Neuroprotective effect of Chinese herbal monomers and extracts *via* activation of Nrf2 signal pathway

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Abstract

BACKGROUND: Large amounts of data have shown that Chinese herbal monomer has a neuroprotective effect, and can improve the quality of life in stroke patients with cerebral nervous system injury. Nuclear-factor-erythroid 2-related (Nrf2) factor has neuroprotective effect on hemorrhagic stroke and ischemic stroke, which is an important way to reverse the damage of nervous system through the natural non-toxic Chinese herbs or composition, but it is rarely reported systemically.

OBJECTIVE: To summarize the neuroprotective effect of Chinese herbal monomers *via* the Nrf2 signal pathway in stroke patients.

METHODS: The first author retrieved the CNKI, VIP, Medline, and PubMed databases by computer. The keywords were “Nrf2, ARE, stroke, traditional Chinese medicine, neuroprotection” in Chinese and English,respectively. Articles concerning the neuroprotective role of Chinese herbal monomer *via* Nrf2 were analyzed and discussed.

RESULTS AND CONCLUSION: Totally 85 articles were retrieved. According to the inclusion and exclusion criteria, 46 articles were included in result analysis. The results show that a variety of monomers can exert neuroprotective effects *via* the Nrf2 pathway. Chinese herbal monomers include organic acids, phenols, saponins, terpenes, flavonoids, alkaloids, and other single or composite components. Traditional Chinese medicine has the clear neuroprotective effect after stroke, but it is lack of regularity, and it is still need to expand the data and further research as the basis.

Subject headings: Tissue Engineering; Stroke; Drugs, Chinese Herbal

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INTRODUCTION

Biological functions of nuclear factor-erythroid 2-related factor 2 (Nrf2)

Nrf2 is an essential factor of oxidant stress, which can up-regulate multiple detoxifying enzymes and antioxidase enzymes, and increase the level of anti-oxidants such as glutathione and superoxide dismutase. Nrf2 combined with antioxidant response element (ARE) can activate the convey of a series of molecules downstream, such as antioxidase enzyme, detoxifying enzyme, heme oxygenase (HO), glutathione synthesis and metabolism-related enzyme, and play a neuroprotective role[1] .

Kelch-like ECH associated protein 1 (Keap1) is composed of three functional domains: an intervening region, a bric-a-brac domain, and a Kelch domain (also named DGR domain)[2]. The Keap1-Nrf2 complex is linked to a functional E3 ubiquitin ligase complex (Rbx1) *via* an adaptor protein, Cullin3. Conjugating Nrf2 with Keap1 by the two DLG and ETGF motifs aligns the seven lysine residues of Nrf2 between the two motifs and facilitates Rbx1 mediated ubiquitination of Nrf2[3].

Classic model of Keap1/Nrf2/ARE signaling includes three parts: (A) Under basal conditions, the Cul3-Keap1 complex sequesters Nrf2 in the cytosol by binding its ETGF and DLG motifs. This facilitates the ubiquitination and proteasomal degradation of Nrf2. (B) The DLG motif of Nrf2 is loosened from the Cul3-Keap1 complex when cells are exposed to reactive oxygen species (ROS) which blocks the ubiquitination and degradation of Nrf2. Following an intricate series of phosphorylations by several kinases, Nrf2 is translocated into the nucleus and subsequently binds to the AREs by forming a heterodimer with Maf protein and initiating the

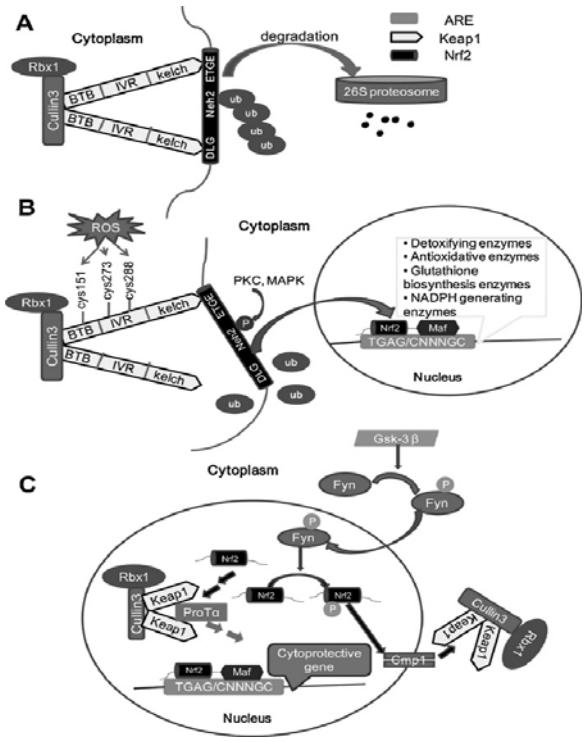

Figure 1 Classic model of Keap1/Nrf2/ARE signaling

Note: (A) Under basal conditions, the Cul3-Keap1 complex sequesters Nrf2 in the cytosol by binding its ETGF and DLG motifs. This facilitates the ubiquitination and proteasomal degradation of Nrf2. (B) The DLG motif of Nrf2 is loosened from the Cul3-Keap1 complex when cells are exposed to ROS which blocks the ubiquitination and degradation of Nrf2. Following an intricate series of phosphorylations by several kinases, Nrf2 is translocated into the nucleus and subsequently binds to the ARE elements by forming a heterodimer with Maf protein and initiating the transcription of phase II genes. (C) Nuclear Nrf2 can be phosphorylated by Fyn and be extruded back to the cytoplasm through the Cmp1 system. On the other hand, nuclear Nrf2 may also be sequestered by several Cul3- Keap1 complexes in the nucleus that are imported by ProTa. Both of these mechanisms help cells return back to basal conditions[4]. ROS: reactive oxygen species; Nrf2: nuclear factor-erythroid 2-related factor 2; ARE: antioxidant response element; Keap1: Kelch-like ECH associated protein 1.

transcription of phase II genes. (C) Nrf2 can be phosphorylated by Fyn and be extruded back to the cytoplasm through the Cmp1 system. On the other hand, Nrf2 may also be sequestered by several Cul3-Keap1 complexes in the nucleus that are imported by ProTa. Both of these mechanisms help cells return back to basal conditions[4].

