Traditional Chinese Medicine for Ischemic Stroke

Subjects: Medical Informatics

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Stroke is associated with the second leading cause of death and the third leading cause of disability among human diseases, and ischemic stroke (IS) specifically accounts for over 80% of all stroke situations. Due to the rapidly growing and aging population, IS incidence increases dramatically with age, this disease has a substantial impact on both afflicted families and society at large. IS is characterized by localized ischemic and hypoxia necrosis of brain tissue caused by infarction and occlusion of cerebral arteries, which is often accompanied by significant physical and cognitive impairment.

Keywords: ischemic stroke ; natural compounds ; traditional Chinese medicine

1. The Signaling Pathways of Active Compounds in the Treatment of Ischemic Stroke (IS)

1.1. JAK/STAT Signaling Pathway

The JAK/STAT signaling pathway is involved in various physiological processes, such as cell proliferation, differentiation, and apoptosis. The JAK protein tyrosine kinase family consists of JAKI, JAK2, JAK3, and Tyk2. To date, seven members of the STAT family have been ascertained: STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 [1]. Extracellular signals such as cytokines and growth factors bind to corresponding receptors on the cell membrane, causing receptor dimerization and bringing receptor-coupled JAK kinases closer together, thus activating them through interactive tyrosine phosphorylation, which then phosphorylates STAT and transports it from the intracellular environment to the nucleus. STAT binds to the promoter region of the gene containing the γ-activation sequence, resulting in changes in the transcription and activity of DNA, which in turn affects essential cellular functions including cell growth, differentiation, and death [2]. After cerebral ischemia, ischemia and hypoxia can directly damage neurons and tissue cells in brain tissue, activate microglia and astrocytes in the ischemic area, and release inflammatory factors (IL-1, IL-6, TNF-α, ICAM-1α) and growth factors (EPO, ECF, PDGF), that can activate JAK/STAT signaling pathways [3]. It was shown that both JAK and STAT expression were upregulated in brain tissue after ischemia and then activated JAK-STAT phosphorylation, which significantly increased p-JAK and p-STAT protein expression and induced brain injury with brain edema, infarct size expansion, and neurological dysfunction. At the same time, downregulation of the JAK/STAT signaling pathway could reduce ischemic brain infarction, restore blood-brain barrier integrity and promote neurological recovery after cerebral ischemic injury [4][5].

Matrine, an alkaloid that is extracted from *Sophora flavescens* Aiton., has been shown to reduce the expression of the p-JAK2 and p-STAT3 proteins and the number of apoptotic cells in the brain tissue of Middle Cerebral Artery Occlusion (MCAO) rats, and plays a neuroprotective role by inhibiting the activation of JAK-STAT signaling pathway and reducing the inflammatory response [6]. Hydroxy saffron yellow A is a flavonoid extracted from *Carthamus tinctorius* L., it can significantly down-regulate the expression of JAK2-mediated signaling due to ischemic injury, while significantly promoting the expression of SOCS3, which is a negative regulator of STAT3. By modulating the cross between JAK2/STAT3, Hydroxy saffron yellow A can confer neuroprotection against focal cerebral ischemia [7]. Catalpol, a terpenoid extracted from *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & C. A. Mey., has multiple pharmacological activities, it can increase blood flow in ischemic brain tissues of MCAO rats, upregulate EPO and EPOR expression, promote STAT3 phosphorylation and inhibit VEGF mRNA expression, thus improving blood supply to ischemic brain tissues, reducing vascular permeability and promoting angiogenesis through the JAK2/STAT3 signaling pathway [8]. Nicotiflorin, a flavonoid extracted from *Carthamus tinctorius* L., can increase the protein expression level of Bcl-2 and downregulate the expression of p-JAK2, p-STAT3, caspase-3 and Bax, and inhibit the JAK2/STAT3 signaling pathway to alleviate apoptosis caused by cerebral ischemia-reperfusion injury (CIRI) [9]. Additionally, in vivo and in vitro experiments showed that Atractylenolide III and Stachydrine could exert antioxidant and anti-inflammatory effects by inhibiting the JAK2/STAT3 signaling pathway, and thus play a neuroprotective role [10][11].

