. |
|--------------------------|-----------|--------------------------|------|
| Leonurine                | ![Leonurine Structure](Leonurine.png) | Yimucao (Herba Leonuri)  | 30   |
| Luteolin                 | ![Luteolin Structure](Luteolin.png) | Multiple plants          | 31   |
| Lycopene                 | ![Lycopene Structure](Lycopene.png) | Tomato                   | 32   |
| Morroniside              | ![Morroniside Structure](Morroniside.png) | Shanzhuyu (Fructus Corni) | 33   |
| Notoginsenoside R1       | ![Notoginsenoside R1 Structure](Notoginsenoside R1.png) | Sanqi (Radix et Rhizoma Notoginseng) | 34   |
| Paeonol                  | ![Paeonol Structure](Paeonol.png) | Mudanpi (Cortex Moutan Radicis) | 35   |
| Resveratrol              | ![Resveratrol Structure](Resveratrol.png) | Grape                    | 36, 37 |
| Salvianolic acid A       | ![Salvianolic acid A Structure](Salvianolic acid A.png) | Danshen (Radix Salviae Miltiorrhizae) | 38–40 |
| Salvianolic acid B       | ![Salvianolic acid B Structure](Salvianolic acid B.png) | Danshen (Radix Salviae Miltiorrhizae) | 41, 42 |
| Scutellarin              | ![Scutellarin Structure](Scutellarin.png) | Huangqin (Radix Scutellariae) | 43   |
| Senegenin                | ![Senegenin Structure](Senegenin.png) | Yuanzhi (Radix Polygalae)  | 44   |
| Tanshinone II B          | ![Tanshinone II B Structure](Tanshinone II B.png) | Danshen (Radix Salviae Miltiorrhizae) | 45   |
| Tetrahydroxystilbene glucoside | ![Tetrahydroxystilbene glucoside Structure](Tetrahydroxystilbene glucoside.png) | Heshouwu (Radix Polygoni Multiflori) | 46   |
| Tetrandrine              | ![Tetrandrine Structure](Tetrandrine.png) | Fangji (Radix Stephaniae Tetrandae) | 47   |
2. Effects of TCM preparation, Chinese materia medica, and active compounds on pathogenesis of cerebral microcirculatory disturbances induced by I/R

2.1. Oxidative stress

Significant amounts of ROS are generated during cerebral I/R, which are widely regarded as the initial step in brain damage after stroke. Numerous clinical and experimental observations have shown increased ROS formation during all forms of stroke injury. ROS are highly active and able to react with DNA, protein, and lipid directly, causing damage and dysfunction of the molecules to various degree. The primary source of ROS during I/R injury is the mitochondria, which produce superoxide anion radicals during the electron transport process. Other potentially important sources of ROS include xanthine oxidase, cyclooxygenase, lipooxygenase, and others, depending on cell types. Oxygen free radicals can also be generated by activated microglia and infiltrating leukocytes via the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway following reperfusion. In recent studies, researchers found that some compound TCM preparations, Chinese materia medica and active components produce positive effects on cerebrovascular diseases partially due to their antioxidant properties. Using dihydrodromamine 123 (DHR), a hydrogen peroxide-sensitive mitochondrial probe, researchers demonstrated in vivo that compound TCM preparations, such as Cerebralcare Granule attenuates DHR fluorescence intensity in gerbil cerebral microvessels, in either the early phase (60 min) or late phase (5 days) of reperfusion after global ischemia. The single active component notoginsenoside R1 was found to prevent oxidative stress by suppressing both mitochondrial and NADPH oxidase-dependent superoxide generation and inhibiting production of malondialdehyde (MDA), protein carbonyl, and 8-hydroxydeoxyguanosine (8-OHdG) in rat with middle cerebral artery occlusion (MCAO) and reperfusion in vivo. This compound also had antioxidant activity in primary cortical neurons stimulated by oxygen-glucose deprivation (OGD) followed by reoxygenation in vitro. Other compound TCM preparations, such as Yiqi Tongluo Jiedu capsule, as well as several active components from Chinese materia medica (including total glycoside from Chishao, Radix Paeoniae Rubra, astragaloside, and tetrahydroxystilbene glucoside) alleviated ROS production in cerebral tissue via inhibiting inducible nitric oxide synthase (iNOS) activation and nitric oxide (NO) overproduction. Other experiments reported that Chinese materia medica Danshen (Radix Salviae Miltiorrhizae) and active component baicalin exert their antioxidant effect by lowering adenosine metabolites hypoxanthine and inhibiting cyclooxygenase, respectively. In addition to ROS source regulation, TCM preparation, Chinese materia medica and active compounds ameliorate I/R-induced oxidative stress by scavenging free radicals directly or by modulating tissue antioxidant potency. In a recently published study, three classical formulas (Huanglian Jiedu Tang, Chaihu Jia Longgu Muli Tang, and Guizhi Fuling Wan) showed scavenging activity for free-radical superoxide anion radicals, hydroxyl radicals and 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl) hydrazyl (DPPH), respectively, while suppressing lipid peroxidation. Another study reported that the single active component salvianolic acid A reduces the MDA contents in the cortex, hippocampus and corpus striatum in I/R rat brain, which may also attribute to its scavenging effect on free hydroxyl radicals in vitro. Shahmok San, another classical formula, improved oxidative damage manifested as suppression of MDA and thiobarbituric acid reactive substance (TBARS) formation and increased superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in brain tissues from rodent global or MCAO models. This treatment was active whether given before ischemia or after re-perfusion. In parallel, aqueous or ethanolic extract from Balsamussauenggou (Embelia ribes Burm) increased the glutathione (GSH), GSH-Px, glutathione reductase and glutathione-S-transferase levels in both hippocampus and frontal cortex with decreases in lactate dehydrogenase (LDH) level in serum and TBARS levels in hippocampus and frontal cortex in rat after MCAO. Lavender oil was also found to reduce the levels of mitochondria-generated ROS, MDA and carbonyl, and to upregulate the ratio of GSH/glutathione disulfide (GSSG), the activities of SOD, catalase (CAT) and GSH-Px in brain tissue after focal ischemia. Similar antioxidant effects have been reported for some other TCM preparation, such as Tongxinluo capsule, Yimucao (Herba Leonuri) injection, and active components, such as Yinxiing (Folium Ginkgo) extract, paenol and 3’-methoxy-puerarin.

