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Polyphenols in Cerebral Ischemia

Novel Targets for Neuroprotection

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Abstract

Plant polyphenols are dietary components that exert a variety of biochemical and pharmacological effects. Recently, considerable interest has been focused on polyphenols because of their antioxidant, anti-inflammatory, and antiproliferative activities. Oxidative stress is thought to be a key event in the pathogenesis of cerebral ischemia. Overproduction of reactive oxygen species during ischemia/reperfusion could cause an imbalance between oxidative and antioxidative processes. Reactive oxygen species can damage lipids, proteins, and nucleic acids, thereby inducing apoptosis or necrosis. There is increasing evidence supporting the hypothesis that plant polyphenols can provide protection against neurodegenerative changes associated with cerebral ischemia. This article reviews the neuroprotective effects of plant extracts and their constituents that have been used in animal models of cerebral ischemia. The use of polyphenols as therapeutic agents in stroke has been suggested.

Index Entries: Polyphenols; flavonoids; cerebral ischemia; antioxidants; neuroprotection; plants; tea; grape; curcumin; ginkgo.

Introduction

Stroke is the third leading cause of death in North America and Europe and a major cause of disability in aging adults *(1,2)*. The pathophysiological processes in stroke are extremely diverse and are dependent on the severity, duration, and localization of the ischemic damage in the brain. Animal models of cerebral ischemia have been frequently used to study mechanisms underlying cellular damage *(3)*. Focal cerebral ischemia can be transient or permanent and usually produced by the occlusion of the middle cerebral artery (MCA). The MCA occlusion leads to rapid cell death in the

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ischemic core. In the periphery of the ischemic region, the blood flow reduction is less marked and neurons in this area, referred as penumbra, can be saved. In this model, infarction develops in cortical areas and sometimes also in the caudate–putamen *(4).* This is the preferred model for studies involving transgenic and knockout mutant mice *(5).* Global ischemia models are the four- and two-vessel occlusion models to produce a reversible forebrain ischemia. In the most commonly used global ischemia model, ischemia is induced by bilateral common carotid artery (CCA) occlusion in gerbils. In this model, ischemia/reperfusion causes injury to selectively vulnerable areas. Using a 5-min CCA ligation, delayed neuronal death occurs in the hippocampal CA1 neurons by apoptosis *(6).*

Oxidative stress has been implicated as one of the potential contributors to ischemic injury *(6–8).* The brain requires and utilizes a high amount of energy. Owing to its high oxygen utilization, the high levels of polyunsaturated fatty acids in neural cell membranes, and the relatively low levels of antioxidant enzymes, the brain is particularly susceptible to oxidative stress. During ischemia/reperfusion, biochemical events are initially triggered by the release of excitatory neurotransmitters *(9),* followed by neuronal membrane depolarization and an increase in intracellular calcium concentration leading to the generation of reactive oxygen species *(10).* The balance between oxidative and antioxidative processes breaks down because of the perturbation in antioxidant defense systems caused by the overproduction of reactive oxygen species. Reactive oxygen species can damage lipids, proteins, and nucleic acids and also alter signaling pathways leading to cell death, either necrosis or apoptosis, or both *(11).* Consequently, the use of antioxidants as therapeutic agents in stroke has been suggested *(12).*

Polyphenols are natural substances with variable phenolic structures and are enriched in vegetables, fruits, grains, bark, roots, flowers, seeds, tea, and wine *(13).* They are generally known to possess potent antioxidant properties *(14–16).* Research on these compounds has started to accelerate with the discovery of the "French paradox" (i.e., the low incidence of coronary heart disease observed in France in association with red wine consumption and intake of a high-fat diet) *(17).* This effect seems to be attributed to the polyphenols in red wine. Recently, important epidemiological studies have found that a high consumption of fruits and vegetables can also lower the risk of ischemic stroke *(18–20).* However, studies examining the health benefit of polyphenols have been primarily focused on heart disease and cancer. Whether polyphenols can offer antioxidant benefits to the brain and have possible preventive effects on neurodegenerative diseases only began to receive increased attention and remains to be studied. For example, initial studies have emerged to show the ability of polyphenolic compounds to improve neurological functions in aging *(21,22)* and to ameliorate oxidative and inflammatory damages owing to chronic alcohol abuse *(23).*

This review is aimed to summarize the available literature pertaining to the beneficial roles of polyphenols in protection against ischemia/reperfusion injury using animal models.