Nrf2/ARE signaling pathways involved in attacks of stroke

Strokes are the leading cause of disability and mortality in the world[4]. Damage to the nerve system is one of the serious pathologic changes and sequelae. Many patients become dementia, action disabled, even deprived of the self-care ability, which brings economic and psychological pressure to the families and society. However, effective therapeutic approaches for post-stroke neuroprotection are still limited. Traditional Chinese medicine for nerve system repair has a broad prospect. Most of Chinese herbs have good clinical outcomes, but the mechanisms of action are still unclear. At present, some effective mechanisms of traditional Chinese medicines have been proved, but not systematically summarized. This paper summarizes the neuroprotective mechanisms of traditional Chinese medicines in patients with post-stroke nerve system damage *via* Nrf2/ARE signaling pathways. Traditional Chinese medicine combined with modern medicine is a better choice for patients with post-stroke nerve system damage, which can be developed deeply and will be accepted by international community.

Ischemic stroke is the most common type of stroke. Multiple pathological processes are involved in the progression of stroke, including excitotoxicity, oxidative stress, inflammation, and mitochondrial dysfunction[4]. Ischemia-reperfusion injuries include ischemic damage and reperfusion damage, and the initiation factor is hypoxia-ischemia. Oxidative stress represents a potential target for treatment because it is one of the most critical insults in stroke. Nrf2 may play a role as an endogenous compensatory adaptation against stroke. Administration of tBHQ or CDDO through either intracerebroventricular or intraperitoneal routes reduces sensorimotor deficits and infarct size after ischemia in the rodent model of middle cerebral artery occlusion[5-6]. Furthermore, $Nrf2^{-/-}$ mice display an exacerbated outcome at 7 days after injury in a focal ischemia model combined with permanent distal middle cerebral artery occlusion[5]. Activation of the Nrf2 pathway is critical for scavenging ROS, which contributes to neuroprotection against ischemic brain injury. For example, $Nrf2^{-/-}$ mice produce more ROS species after brain injury[7].

Depending on where the bleeding occurs and blood accumulates, hemorrhagic strokes can be divided into intracerebral hemorrhage in which the focus is bleeding into brain parenchyma and subarachnoid hemorrhage in which the focus is bleeding into the subarachnoid space. With hypertension as the major cause, intracerebral hemorrhage is associated with high mortality due to its acute onset and mass effects of hematoma and edema, which lead to intracranial hypertension and brain herniation[8]. Subsequently, ischemia and oxidative stress also contribute to brain injury after intracerebral hemorrhage. The Nrf2 pathway is activated in the brain following intracerebral hemorrhage, as indicated by increased HO-1 expression. In mice, HO-1 upregulation begins at 24 hours after intracerebral hemorrhage, peaks at day 5 and subsides at day 8[9]. It has been reported that curcumin can protect against intracerebral hemorrhage by activating Nrf2 and reducing oxidative stress, brain edema and neuroinflammation[10].

It has been also reported that Nrf2 activation plays a protective role against subarachnoid hemorrhage. For example, the administration of curcumin reduces vascular inflammation and cerebral vasospasm in mice after intracerebral hemorrhage[11] and decreases both oxidative

stress and mortality in rats[12]. SFN also activates Nrf2, up-regulates downstream enzymes such as HO-1 and quinone oxidoreductase 1, and reduces cortical apoptosis, brain edema and blood-brain barrier impairment[1]. In addition to these classic Nrf2 inducers, two hormones have also been reported to protect the brain from subarachnoid hemorrhage by activating the Nrf2 pathway-erythropoietin[13] and melatonin[14]. These hormones up-regulate phase 2 enzymes such as HO-1 and quinone oxidoreductase 1, reduce early brain injury such as cortical apoptosis and protect the blood-brain barrier[13-14].

Chinese herbs as neuroprotective agents in Nrf2 signal pathway

Chinese herbs are widely from nature plants such as flowers, grasses, trees, leaves, fruits and seeds. Some traditional herbs and their preparations have the pharmacological effects on blood vessels and nerve protection by anti-inflammation and antioxidant, and their biological active ingredients include alkaloids, organic acids, phenolic substances, terpenoids, saponins, flavonoids, essential oils, *etc*. Chinese herbal active parts or effective components, monomers, extracts and compounded prescriptions offer many unique antioxidant advantages. The usage of Chinese herbs as routine neuroprotection agents will require a better understanding of the underlying mechanisms of action and the identification of pathways impacted by active components of Chinese herbs.

Here, we review the articles addressing the effect of various Chinese herbal monomers on post-stroke nerve injury to explore the neuroprotective effect of Chinese herbal monomers and to provide ideas for drug guiding.

MATERIALS AND METHODS

Information retrieval strategy

The first the author retrieved CNKI, VIP, Medline database, and PubMed databases by computer. The key words were “Nrf2, ARE, stroke, traditional Chinese medicine, neuroprotection” in Chinese and English, respectively.

