

Free Radicals, the Natural Antioxidant Buyang Huanwu Decoction and Ischemic Stroke

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ABSTRACT

Ischemic stroke is a widespread health issue worldwide, and a major cause of death and disability in an aging population. Research shows that reactive oxygen and free radicals generated by oxidative stress play an important role in cell apoptosis and often leads to stroke, one of the main causes of brain damage. Excessive free radicals also cause lipid peroxidation, leading to irreversible damage to cell membranes. The presence of free radical scavengers provides protection to brain tissues and can reduce the risk of stroke. Natural antioxidants have proved to be effective in the prevention and treatment of stroke, while also displaying fewer side effects. Buyang Huanwu Decoction (BYHWD), a traditional Chinese medicine prescription is commonly used for the prevention and treatment of ischemic cerebral stroke by inhibiting the formation of reactive oxygen species (ROS). Understanding the role of free radicals and the mechanisms by which natural antioxidants are used for the prevention and treatment of stroke can provide new insight into the research and clinical treatment of ischemic stroke.

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Key words: Free Radicals; Natural Antioxidant; Reactive oxygen species; Buyang Huanwu Decoction; Ischemic Stroke

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INTRODUCTION

Oxygen free radicals (OFR) are molecules containing one or more unpaired valence electrons, which makes it highly unstable, and result in the strong reaction and oxidation of DNA and lipids. OFR, including superoxide anion (O_2^-), hydroxyl radical ($\cdot OH$), nitric oxide ($NO\cdot$) and hydrogen peroxide (H_2O_2) are related to many neurological diseases and brain dysfunction. However, the presence of OFR is also crucial for electron transfer in the mitochondrial respiratory chain, the immune response and cell differentiation and growth. For instance, the NO free radical plays a key role in the relaxation of blood vessels, blood pressure regulation and during learning and memorization. Nevertheless, excessive free radicals can initiate lipid peroxidation and result in protein oxidation and DNA damage in cells, and are often implicated in diseases with high mortality rates, including cardiovascular and cerebrovascular diseases, cancer and neurodegenerative diseases^[1-4].

FREE RADICALS AND ISCHEMIC STROKE

Stroke can be divided into ischemic and hemorrhagic stroke, with ischemic stroke being far more prevalent. There are generally only two treatments for ischemic stroke: increasing blood flow in order to improve oxygen and glucose uptake in the arteries, or protecting neurons in order to reduce the likelihood of brain ischemia and neuronal excitotoxicity. Neuroprotectants commonly used in clinical treatment include calcium channel blockers, glutamate receptor antagonists and N-methyl-D-aspartic acid receptor (NMDA) antagonists. Research has shown that oxidative stress plays an important role in the pathophysiology of cerebral ischemia and that excessive ROS is produced during cerebral ischemia and

reperfusion^[1]. Studies have also suggested that oxidative stress and damage caused by free radicals lead are key factors for triggering stroke and other neurodegenerative diseases. Furthermore, the generation of large numbers of oxygen free radicals following a stroke results in peroxidation and irreversible damage to nearby tissues and cells, leading to permanent brain neuronal damage^[5,6]. Clinical treatment utilizing antioxidants and free radical scavengers can extend the optimal treatment time following oxidative damage, while natural antioxidants have the added advantage of reduced side effects. Therefore, the development and utilization of natural antioxidants and free radical scavengers for stroke prevention is of significant importance.