1.2. NF-κB Signaling Pathway

The NF-κB signaling pathway is a classic signal transduction pathway mediated by cytokines. It plays an important role in several physiological and pathological activities, including inflammation, oxidative stress, endothelial cell injury, and cell death [12]. NF-κB is a significant transcriptional regulatory factor, comprising NF-κB1 (p50), NF-κB2 (p52), Rel A (p65), Rel B, and c-Rel [13]. Under normal conditions, NF-κB is inhibited and exists in the cytoplasm as a dimer in a complex with its inhibitory protein IκB. IκB can obscure the nuclear localization signal of NF-κB, making it inactivated. After the cerebral ischemic injury, cells are stimulated by factors such as inflammation and oxidation, and IκB proteins are degraded by phosphorylation, resulting in the dissociation of NF-κB dimers from the inactive complex to the activated state. Activated NF-κB migrates into the nucleus due to nuclear localization signal exposure and exerts its transcriptional regulatory role to induce the transcriptional synthesis and expression of relevant inflammatory factors, ultimately aggravating the degree of brain injury [14][15].

Artesunate, a derivative of artemisinin, reduces tissue damage caused by traumatic brain injury and protects MCAO mice from inflammatory injury by inhibiting NF-κB, releasing the pro-inflammatory cytokines IL-1β and TNF-α, reducing neutrophil infiltration, and inhibiting microglia activation [16]. Skullcapflavone II, a flavonoid from *Scutellaria baicalensis* Georgi, exerts protective effects against cerebral ischemia by inhibiting TLR4/NF-κB signaling pathway and suppressing mitochondrial apoptosis, inflammation, and oxidative stress [17]. Syringin, a lignan isolated from *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., can promote FOXO3a phosphorylation and inhibit NF-κB nuclear translocation, which in turn reduces the levels of pro-inflammatory cytokines IL-1β, IL-6, TNF-α and MPO, and exerts a protective effect against ischemic brain injury by reducing the inflammatory response through the FOXO3a/NF-κB signaling pathway [18]. Schisandrin B, a lignan derivative isolated from *Schisandra chinensis* (Turcz.) Baill., can inhibit TLR4 expression and NF-κB activation and reduces TNF-α, IL-6 and IL-1β levels, exerts protective effects against cerebral ischemia by inhibiting TLR4/NF-κB signaling pathway [19]. Ephedrine, an alkaloid isolated from *Ephedra sinica* Stapf, has been shown to decrease oxidative stress, prevent inflammation, increase immunological function, and decrease CIRI, which may be due to their suppression of NF-κB-NLRP3 signaling [20]. Salvianolic acid D, a polyphenol component of *Salvia miltiorrhiza* Bunge, inhibits NF-κB activation and inflammatory factor release mediated by HMGB1-TLR4 signaling and attenuates HMGB1-mediated inflammatory response by inhibiting TLR4/MyD88/NF-κB signaling pathway [21]. Furthormore, other natural compounds such as triptolide, β-patchoulene, ginkgetin, tanshinone IIA, breviscapine, diosgenin, icariin, and berberine can also exert a protective effect against ischemic brain injury by inhibiting the NF-κB signaling pathway [22][23][24][25][26][27][28][29].

1.3. MAPK Signaling Pathway

Recent research demonstrated that the MAPK pathway plays an essential role in the initiation and progression of IS [30]. The MAPK family comprises conserved serine/threonine protein kinases in eukaryotes that function as crucial regulators of cell physiology and immune responses. MAPK transmits signals from the cytoplasm to the nucleus and activates various biological reactions, such as cell proliferation, differentiation, apoptosis, oxidative stress, inflammation, and innate immunity [31][32][33]. First, extracellular stimulation activates MAPK on the cell membrane via autophosphorylation. Once MAPK is activated, MAPK3 phosphorylates and activates MAPK2. MAPK2 then phosphorylates MAPK threonine/tyrosine residues, eventually activating and transferring MAPK into the nucleus, interacting with transcription factors such as c-Jun and c-Fos. Finally, MAPK upregulates the expression of target genes or acts on downstream kinases in the cytoplasm and regulates cellular activity [34][35]. Numerous experiments have shown that the MAPK signaling pathway is involved in multiple stages of cerebral ischemic and hypoxic injury. MAPK3 phosphorylation is inhibited during cerebral ischemic injury, and the application of MAPK pathway-specific inhibitors reduces phosphorylated MAPK3 expression and increases the number of cells in the ischemic area, suggesting that MAPK signaling pathway is involved in the protection of neurons after ischemia and plays an anti-modulatory role in cerebral ischemia-reperfusion [36].