Inclusion/exclusion criteria

The inclusion criteria: (1) Originality: Nrf2 signal pathway is involved in neuroprotection after stroke based on reliable evidence. (2) Clear view: Post-stroke neuroprotection of Chinese herbal monomers *via* Nrf2 signaling pathway is comprehensively analyzed. (3) Articles addressing the neuroprotective role of Chinese herbal monomers through Nrf2 signal pathway after stroke.

Exclusion criteria: (1) Meta analysis. (2) Articles addressing no neuroprotective role of Chinese herbal monomers. (3) Stroke without nerve injury. (4) Repetitive studies.

Screening data

Original studies on Chinese herbal monomers for nerve injury *via* Nrf2 signaling pathway after stroke were retrieved.

RESULTS

The general information of the included articles

There are 14 articles included because of background and mechanism[1-14]; 3 articles of alkaloids[15-17]; 5 articles of organic acids[18-22]; 2 articles of phenols[23-24]; 1 articles of saponins and terpenes[25]; 12 articles of flavonoids[26-37]; 7 articles of alkaloids[38-44]; 2 articles of other single and composite components[45-46].

The evaluation methods involved in the included studies

Methods in the included studies include immunohistochemistry, PCR, western blot and wlectron microscope, *etc*.

Neuroprotection of alkaloids as monomers

Oxymatrine

Oxymatrine is the major quinolizidine alkaloid extracted from the root of sophora flavescens ait, and has been proved to be protective function after brain ischemia in recent studies. The results indicated that the ischemic infarct and edema were significantly reduced in rats that received oxymatrine, with a corresponding improvement in neurological function after cerebral ischemia-reperfusion. In immunohistochemistry and western blot analyses, Nrf2 and HO-1 were up-regulated in the ischemic cortex, beginning at 6 hours, peaking at 48 hours and declining at 72 hours after cerebral ischemia-reperfusion. Intraperitoneal injection of oxymatrine inhibited the production of lipid peroxidation and increased the activities of Nrf2 and HO-1 in rat’s brain after cerebral ischemia-reperfusion. Taken together, these results suggest that oxymatrine administered systemically protects the brain against focal ischemia-reperfusion damage at the early stage of stroke, and activating Nrf2/HO-1 pathway may contribute to the neuroprotective action of oxymatrine in the rat focal brain ischemia-reperfusion model. Thus, treatment of stroke with oxymatrine may prevent severe consequences after brain attack[15].

Sulforaphane

Sulforaphane is a multifunctional enzyme inducer and induce the body to produce a II type detoxification enzyme of glutathione transferase and quinone reductase against free radicals injuries. It commonly exists in cruciferous vegetables such as carrots, cabbage, broccoli and other vegetables and in one of Chinese herbs of radish seed. We have tested the hypothesis that sulforaphane, a naturally occurring isothiocyanate that is also a known activator of the ARE/Nrf2 antioxidant pathway, can protect immature neurons from oxidative stress-induced death. The hypothesis was tested with primary mouse hippocampal neurons exposed to either oxygen and glucose deprivation or hemin. Treatment of immature neurons with sulforaphane immediately after the oxygen and glucose deprivation during reoxygenation was effective in protecting immature neurons from delayed cell death. Exposure of immature hippocampal neurons to hemin induced significant cell death, and both pre- and

co-treatment with sulforaphane were remarkably effective in blocking cytotoxicity. RT-PCR analysis showed that several Nrf2-dependent cytoprotective genes, including NAD(P)H quinone oxidoreductase 1, HO-1, and glutamate-cysteine ligase modifier subunit, which are involved in glutathione biosynthesis, were up-regulated following sulforaphane treatment both in control neurons and following exposure to oxygen and glucose deprivation and hemin. These results indicate that sulforaphane activates the ARE/Nrf2 pathway of antioxidant defense and protects immature neurons from death caused by stress paradigms relevant to those associated with ischemic and traumatic injury to the immature brain[16].

Berberine

Berberine is one of the major alkaloids and has been reported to have a variety of pharmacologic effects, including inhibition of cell cycle progression. Here, we investigated the mechanisms of berberine protection of neuronal cells from cell death induced by the Parkinson's disease-related neurotoxin 6-hydroxydopamine (6-OHDA). Pretreatment of SH-SY5Y cells with berberine significantly reduced 6-OHDA-induced generation of ROS, caspase-3 activation, and subsequent cell death. Berberine also upregulated HO-1 expression, which conferred protection against 6-OHDA-induced dopaminergic neuron injury and besides, effect of berberine on HO-1 was reversed by siRNA-Nrf2. Furthermore, berberine induced PI3K/Akt and p38 activation, which is involved in the induction of Nrf2 expression and neuroprotection. These results suggest that berberine may be useful as a therapeutic agent for the treatment of dopaminergic neuronal diseases[17].

Neuroprotection of organic acids as monomers

Ferulic acid

Angelica, prepared from the rhizome of the plant Angelica sinesis, is a Chinese herb traditionally used in the treatment of women's menopausal symptoms and is one of the herbs in *Shiquan Dabu Tang*. Angelica has been shown to hold anti-inflammatory properties and antioxidant activities, especially when used concurrently with other herbs[18-19]. It has also been found that Angelica may exert protection against neuronal oxidative stress on rat cerebral ischemia/reperfusion models[20]. Dietz and colleagues have reported that the major lipophilic constituent of Angelica, Z-lingustilide, reduces oxidative stress through up-regulation of antioxidant enzymes such as quinone oxidoreductase 1, a Nrf2 pathway gene[21]. The study showed that both angelica extract and Z-lingustilide induced a dose-dependent increase of quinone oxidoreductase 1 in an ARE luciferase reporter assay. Further matrix assisted laser desorption/ionization time-of-flight mass spectrometry and liquid chromatography-tandem mass spectrometry analysis of the incubation mixture reveals that Z-lingustilide is able to alkylate cysteine residues in the KEAP1 protein which allows Nrf2 to bind to the ARE and activate transcription of Nrf2-ARE genes[21]. In addition, this Chinese herb can inhibit cyclooxygenase-2 expression at mRNA and protein levels[19].