2.2. Inflammatory mediators

Inflammation is increasingly recognized to be the key element in the pathological progression of ischemic stroke, as early inflammatory responses may potentiate reperfusion injury in post-ischemic brain tissue. There are several resident cell populations within brain tissue that are able to secrete proinflammatory mediators after an ischemic insult, including endothelial cells, leukocytes, astrocytes, microglia and neurons. Activation of transcription factors via nuclear factor-κB (NF-κB), mitogen activated protein kinase (MAPK), and activator protein-1 (AP-1) inflammatory signaling pathway causes an increased production of cytokines, chemokines, adhesion molecules and other proinflammatory mediators. Several agents that can regulate inflammatory mediators or transcription factors reduce infarct size and neurological deficits following focal stroke in rodents; such treatments are considered to be an alternative therapeutic approach in stroke patients. In this regard, Guizhi Fuling capsules, a classical formula, were reported to down-regulate the expression of pro-inflammatory cytokines [including interleukin-1β (IL-1β) and tissue necrosis factor-α (TNF-α)] and markedly up-regulate the expression of anti-inflammatory cytokines interleukin-10 (IL-10) and IL-10 receptor at both mRNA and protein levels in rats with focal cerebral I/R. The serum levels of these inflammatory cytokines were regulated in the same way. In other in vivo MCAO experiments, TCM Naomaitong preparation and the single active component tetrandrine have proven to decrease the expression and mRNA level of TNF-α, vascular adhesion molecule-1 (VCAM-1), ICAM-1 in brain tissue 1 or 3 days after reperfusion, partly due to suppressing NF-κB activation. Similar results were found for TCM preparations, such as Taohong Siwu Tang, FBD formula [a herbal formula composed of Fuling (Porzia), Baizhu (Rhizoma Atractylodis Macrocephalae) and Danggui (Radix Angelicae Sinensis)] as well as Chinese materia medica Danshen (Radix Salviae miltiorrhizae) aqueous extract. A broad spectrum of cytokines and chemokines were abrogated by treatment with these TCM, including high-sensitivity C-reactive protein (hs-CRP), hypoxia-inducible factor (HIF)-1α, interleukin-8 (IL-8), TNF-α, iNOS levels in serum or brain, and TNF-α mRNA and transforming growth factor β1 (TGF-β1) expression in cerebral tissue; these effects were partly due to down-regulation of cerebral Inhibitory-xB-a (IκBα) and NF-κB phosphorylation. In a global cerebral ischemia model, treatment with the single active component resveratrol before insult reduced astroglial and microglial activation as well as cyclooxygenase-2 (COX-2) and iNOS expression 7 days after I/R. These effects...
were attributed to suppression of NF-κB and Jun N-terminal kinase (JNK) activation\(^{56}\). In addition to in vivo results, in vitro experiments demonstrated that another active component honokiol reduced TNF-α and NO level in the primary cultured microglia medium and in the microglia and astrocytes co-culture medium. Also, honokiol was shown to decrease the level of RANTES (regulated upon activation normal T-cell expressed and secreted) protein in medium of microglia or astrocytes, which was related to its effect on microglia NF-κB p65 nuclear translocation\(^{57,58}\).