Nobiletin, a flavonoid extracted from *Citrus reticulata* Blanco, can reduce ischaemic/reperfusion-induced brain apoptosis by upregulating Bcl-2 expression, downregulating Bax and caspase-3 expression, and reducing the levels of pro-inflammatory factors TNF-*α* and IL-6 and the expression of *p*-p38 and MAPAP-2 in MCAO rats. This mechanism is related to the MAPK signaling pathway [37]. Coriolus versicolor polysaccharides (CVP) can inhibit the phosphorylation of p38 MAPK, up-regulate Bcl-2 expression, down-regulate Bax and Caspase-3 activity, reduce the number of CIRI neuronal apoptosis; reduce the area of cerebral infarction, through the regulation of MAPK signaling pathway to achieve the role of protecting neuronal cells and restoring brain function [38]. Scrophularia ningpoensis polysaccharides can regulate the brain injury of CIRI rats by improving the antioxidant capacity of brain tissue, inhibiting the excessive production of inflammatory cytokines, inhibiting the expression of the JNK, p38, ERK, and other MAPK pathway proteins [39]. Emodin, a quinone isolated from *Rheum palmatum* L., can induce the expression of Bcl-2 and GLT-1 through the ERK-1/2 signaling pathway,

inhibits neuronal apoptosis and ROS production, reduces glutamate toxicity, and alleviates nerve cell injury in a rat model of MCAO [40]. Furthermore, ginsenoside Rg1, baicalin and curcumin also have significant neuroprotective effects in IS by inhibiting the MAPK signaling pathway [41][42][43].

1.4. Notch Signaling Pathway

Notch is a highly conservative signaling pathway that plays a critical role in cell proliferation, differentiation, and apoptosis [44], it is activated by ischemia and hypoxia in brain tissue during IS. The activated Notch pathway promotes the proliferation of neural stem cells and recovers the neural function defect after ischemia and promotes the neovascularization in the ischemic area, improves the ischemic and anoxic state of brain tissue, and effectively protects the recovery of neural function [45]. The Notch signaling pathway mostly comprises Notch receptors (Notch1~4), ligands (Jagged1/2 and Delta-like-1/3/4), and intracellular effector molecules (CSL) and Notch effector. Notch signaling is activated following Notch receptor-ligand binding on contacting cells [46]. The Notch receptor protein undergoes 3 cleavage and is released from the Notch intracellular domain (NICD) into the cytoplasm to form the NICD/CSL transcription activation complex, which enters the nucleus and binds to the transcription factor CSL, thereby activating the target genes of the transcriptional repressor family such as HES, HEY, HERP and so on. to play a biological role [47].

1.5. Nrf2 Signaling Pathway

The Nrf2 signaling pathway plays a significant role in the occurrence and development of IS, and it can regulate the ability of cells to resist oxidative stress and protect brain tissue [48]. Nrf2 belongs to the CNC basic leucine zipper transcriptional activator family, containing seven highly conserved functional structures. When stimulated by oxygen radicals, each of these structural domains plays a role in regulating the activation of Nrf2 and initiating the transcription of downstream genes, thereby protecting the cell from damage. In the resting state, Nrf2 can be coupled with its inhibitory factors, so that the antioxidant capacity of the cell is at the most basic level. After ROS attack, Nrf2 is decoupled and released into the cytoplasm in large quantities.

Biochanin A, the main flavonoid component of *Trifolium pratense* L., promotes the nuclear translocation of Nrf2 and induces the expression of HO-1 by regulating the Nrf2/HO-1 signaling pathway, it protects the rat brain from ischemic injury through antioxidant and anti-inflammatory effects [49]. Rosmarinic acid, a water-soluble polyphenol compound widely found in the plant species of Lamiaceae and Boraginaceae [50], can up-regulate Bcl-2 and down-regulate the level of Bax and Caspase-3 to exert its anti-apoptotic effect. This effect is related to activating the Nrf2/HO-1 pathway and inhibiting the p53 gene [51]. Adenosine monophosphate (AMPK) is an important intracellular metabolic and stress receptor, and is a key regulatory protein of autophagy. Palmatine, the main alkaloid of *Coptis chinensis* Franch., can reduce oxidative stress, inflammatory response, and neuronal apoptosis in MCAO mice by activating the AMPK/ Nrf2 pathway [52]. Taraxasterol, the main terpenoid ingredient of *Taraxacum mongolicum* Hand.-Mazz., can significantly inhibit the generation of ROS and MDA in hippocampal neurons induced by OGD/R, leading to a decrease in caspase-3 and Bcl-2 expression, and a concurrent increase in the expression of Bax, HO-1, NQO-1, and GPX-3. Taraxasterol can protect hippocampal neurons from OGD/R-induced injury by activating the Nrf2 signaling pathway [53]. In addition, senkyunolide I and ginkgolide B can also protect brain tissue from ischemic injury by inhibiting the Nrf2 signaling pathway [54][55].