Polyphenols

Polyphenols are found in most plant-derived foods and beverages. There are over 8000 polyphenolic structures identified in plants. Edible plants contain only several hundred polyphenolic structures *(13,24,25).* Polyphenols add to the sensory and nutritional qualities of plant foods. Polyphenols are often involved in the plant's defensive response against different types of stress such as ultraviolet radiation, pathogens, and physical damage *(13,25).* Because plants usually produce these polyphenols as a defensive mechanism, environmental conditions such as soil type, sun exposure, and rainfall along with other factors such as genetics factors, germination, degree of ripeness, processing and storage, and species variety can have effect on the polyphenol concentration

Table 1
Major Subclasses of Polyphenols, Compounds, and Food Sources

Polyphenol group	Name	Source
Flavonoid		
Flavonols	Quercetin, myricetin, kaempferol	Onions, apples, kale, red wine, green and black tea, broccoli, berries
Flavanones	Hesperetin, naringenin, eriodictyol	Citrus fruit, tomatoes, mint
Flavanols (proanthocyanidins and catechins)	Epicatechin, epigallocatechin, epicatechin-3-gallate, epigallocatechin-3-gallate	Apricots, green and black tea, red wine, chocolate
Anthocyanins	Cyanidin, pelargonidin, malvidin	Strawberries, other berries, grapes, wine, tea, eggplant, cabbage, beans, onions, radishes
Isoflavonoids	Genistein, diadzein, glycitein (phytoestrogens)	Lentils, chickpeas, alfalfa, clover, flaxseed, soybeans
Flavones	Apigenin, luteolin, diosmetin, tangeretin, nobiletin, sinensetin, wogonin	Parsley, celery, skin of citrus fruit
Phenolic acid		
Benzoic acid derivative	Gallic acid, vanillic, syringic, hydroxybenzoic	Tea, strawberries, raspberries, blackberries, black radish, onions
Cinnamic acid derivative	*p*-Coumaric, caffeic, ferulic, sinapic acids, vanillin, syringaldehyde, *p*-hydroxybenzaldehyde	Blueberries, kiwis, plums, cherries, apples, cereal grains, coffee
Lignan	Secoisolariciresinol	Linseed, lentils, cereals, garlic, asparagus, carrots, pears, prunes
Stilbene	Resveratrol	Grapes, red wine
Saponin	Ginsenoside	Ginseng root
Other polyphenols	Curcumin	Turmeric

(13,24,25). Even pieces of fruit from the same tree can have significant differences in their polyphenol concentrations owing to different exposures to sunlight or other environmental factors. Because of the large variability, the content of polyphenols in foods is usually poorly characterized *(25).* All polyphenols contain an aromatic ring with one or more hydroxyl group. Most also have at least one sugar residues (glycosides) attached to the hydroxyl groups. They are classified into different groups depending on the number of phenol rings and chemical groups bound to the rings *(13,24,25).*

Polyphenols contain a wide range of molecule sizes. Polyphenols, such as phenolic acids, are simple compounds, whereas the tannins are highly polymerized molecules *(13).* Flavonoids make up most of the polyphenols and they form the most important single group of polyphenols *(13).* Table 1 summarizes the main classes of polyphenols, some representative phenolics in the groups, and their dietary sources.