2.3. Leukocyte infiltration

A growing body of evidence indicates that recruitment of leukocytes contributes to the initiation and evolution of brain injury after ischemic stroke. The adhesion and migration of neutrophils is evident in cerebral venules from several minutes to a few hours following reperfusion. The population of recruited cells shifts from polymorphonuclear (PMN) to venules from several minutes to a few hours following reperfusion. The stroke. The adhesion and migration of neutrophils is evident in cerebral venules from several minutes to a few hours following reperfusion. The population of recruited cells shifts from polymorphonuclear (PMN) to mononuclear leukocytes and lymphocytes, and leukocyte recruitment persists for days to weeks following ischemia\(^{72,73}\). Rodents with reduced PMN or T-lymphocyte accumulation show reduced infarct volumes and improved neurological outcomes. Prevention of leukocyte-endothelial cell adhesion with adhesion molecule antibodies also protects against stroke injury\(^{74}\). In a rat MCAO model, leukocyte adhesion was observed continuously in cerebral microvessels by infusion of rhodamine 6G, and the number of adherent leukocytes after reperfusion increased immediately and remained increased during 60 min after reperfusion. Pretreatment with TCM preparation Cerebralcare Granule\(^{80}\), attenuated this I/R-elicted enhancement of leukocyte adhesion; the effects of the higher dose (0.8 g/kg) were more significant than those following the lower dose (0.4 g/kg)\(^{75}\). Consistently, Cerebralcare Granule\(^{80}\) inhibited leukocyte adhesion either during the early phase (60 min) or in the late phase (5 days) of reperfusion after global ischemia in gerbils\(^{76,77}\). Using \(^{51}\)Cr-labeled neutrophil, researchers also demonstrated that tetraderine decreased neutrophils recruitment in brain tissue 24 h after reperfusion in rat with MCAO\(^{78}\). The classic formula Huanglian Jiedu Tang and its constituents inhibited myeloperoxidase (MPO) activity, an indication of neutrophil infiltration, in ischemic brain tissue by 30% after focal I/R\(^{79}\). The same result was observed when using other TCM preparations, such as Shengmai San\(^{80}\), Tongxinluo capsule\(^{81}\), and Chinese materia medica Zhihun (Rhizoma Anemarrhenae)\(^{82}\), either in rodent global or focal I/R injury. FBD formula inhibited PMNs infiltration in ICR mouse brain subjected to repetitive 10 min of common carotid arteries occlusion followed 24 h reperfusion, and in vitro results showed that FBD formula could inhibit TNF-α-triggered PMNs adhesion to ECV304 endothelial cells\(^{83}\). In another in vitro experiment, the single active compound salvianolic acid A was proven to inhibit the adherence of granulocytes on brain microvascular endothelial cells (BMEC); the effect was attributed to decreasing the expression of ICAM-1 on BMEC at the gene and protein levels\(^{84}\).

2.4. Brain blood barrier (BBB) disruption

BBB consists of microvascular endothelial cells, basal lamina, pericytes and astrocyte endfeet. Microvessel hyperpermeability disrupts the normal BBB function during cerebral I/R injury. Microvessel permeability is regulated by both paracellular and transcellular pathways\(^{85}\). Paracellular pathways are mainly governed by tight junctions; the loss of tight junction integrity occurs and directly contributes to cerebral BBB disruption under ischemic stroke conditions\(^{86}\). An alternative mechanism for BBB opening involves upregulation of caveolae, including the expression and phosphorylation of the structural component caveolin-1 (Cav-1), as demonstrated by transmission electron microscopy in endothelial cells in several stroke models\(^{87}\). In a rat MCAO model, recent in vivo studies found that both pre- and post-treatment with the TCM preparation Cerebralcare Granule\(^{88}\) reduces FITC-labeled albumin leakage from cerebral venules evoked by I/R, in either the early or late phase after reperfusion\(^{75,76}\); the same results were found in a gerbil global I/R model\(^{80,81}\). Further study using confocal microscopy revealed that the continuous distributions of tight junction proteins including claudin-5, occludin, junctional adhesion molecule-1 (JAM-1) and zonula occludens-1 (ZO-1) were disrupted after reperfusion for 3 h and 6 days, concomitant with reduced immune staining. Western blotting indicated the degradation of tight junction proteins in response to I/R. Interestingly, these losses in structural integrity were reversed by Cerebralcare Granule\(^{88}\) treatment. In addition, I/R-induced increases in the cytoplasmic caveolae of capillary endothelial cells and cerebral Cav-1 expression were both down-regulated by Cerebralcare Granule\(^{88}\), as observed by electron microscopy and Western blotting, respectively. Taken together, these findings suggest involvement of both the paracellular and transcellular pathways in the beneficial effects of Cerebralcare Granule on BBB disruption following cerebral I/R injury\(^{82}\). Similarly, treatment with the TCM preparation Tongxinluo capsule increased ZO-1 and occludin expression in cerebral microvessels 24 h after MCAO in mice\(^{77}\). In addition, enzymatic degradation of the extracellular matrix by matrix metalloproteinases (MMPs), secretion of vascular endothelial growth factor (VEGF), ROS production, leukocyte infiltration, and inflammatory mediator release within the ischemic core or peri-infarct area have all been postulated to trigger BBB disruption directly or indirectly during I/R process. To this end, TCM preparations such as naomaoting preparation\(^{83}\), and Panax notoginseng saponins combined with astragaloside\(^{15}\), were reported to protect against cerebral microvessel basement membrane injury via modulating gelatinase system, inhibiting MMP-2 and MMP-9 expression and improving tissue inhibitor of metalloproteinase-1 (TIMP-1) protein level in rodent brain tissue after I/R. Using immunohistochemistry, the single active component salvianolic acid B was shown to alleviate the extravasation of immunoglobulin and attenuate MMP-9 expression induced by cerebral I/R, which was related to the inhibition on p38MAPK activation and extracellular signal-regulated kinase (ERK) 1/2 phosphorylation\(^{44}\). Other TCM preparations, such as Weinakang preparation\(^{87}\) and Huatuo Zaizao extractum\(^{85}\), may also effectively recover BBB ultrastructure damage induced by I/R via inhibiting expressions of MMP-2 and MMP-9, which might be associated with reduction of G protein-coupled receptor kinase 2 (GKR2) in membrane translocation and activation. Another experiment showed that aromatic resuscitation drugs have the protection effect on BBB by decreasing the level of VEGF in addition to MMP-9\(^{86}\). Besides, the effects of TCM preparation, Chinese materia medica and active compounds on ROS production, leukocyte infiltration and inflammation all contribute to ameliorating BBB disruption to some extent, as discussed above.