Acute cerebral infarction (ACI), which accounts for 80 to 85 percent of all cerebrovascular diseases, is the result of cerebral artery occlusion caused by acute cerebral tissue necrosis. The brain has one of the highest oxygen consumption rates in the body. Only a few minutes of ischemia can cause irreversible damage to the brain, therefore blood supply must be restored as soon as possible in order to prevent permanent injury. At present, treatment of ACI involves thrombolysis using recombinant tissue type plasminogen activator (t-PA). However, there are shortcomings and risks involved with this treatment, including complications due to bleeding and a limited time window for optimal treatment^[7]. Further study into the mechanism and prevention of ischemia reperfusion injury could help in improving the treatment of stroke. Many factors contribute to the process of cerebral ischemia-reperfusion, including excessive glutamate release, calcium overload, neuronal depolarization, energy shortage and most significantly, the large scale generation of ROS^[8]. During cerebral ischemia-reperfusion, many factors contribute to the generation of ROS, including mitochondrial respiratory chain, fatty acid metabolism and activation of xanthine and hypoxanthine oxidase. Brain tissue is characterized by a high rate of oxidative phosphorylation metabolism, high concentration of unsaturated fatty acids, relatively low oxidation resistance, low neuronal repair capacity and low cell division activity, thus it is extremely sensitive to oxidative damage. The large scale generation of ROS within a short time frame can cause damage to intracellular protein, lipid membranes and DNA. Furthermore, generation of ROS inhibits the mitochondrial respiratory chain, affects cell energy supply and disturbs cellular signaling pathways and gene transcription. Large quantities of oxygen free radicals in the brain's blood supply can also cause damage to endothelial and vascular smooth muscle cells, inducing platelet aggregation, changes in vascular permeability and ultimately leading to brain edema.

Transgenic and gene knockout experiments have demonstrated that ROS plays a vital role during the course of ischemia-reperfusion injury in the brain. Recent studies have focused on the use of antioxidants in protecting against cerebral ischemia-reperfusion injury, whereby free radical scavengers such as superoxide dismutase and peroxidase have been shown to be effective in reducing the risk of cerebral ischemia-reperfusion injury. Natural antioxidants extracted from plants, such as Extract of Ginkgo Biloba 761 (EGB76) and green tea polyphenols have also demonstrated to be effective in protecting against cerebral ischemia-reperfusion injury. Antioxidants offer protection of neurons in the CA1 area of the hippocampus by scavenging reactive oxygen free radicals following ischemia-reperfusion injury, suggesting that antioxidants can cross the blood-brain barrier (BBB) in order to protect the brain^[9].

NO is a key signaling molecule in the regulation of cerebral blood flow^[10]. Studies have shown that NO free radicals play a crucial role in brain ischemia-reperfusion injury. The reaction of NO

and superoxide anion radicals can generate peroxynitrite, a strong oxidizing agent, which can interfere with normal ion metabolism, thereby promoting oxidative damage during ischemia and reperfusion. NO can inhibit the mitochondrial electron transport chain both directly and indirectly, which causes disturbances in cellular energy and occurrence of DNA damage, eventually resulting in cell death. NO also accelerates the release of excitatory amino acids, such as glutamic acid following ischemia, which contributes to ischemic brain injury and neuronal excitotoxicity. However, there is evidence that NO acts like a double-edged sword, whereby in addition to its cytotoxicity and damage to cells, NO also has a cytoprotective effect due to its potential as a strong vasodilator and inhibitor of platelet aggregation and leukocyte adhesion. NO can therefore improve the blood supply to the brain following cerebral ischemic-reperfusion injury and protect brain tissue by inhibiting leukocytes and platelets which cause blockages in capillaries.

USE OF NATURAL ANTIOXIDANTS IN THE PREVENTION AND TREATMENT OF ISCHEMIC STROKE

Cerebral ischemia is a complex pathological process involving a series of mechanisms that includes generation of free radicals, oxidative stress, cell membrane dysfunction, release of neurotransmitters and cell apoptosis. Currently, the most effective treatment for cerebral ischemia is thrombolytic therapy, although there are significant risks and complications involved. The development of new drugs and increased awareness on the benefits of natural products has generated renewed interest in the use of traditional Chinese herbs in protecting against cerebral ischemia and nerve injury^[11,12].