Ursolic acid

We demonstrated the hypothesis that ursolic acid, a natural pentacyclic triterpenoid acid, isolated from edible plants in the Oleaceae family, a well-know antioxidative and anti-inflammatory reagent, protects the brain against ischemic injury by activating the Nrf2 pathway. Nrf2-/- and wild-type mice were induced into focal cerebral ischemia by transient middle cerebral artery occlusion, and received ursolic acid treatment immediately after middle cerebral artery occlusion. The behavioral dysfunction, infarct size, and the expression of Nrf2, HO-1 and inflammatory factors (Toll-like receptor 4 and nuclear factor-κB) in ischemic brain were measured at 24 hours after stroke. Ursolic acid treatment significantly improved neurological deficit and reduced infarct size in wild-type mice after middle cerebral artery occlusion. Administration of ursolic acid also decreased the product of lipid peroxidation, promoted the actibation of Nrf2 pathway and decreased the expression of Toll-like receptor 4 and nuclear factor-κB after stroke in wild-type mice. However, Nrf2-/- mice demonstrated more severe neurologic deficits, infarct size and inflammatory damage after middle cerebral artery occlusion, and did not benefit from the protective effect of ursolic acid. The results indicated that ursolic acid protected the brain against ischemic injury in mice by anti-oxidative and anti-inflammatory effects after middle cerebral artery occlusion. Activation of the Nrf2 pathway contributes to the neuroprotective effects induced by ursolic acid in cerebral ischemia[22].

Neuroprotection of phenolic substances as monomers

Curcumin

Oxidative and cytotoxic damage plays an important role in cerebral ischemic pathogenesis and may represent a target for treatment. Curcumin is proved to elicit a vanity of biological effects through its antioxidant and anti-inflammatory properties. Curcumin protect the brain from damage caused by middle cerebral artery occlusion, and this effect may be through upregulation of the transcription factor Nrf2 expression. Nrf2 may be one of the strategic targets for cerebral ischemic therapies[23].

Epicatechin

Catechin is also called tea tannin. The polyphenols content of catechin in tea is 75% to 80%. In one study, wild-type mice pretreated orally with 5, 15, or 30 mg/kg epicatechin before middle cerebral artery occlusion had significantly smaller brain infarcts and decreased neurologic deficit scores than did the vehicle-treated group. Mice that were posttreated with 30 mg/kg of epicatechin at 3.5 hours after middle cerebral artery occlusion also had significantly smaller brain infarcts and decreased neurologic deficit scores. Similarly, wild-type mice pretreated with 30 mg/kg of epicatechin and subjected to N-methyl-D-aspartate-induced excitotoxicity had significantly smaller lesion volumes. Cell viability assays with neuronal cultures further confirmed that epicatechin could protect neurons against oxidative insults. Interestingly, the epicatechin-associated neuroprotection

was mostly abolished in mice lacking the HO-1 or Nrf2, and in neurons derived from these knockout mice. These results suggest that epicatechin exerts part of its beneficial effect through activation of Nrf2 and an increase in the neuroprotective HO-1[24].

Neuroprotection of terpenoids and saponins as monomers

Ginsenoside

Intestinal ischemia reperfusion is a serious clinical condition associated with simultaneous multiple organ dysfunction. In a study aiming to investigate the effects of ginsenoside Rb1 on intestinal ischemia reperfusion induced renal injury in mice, an intestinal ischemia reperfusion mouse model was established by superior mesenteric artery occlusion for 45 minutes, followed by reperfusion for 2 hours. Intestinal ischemia reperfusion induced renal injury characterized by increase of blood urea nitrogen, creatinine and neutrophil gelatinase associated lipocalin in serum, malonaldehyde levels and decrease of superoxide dismutase levels in the renal tissues. Ginsenoside Rb1 (30, 60 mg/kg) given intraperitoneally before reperfusion attenuated renal injury, which was associated with decrease of blood urea nitrogen, creatinine and neutrophil gelatinase associated lipocalin in serum, malonaldehyde levels and increase of superoxide dismutase levels in the renal tissues. Furthermore, the immunohistochemistry and western blot data showed that ginsenoside Rb1 dramatically reversed intestinal ischemia reperfusion induced renal injury, associated with upregulated Nrf2 and HO-1 in renal tissues. These data suggests that ginsenoside Rb1 attenuates acute renal injury induced by intestinal ischemia reperfusion by activating the Nrf2/ARE pathway[25].

Neuroprotection of flavonoids as monomers

Quercetin

Some research showed rapid quercetin internalization into neurons, reaching the nucleus after its addition to the culture. Quercetin pretreatment increased total glutathione levels, but did not increase Trx2. Interestingly it caused Nrf2 nuclear translocation and significantly increased GCLC gene expression. At the moment of hydrogen peroxide addition, intracellular quercetin or related metabolites were undetectable in the cultures although quercetin pretreatment prevented neuronal death from the oxidant exposure. The findings suggest alternative mechanisms of quercetin neuroprotection beyond its long-established ROS scavenging properties, involving Nrf2-dependent modulation of the glutathione redox system[26].

