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Natural Compounds in The Treatment of Cerebral Ischemia-Reperfusion Injury - A Systematic Review

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ABSTRACT

More and more attention in the field of drug discovery has been focused on the neuroprotection of natural compounds from traditional medicinal herbs. Cerebral ischemia is a complex pathological process involving a series of mechanisms, and a framework for the development of neuroprotectants from traditional herb medicine is a promising treatment for cerebral ischemia. Natural compounds with the effects of anti-oxidation, anti-inflammation, calcium antagonization, anti-apoptosis, and neurofunctional regulation exhibit preventive or therapeutic effects on experimental ischemic brain injury. According to the pharmacological mechanisms underlying neuroprotection, we evaluated natural products from traditional medicinal herbs that exhibit protective effects on ischemic brain injury and characterized the promising targets.

INTRODUCTION

Cerebral ischemia causes disturbances in a variety of cellular and molecular mechanisms, including oxidative phosphorylation, membrane function, neurotransmitter release, and free radical generation. It has been years since tissue-type plasminogen activator (tPA) became the first medication approved by the FDA for the management of stroke, with limited success. Thrombolytic therapy is the most effective therapeutic strategy for the prevention of brain injury and the reduction of mortality in patients with cerebral infarction. However, reperfusion after thrombolytic therapy often leads to a series of cellular, biochemical and metabolic consequences of cerebral ischemia, including intracellular reactive oxygen species generation, calcium overload, excitotoxic cell injury and, inflammation, which ultimately lead to irreversible brain injury. Many neuroprotective agents are designed to protect the brain from irreversible injury after ischemia-reperfusion or to retard the pathological process. Therefore, a combination of established thrombolytic therapy and effective neuronal protection therapy may have more beneficial effects for patients with ischemic stroke.[1] Because clinical trials of pharmacological neuroprotective strategies in stroke have been disappointing, more attention has turned towards approaches that include herbal drugs that can be used in limiting the neurological damage associated with stroke. Herbal drugs can also be used as a prophylactic treatment in patients with a high risk of stroke. Herbals drugs have been described in ancient systems of medicine for the treatment of various ailments associated with stroke and have more recently been reported to be beneficial in treating stroke.[2]

Epigallocatechin Gallate (EGCG): A natural polyphenol found in green tea, is a potent free radical scavenger. It has been demonstrated that EGCG significantly protected neurons from cerebral ischemia-reperfusion injury by reducing infarction volume, improving neurological deficit score, and significantly attenuating the ischemia-reperfusion induced increase in malondialdehyde and oxidized/total glutathione ratio. [3]

Cinnamophilin: It is isolated from the *Cinnamomum Philippines.* Pre and post-ischemic treatment with cinnamophilin effectively reduced brain infarction and improved neurobehavioral outcome following cerebral ischemia-reperfusion injury in mice. Additionally, cinnamophilin administration significantly attenuated *in situ* accumulation of superoxide anions (O^{2-}) in the boundary zones of infarct after reperfusion and also

significantly decreased the levels of oxidative damage, as assessed by immunopositive reactions for 8-hydroxy-2'-deoxyguanosine (8-OHDG) and 4-hydroxynonenal (4-HNE).[4]

Theaflavin: It is a natural polyphenol found in black tea, possesses biological functions such as antioxidative, and anti-inflammatory actions. It has been demonstrated that theaflavin significantly protected neurons from cerebral ischemia-reperfusion injury by limiting leukocyte infiltration and expression of ICAM-1, and suppressing upregulation of inflammatory-related prooxidative enzymes (iNOS and COX-2) in the ischemic brain via, at least in part, reducing the phosphorylation of STAT-1.[5]

Garlic: Aqueous extract of *Allium sativum L*. fruits has been shown to reduce brain damage after cerebral ischemia-reperfusion injury. In addition, it improves neurobehavioral outcomes and decreases glutamate and aspartate levels, possibly by increasing the endogenous defensive capacity of the brain.[6]

Honokiol: A component of the herb *Magnolia officinalis*, exhibits antioxidant, anti-inflammatory properties. It was studied for neuroprotection against ischemic-reperfusion injury. Administration of honokiol resulted in a significant reduction in brain infarct volume and production of reactive oxygen species, also attenuated the decrease in mitochondrial membrane potential, mitochondrial metabolic function, and tissue Na^+ K^+-ATPase activities observed in the ischemic brains.[7]

Curcumin: It is a member of the curcuminoid family of compounds, is a yellow-colored phenolic pigment obtained from the powdered rhizome of *C. longa Linn*. It is reported that a single injection of curcumin (1 and 2 mg/kg, i.v.) 30 min after focal cerebral ischemia in rats significantly diminished infarct volume, improved neurological deficit, decreased mortality, reduced the water content of the brain, and the extravasation of Evans blue dye in the ipsilateral hemisphere in a dose-dependent manner. Moreover, in cultured astrocytes, curcumin significantly inhibited inducible nitric oxide synthase (iNOS) expression and NO(x) (Nitrites/nitrate contents) production induced by lipopolysaccharide (LPS) /TNF-α.[8]