2.5. Capillary hypoperfusion

It has been known for some time that loss of microvascular patency impairs cerebral vascular re-perfusion following global or
focal cerebral ischemia. After reperfusion, adhered leukocytes, entrapped erythrocytes, and fibrin-platelet deposits obstruct capillary lumens. Moreover, swollen astrocyte endfeet, pericyte contraction, and microvessel hyperpermeability all contribute to capillary occlusion after reperfusion. A recent clinical study showed that perfusion status, rather than successful recanalization, has a significant impact on the outcome of stroke patients, suggesting that improvement of microcirculatory reperfusion seems to be a promising strategy in stroke patients after thrombolysis. With the use of the intravital microscopy equipped with a high-speed video camera in a rat MCAO model, researchers demonstrated that the number of open capillaries reduced considerably 60 min after reperfusion, and pre-treatment with TCM preparation Cerebralcare Granule attenuated this alteration. In addition, cerebral blood flow (CBF) in cortex decreased 3 h after I/R, and this reduction remained for 6 days. Cerebralcare Granule post-treatment after reperfusion attenuated the I/R-evoked decrease in CBF. Consistent with the result observed by intravital microscopy and laser Doppler, transmission and scanning electron microscopy clearly identified that pre- or post-treatment with Cerebralcare Granule ameliorated cerebral microvasculature changes, including narrowed lumen, rough inner surface, swelling endothelial cells and perivascular astrocyte end feet. The treatment also restored the decrease in the number of open capillaries 24 h or 6 days after reperfusion. Similar results were found when using Cerebralcare Granule in global I/R injury. Assessment of hemorheology by a full-automatic hemorheolometer revealed that acupoint-injection of compound Angelica-root injection (Fangfumangui injection) downregulated blood viscosity including high, medium and low shear rates, erythrocyte aggregation index, and rigidity index; deformity index was up-regulated, facilitating cerebral blood circulation. Other TCM preparations, such as Erigeron injection (Dengzhanguansu injection), Astragalus injection (Huangqi injection), Naosaitong preparation, and icarin combined with P. notoginseng saponins were also reported to improve blood rheology and CBF after cerebral I/R injury. In addition, Guaisol Guizhi decoction reduced cerebral ischemic spasticity, improved the screen test and Hoffman’s reflex scores, which might be related to modulation of glutamate (Glu) levels and α-amino-3-hydroxy-5-methyl-4-isoaxazolpropionic acid (AMPA) receptor expression.

3. Effects of TCM on brain injury and neuron damage induced by I/R

Within a few minutes of the onset of cerebral ischemia in stroke patients, the core of brain tissue exposed to the most dramatic blood flow reduction is mortally injured, and subsequently undergoes necrotic cell death. The reduction of oxygen and nutrient supply induces a series of metabolic dysfunctions such as reduction in ATP formation and energy failure, loss of cell ion homeostasis, acidosis, membrane depolarization, Ca2+ influx, excessive release of excitatory amino acids, free radical-mediated toxicity, all resulting in brain and neuron necrosis. Thrombolysis strategies have proven to be the most effective therapies for stroke treatment. However, early reperfusion of ischemic brain tissue can result in harmful consequences, including ROS outburst, overproduction of inflammatory mediators, leukocyte infiltration, microvessel hyperpermeability, all of which lead to BBB disruption and capillary hyperperfusion. Ultimately, activation of these cascades culminate in cerebral edema and/or brain hemorrhage and exacerbate brain injury. On the other hand, cerebral microcirculatory disturbances, together with other neurotoxicity mediators such as Na+, Ca2+, and Glu, are detrimental to neuronal survival in the ischemic penumbra or peri-infarct zone; apoptotic neuron death is a common outcome. As discussed above, TCM has multiple beneficial roles in cerebral I/R-induced microcirculatory disturbances. Among these are positive effects on brain injury, such as brain infarction, perivascular edema and hemorrhage after I/R. In this aspect, HE staining demonstrated a histopathological damage after global or focal cerebral I/R, which could be inhibited by some TCM preparations, such as Shengmai San, Naomaitong preparation and active component baicalin. By virtue of magnetic resonance imaging (MRI), rat brain edema was observed 3 h after MCAO and remained unchanged 6 days after reperfusion. Interestingly, TCM preparation Cerebralcare Granule reduced cerebral edema even when administered after the initiation of edema. Likewise, scanning electron microscopy revealed swollen glia and edema around cerebral microvessels which were abrogated by Cerebralcare Granule and the active component apigenin after global or focal I/R injury. By means of high performance liquid chromatography (HPLC) and atomic absorption spectrophotometry, the TCM preparation Shenfu injection was found to decrease glutamate and Ca2+ in brain tissue and reduce excitatory amino acid toxicity, effects which can alleviate tissue edema. Using Evan’s blue dye extravasation and/or brain water content assessment, large doses of TCM preparation, Chinese materia medica, and active compounds were demonstrated to restrain BBB disruption and brain tissue edema after I/R, although the mechanisms for the effects of each TCM may differ. For example, combinations of total alkaloids from Gouteng (Ramulus Uncariae cum Uncis) and Xiatianwu (Rhizoma Corydalis Decumbentis) as well as the active compound sodium tanshinone B were thought to act through anti-oxidation, whereas the TCM preparation Naomai-tong preparation and the active compound sodium tanshinone B may produce beneficial effects by anti-inflammatory mechanisms. In contrast, FBD formula and Chinese materia medica Zhuimu (Rhizoma Anemarrhenae) may act through inhibition of leukocyte infiltration, whereas the TCM preparation Shexiang Xingnaoning preparation as suggested may act by anti-thrombotic mechanisms. Finally, Cerebralcare Granule and Tongxinluo capsule are thought to maintain the BBB. Consistent with anti-edema effects, the beneficial effects of TCM preparations, Chinese materia medica and active compounds on cerebral infarction after I/R injury have also been reported. For example, 2,3,5-triphenyltetrazolium chloride (TTC) staining showed that the TCM preparation Xuezhikang capsule, total flavones of Chinese materia medica Huangshuiku (Abelmoschus manihot) and active compounds, such as calycosin and astraguloside, inhibited brain infarction mainly by anti-oxidative effect. On the other hand, the classical formula Buyang Huanwu Tang, and TCM preparations, such as Danhong injection, Astragalus injection (Huangqi injection), and Naosaitong preparation were shown to inhibit cerebral tissue necrosis via promoting blood vessel repair, anticoagulant and anti-fibrinolytic activity, and improvement of capillary perfusion. In contrast studies on edema and infarction, few studies have been published showing the ameliorating effect of TCM preparations, Chinese materia medica, and active compounds on cerebral hemorrhage induced by I/R injury. However, these medicinals can
alleviate intracerebral hemorrhage in some situations, such as surgery, traumatic intracranial hematoma, and artificial cerebral hemorrhage in animal models.