EGb 761

Ginkgo biloba/EGb 761® (EGb 761) is a popular and standardized natural extract used worldwide for the treatment of many ailments. Although EGb 761 is purported to have a plethora of benefits, scholars are interested to study the neuroprotective properties of EGb 761 and its components and determine whether Nrf2/HO-1 induction of the collapsin response mediator protein 2 pathway contributes to neuroprotection. Mice were pretreated with EGb 761 or one of its constituents (bilobalide, ginkgolide A, ginkgolide B, and terpene free material) for 7 days and then subjected to transient middle cerebral artery occlusion and 48 hours of reperfusion. All components except terpene free material significantly reduced infarct volumes and neurologic deficits. The antioxidant and neuritogenic properties of EGb 761 in primary neurons were examined. Compared with vehicle-treated cells, pretreatment with EGb 761 significantly enhanced the survival of neurons exposed to tertiary butylhydroperoxide, hydrogen peroxide, and N-methyl-D-aspartate. Bilobalide and ginkgolide A also protected cells against N-methyl-D-aspartate-induced excitotoxicity. Immunofluorescence and Western blot analysis showed that EGb 761 pretreatment significantly increased the protein expression levels of Nrf2, HO-1, GAPDH, β-actin, collapsin response mediator protein 2, and histone H3 during tertiary butylhydroperoxide-induced oxidative stress. These findings suggest that EGb 761 not only has antioxidant activity but also possesses neuritogenic potential. Demonstrating such effects for possible drug discovery may prove beneficial in stroke and ischemic brain injury[27].

Tanshinone

The root of the plant Salvia miltiorrhiza, known as *Danshen* or red sage root, is a widely used in TCM clinic for treatment of cardiovascular disorders by improving blood circulation[28]. Its major chemical constituents have been found to be lipophilic tanshinones, mainly tanshinone IIA and the hydrophilic compounds tanshinol (also known as salvianolic acid A) and salvianolic acid B[29]. Recent studies have also shown that *Danshen* and its constituents possess anti-inflammatory effects through the inhibition of inducible nitric oxide synthase expression and cytokine secretion[30-31]. Some studies have shown that tanshinone IIA induces apoptosis and inhibits growth in leukemia THP-1 and human colon adenocarcinoma cells as well[32]. Tanshinone IIA also displays antioxidant protection against ROS-nduced oxidative stress through stress-activated kinases JNKs and p38 MAPK and by an increase in scavenging of oxygen free radicals[33-34]. The Nrf2 pathway has been known to interact with JNKs and p38 MAPK kinases which indirectly regulate the Nrf2 pathway through phosphorylation of Nrf2[33-34]. Zhang and colleagues showed that Nrf2 is involved in the effects of tanshinone IIA by reversing tumor necrosis factor-induced down-regulation of glutathione, NADPH, and glucose 6-phosphate dehydrogenase[35]. Small interfering RNA silencing of Nrf2 abolishs tanshinone IIA-induced up-regulation of glutathione and glucose 6-phosphate dehydrogenase[35]. It has also been found that the anti-inflammatory effects of tanshinone IIA directly result from the up-regulation of HO-1 through PI3-K/Akt and ERK pathways induced higher levels of Nrf2[36]. Tanshinone IIA activates the Nrf2 pathway through disruption of the Nrf2-KEAP1 complex and through kinase signaling pathways such as JNKs, p38 MAPK, PI3-K/Akt, and ERK to assist in the release of Nrf2. Based on the

effectiveness on Nrf2, *Danshen* is also a promising medicine for the treatment of neuroprotection after stroke.

Naringin

In order to investigate neuroprotective role of naringin and delineate the mechanismof action on 3-nitropropionic acid-induced neurodegeneration, the rats were injected with 3- nitropropionic acid (10 mg/kg body weight/day, i.p.) for 2 weeks to develop neurodegeneration, while naringin (80 mg/kg body weight/day, orally) was administered throughout the experimental period, 1 hour prior to 3-nitropropionic acid exposure. Thereafter rats were euthanized for biochemical, histological, and molecular studies. Treatment with naringin ameliorated the reduced glutathione/oxidized glutathione ratio with concomitant decrease in the levels of hydroxyl radical, hydroperoxide and nitrite in 3-nitropropionic acid-induced rats. Nissl staining and transmission electron microscopic studies showed that naringin modulated 3-nitropropionic acid-induced histological changes. Naringin induces NAD(P)H: quinone oxidoreductase-1, HO-1, glutathione S-transferase P1 and gamma-glutamylcysteine ligase mRNA expressions through the activation of Nrf2 and decreases the expressions of pro-inflammatory mediators, such as tumor necrosis factor-alpha, cyclooxygenase-2 and inducible nitric oxide synthase. These results indicate that naringin may be beneficial in mitigating 3-nitropropionic acid-induced neurodegeneration through the enhancement of phase II and antioxidant gene expressions *via* Nrf2 activation, thereby modulating the oxidative stress and inflammatory responses[37].

Neuroprotection of alkaloids of essential oils as monomers

Borneol

Borneol is proved to protect human neuroblastoma cells (SH-SY5Y) against Aβ-induced toxicity, exert an antioxidative effect and suppress apoptosis, which is hopeful to be a candidate compound for developing therapeutic drug for the prevention and treatment of Alzheimer's disease and other Aβ-related neurodegenerative diseases[38].

