Neuroprotective effects of Buyang Huanwu decoction on cerebral ischemia-induced neuronal damage

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Abstract
Among the various treatment methods for stroke, increasing attention has been paid to traditional Chinese medicines. Buyang Huanwu decoction is a commonly used traditional Chinese medicine for the treatment of stroke. This paper summarizes the active components of the Chinese herb, which is composed of Huangqi (Radix Astragali seu Hedysari), Danggui (Radix Angelica sinensis), Chishao (Radix Paeoniae Rubra), Chuanxiong (Rhizoma Ligustici Chuanxiong), Honghua (Flos Carthami), Taoren (Semen Persicae) and Diling (Pheretima), and identifies the therapeutic targets and underlying mechanisms that contribute to the neuroprotective properties of Buyang Huanwu decoction.

Key Words: nerve regeneration; Buyang Huanwu decoction; traditional Chinese medicine; cerebral ischemia; clinical application; neuroprotection; review; neural regeneration

Funding: This work was supported by grants from Health and Family Planning Commission of Heilongjiang Province Research Projects, No. 2014-195 and Science and Technology Research Projects of Mudanjiang Medical University, No. ZS201305.

Mu QC, Liu PF, Hu XT, Gao HJ, Zheng X, Huang HY. Neuroprotective effects of Buyang Huanwu decoction on cerebral ischemia-induced neuronal damage. Neural Regen Res. 2014;9(17):1621-1627.

Introduction
Cerebrovascular diseases are ranked as the third leading cause of death and disability after cancer and heart disease (Feigin et al., 2003; Pandya et al., 2011). Both ischemia and hemorrhage are pathologic causes of cerebrovascular disease, with ischemic injury contributing to approximately 85% of all cases. Until recently, tissue plasminogen activator (t-PA) is the only FDA authorized drug that can promote vessel rebuilding after ischemic injury and facilitate neural recovery (Jaffer et al., 2011). However, several disadvantageous limit its clinical application (Bambauer et al., 2006). The therapeutic window is limited to the first 4.5 hours after the indication of symptoms. Only 3–8.5% of patients are treated with t-PA because of its potential to cause hemorrhage and second injury (Bambauer et al., 2006). Moreover, the diffusion of t-PA into the brain parenchyma increases vascular permeability (Yepes et al., 2003) and can cause neurotoxicity (Goto et al., 2007). Therefore, a toxin-free therapeutic method is urgently needed for the treatment of cerebral ischemic injury.

Recent studies have confirmed the beneficial effects of traditional Chinese medicine (TCM) in the treatment of cerebral ischemic injury (Yang et al., 2011a; Zhao et al., 2012a). Among the investigated TCM prescriptions, Buyang Huanwu decoction (BHD) is a well-known Chinese herb prescription which is functionally characterized by Qi supplement, and blood and meridian circulation (Fan et al., 2014). This TCM prescription originated from the old record Yi Lin Gai Cuo (corrections on the errors of medical works), which was compiled by Qingren Wang, a famous doctor in the Qing dynasty. BHD is composed of seven kinds of Chinese herbs, including Huangqi (Radix Astragali seu Hedysari), Danggui (Radix Angelica sinensis), Chishao (Radix Paeoniae Rubra), Chuanxiong (Rhizoma Ligustici Chuanxiong), Honghua (Flos Carthami), Taoren (Semen Persicae), and Diling (Pheretima). Because of drug-like properties of each herb, BHD is the primary prescription for the treatment of symptoms for hemiplegia and paraplegia (Wang and Jiang, 2009). In particular, BHD has been extensively used for the treatment of cerebral ischemic injury (Sun et al., 2007a), with accumulating experimental evidence indicating that BHD can improve recovery of behavioral scores, reduce the rate and area of infarction, and decrease ischemia-reperfusion injury (Yang et al., 2011a; Zhao et al., 2012a). Additionally, BHD has the ability to promote neurogenesis, increase vascular endothelial growth factor (VEGF) expression (Cai et al., 2007) and neural growth and differentiation, and inhibit apoptosis (Chen et al., 2008; Wang and Jiang, 2009). Although the neuroprotective properties of BHD are known, a systematic review of the mechanisms underlying this neuroprotective effect is still lacking. Here, the active components, the therapeutic targets, the clinical application, and the mechanisms underlying the neuroprotective properties of BHD in stroke are reviewed.