3.2. Effects of TCM on neuron damage induced by I/R

A variety of tests are available for evaluation of neurobehavioral function, such as Long’s test, Morris water maze test, eight-arm radial maze test, step down and step through test, beam-walking test, forced swimming test, and tail suspension test. Using these tests, many TCM preparations, Chinese materia medica and active compounds have been shown to alleviate neurological deficits and improve learning and memory after I/R injury. These include TCM preparations such as Xiaoxuming decoction, Yizhi capsule, and Chinese materia medica such as Sanqi (Radix et Rhizoma Notoginseng), Xuesaitong injection, Naoshuantong capsule, Yinxing toxylin and eosin (HE), TUNEL and Hoechst 33258 was used to ginsenoside Re2 were reported to attenuate I/R-induced mitochondrial disturbances, brain injury and neuron damage. TCM preparation, Chinese materia medica and active compounds inhibit I/R-induced multiple insults in cerebral microcirculation, including ROS outburst, inflammatory mediators overproduction, leukocyte infiltration, microvessel hyperpermeability, platelet aggregation, etc., leading to BBB disruption and capillary hypoperfusion, which culminate in ameliorating cerebral edema and/or brain hemorrhage, and brain injury. On the other hand, by improving cerebral microcirculation, together with anti-apoptosis, anti-excitotoxicity and neurogenesis, protection against I/R. BBB, blood brain barrier; EC, endothelial cell; H2O2, hydrogen peroxide; I/R, ischemia/reperfusion; MMPs, matrix metalloproteinases; NADPH, nicotinamide adenine dinucleotide phosphate; O2•−, superoxide anion; OH•, hydroxyl radicals; ONOO•, peroxynitrite anion; ROS, reactive oxygen species, TCM, traditional Chinese medicine; XO, xanthine oxidase; denotes inhibition.

ginsenoside Rg3 displayed similar neuroprotective actions via anti-apoptotic effects.

3.2.2. Effects of TCM on excitotoxicity

Excitatory amino acids accumulate in the extracellular space following ischemia and activate their receptors, leading to intracellular Ca2+ overload followed by neuronal damage. HPLC results demonstrated that the classical formula Buyang Huanwu decoction, Tianma Gouteng Fang, and Chinese materia medica Yinxing (Folium Ginkgo) extract displayed similar neuroprotective actions via anti-apoptotic effects.
### Table 3  Effect of TCM preparation, Chinese materia medica and active compounds on pathogenesis of cerebral microcirculatory disturbances induced by I/R.