Polyphenols are usually recognized for their antioxidant capabilities. Phenolic antioxidants have been shown to inhibit the oxidation of lipids and other molecules and protect against free radicals *(13,26).* Polyphenols can react with radicals to form polyphenol radicals. The polyphenol radical is more stable and less reactive because of the ability of the phenol group

to absorb extra electrons. Most polyphenols are conjugated by methylation, sulfation, or glucuronidation during metabolism. The antioxidant capability could be determined by the type of conjugate and its location on the polyphenol structure. This might be why certain polyphenols are better at scavenging superoxides, whereas others can scavenge the highly reactive oxygen-derived radical peroxynitrite *(13,25,27–30).* Their antioxidant capacity may also correlate with their ability to chelate metals. Specific polyphenols can chelate iron and possibly prevent the formation of free radicals by iron *(26,27).*

Polyphenols have been shown to have several other actions in addition to their antioxidant ability. Evidence show that they can inhibit the activities of several enzymes, including lipoxygenase, cyclo-oxygenase, xanthine oxidase, phospholipase A_2, ATPases, aldole reductase, phoshodiesterases, topoisomerase I and II, protein kinase C, phosphoinositide 3-kinase, Akt/PKB, (protein kinase B) and mitogen-activated protein (MAPKs) kinases (MAPs) *(26–28,30,31).* Some polyphenols have weak estrogenic properties and others can inhibit the enzymes involved in estrogen metabolism, aromatase, and 17β-hydroxysteroid oxidoreductase *(31).*

The reduction of several diseases has been linked to polyphenols. Cardioprotection and a reduction in certain types of cancer have correlated with consumption of phenolic antioxidants *(13,24,31).* There is also evidence for polyphenols to be antiallergic, antiviral, antibiotic, antidiarrheal, antiulcer, and anti-inflammatory agents. Polyphenols have been used to treat hypertension, vascular fragility, allergies, and hypercholesterolemia *(13,26,27).*

Polyphenols have also been implicated in the prevention of neurodegenerative diseases. Polyphenols protect neurons against oxidative stress thought to one of the main causes of neurodegenerative diseases. Even a 10-fold higher concentration of ascorbate did not protect neurons similar to polyphenols *(28,30).* Polyphenols attenuate ischemia–reperfusion injury by interfering with inducible nitric oxide synthase activity, inhibiting lipid peroxidation, decreasing the number of immobilized leukocytes during reperfusion, and reducing complement activation which results in a diminished inflammatory response *(27).* Most importantly, in addition to their antioxidant actions, they also influence neuroprotective and neurorestorative signal transduction mechanisms *(30).* Epidemiological studies show an inverse relationship between stroke and polyphenol consumption *(24).*

The dietary intake of polyphenols varies greatly among different societies. Isoflavone intake as a result of soy consumption ranges from 20 to 240 mg for Asians and from 1 to 9 mg in the United States and Western populations *(25,31).* Agriculture practices can also affect dietary intake of polyphenols. The region where a particular plant is grown will probably have the greatest consumption *(25).* The total consumption of flavonols, flavanones, flavanols, and isoflavones in Western cultures is estimated to be 100 to 150 mg/d. An accurate estimate of dietary intake of all polyphenol ingested is difficult to achieve because of poor characterization of polyphenols in foods and the great variability of polyphenol concentration within foods *(25).*

Most polyphenols occur naturally as a form of ester, glycoside, or polymer rather than the aglycone form *(25,27).* Because no known digestive enzymes can hydrolyze the polyphenols, originally it was assumed that only the aglycone form and free simple phenolic compounds were able to penetrate the intestinal wall, but recent evidence shows intestinal bacteria hydrolyzes polyphenols to the aglycone form *(13,24,25,31,32).* For some glycosides, hydrolysis can be achieved in the oral cavity *(32).* There is also evidence for absorption of glycosides using transporters without hydrolysis to aglycones. The transporters that are implicated include the absorptive transporter (SGLT1), monocarboxylate transporter (MCT), and multiresistant protein 2 transporter (MRP2) *(25,32).* The bioavailability of polyphenols is determined by their molecular structure. Glycosylation, conjugation, molecular size, poly-

merization, and solubility can influence the rate of absorption *(13,27,31).*

Polyphenols are subject to conjugation by methylation, sulfation, and glucuronidation by the liver and intestinal bacteria *(13,31).* The formation of certain conjugates is favored when the intake of polyphenols is consistent, such as sulfation favored in low concentrations and a shift toward glucuronidation at higher concentrations *(25,27).* As a result of liver metabolism, the concentration of free aglycone is usually very low in the blood after the consumption of polyphenols *(25).* Almost all of the conjugated polyphenols are bound to albumin. Up to 99.1% of quercetin is bound to plasma proteins *(25,31,32).* Binding of conjugated polyphenols to albumin extends their half-life (23–28 h). Because of the long half-lives, regular intakes could accumulate polyphenols to active concentrations *(27).*