Embelia ribes: *Embelia ribes* Burm (Myrsinaceae), commonly known as Vidanga, is a large woody climbing shrub and is widely distributed throughout India. Pretreatment with aqueous extract of *Embelia ribes* Burm fruits ameliorates cerebral infarction and enhances the antioxidant defense against middle cerebral artery occlusion induced cerebral ischemia-reperfusion injury in rats, observed by investigating markers of oxidative damage;

thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), and, glutathione-S-transferase (GST).[9]

***Phyllanthus emblica**:* It is also known as amla. It has been used in Ayurveda, the ancient Indian system of medicine. It is a very effective free-radical scavenger. Alcoholic extract of amla fruits provided significant protection against cerebral ischemia-reperfusion injury in a rat model. The authors concluded that amla has antioxidant properties, which are responsible for beneficial effects in cerebral ischemia-reperfusion injury.[10]

Ocimum basilicum: The genus Ocimum (Lamiaceae) has a long history of use as a culinary and medicinal herb. Many species are used for their antioxidant and neuroprotective activity in various parts of the world. Pre-treatment with a standardized ethyl acetate extract of *Ocimum basilicum* markedly reduced cerebral infarct size and lipid peroxidation restored GSH content, and attenuated impairment in short-term memory and motor coordination in the model of cerebral ischemia and reperfusion.[11]

***Wedelia calendulacea*:** Coumestan derivative wedelolactone and nor-wedelolactone are the main active constituents of the *Wedelia calendulacea*. Treatment with *W. calendulacea* is shown to have neuroprotective activity against cerebral ischemia-reperfusion-induced oxidative stress in the rats. It significantly attenuated ischemia-reperfusion induced oxidative stress, decreased the concentration of MDA, hydrogen peroxide, and followed by increased GPx, GR, and GST activity.[12]

***Phoenix dactylifera**:* Pretreatment with methanolic extract of *P. dactylifera* fruits significantly attenuated cerebral ischemia-reperfusion induced alterations of superoxide dismutase, catalase, glutathione, glutathione peroxidase, glutathione-S-transferase, glutathione reductase, and the significant increase in lipid peroxidation along with severe neuronal damage in the brain.[13]

***Eclipta alba*:** *Eclipta alba* pretreatment has been shown to reduce cerebral ischemia-reperfusion injury by enhancing the antioxidant defenses, as demonstrated by a significant increase in SOD, GPx, GSH, catalase, GST, GR, and a significant decrease in MDA in the brain. [14]

Galangin: Galangin is a natural flavonoid isolated from the rhizome of *Alpina officinarum* Hance, which has been widely used as an antioxidant agent. It shows that Galangin alleviated

the neurological impairments, reduced cerebral infarct size, and exerted a protective effect on the mitochondria with decreased production of mitochondrial reactive oxygen species (ROS) after cerebral ischemia and reperfusion injury.[15]

Xanthohumol: Xanthohumol is the principal prenylated flavonoid in *Humulus lupulus* L., an ingredient of beer. Xanthohumol was found to be a potent anti-oxidant and chemopreventive agent. It has been found to have anti-inflammatory, anti-apoptotic, antioxidant, and antithrombotic properties. This has been shown to reduce infarct volume and improve neurobehavior in rats with cerebral ischemia-reperfusion injury. This activity is mediated by inhibition of inflammatory responses (i.e., hypoxia-inducible factor-1α, iNOS expression, and free radical formation), apoptosis (active caspase-3), and platelet activation.[16]

Resveratrol: Resveratrol is a natural flavonoid found in grape skin and possesses potent antioxidant, anticancer, anti-inflammatory, and vasodilating actions. It has significant Cerebro-protective action against cerebral infarction induced by ischemia-reperfusion in rats, demonstrated by a significant reduction in oxidative stress and inflammatory markers like malondialdehyde, TNF-α, and myeloperoxidase and the significant increase in antioxidant and anti-inflammatory markers like superoxide dismutase, catalase, and IL-10 levels.[17]

Asiatic Acid: Asiatic acid (AA) is a triterpene isolated from *Centella asiatica*, is widely used as an antioxidant and anti-inflammatory agent. It has been shown to reduce infarct size and improve neurologic scores after cerebral ischemia and reperfusion, possibly by suppression of mitochondrial damage, BBB disruption, and MMP-9 activation.[4]

Centella asiatica: *Centella asiatica* has been used as psychoactive and antioxidant herbal medicine since ancient times. An extract of *Centella asiatica* has been shown to attenuate the neurobehavioral, neurochemical, and histological changes in cerebral ischemia and reperfusion of rats. It effectively reduced the level of thiobarbituric acid reactive species, restored glutathione content, and augmented the activities of antioxidant enzymes; catalase, glutathione peroxidase, glutathione reductase, glutathione-S-transferase, and superoxide dismutase in a dose-dependent manner.[18]