| Effect                                      | Target                          | TCM                                                                 | Refs. |
|---------------------------------------------|--------------------------------|----------------------------------------------------------------------|-------|
| Amelioration of oxidative stress            | ROS source                     | Cerebralcare Granules®                                               | 50,51 |
|                                             |                                | Yiqi Tongluo Jiedu capsule                                           | 52    |
|                                             |                                | Chishao total glycoside                                              | 53    |
|                                             |                                | Danshen                                                             | 54    |
|                                             |                                | Astragaloside                                                        | 13,14 |
|                                             |                                | Baicalin                                                             | 19    |
|                                             |                                | Notoginsenoside R1                                                  | 34    |
|                                             |                                | Tetrahydroxystilbene glucoside                                       | 46    |
|                                             | Free-radical scavenger         | Chaihu Jia Longgu Muli Tang                                          | 55    |
|                                             |                                | Guizhi Fuling Wan                                                   | 55    |
|                                             |                                | Huanglian Jiedu Tang                                                 | 55    |
|                                             |                                | Salvianolic acid A                                                  | 38    |
|                                             | Anti-oxidase activity          | Shengmai San                                                        | 56–58 |
|                                             |                                | Baihuasuantenggou extract                                           | 59,60 |
|                                             |                                | Lavender oil                                                        | 61    |
|                                             |                                | Yimucao injection                                                   | 63    |
|                                             |                                | Yinxing extract                                                     | 64    |
|                                             |                                | 3’-Methoxy-puerarin                                                 | 12    |
|                                             |                                | Paenol                                                              | 35    |
| Amelioration of inflammation                | Cytokines/chemokines           | FBD formula                                                          | 71    |
|                                             |                                | Guizhi Fuling capsule                                               | 67    |
|                                             |                                | Naomaitong preparation                                              | 68    |
|                                             |                                | Taohong Siwu Tang                                                   | 69    |
|                                             |                                | Danshen                                                             | 71    |
|                                             |                                | Honokiol                                                            | 28    |
|                                             |                                | Tetrandrine                                                         | 47    |
|                                             | Adhesion molecules             | Naomaitong preparation                                              | 68    |
|                                             |                                | Tetrandrine                                                         | 47    |
|                                             | Other mediators (TGF-β1, iNOS, Cox-2) | Taohong Siwu Tang                                                | 69    |
|                                             |                                | Danshen                                                             | 71    |
|                                             |                                | Honokiol                                                            | 28    |
|                                             |                                | Resveratrol                                                         | 36    |
| Amelioration of leukocyte infiltration      | Leukocyte-endothelial interaction | Cerebralcare Granules®                                           | 50,51,75 |
|                                             |                                | FBD formula                                                          | 70    |
|                                             |                                | Salvianolic acid A                                                  | 39    |
|                                             |                                | Tetrandrine                                                         | 47    |
|                                             | MPO increase                   | Huanglian Jiedu Tang                                                | 76    |
|                                             |                                | Shengmai San                                                        | 56    |
|                                             |                                | Tongxinluo capsule                                                  | 77    |
|                                             |                                | Zhimu                                                               | 78    |
| Amelioration of BBB disruption              | Endothelial cell junction      | Tongxinluo capsule                                                  | 77    |
|                                             |                                | Cerebralcare Granules®                                              | 82    |
|                                             |                                | Huatuo Zaizao extractum                                             | 85    |
|                                             |                                | Naomaitong preparation                                              | 83    |
|                                             |                                | Weinaokang preparation                                              | 84    |
|                                             |                                | Aromatic resuscitation drugs                                        | 86    |
|                                             |                                | Panax notoginseng saponins plus astragaloside                       | 15    |
|                                             |                                | Salvianolic acid B                                                  | 41    |
| Amelioration of capillary hypoperfusion     | CBF                            | Naosaitong preparation                                              | 94    |
|                                             |                                | Cerebralcare Granules®                                              | 50,75,82 |
|                                             |                                | Astragalus injection                                                | 93    |
|                                             |                                | Erigeron injection                                                  | 92    |
|                                             |                                | Compound Angelica-root injection                                   | 91    |
|                                             |                                | Icariin plus Panax notoginseng saponins                             | 29    |
|                                             |                                | Gualou Guizhi decoction                                             | 95    |
### Table 4  Effect of TCM preparation, Chinese materia medica and active compounds on brain injury induced by I/R.

| Effect                          | Mechanism               | TCM                                   | Refs. |
|---------------------------------|-------------------------|---------------------------------------|-------|
| **Amelioration of brain edema** | Inhibiting excitotoxicity| Shenfu injection                       | 100   |
| Anti-oxidation                  |                         | Shengmai San                          | 56    |
|                                 |                         | Cerebralcare Granule®                 | 50    |
|                                 |                         | Gouteng total alkaloids               | 101   |
|                                 |                         | Xianianwu total alkaloids             | 101   |
|                                 |                         | Sodium tanshinone B                   | 45    |
|                                 | Anti-inflammation       | Guizhi Fuling capsules                 | 67    |
|                                 |                         | Naomaitong preparation                 | 68    |
|                                 |                         | Shengmai San                          | 56    |
|                                 |                         | Baicalin                               | 20    |
|                                 | Inhibiting leukocyte infiltration | FBD formula                           | 70    |
|                                 |                         | Cerebralcare Granule®                 | 50, 75|
|                                 |                         | Zhiimu                                | 78    |
|                                 | Anti-thrombosis         | Shexiang Xingnaoning preparation       | 102   |
|                                 | Maintaining BBB         | Tongxinluo capsule                     | 77    |
|                                 |                         | Cerebralcare Granule®                 | 82    |
| **Amelioration of brain infarction** | Anti-oxidation       | Xuezhikang capsule                     | 103   |
|                                 |                         | Huangshuikui total flavones            | 104   |
|                                 |                         | Astragaloside                          | 16    |
|                                 |                         | Calycosin                             | 24    |
|                                 | Anti-thrombosis         | Danhong injection                      | 106   |
|                                 | Improve capillary perfusion | Naosaitong preparation               | 94    |
|                                 | Promote blood vessel repair | Buyang Huanwu Tang                   | 105   |