There exists a balance between the generation and removal of free radicals *in vivo*, in other words, the balance between oxidation and reduction ensures a healthy body. This may be parallel to the Yin and Yang theory of Chinese traditional medicine. In theory, if there is excess generation of free radicals or a decrease in the scavenging ability of free radicals, this imbalance will result in a buildup of free radicals in the body. Most importantly, excess oxygen free radicals can significantly damage cellular components and act a precursor to diseases and aging. In order to eliminate excess free radicals, antioxidants must be used to preserve the balance between oxidation and reduction *in vivo*, thereby preventing the development of diseases. Studies have found that natural antioxidants such as hawthorn flavonoids, green tea polyphenols, resveratrol and the traditional Chinese medicine prescription Buyang Huanwu Decoction (BYHWD) are effective in the prevention and treatment of cardiovascular and cerebral diseases^[13-19].

Preventative and therapeutic effect of Buyang Huanwu Decoction (BYHWD) on ischemic stroke

Buyang Huanwu Decoction, which consists (Astragalus, Angelica root tail, red peony root, earthworm, Szechwan Lovage Rhizome, safflower, walnut kernel) is a classic traditional Chinese medicine prescription. Long-term clinical applications have demonstrated the effectiveness of BYHWD in the treatment of ischemic stroke. In recent years, the therapeutic mechanisms of BYHWD have been widely studied and its method of action involves increasing the scavenging ability of free radicals and anti-lipid peroxidation. We measured the level of plasma lipid peroxidation in patients with ischemic stroke, using ultraviolet spectrophotometry and

found that the level of acylhydroperoxide (AHP), an intermediary metabolic product of plasma lipid peroxidation was significantly higher in patients with acute ischemic stroke than that of healthy controls. However, following one month of continuous treatment with BYHWD, patients with acute ischemic stroke had significantly reduced levels of plasma lipid AHP ($P < 0.01$). Furthermore, patients with chronic ischemic stroke also had significantly decreased levels of plasma lipid AHP following two months of continuous treatment with BYHWD ($P < 0.05$). This suggests that BYHWD acts by inhibiting lipid peroxidation in the treatment of ischemic stroke^[14]. Li Tingfeng *et al* studied the generation of ROS in the brain tissues of rats following stroke using low temperature electron paramagnetic resonance (EPR) and spin trapping technique, and also measured the effects of BYHWD on ROS generation and levels of NO within the cortex and cerebellum in a rat stroke model. They also examined the effectiveness of BYHWD in the removal of superoxide anion (O_2^-) and hydroxyl radicals ($\cdot OH$) produced by a chemical simulation system in vitro. Their results revealed that the generation of ROS within the cortex and cerebellum of rats increased significantly following stroke ($P < 0.05$) and the level of NO was also significantly increased in the brain tissue of rats following stroke ($P < 0.001$) compared to the control groups. They also determined that BYHWD treatment can inhibit the generation of ROS within the cortex and cerebellum of rats following stroke and significantly decrease the level of NO in the brain tissue of rats following stroke, compared to the control groups without BYHWD treatment ($P < 0.05$). BYHWD was also shown to be efficient in scavenging O_2^- and $\cdot OH$ radicals produced by chemical simulation reaction in vitro. Thus, these results suggest that BYHWD also acts by inhibiting ROS generation in the treatment of ischemic stroke^[15]. Reperfusion of ischemic tissue is essential for survival, but it also initiates oxidative damage, cell death and aberrant immune responses through the generation of mitochondrial ROS^[20]. Tan Wencong *et al.* demonstrated that BYHWD prescription can increase superoxide dismutase (SOD) activity, which can assist in clearing free radicals, as well as decreasing malondialdehyde (MDA) content, an indicator of lipid peroxidation, and thereby reducing the risk of ischemia-reperfusion injury in rat brain^[21].

Individual components of BYHWD and its active ingredients in the prevention and treatment of stroke (Table 1).

Angelica root tail is one of the main components of BYHWD and is commonly used in traditional Chinese medicine for blood rejuvenation and renewal. The main chemical components of angelica root tail include phthalides, coumarins, flavonoids, polysaccharides and volatile oil compounds, many of which are associated with resistance to ischemic and hypoxic injury. These components have also been shown to decrease malondialdehyde (MDA) activity,

enhance SOD activity and assist in reversing the damage caused by free radicals, protease activation and NO generation^[22-23].