Naringenin: Naringenin, a potent flavonoid reported to have potent antioxidant activity. Prophylactic treatment with naringenin improved functional outcome and reduced the ischemic brain injury by suppressing NF-κB mediated neuroinflammation.[19]

Quercetin and Rutin: Quercetin and Rutin are members of the class of flavonoids termed as flavonols. These were evaluated for their cerebroprotective role in experimental ischemia-reperfusion-induced cerebral infarction in rats. Quercetin and rutin administered 10 min before reperfusion resulted in a significant reduction of infarct size, MDA, and MPO levels and a significant increase in SOD and CAT levels.[20]

Allium cepa: Methanolic extract of Flavonoid-rich fraction of *Allium cepa* L. (outer scales, bulb) reduced the cerebral damage and oxidative stress after ischemia and reperfusion in rats.[21]

Pomegranate Extract: Protects against cerebral ischemia-reperfusion injury in rats, demonstrated by decreased brain levels of NF-κB, p65, TNF-α and increased level of IL-10 in stroke condition. [22]

Madecassoside: A triterpenoid derivative isolated from *Centella asiatica*, exhibits anti-inflammatory and antioxidant activities. It is shown to have a neuroprotective effect against focal cerebral ischemia-reperfusion injury in rats, as demonstrated by a significant decrease in levels of malondialdehyde, nitric oxide, pro-inflammatory cytokines, and NF-κB. [23]

Caffeic Acid: It is an active component of Propolis extract and also in a wide variety of plants. Caffeic acid has neuroprotective effects on global cerebral ischemia-reperfusion injury in rats, as shown by the decrease in MDA overproduction, increase in SOD activity, and also through the inhibition of 5-lipoxygenase.[24]

Mangiferin: It is a natural glucosyl xanthone found in both mango and papaya. It has been shown to ameliorate neurologic deficit, infarct volume, and brain water content after cerebral ischemia-reperfusion. Mangiferin also reduced the content of malondialdehyde (MDA), IL-1β, and TNF-α, and up-regulated the activities of superoxide dismutase (SOD), glutathione (GSH), and IL-10 levels in the brain tissue of rats with the cerebral ischemia-reperfusion injury. Moreover, mangiferin up-regulated the expression of nuclear factor erythroid 2-related factor 2 (Nrf2) and its downstream anti-oxidant protein heme oxygenase-1 (HO-1). The Cerebro-protective effect of mangiferin may be related to the improvement of the antioxidant capacity of brain tissue and the inhibition of the overproduction of inflammatory cytokines. The mechanisms are associated with enhancing the oxidant defense systems via the activation of the Nrf2/HO-1 pathway. [25]

Icariin (ICA), an active flavonoid extracted from Chinese medicinal herb *Epimedi.* It was found that pretreatment with ICA could decrease neurological deficit score, diminish the infarct volume, and reduce the protein levels of IL-1β and TGF-β_1. Moreover, ICA suppressed IκB-α degradation and NF-κB activation induced by I/R. Furthermore, the present study also showed that ICA up-regulated PPARα and PPARγ protein levels. These findings suggest that ICA has a neuroprotective effect on ischemic stroke in rats through inhibition of inflammatory responses mediated by NF-κB and PPARα and PPARγ.[26]

Galuteolin is a substance extracted and purified from honeysuckle. The results showed that galuteolin could significantly reduce the cerebral infarction volume, neurologic score, and cerebral water content in a dose-dependent manner. In addition, galuteolin reduced the apoptosis rate of nerve cells and the expression levels of caspase-3 and Bax, meanwhile up-regulated the expression levels of p-Akt and Bcl-2. Furthermore, galuteolin inhibited the levels of LPO, Sod1, Sod2, and catalase in the cerebral infarction tissues. Moreover, galuteolin also significantly reduced the levels of pro-inflammatory factors IL-1β and TNF-α in the cerebral infarction tissues. Finally, Galuteolin markedly inhibited the expression of VEGF in cerebral infarction tissues. Galuteolin exerts neuroprotective effects against CIRI by inhibiting apoptosis, oxidation, and inflammation.[27]

CONCLUSION

Brain ischemia is a complex progressive injury involving a series of mechanisms such as cell survival and apoptosis, ROS accumulation, Ca2+ overload, inflammation, microglia aggregation, etc. Considering that multifactorial and progressive pathophysiological processes are involved in cerebral ischemia, therefore, suggests that drugs binding to multiple targets or those combinations of drugs that act on single targets may be more effective in curing ischemia and related cerebral injury. In conclusion, the development of protective agents from traditional herb medicine is a promising direction in the treatment of ischemic cerebral injury and related neurodegenerative diseases. In the future, more attention should be paid to natural compounds that can transverse the BBB and have wide therapeutic time windows, clear pharmacological targets, and fewer side effects.

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