### Table 5  Effect of TCM preparation, Chinese materia medica and active compounds on neuron damage induced by I/R.

| Effect                     | Mechanism                     | TCM                                   | Refs. |
|----------------------------|-------------------------------|---------------------------------------|-------|
| **Anti-neuronal apoptosis**| Down-regulating caspases      | Buyang Huanwu Tang                     | 117   |
|                            |                               | Ginsenoside Rg3                       | 27    |
|                            |                               | Morroniside                           | 33    |
|                            | To balance Bcl-2/Bax          | Naosaitong capsule                    | 115   |
|                            |                               | Xiaoxuming decoction                  | 112   |
|                            |                               | Cerebralcare Granule®                 | 51    |
|                            |                               | Lycopene                              | 32    |
|                            | Restoring mitochondrial membrane potential | Leonurine                             | 30    |
|                            |                               | Ginsenoside Re                        | 26    |
|                            | Regulating p53-PUMA pathway   | Cerebralcare Granule®                 | 75    |
|                            | Regulating MAPK/Akt pathway   | Dihuang Yinzi                         | 116   |
|                            | Regulating PARP-AIF pathway   | Astragalus injection                   | 93    |
|                            |                               | Astragaloside                          | 17    |
|                            |                               | Eupatilin                             | 25    |
|                            |                               | Resveratrol                           | 37    |
|                            |                               | Tetrahydroxystilbene glucoside        | 46    |
|                            |                               | Scutellarin                           | 43    |
| **Anti-neurotoxicity**     | Decreasing excitotoxicity amino acids | Buyang Huanwu Tang                   | 121   |
|                            |                               | Tianma Gouteng Fang                    | 122   |
|                            |                               | Gegen ethonal extract                  | 114   |
|                            |                               | Yinxing extract                       | 120, 123|
|                            | Decreasing excitotoxicity amino acid receptor | Buyang Huanwu Tang              | 126   |
|                            |                               | Huanshaodan decoction                 | 124   |
|                            |                               | Tianma Cuozhi granules                | 125   |
|                            |                               | Shishu leaves flavonoids extract       | 127   |
|                            |                               | Yinxing extract                       | 128   |
|                            |                               | Senegenin                             | 44    |
|                            |                               | Sodium tanshinone B                   | 45    |
|                            | Decreasing [Ca^{2+}]{sub i}    | Shenqi Fuzheng injection              | 129   |
|                            |                               | Yinxing extract                       | 123   |
Shishu (Diospyros kaki) leaves\(^{127}\) and Yinxing (Folium Ginkgo) extract\(^{128}\) protected neurons from Glu- or NMDA receptor-induced excitotoxicity. Also, microfluorometry showed that the Chinese materia medica Yinxing (Folium Ginkgo) extract decreased intracellular Ca\(^{2+}\) concentrations in primary cultured hippocampal neurons treated with Glu\(^{123}\). In addition, injections of the TCM preparation Shenqi Fuzheng improved neuronal deficits, an effect which was suggested to be related to inhibition of Ca\(^{2+}\) aggregation in brain\(^{129}\).

### 3.2.3. Effects of TCM on neurogenesis

Given that the proliferation of endogenous neuron stem cells and neurogenesis occurs in rodents\(^{130}\), as well as in primate\(^{131}\) and patients\(^{132}\) after ischemic stroke, pharmacological interventions related to neurogenesis are believed to be a key strategy to improve the neuron functions after cerebral I/R injury. TCM finds its role in this field as well. It was reported that the TCM preparation Tongxinluo capsule increased the number of nestin-positive neurons and VEGF mRNA expression in the rat subventricular zone and hippocampal subdentate gyrus zone of the ischemic hemisphere after MCAO, indicating a capacity of promoting differentiation and proliferation of the neural stem cells\(^{133,134}\). In addition to VEGF, brain-derived neurotrophic factor (BDNF)\(^{12,12}\), basic fibroblast growth factor (bFGF)\(^{135}\) and nerve growth factor (NGF)\(^{13}\) may also be targets for the pro-neurogenic potential of some TCM preparations (e.g., Naoluo Xintong recipe\(^{135}\), Chinese materia medica (e.g., Sanqi (Radix et Rhizoma Notoginseng))\(^{13}\), and active components (baicalin\(^{19,23}\), luteolin\(^{11}\) and astragaloside\(^{12}\)). In an in vitro study, \(P.\) *notoginseng* saponins, active components extract from Sanqi (Radix et Rhizoma Notoginseng), was shown to promote rat hippocampal neural stem cells (NSC) proliferation and the expression of nestin/BrdU. mRNA levels of class III \(\beta\)-tublin (Tuj-1), vimentin, and nestin were also enhanced. Also, \(P.\) *notoginseng* saponins increased area density, optical density and numbers of nestin/BrdU, nestin/vimentin, and nestin/Tuj-1 positive NSC following OGD\(^{136}\). The TCM preparation Weinaokang preparation\(^{137}\) and the single active component salvianolic acid B\(^{42}\), among others, were reported to improve neurogenesis and to promote the repair of ischemic areas.