Astragalus is another component of BYHWD, which contains isoflavone, polysaccharides, and Huang Qidai as the main active ingredients^[22,24]. Astragalus also enhances SOD activity and improves the body's autoxidation performance by scavenging OFR, hydroxyl free radicals ($\cdot OH$), hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), as well as protecting against excessive tissue damage due to oxidative stress. Studies show that mice fed with Astragalus extract had an increase in SOD activity in the blood and a decrease in MDA content in tissues^[22,25]. Calycosin, an active ingredient isolated from Astragalus can protect against nerve injury caused by cerebral ischemia and reperfusion^[26]. Astragaloside IV is one of the primary active ingredients extracted from the Astragalus. Luo *et al.* showed that rats treated with astragaloside IV (20 and 40 mg/kg/day) prior to focal cerebral ischemia and transient middle cerebral artery occlusion (MCAO) and reperfusion had significantly reduced MDA content and lipid peroxidation in the blood. In addition, astragaloside IV increased the levels of antioxidant enzymes, glutathione peroxidase (GSH-Px) and SOD in ischemic tissue. These results suggest that the ischemic protection of astragaloside IV may be partly attributed to its antioxidant properties^[27].

Hydroxy Safflower Yellow pigment A (HSYA) is a chemical compound that contains a single chalcone glycoside structure and is the active ingredient derived from the water soluble part of safflower (*Carthamus tinctorius*). Tian Jingwei *et al* found that HSYA can significantly enhance SOD activity and reduce MDA content in brain mitochondria of rats following stroke. This suggests that HSYA offers protection against mitochondrial injury in cerebral ischemic due to scavenging of oxygen free radicals, inhibition of lipid peroxidation and antagonism of Ca^{2+} ^[28]. It should be noted that quinochalcone contents in safflower and its antioxidant activities vary considerably as a result of fluctuating flowering stages^[29].

The extract of Rhizoma Chuanxiong is a robust scavenger of superoxide anions and inhibitor of lipid peroxidation, with a strong dose-response relationship within a narrow concentration range. Chuanxiong can inhibit 96.93% of lipid peroxidation at an extract concentration of 6.25mg/mL, as well as scavenging 83.33% of superoxide anion radicals at an extract concentration of 15.625 mg/ml. The active ingredients in Chuanxiong include ferulic acid, alkaloids, volatile oil compounds and polysaccharides. The effect of Chuanxiong extract on the ability to scavenge superoxide anions and inhibit lipid peroxidation is likely due to the combined interaction of its active ingredients^[30]. Studies have revealed that the chemical composition of Chuanxiong includes phenolic composition and organic acids (ferulic acid, chrysophanol), alkaloids (chuanxiongzine), phthalide lactone (ligustilide, a specific bioactive ingredient in Chuanxiong) and volatile oil compounds. Modern

Table 1 Functions of individual component of BYHWD and its active ingredients.

Component	Active Ingredients	Main Functions	References
Angelica root tail	Phthalides, coumarins, flavonoid, volatile oil	inhibit free radicals, protease activation, NO generation	[22, 23]
Astragalus	Isoflavone, Huang Qidai	increase SOD activity, scavenge OFR, $\cdot OH$, H_2O_2 , O_2^- and MDA	[22, 24, 25, 26, 27]
Safflower	Hydroxyl safflower yellow pigment A (HSYA)	increase SOD activity, reduce MDA, inhibit LPO, antagonism of Ca^{2+}	[28, 29]
Chuanxiong	Ferulic acid, alkaloids, phthalide lactone, volatile oil	scavenge OFR, antagonism of Ca^{2+} , anti-platelet aggregation, inhibit XO, increase SOD activity	[30, 31, 32]
Earthworm	Free amino, fibrinolytic enzyme	reduce SOD and MDA, scavenge $\cdot OH$, O_2^- , NO generation	[33, 34, 35]
Radix Paeoniae Rubra	Glycosides	scavenge free radicals, increase GSH Px and SOD	[36, 37, 38, 39]
Walnut kernel	Polyphenols	scavenge OFR, inhibit LPO	[40, 41, 42, 43]