Active components in BHD and their therapeutic targets
BHD is a combination of several Chinese herbs and each
herb has its own bioactive components. Although the effect of the active components of BHD in other diseases has been widely reported (Chun-sheng et al., 1978; Grdisa et al., 2001; Fang et al., 2002; Cheng et al., 2006, 2007; Ren et al., 2006; Chen et al., 2008; Chi et al., 2009; Wei et al., 2009; Chang et al., 2011; Li et al., 2012; Liu et al., 2012; Tang et al., 2012; Zhang et al., 2012, 2014a, b; Zhao et al., 2012b; Jin et al., 2013; Li et al., 2013; Gong et al., 2014; Kim et al., 2014; Koushki et al., 2014; Qi et al., 2014; Yan et al., 2014; Yang et al., 2014; Zeng et al., 2014b). There is also evidence indicating that the neuroprotection afforded by Chuanxiong is because of inhibition of Bcl-2 and caspase-dependent apoptosis, as observed in PC12 cells subjected to oxidative stress (Cheng et al., 2007) and in animal models of cerebral ischemic injury (Cheng et al., 2006).

Pheretima aspergillum (PA) is one type of Dilong and stroke treatment with PA has been confirmed previously (Fang et al., 2002; Ren et al., 2006). Wei et al. (2009) reported that PA possesses pharmacological activity to promote regeneration of the peripheral nervous system after injury. Several studies have demonstrated that PA has anticoagulant and antioxidative properties (Grdisa et al., 2001) and promotes the growth of Schwann cells (Chang et al., 2011). Liu et al. (2012) reported that oral application of PA could ameliorate cerebral ischemic injury through decreasing the expression of glial fibrillary acidic protein (GFAP) and S-100B. Additionally, ferulic acid in Danggui (Zeng et al., 2014), hydroxysafflor yellow A in Honghua (Qi et al., 2014), benzoic acid in Chishao (Tang et al., 2012), and amygdalin in Taoren (Yan et al., 2014) also have beneficial effects on cerebral ischemic injury. Although the active compounds in BHD are not completely known, the active components already identified contribute to the multiple therapeutic targets of BHD against cerebral ischemic injury. This multi-targeted therapy most likely enhances the efficacy of BHD in fighting against cerebral ischemic injury.

**Clinical application of BHD in cerebral ischemic stroke**

BHD has been used for the treatment of several diseases, especially paralysis (Wang and Jiang, 2009) and stroke (Sun et al., 2007a) for many years because the formula was formed in the Qing dynasty (approximately 400 years ago). Based on the theory of TCM, BHD has advantages in invigorating the body, blood circulation, Qi supplement, and blood.
and meridian activation (Liu and Zhou, 1993; Zhang et al., 2010a; Ren et al., 2011). The hundreds of years of clinical experience, as well as modern experimental research, indicates the neuroprotective activity of BHD (Zhao et al., 2012a). In a clinical study, Cai and Lui (2010) found that BHD could promote functional recovery, enhance serum VEGF content, and ameliorate patient’s quality of life during the recovery period after stroke. BHD was also effective in treating coronary disease and syndrome of Qi deficiency and blood stasis by decreasing blood viscosity and plasma fibrinogen. For example, Wang et al. (2011b) reported that BHD ameliorated coronary disease through increasing blood circulation and energy metabolism. Zhang et al. (2010a) verified that BHD could inhibit C-reactive protein and cluster of differentiation 40 (CD40L) in white blood cells to treat coronary disease. In addition, BHD also has the ability to maintain blood glucose levels (Wang et al., 2011b).