### 3.2.4. Other neuroprotective mechanisms of TCM preparation, Chinese materia medica and active compounds

In addition to aforementioned mechanisms, TCM preparations, Chinese materia medica and active compounds are known to alleviate neuron damage after cerebral I/R indirectly via anti-oxidant, anti-inflammatory roles and promotion of capillary perfusion around neurons. For example, the classical formula oren-gedoku-to (Huanglian Jiedu Tang) lowered neuronal death by increasing the expression of Cu/Zn-SOD in the hippocampus of ischemic mice\(^{138}\). The TCM preparation Breviscapine injection (Dengzhanhuasu injection)\(^{139}\), and active compounds, such as total glycoside from Chishao (Radix Paeoniae Rubra)\(^{53}\) and luteolin\(^{31}\), ameliorated neurological deficit and improved neuronal function through anti-oxidant actions as well. In addition, electron microscopic studies found that TCM preparations, such as Cerebralcare Granules\(^{51,75}\), Shexiang Xingnaonin preparation\(^{102}\) and the single active compound apigenin\(^{18}\), exhibited a potential to decrease neurological scores and improve pathomorphology and neuron ultrastructure of ischemic cortex and hippocampal CA1 region after I/R. Suggested mechanisms for these effects include inhibition of cytokine production and platelet aggregation\(^{102}\), or promotion of capillary opening\(^{51,75}\). Similarly, HE staining was used to show that

| Effect | Mechanism | TCM | Refs. |
|--------|----------|-----|-------|
| Neurogenesis | Promoting NSC proliferation | Tongxinluo capsule | 133,134 |
| | Increasing neurotrophic factors (VEGF, BDNF, bFGF, NGF) | Naoluo Xintong recipe | 135 |
| | Promoting neuronal self-repair | Sanqi | 113 |
| | | Astragaloside | 13 |
| | | Baicalin | 21,22 |
| | Amelioration of microcirculatory disturbances | Weinaokang preparation | 137 |
| | | Salvianolic acid B | 42 |
| | Anti-inflammation | Huanglian Jiedu Tang | 138 |
| | | Xingnaojing plus Xuesaotong injection | 118 |
| | | Breviscapine injection | 139 |
| | | Chishao total glycoside | 80 |
| | | Yinxing extract | 120 |
| | | Icarin plus \(P.\) *notoginseng* saponins | 29 |
| | | Luteolin | 31 |
| | | Lycopen | 32 |
| | | Salvianolic acid A | 40 |
| | | Shexiang Xingnaonin preparation | 102 |
| | | Yinxing extract | 120 |
| | | Apigenin | 18 |
| | | Baicalin | 19,23 |
| | | Lycopen | 32 |
| | Promoting capillary perfusion | Cerebralcare Granules\(^{5}\) | 51,75 |

Table 5 (continued)
the active compound baicalin increased neurons with pycnotic shape and condensed nuclei in cortex and hippocampus, effects which were related to suppression of NF-κB p65 activation\(^2\).

4. Summary

The present paper provides an overview of the ameliorating effects of compound TCM preparations, Chinese materia medica and active components on I/R-induced cerebral microcirculatory disturbances, brain injury and neuron damage, as summarized in Fig. 1.

(1) Many studies demonstrated that compound TCM preparations, Chinese materia medica and their active components can ameliorate I/R-induced cerebral microcirculatory disturbances, including enhanced ROS production, release of cytokines/chemokines/adhesion molecules, leukocyte recruitment, microvessel hyperpermeability, BBB disruption and capillary hypoperfusion (Table 3).

(2) Compound TCM preparations, Chinese materia medica and their active components are capable of ameliorating I/R-induced brain injury, such as brain infarction and perivascular edema. Such effects are attributable to beneficial actions on cerebral microcirculatory disturbances, together with attenuation of excitotoxicity (Table 4).

(3) Compound TCM preparations, Chinese materia medica and their active components have the potential to alleviate neurological deficits and improve learning and memory capacity after I/R injury. Underlying mechanisms for these effects include inhibition of apoptosis, excitotoxicity, oxidation and inflammation, along with stimulation of pro-neurogenesis (Table 5).

(4) In spite of a number of clinical studies illustrating the ameliorating effects of TCM preparations, Chinese materia medica and active compounds on cerebral I/R-related diseases, strictly randomized, double-blind, placebo-controlled, and multicenter clinical trials remain to be conducted with large samples. More evidence-based data are required to confirm the effects of TCM preparation, Chinese materia medica and active compounds, especially Chinese classic formulas, on ischemic stroke in clinical studies.

(5) Majority of the studies concerning the protective effects of TCM preparation, Chinese materia medica and active compounds on I/R-induced cerebral injury and neuron damage focus on ROS production, cytokine release, leukocyte recruitment and microvessel hyperpermeability. More attention should be paid to other microcirculatory insults, including enhanced ROS production, cytokine release and leukocyte recruitment? Moreover, additional mechanisms that participate in ischemic brain injury and neuron damage should be addressed further in studying the role of TCM preparation, Chinese materia medica and active compounds in cerebral I/R injury. These energize metabolism, microglia activation, aquaporin molecules, and cyclic adenosine monophosphate (cAMP).

Ischemic stroke, post I/R-induced cerebral microcirculatory disturbances, subsequent brain injury, and neuron damage are complicated processes, and require interventions at multiple targets for successful treatment. TCM preparations and Chinese materia medica contain multiple active ingredients which have been widely used for decades in the clinic as a potential therapeutic strategy for I/R-related cerebral diseases. Further clinical and experimental research is needed to understand their actions.

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