Notopterygium forbesii Boiss extract

Qianghuo is one of Chinese herbs and prepared from the root and rhizome of the plant Notopterygium forbesii Boiss, which belongs to the umbelliferae family. *Qianghuo* has been used to treat the common cold, headache, rheumatism. Its extract contained sweet bean pure compounds and may possibly possess anti-inflammatory, diaphoretic, and analgesic properties[39-41]. Nine chemical constituents of Notopterygium forbesii Boiss have been isolated and identified[42]. Tang and co-workers found that the mechanism for *Notopterygium forbesii* Boiss underlying cancer preventive effects is correlated to the up-regulation of HO-1 and *Notopterygium forbesii* Boiss also has effects on oxidative stress[43-44]. It is found that *Notopterygium forbesii* Boiss induces oxidative stress and a rise in HO-1 proteins in human fetal hepatocytes through the activation of the Nrf2 pathway by the p38 MAPK pathway and reactive species. The active constituents are identified to be phenethyl ferulate, bergaptol, and isoimperatorin which contribute to the increased HO-1 levels. Extracts of *Notopterygium forbesii* Boiss also induce a six-fold increase in quinone oxidoreductase 1 levels. *Notopterygium forbesii* Boiss and its constituents also attenuate lipopolysaccharide-induced pro-inflammatory responses and inducible nitric oxide synthase and cyclooxygenase-2 overexpression[44].

Neuroprotection of other monomers and compound preparations

Lipoic acid

R-a-lipoic acid has a dramatic neuroprotective effect against oxidative stress-induced death of the retinal neuronal RGC-5 cell line. R-a-lipoic acid induces the expression of HO-1 by promoting the translocation of Nrf2 to the nucleus, and the mechanism underlying HO-1 induction by R-a-lipoic acid is examined by focusing on downstream signaling pathways. R-a-lipoic acid activates Akt, and HO-1 induction by R-a-lipoic acid (involving Nrf2 translocation to the nucleus) suppressed by PI3-K inhibitors. In addition, R-a-lipoic acid produces ROS, including hydrogen peroxide. Pretreatment with a ROS scavenger or a NADPH oxidase inhibitor can suppress R-a-lipoic acid-induced Nrf2 translocation to the nucleus and HO-1 induction. These results suggest that ROS production triggered by R-a-lipoic acid may modify keap1, which in turn induces HO-1 expression through the PI3K signaling pathway. Furthermore, R-a-lipoic acid significantly attenuates cell death and accumulation of 4-hydroxy-2-nonenal in the ertina induced by optic nerve injury in vivo through an HO-1 activity-dependent mechanism. These data demonstrate for the first time that R-a-lipoic acid exerts a neuroprotective effect against oxidative stress in retinal neurons *in vitro* and *in vivo* by inducing HO-1 through keap1/Nrf2 signaling[45].

Shiquan Dabu Tang

Shiquan Dabu Tang is a TCM formula consisting of 10 ingredients, originally formulated in the China Song Dynasty in AD 1200. It has been used to help treat anemia, anorexia, exhaustion, fatigue, and general weakness[46].

CONCLUSION

Nrf2/ARE pathway plays a particularly important and explicit role in neuroprotection, A lot of traditional Chinese medicines have been proved to have the neuroprotective effects *via* the pathway. So the patients with post-stroke nerve injury may have a board range of choices. As many Chinese herbs are proved to have the chemoprevention and anti-inflammation roles *via* the Nrf2/ARE pathway, there is a possibility that the Chinese herbs may also have the effectiveness of neuroprotection. Therefore, Chinese herbs have a broad prospect. But relevant experiments on Chinese herbal monomers are inadequate. Studies on traditional Chinese medicine are of dispersion and variable quality. Here, we retrieve and summarize the articles addressing Chinese

herbal monomers for post-stroke nerve injury and provide certain basis for the different formula of Chinese Herbs.

REFERENCES

[1] Chen G, Fang Q, Zhang J, et al. Role of the Nrf2-ARE pathway in early brain injury after experimental subarachnoid hemorrhage. J Neurosci Res. 2011;89(4):515-523.

[2] Motohashi H, O'Connor T, Katsuoka F, et al. Integration and diversity of the regulatory network composed of Maf and CNC families of transcription factors. Gene. 2002;294(1-2):1-12.

[3] Baird L, Dinkova-Kostova AT. The cytoprotective role of the Keap1-Nrf2 pathway. Arch Toxicol. 2011;85(4):241-272.

[4] Zhang MJ, An CR, Gao YQ, et al. Emerging roles of Nrf2 and phase II antioxidant enzymes in neuroprotection. J Prog Neurobiol. 2013;(100):30-47.

[5] Shih AY, Li P, Murphy TH. A small-molecule-inducible Nrf2-mediated antioxidant response provides effective prophylaxis against cerebral ischemia in vivo. J Neurosci. 2005;25 (44):10321-10335.

[6] Zhang F, Wang S, Zhang M, et al. Pharmacological induction of heme oxygenase-1 by a triterpenoid protects neurons against ischemic injury. Stroke. 2012;43(5):1390-1397.

[7] Zhao X, Sun G, Zhang J, et al. Transcription factor Nrf2 protects the brain from damage produced by intracerebral hemorrhage. Stroke. 2007;38:3280-3286.

[8] Keep RF, Hua Y, Xi G. Intracerebral haemorrhage: mechanisms of injury and therapeutic targets. Lancet Neurol. 2012;11:720-731.

[9] Chen M, Regan RF. Time course of increased heme oxygenase activity and expression after experimental intracerebral hemorrhage: correlation with oxidativeinjury. J Neurochem. 2007;(103):2015-2021.

[10] Sun KH, Chang KH, Clawson S, et al. Glutathione-S-transferase P1 is a critical regulator of Cdk5 kinase activity. J Neurochem , 2011;118:902-914.

[11] Wakade C, King MD, Laird MD, et al. Curcumin attenuates vascular inflammation and cerebral vasospasm after subarachnoid hemorrhage in mice. Antioxid Redox Signal. 2009;11:35-45.