**BHD inhibits excitotoxicity following cerebral ischemic injury**

Excitatory amino acids are up-regulated in blood serum and cerebrospinal fluid after ischemic injury, which suggests that inhibiting excitotoxicity may be an effective strategy to inhibit neurological deficits after stroke (Castillo et al., 1996; Oja and Saransaari, 2013). Glutamate is the most important excitatory amino acid, performing critical roles in sustaining neuronal function. However, excitotoxicity due to over-release of glutamate is one of the pathological mechanisms of stroke (Eweka et al., 2010). Under normal physiological conditions, intracellular glutamate is at a resting state (Danbolt, 2001). However, following over-release, a large amount of glutamate is released outside the cell and binds to its receptors to cause depolarization and cell death during ischemic injury (Bonde et al., 2005). In a rat model of middle cerebral artery occlusion (MCAO), Wang et al. (2013) measured the content of excitatory amino acids in cerebrospinal fluid using microdialysis-high performance liquid chromatography-fluorescence detection. They showed that glutamate and aspartic acid were released 40 minutes post ischemia and peaked at 120 and 80 minutes after ischemia, respectively. Glycine, taurine and γ-aminobutyric acid also increased after ischemia and peaked at 120 minutes. By contrast, BHD application could decrease the levels of these excitatory amino acids and increase inhibitory amino acids to neutralize excitotoxicity. Consistently, Zhao et al. (2012a) also found that BHD inhibited ischemic injury-induced elevations of excitatory amino acids. Additionally, BHD also neutralized the increase of metabolotropic glutamic acid receptor-1 (m-GluR1) expression in a rat MCAO model (Zhao et al., 2012a). Importantly, the inhibition was related to neurological recovery and a decrease in infarct area. This evidence suggests that inhibition of excitotoxicity is one of the mechanisms involved in the neuroprotective effect of BHD against cerebral ischemic injury.

**BHD promotes angiogenesis after cerebral ischemic injury**

Induction of angiogenesis, especially in the ischemic boundary area, enhances oxygen and nutrient supply to the infarcted tissue (Wei et al., 2001). Generation of new blood vessels facilitates highly coupled neurorestorative processes including neurogenesis and synaptogenesis, which in turn leads to improved functional recovery (Chen and Chopp, 2006; Beck and Plate, 2009). Therefore, promoting angiogenesis represents an effective way to facilitate neurological functional recovery. Although angiogenesis is not sufficient to satisfy the requirement of new blood vessels in an MCAO model, BHD administration before modeling not only elevates Ang-1 expression, but also extends the expression period (Shen et al., 2014). The changes in Ang-1 levels following BHD administration increase blood vessel density, which contribute to the decrease in infarct area and recovery of the nervous system. Hence, angiogenesis is a mechanism underlying the effect of BHD on neurological recovery after ischemic injury. Consistently, BHD administration also increases the expression of angiogenesis-related proteins (ARP), such as VEGF and its receptor and F1K1 at later recovery phases after ischemic injury (Cai et al., 2007). Although there was a report indicating that in the early phase after injury, BHD restricts the expression of angiogenesis-related proteins (Wang et al., 2011a), further studies on how BHD regulates these proteins is required. The up-regulation of ARPs provides a basis for new blood vessel generation at later recovery phases. The increase in expression of VEGF at the early phase after ischemic injury increases the permeability of the blood-brain barrier and elicits secondary damage (Vandenbroucke et al., 2008). Based on these results, we infer that like VGA1155 (Chiba et al., 2008), an antagonist of VEGF, BHD may also restrict ARP expression to avoid secondary damage following cerebral ischemic injury.