Plant Extracts/Polyphenols Used in Models of Cerebral Ischemia

Tea Extract

Many varieties of tea *(Camellia sinensis)* are cultivated and consumed around the world. Tea extracts are particularly enriched in catechins but also contain flavonols and proanthocyanidins *(33,34).* The tea catechins are (–)-epigallocatechin-3-galleta (EGCG, as the major constituent), (–)-epigallocatechin (EGC), (–)-epicatechin (EC), and (–)-epicatecin-3-galleta (ECG). The bioavailability of the catechins has been studied in both humans and rodents and brain incorporation was demonstrated *(24,32).* Significant data have been accumulated on the radical scavenging, metal chelating, and anti-inflammatory activities of tea flavonoids *(35).* However, many investigations showed that in addition to their antioxidant characteristics, these compounds influence signaling pathways responsible for cell death, growth, and survival *(30,36).* One epidemiological study reported on lower stroke incident in people drinking more than five cups of green tea daily *(37).* However, another study reported no association between catechin intake (highest intake among polyphenols in human diet) and stroke incident or mortality *(38).*

Using a mouse model of CCA occlusion, Matsuoka et al. *(39)* showed that intravenous administration of catechin or EC (100 mg/kg) before ischemia can improve the behavioral deficit measured by a step-down inhibitory avoidance paradigm. Several studies used the gerbil model of transient global cerebral ischemia. Inanami et al. *(40)* observed a dose-dependent protection against hippocampal neuronal death after ad libitum oral administration of catechin in the drinking water for 2 wk. EGCG injection immediately after ischemia was also shown to reduce neuronal damage in the CA1 region in a dose-dependent manner *(41).* EGCG (50 mg/kg intraperitoneally) was effective even when it was administered 3 h after the ischemic insult *(42).* Hong et al. *(43)* used green tea extract in the drinking water ad libitum for 3 wk before ischemia in gerbils. This treatment reduced the infarct volume, the number of apoptotic cells, and lipid peroxidation and inhibited the ischemia-induced hyperactivity. Interestingly, catechin injection (30 mg/kg intraperitoneally) after ischemia did not give protection using a permanent MCA occlusion model in rats *(44).*

Grapes/Wine

Grapes and wine contain considerable amount of polyphenols such as resveratrol, catechins, flavonols, and proanthocyanidins. The flavonoid concentration in red wine can be 20-fold the concentration found in the white wine *(45).* There is extensive literature describing the biological effects including the antioxidant activity of these compounds in vitro *(45–47).* Their absorption and metabolism have also been intensively studied although future studies are required, particularly in humans *(32).*

Even though many studies have indicated the neuroprotective roles of grape polyphenols, only a few studies examined their in vivo effects on cerebral ischemia. Using a focal

ischemia model in rats by MCA ligation together with bilateral CCA occlusion for 1 h, intravenous injection of resveratrol decreased cerebral infarct volume in a dose-dependent manner given either before or after ischemia. The effective dose was 0.1 μg/kg. Resveratrol did not induce changes in hemodynamic and blood gas parameters *(48).* In another focal ischemia model using only MCA occlusion in rats, the protective effect of resveratrol was shown with pretreatment for 21 d (20 mg/kg intraperitoneally per day). The treatment reduced the infarct volume, prevented motor impairment, and inhibited lipid peroxidation *(49).* A single dose of resveratrol (20 mg/kg) given orally 1 h before permanent MCA ligation in mice did not protect against ischemic damage. However, when given daily for 3 d before ischemia, resveratrol significantly reduced the infarct size. The authors had attributed this effect to the activation of peroxisome proliferator-activated receptor α (PPARα) because resveratrol was ineffective in PPARα knockout mice *(50).* In another study, the effect of resveratrol on transient global cerebral ischemic injury was examined in gerbils. Resveratrol (30 mg/kg intraperitoneally) was injected either during or shortly after CCA ligation and 24 h later. Resveratrol decreased neuronal death in the hippocampus and also inhibited glial cell activation. It was demonstrated that resveratrol could cross the blood–brain barrier and incorporate into the brain tissue *(51).* Dietary supplementation of grape powder or an extract of the powder ameliorated ischemia-induced delayed neuron death and glial cell activation. The grape extract showed effective protection even when it was given after ischemia *(52).*