1.6. PI3K/Akt Signaling Pathway

There are many experimental ones on the regulatory role of the PI3K/Akt signaling pathway in IS [56][57][58]. The PI3K/Akt/mTOR signaling pathway plays a neuroprotective role in ischemic reperfusion injury by upregulating the expression of PI3K, p-Akt, and p-mTOR in brain tissue, which significantly reduces the brain infarct size in MCAO rats and the pathological changes of brain tissue, thus alleviating CIRI [59]. PI3K can be further divided into PI3KⅠ, PI3KⅡ, and PI3KⅢ according to its structure and substrate specificity [60]. Akt is an essential active signaling target downstream of PI3K and is a serine/threonine protein kinase [61][62]. PI3K activation leads to the formation of PIP3 on the plasma membrane, which induces a conformational change in Akt.

Resveratrol is a natural polyphenol isolated from plants such as *Reynoutria japonica* Houtt. and *Vitis vinifera* L., it reduces the expression of IL-1β, COX-2 and TNF-α by stimulating the PI3K/Akt signaling pathway as well as decreasing infiltration of neutrophils, thereby reducing the inflammatory response in rats with ischemic stroke [63]. Ligustrazine, the main alkaloid ingredient of *Ligusticum chuanxiong* Hort., can significantly increase the levels of p-Akt and p-eNOS in the brain tissue of MCAO rats, and play a neuroprotective role on the brain of ischaemic/reperfusion injury rats by stimulating the PI3K/Akt pathway [64]. Polygalasaponin F, the main terpenoid of *Polygala tenuifolia* Willd., can downregulate the expression of Bcl-2/Bax and caspase-3 in PC12 cells and prevent OGD/R-induced injury by stimulating the PI3K/Akt signaling pathway [65].

Puerarin, a flavonoid isolated from *Puerariae Lobata* (Willd.) Ohwi, can significantly increase the expression of Akt1, GSK-3β, and MCL-1 p62 as well as decrease caspase-3 expression levels in MCAO rats.

2. The Target Protein of Natural Compounds in the Treatment of IS

2.1. SIRT1

SIRT1 is a nicotinamide adenine dinucleotide-dependent histone deacetylase with deacetylation of various histones and non-histones [66], which can regulate pathological processes such as oxidative stress, inflammatory response, and apoptosis by regulating FOXO, NF-κB PARP-1, PGC-1, PPAR-γ, and eNOS deacetylation, exerting a role in regulating pathological processes such as oxidative stress, inflammatory response, and apoptosis [67]. In SIRT1-deficient mice, CIRI is manifested by increased levels of inflammation, oxidative stress, and apoptosis, suggesting that SIRT1 may play a neuroprotective role [68]. Ginsenosides activate SIRT1 protein expression in the ischemic penumbra of MCAO rats, and SIRT1 can directly deacetylate the p65 subunit of NF-κB and reduce its acetylation level, thereby inhibiting the transcriptional activity of NF-κB and the expression of IL-1β, IL-6, and TNF-α, and reduce the ischemic injury and neurological deficits in MCAO rats [69]. Magnolol (a phenolic compound derived from *Magnolia officinalis* Rehd. Et Wils) and Salvianolic acid B (a phenolic compound derived from *Salvia miltiorrhiza* Bunge.) can regulate brain injury induced by cerebral ischemia by activating SIRT1, deacetylating to inhibit Ac-FOXO1 expression, and suppressing inflammatory cytokines and apoptosis [70][71]. Calycosin-7-*O*-*β*-*D*-glucoside, a flavonoid isolated from *Astragalus penduliflorus subsp. mongholicus var. dahuricus* (Fisch. ex DC.) X. Y. Zhu, can attenuate OGD/R-induced oxidative stress and neuronal apoptosis by activating SIRT1 and upregulating FOXO1 and PGC-1 α expression [72]. Moreover, the inhibitor of Sirt1 can reverse these neuroprotective effects.