**BHD promotes migration of neural precursor cells (NPCs) to the infarct zone**

NPCs, located in the subventricular zone (SVZ) and subgranular zone (SGZ), have the potential to renew and differentiate into various types of neuronal cells in adult animals (Gage, 2000; Ma et al., 2009). After ischemic injury, endogenous NPCs proliferate, migrate to the ischemic zone and differentiate into neurons (Nakatomi et al., 2002). This process appears to be a means of neurological functional recovery after ischemic injury because newborn neurons replace the damaged cells. However, the newborn neurons are insufficient to facilitate recovery of the injured tissue. Interestingly, advanced studies indicate that proliferation, migration and differentiation of neural precursors can be up-regulated by exogenous interference, which promotes neurological recovery following ischemic injury (Bonde et al., 2005; Nakano-Doi et al., 2010; Osman et al., 2011; Sejersted et al., 2011; Zhuang et al., 2012; Ara and De Montpellier, 2013). In an MCAO model, Kong et al. (2014) verified that BHD could promote proliferation of neural precursors in the SVZ, SGZ and corpus striatum of the infarcted brain. Additionally, expression of migration-related proteins such as stromal cell-derived factor 1 and chemokine receptor type 4 were also up-regulated after BHD administration. These data provide evidence that BHD may exert its neuroprotective effect partially by promoting NPC migration to ischemic brain areas.
BHD facilitates the proliferation and differentiation of NPCs

BHD may facilitate NPC proliferation in a mouse ischemic model (Cai et al., 2007). Cellular calcium concentration is critical for neuronal proliferation and differentiation (Catterall, 2000). Although calcium overload could lead to cell death following cerebral ischemic injury, a low calcium concentration by contrast is beneficial for axon growth (Sun et al., 2007a). With the assistance of serum pharmacological method, the effects of BHD on the growth of hippocampal NPCs was investigated (Sun et al., 2007a, b). Compared with controls, BHD could clearly increase the length of axons, and the expression of neurofilament and GFAP. Consistently, calcium concentrations decreased after application of BHD-containing serum. Extracellular signal regulated kinase 2 (ERK2) is an important component of the MAPK signaling pathway. The ERK2-mediated signaling pathway is known to regulate neural regeneration, neural growth, and differentiation and restoration after neurological injury (Nishimoto and Nishida, 2006; Berwick et al., 2009; Huang et al., 2011; Duan et al., 2013; Ishii et al., 2013). For example, Jinglong et al. (2013) verified that chronic BHD treatment for 30 days could activate ERK2 expression and promote neuronal growth and differentiation in the ischemic area. Based on these results, inhibition of calcium concentrations, as well as activation of ERK2 expression may underlie the effects of BHD on growth and differentiation of NPCs. Additionally, Wang et al. (2011a) employed gene set enrichment analysis and confirmed that BHD enhanced the expression of neural regeneration-related genes (Dcx, Fgfr3, Ctnnbp2, Rorb, Abi2 and Miat) and neural development-related genes (Ptprf, Ift172 and Nfib). Hence, promoting NPC regeneration is a potential mechanism underlying the neuroprotective effects of BHD against cerebral ischemic injury.

BHD inhibits inflammation in cerebral ischemic injury

Diapedesis and proinflammatory cytokine release in the ischemic region elicits an inflammatory reaction, which leads to early functional defects to the blood-brain barrier (Jin et al., 2010). Transcription factors, such as nuclear factor-kappaB play critical roles in regulating the post-ischemic inflammatory reaction (Nurmi et al., 2004; Zhang et al., 2005). The up-regulation of related inflammatory cytokines determines neuronal fate. BHD application effectively inhibits cerebral ischemic injury-activated TLR4 expression (Wang et al., 2011a). Additionally, gene expression-mediated diapedesis is significantly attenuated after BHD administration. This evidence suggests that BHD not only antagonizes the inflammation-related signaling pathway, but also inhibits the diapedesis-regulated inflammatory reaction in the cerebral ischemic region, thus preventing cell death.