studies have demonstrated that the active ingredients in Chuanxiong, in particular chuanxiongine and ferulic acid, have various important pharmacological roles. Chuanxiongine can inhibit xanthine oxidase (XO) activity and increase SOD activity, thereby reducing the generation of OFR and myocardial oxygen consumption in order to protect against ischemic damage. Furthermore, ferulic acid can inhibit platelet aggregation and atherosclerosis, prevent vein thrombosis, scavenge free radicals and strengthen immune function^[31-32].

Earthworm (*Eisenia foetida*) extract is another component of BYHWD, which can effectively reduce lipid peroxidation in mice and prevent oxidative damage induced by free radicals *in vivo*, thereby improving the activity of antioxidant enzymes^[33]. Wu Jinxia *et al* showed that lyophilized powder of earthworm can notably reduce SOD and MDA content in mice. In addition, earthworm extract showed varying degrees of inhibition for hydroxyl free radicals and superoxide anions *in vitro*^[34]. The large extracellular hemoglobin protein (erythrocrurin) from the earthworm (*Lumbricus terrestris*) has shown promise as a potential hemoglobin-based oxygen carrier (HBOC) *in vivo* studies. Another important factor is the ability for erythrocrurin to either generate or preserve NO bioactivity in response to decreased levels of NO in the blood^[35].

The extract of red peony root (*Radix Paeoniae Rubra*) is also a component of BYHWD and acts an effective natural antioxidant^[36-37]. *Radix Paeoniae Rubra* extract can suppress the depletion of antioxidant enzymes, glutathione and SOD^[38]. Pu Tian *et al* studied and demonstrated the total glycosides (TG) activity in *Radix Paeoniae Rubra* extract, as well as its anticoagulant activity and ability to scavenge free radicals *in vitro*. The capability of TG in scavenging free radicals was confirmed using multiple methods, including diphenyl trinitrobenzene hydrazine (DPPH) free radical system, superoxide anion free radical system and hydroxyl free radical system. In addition, the TG extract was shown to have notable anticoagulant activity by analyzing the effects and durations of prothrombin time (PT), partial thrombin time (APTT) and thrombin time (TT)^[39].

The active ingredients in Walnut Kernel extract also has superior anti-oxidation performance and are adept at scavenging free radicals^[40-41]. Fang Meishan *et al.* showed that the ethanol extract of Walnut Kernel is a natural antioxidant that can effectively scavenge oxygen free radicals and protect against cerebral oxidative damage in mice with dementia^[42]. Aged rats that were fed a diet containing walnuts for 3 months showed a marked increase in SOD activity and decrease in lipid peroxidation in the brain, plasma and red blood cells. Furthermore, walnut extract can inhibit lipid peroxidation generated by cysteine and ferrous sulfate in tissue culture, which further demonstrates its antioxidant and anti-aging properties^[43].

As a result, almost all components of BYHWD contain active ingredients that can scavenge free radicals and have anti-oxidation properties. Therefore, the synergy of these active ingredients makes BYHWD an effective prescription in the prevention and treatment of ischemic stroke.

CONCLUSION

Free radicals play an important role in the pathogenesis of ischemic stroke. Natural antioxidants can act as neuroprotective agents and have proved to be effective to the prevention and treatment of ischemic stroke. The traditional Chinese medicine prescription, BYHWD and each of its individual components have been shown to be effective scavengers of free radicals. One of the main methods of action of BYHWD in the prevention and treatment of ischemic

stroke is the reduction of ROS generation in brain tissues. These studies provide new insight and experimental evidence for the better understanding and clinical treatment of ischemic stroke.

CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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