2.2. MMP9

MMP9 is a member of the zinc-dependent protein hydrolase family and can degrade extracellular matrix, including collagen IV, laminin, and fibronectin [73]. MMP9 expression increased during cerebral ischemia [74]. Up-regulated MMP9 destroys the structural integrity of brain microvessels and the blood-brain barrier by degrading the extracellular matrix, resulting in secondary brain edema and brain injury [75], while knockout of MMP9 in mice or the use of MMP9 inhibitors can reduce brain edema [76]. Therefore, MMP9 is expected to be a target for treating ischemic brain injury. TIMP1 is an endogenous inhibitor that regulates the activity of MMP9 and can inhibit the activity of MMP9 through non-covalent binding to the catalytic domain of MMP9. The imbalance between MMP-9 and TIMP-1 can lead to secondary brain damage. Icariside II (a flavonoid derived from *Epimedium brevicornu* Maxim.) and ursolic acid (a pentacyclic triterpene derived from many plants, such as *Scleromitrion diffusum* (Willd.) R. J. Wang and *Actinidia chinensis* Planch.) could further inhibit neuronal apoptosis by regulating the balance of MMP9/TIMP1, thereby significantly improving the ischemia-reperfusion induced BBB disruption in MCAO rats, preventing cerebral ischemia-reperfusion injury [77][78]. Calycosin-7-O-β-D-glucoside (a flavonoid extracted from *Astragalus penduliflorus subsp. mongholicus var. dahuricus* (Fisch. ex DC.) X. Y. Zhu) and oxymatrine (an alkaloid derived from *Sophora flavescens* Aiton) can reduce the expression of MMP9 protein by downregulating the expression of CAV1, thereby improving the integrity of the BBB after CIRI [79][80].

2.3. TLR4

TLR4, also known as CD284, is a transmembrane protein in the Toll-like receptor family [81]. During cerebral ischemia, damaged tissues and cells release damage-associated molecular patterns (DAMPs), such as S100 protein and HMGB1, DAMPs can bind and activate TLR4, TLR4 can activate NF-κB through MyD88 and TRIF pathways, thereby activating inflammatory responses and aggravating brain tissue damage [82][83]. Compared with wild-type mice, the infarct area and volume of TLR4 knockout mice after ischemia/reperfusion are obviously smaller, and the neurological deficit is improved, indicating TLR4 may be one of the targets for the treatment of cerebral ischemia injury [84]. Gentianine, an alkaloid isolated from *Gentiana scabra* Bunge., can inhibit and attenuate the expression of TLR4, MyD88 mRNA, and nuclear translocation of NF-κB in brain tissue, as well as the levels of IL-1β, TNF-α, and IL-6 in serum, suggesting that gentianine may reduce brain tissue injury due to ischemia/reperfusion by inhibiting TLR4 pathway-mediated inflammatory response [85]. Procyanidins, polyphenols extracted from grape seeds, suppress the activation of the NLRP3 inflammasome by inhibiting the expression of TLR4, thereby reducing the inflammatory response and improving cerebral ischemia-reperfusion injury [86].

2.4. HIF-α

HIF-1α is a transcription factor that is widely distributed in mammals under hypoxic conditions and can activate a variety of hypoxia-response genes (HRGs) expression to regulate the oxygen homeostasis and energy metabolism balance of cells

and organism [87]. HIF-1α-induced gene expression can improve glucose transport and blood circulation in the ischemic penumbra after cerebral infarction, mediating hypoxia tolerance after hypoxia, regulating the immune response, and has a significant protective effect on ischemia-hypoxic neurons [88]. In addition, HIF-1α can inhibit PTP by reducing ROS and Ca^{2+} generated during cerebral ischemia-reperfusion, thereby reducing brain cell apoptosis [89], and can also activate various brain protective signaling pathways, such as PI3K/AKT and JAK2/STAT3 pathway to improve mitochondrial respiratory function to protect brain tissue after ischemia-reperfusion [90]. Catalpol (an iridoid glycoside extracted from *Rehmannia glutinosa* (Gaertn.) Libosch. ex Fisch. & C. A. Mey.) and Cardamonin (a chalcone component extracted from the seeds of *Amomum villosum* Lour..) activates the HIF-1α/VEGF signaling pathway in rats with ischemia-reperfusion injury, and upregulate the protein expression of HIF-1α and VEGF, thereby increasing cerebral microvascular density and promoting intracerebral revascularization, and promoting angiogenesis, neural repair and functional recovery in MCAO rats [91][92].

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