BHD inhibits apoptosis in ischemia injury

Apoptosis has been reported to contribute to cell death following cerebral ischemic injury (Chen et al., 1998; Lee et al., 2000; Zeng and Xu, 2000; Sugawara et al., 2002). Caspases are a family of cysteine proteases that play an important role in apoptosis, particularly the "initiator" (caspase-9) and "effector" (caspase-3) caspases (Hengartner, 2000). Caspase 3 is the "effector" protease in apoptosis (Deshmukh et al., 1996; Schulz et al., 1996) and is activated during nutrient deficiency, potassium loss and glutamate elicited excitotoxicity (Chen et al., 1998; Sugawara et al., 2002). Accordingly, regulation of caspase 3 though gene deletion or antagonists decreases ischemic injury-induced cell death.

In a rat model of transient ischemic injury produced by the four-vessel occlusion method, neurological function deficits were coupled with damage to neurons and cell loss (Li et al., 2003). Additionally, transferase-mediated biotin-dUTP nick-end labeling identified apoptotic cells in the model group (Gavrieli et al., 1992; Chen et al., 1997). Interestingly, BHD administration post-ischemia markedly reversed the extent of apoptosis and rescued neural function deficits. Concomitantly, ischemic injury-induced caspase-3 activation was attenuated by BHD administration. Therefore, the blockade effect of BHD on ischemic injury-induced apoptosis is an effective way to rescue neuronal deficits. Using genome-wide transcriptome analysis, Wang et al. (2011a) screened 15 genes that may be involved in the protective effect of BHD on ischemic injury-induced apoptosis.

Conclusion

The protective effects of BHD on ischemic injury were confirmed by various experimental models (Zhang et al., 2001, 2005, 2006, 2010b, 2011; Deng et al., 2002; Lai et al., 2002; Shao et al., 2003; Liao et al., 2004; Qu et al., 2004, 2014; Fan et al., 2006; Tan et al., 2006; Tang et al., 2006; Tong et al., 2007; Wu et al., 2008, 2011, 2012; Zhou et al., 2008, 2011, 2012; Wang and Jiang, 2009; Yi et al., 2010; Zhao et al., 2010; Ren et al., 2011; Yang et al., 2011a; Gu et al., 2013; Wang et al., 2013; Kong et al., 2014). Most investigators preferred the SD rat model of MCAO. The effect of time after BHD administration and the dose of BHD administered were also studied (Zhao et al., 2012a). A dose of 40 mg/kg BHD had a greater effect than 20 mg/kg BHD. Additionally, the therapeutic window was also important, as application of BHD 2 hours after injury had a more prominent effect than application at 4 or 6 hours. Therefore, the therapeutic window of BHD administration is critical for effective treatment. Due to the limited number of studies on BHD, further research related to the time and dose of BHD required for the treatment of ischemic injury is required.

Based on literature, BHD has a therapeutic effect on ischemic injury, primarily through ameliorating blood circulation, reducing calcium overload, promoting neural precursor migration, increasing growth of NPCs, reducing the inflammatory response and inhibiting neuronal apoptosis. In addition to the above mechanisms, BHD has also been reported to ameliorate ischemic injury in cardiac tissue, the spinal cord and the peripheral nervous system via its antioxidant properties (Fan et al., 2006; Yang et al., 2011b). To the best of our knowledge, there have been no studies regarding the anti-oxidation of BHD in cerebral ischemia injury. Additionally, an in-depth
investigation on blood circulation after BHD application is required to further clarify its vessel rebuilding properties.

**Author contributions:** Huang HY, Hu XT, Gao HJ and Zheng X helped in reviewing the study and experimental data. Mu QC and Liu PF wrote the paper. All authors approved the final version of this paper.

**Conflicts of interest:** None declared.

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Mu QC, et al. / Neural Regeneration Research. 2014(9(17):1621-1627.