[12] Kuo CP, Lu CH, Wen LL, et al. Neuroprotective effect of curcumin in an experimental rat model of subarachnoid hemorrhage. Anesthesiology. 2011;115:1229-1238.

[13] Zhang J, Zhu Y, Zhou D, et al. Recombinant human erythropoietin (rhEPO) alleviates early brain injury following subarachnoid hemorrhage in rats: possible involvement of Nrf2-ARE pathway. Cytokine. 2010;52:252-257.

[14] Wang Z, Ma C, Meng CJ, et al. Melatonin activates the Nrf2-ARE pathway when it protects against early brain injury in a subarachnoid hemorrhage model. J Pineal Res. 2012;53(2):129-137.

[15] Li M, Zhang XJ, Cui LL, et al. The Neuroprotection of oxymatrine in cerebral ischemia/reperfusion is related to nuclear factor erythroid 2-related factor 2 (nrf2)-mediated antioxidant response: role of nrf2 and hemeoxygenase-1 expression. Biol Pharm Bull. 2011;34(5):595-601.

[16] Soane L, Li Dai W, Fiskum G, et al. Sulforaphane protects immature hippocampal neurons against death caused by exposure to hemin or to oxygen and glucose deprivation. J Neurosci Res. 2010;88:1355-1363.

[17] Bae J, Lee D, Kim YK, et al. Berberine protects 6-hydroxydopamine -induced human dopaminergic neuronal cell death through the induction of heme oxygenase-1. Mol Cells. 2013;35(2):151-157.

[18] Yang WJ, Li DP, Li JK, et al. Synergistic antioxidant activities of eight traditional Chinese herb pairs. Biol Pharm Bull. 2009;32(6):1021-1026.

[19] Chu Q, Hashimoto K, Satoh K, et al. Effect of three herbal extracts on NO and PGE2 production by activated mouse macrophage-like cells. In vivo. 2009;23(4):537-544.

[20] Lin Z, Zhu D, Yan Y, et al. An antioxidant phytotherapy to rescue neuronal oxidative stress. Evid Based Complement Alternat Med. 2011;2011:519-517.

[21] Dietz BM, Liu D, Hagos GK, et al. Angelica sinensis and its alkylphthalides induce the detoxification enzyme NAD(P)H: quinine oxidoreductase 1 by alkylating Keap1. Chem Res Toxicol. 2008;21(10):1939-1948.

[22] Li L, Zhang X, Cui L, et al. Ursolic acid promotes the neuroprotection by activating Nrf2 pathway after cerebral ischemia in mice. Brain Res. 2013;1497:32-39.

[23] Yang C, Zhang X, Fan H, et al. Curcumin upregulates transcription factor Nrf2, HO-1 expression and protects rat brains against focal ischemia. Brain Res. 2009;1282:133-141.

[24] Shah ZA, Li RC, Ahmad AS, et al. The flavanol (-)-epicatechin prevents stroke damage through the Nrf2/HO1 pathway. J Cereb Blood Flow Metab. 2010;30:1951-1961.

[25] Sun Q, Meng QT, Jiang Y, et al. Ginsenoside Rb1 Attenuates Intestinal Ischemia Reperfusion Induced Renal Injury by Activating Nrf2/ARE Pathway. Molecules. 2012;17:7195-7205.

[26] Arredondo F, Echeverry C, Abin-Carriquiry JA, et al. After cellular internalization, quercetin causes Nrf2 nuclear translocation, increases glutathione levels, and prevents neuronal death against an oxidative insult. Free Radical Biol Med. 2010;49(5):738-747.

[27] Nada SE, Shah ZA. Preconditioning with Ginkgo biloba (EGb 761®) provides neuroprotection through HO1 and CRMP2. Neurobiol Dis. 2012;46:180-189.

[28] Cheng TO. Danshen: what every cardiologist should know about this Chinese herbal drug. Int J Cardiol. 2006;110(3):411-412.

[29] Zhou L, Zuo Z, Chow MS. Danshen: an overview of its chemistry, pharmacology, pharmacokinetics, and clinical use. J Clin Pharmacol. 2005;45(12):1345-1359.

[30] Park EJ, Zhao YZ, Kim YC, et al. Preventive effects of a purified extract isolated from Salvia miltiorrhiza enriched with tanshinone I, tanshinone IIA and cryptotanshinone on hepatocyte in jury in vitro and in vivo. Food Chem Toxicol. 2009;47(11):2742-2748.

[31] Fan GW, Gao XM, Wang H, et al. The anti-inflammatory activities of Tanshinone IIA, an active component of TCM, are mediated by estrogen receptor activation and inhibition of iNOS. J Steroid Biochem Mol Biol. 2009;113(3-5):275-280.

[32] Su CC, Chen GW, Kang JC, et al. Growth inhibition and apoptosis induction by tanshinone IIA in human colon adenocarcinoma cells. Planta Med. 2008;74(11):1357-1362.

[33] Yang R, Liu A, Ma X, et al. Sodium tanshinone IIA sulfonate protects cardiomyocytes against oxidative stress-mediated apoptosis through inhibiting JNK activation. J Cardiovasc Pharmacol. 2008;51(4):396-401.

[34] Fu J, Huang H, Liu J, et al. Tanshinone IIA protects cardiac myocytes against oxidative stress-triggered damage and apoptosis. Eur J Pharmacol 2007;568(1-3):213-221.

[35] Zhang HS, Wang SQ. Nrf2 is involved in the effect of tanshinone IIA on intracellular redox status in human aortic smooth muscle cells. Biochem Pharmacol. 2007;73(9): 1358-1366.

[36] Chen TH, Hsu YT, Chen CH, et al. Tanshinone IIA from Salvia miltiorrhiza induces heme oxygenase-1 expression and inhibits lipopolysaccharide-induced nitric oxide expression in RAW 264.7 cells. Mitochondrion. 2007;7(1-2):101-105.

[37] Gopinath K, Sudhandiran G. Naringin modulates oxidative stress and inflammation in 3-nitropropionic acid-induced neurodegeneration through the activation of nuclear factor-erythroid 2-related factor-2 signalling pathway. Neuroscience. 2012;227:134-143.

[38] Hur J, Pak SC, Koo BS, et al. Borneol alleviates oxidative stress via upregulation of Nrf2 and Bcl-2 in SH-SY5Y cells. Pharm Biol. 2013;51(1):30-35.
[39] Guo LQ, Taniguchi M, Chen QY, et al. Inhibitory potential of herbal medicines on human cytochrome P450-mediated oxidation: Properties of umbelliferous or citrus crude drugs and their relative prescriptions. Jpn J Pharmacol 2001; 85(4):399-408.
[40] Yang XW, Gu ZM, Wang BX, et al. Comparison of anti-lipid peroxidative effects of the underground parts of Notopterygium incisum and N. forbesii in mice. Planta Med. 1991;57(5):399-402.
[41] Okuyama E, Nishimura S, Ohmori S, et al. Analgesic componentof Notopterygium incisum Ting. Chem Pharm Bull (Tokyo). 1993;41(5):926-929.
[42] Wang S, Wang T. Chemical constituents of Notopterygium forbesii Boiss. Zhongguo Zhong Yao Za Zhi. 1996;21(5): 295-296.
[43] Tang SY, Wang H, Zhang W, et al. Notopterygium forbesii boiss extract and its active constituents increase reactive species and heme oxygenase-1 in human fetal hepatocytes: Mechanisms of action. Chem Res Toxicol. 2008; 21(12): 2414-2423.
[44] Tang SY, Cheah IK, Wang H, et al. Notopterygium forbesii Boiss extract and its active constituent phenethyl ferulate attenuate pro-inflammatory responses to lipopolysaccharide in RAW 264.7 macrophages. A “protective” role for oxidative stress? Chem Res Toxicol. 2009; 22(8):1473-1482.
[45] Koriyama Y, Nakayama Y, Matsugo S, et al. Protective effect of lipoic acid against oxidative stress is mediated by Keap1/Nrf2-dependent heme oxygenase-1 induction in the RGC-5 cell line. Brain Research 2013;1499:145-157.
[46] Zee-Cheng RK. Shi-quan-da-bu-tang (ten significant tonic decoction), SQT. A potent Chinese biological response modifier in cancer immunotherapy, potentiation and detoxification of anticancer drugs. Methods Find Exp Clin Pharmacol. 1992;14(9):725-773.

中药单体及提取物的神经保护作用：Nrf2 信号通路研究进展

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文章亮点：

1 此问题的已知信息：很多具体单一中医中药对脑卒中后神经损伤方面的作用已经明确，但目前并无对这些中药进行归纳总结。

2 文章增加的新信息：Nrf2 信号通路在参与神经修复中有关键作用，中药在治疗脑卒中神经损伤后修复有明确作用，但目前尚无对此类中药的归纳总结。

3 临床应用的意义：文章归纳了各类中药通过 Nrf2 信号通路对脑卒中后神经修复方面的作用，对在脑卒中后神经修复方面中药的研究及治疗方法研究提供依据。

关键词：
组织构建；组织工程；中医药；Nrf2；ARE；脑卒中；神经保护；修复
主题词：
组织工程；脑卒中；中草药

摘要

背景：大量数据表明中药单体具有神经保护作用，可以提高脑神经系统损伤患者生活质量。Nrf 信号通路在出血性与缺血性卒中具有神经保护作用，它是通过无毒的天然或合成的中药发挥神经系统修复作用，但是目前很少对这方面中药进行系统报道。

目的：从 Nrf2 信号通路的角度对脑卒中后神经损伤发挥作用的中医单体进行综述。

方法：第一作者计算机检索中国知网，维普，MEDLINE 和 PubMed 数据库。检索词是：“Nrf2、ARE、脑卒中、中药、神经保护”和“Nrf2，ARE，Stroke，Traditional Chinese Medicine，Neuroprotection”。检索语言设置为中文和英文。探讨 Nrf2 和神经保护的研究进展及中药单体的脑卒中后神经保护作用。

结果与结论：共检索到 85 文章，根据纳入和排除标准，对文献进行筛选，最终共纳入 46 篇。结果表明，多种中药单体可以通过 Nrf2 通路发挥神经保护作用。中药单体研究涉及有机酸、酚类、皂甙、黄酮类、萜类、生物碱类和其他单一或复合成分。中药对脑卒中后神经损伤有明确修复作用，但此类中药缺乏规律性，它仍然需要扩大数据和进一步的研究作为基础。

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利益冲突：文章及内容不涉及相关利益冲突。

伦理要求：没有与相关伦理道德冲突的内容。

学术术语：转录因子 NF-E2 相关因子 2(NF-E2-related factor 2，Nrf2)-是细胞氧化应激反应中的关键因子，是细胞抗氧化还原的中枢调节者，Nrf2 通过与抗氧化反应元件(antioxidant response element，ARE)相互作用，诱导编码抗氧化蛋白和Ⅱ相解毒酶的表达，在细胞的防御保护中发挥重要作用。Nrf2/ARE 通路在抗炎症、免疫、抗凋亡、抗肿瘤、抗动脉粥样硬化、抗缺血再灌注损伤、肺纤维化和神经保护等方面起着重要的作用。

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