Quercetin was also shown to be able to scavange superoxide anions released during reperfusion after forebrain ischemia using a four-vessel occlusion model in rats *(53).* Consequently, quercetin (30 mg/kg intraperitoneally) given after MCA ligation in a permanent MCA occlusion model in rats significantly decreased the infarct volume *(44).* Although proanthocyanidin exhibited a dose-dependent inhibition of lipid peroxidation in brain *(54),* no study has been investigated its in vivo effect on cerebral ischemia until now.

Ginkgo Biloba

Ginkgo biloba has been used for medicinal purposes for centuries. EGb 761 is a fully standardized extract obtained from the leaves of ginkgo biloba. The extract contains two major groups of substances (the flavone glycosides [flavonoid, 24%] and the terpene lactones [terpenoid fraction, 6%]), certain low-molecular-weight organic acids, and proanthocyanidins. The flavonoid fraction is mainly composed of quercetin, kaempferol, and isorhamnetin glycosides. The terpenoid constituents are ginkgolides, such as the ginkgolides A, B, C, and J, and bilobalide *(55).* Several studies examined the pharmacokinetics and bioavailability of EGB 761 *(56,57).* The effect of ginkgo biloba extract on cerebral activity has been demonstrated by using electroencephalogram measures in humans *(56).*

In vivo and vitro studies have indicated that EGb 761 and its constituents produce neuroprotective effects in different ischemia models and these effects were associated with a variety of mechanisms (see ref. *58* for a review). Clinical studies have reported on the efficacy and safety of ginkgo biloba extract in treating different symptoms of some neurological disorders, including stroke *(56).*

Pretreatment with oral administration of ginkgo biloba extract attenuated delayed neuronal death in the hippocampal CA1 in a dose-dependent way using the gerbil transient global ischemia model *(59,60).* The neuroprotective effect was partially mediated by the reduction of lipid peroxidation and nitric oxide formation *(60).* Similar neuroprotection was demonstrated in mice after MCA occlusion either with oral or daily intraperitoneal treatments *(61–63).* Acute administration of EGb 761 (100–200 mg/kg intraperitoneally) also afforded neuroprotection against permanent and transient focal cerebral ischemia in rats. Both pretreatment and posttreatment were

effective. In the transient ischemia model, a delayed treatment up to 2 h improved the neurobehavioral scores as well *(64).*

Because EGb 761 is a complex mixture, it is necessary to know which of its constituents mediates the protective activity of EGb 761. Many studies have demonstrated that the nonflavone fraction was responsible for the antihypoxic activity of EGb 761. In focal cerebral ischemia models, the administration of bilobalide (5–20 mg/kg subcutaneously) 60 min before ischemia dose-dependently reduced the infarct area in mouse brain and the infarct volume in rat brain 2 d after the onset of the injury. Ginkgolide A (50 mg/kg subcutaneously) and ginkgolide B (100 mg/kg subcutaneously) reduced the infarct area in the mouse model of focal ischemia but ginkgolides C and J did not *(65).* To compare the protective effect of bilobalide and EGb 761 against ischemic injury, oral administration of EGb 761 at 25, 50, and 100 mg/kg/d and bilobalide at 3 and 6 mg/kg/d for 7 d before ischemia was used in gerbils. Bilobalide exhibited the same level of protection against ischemic neuronal death as EGb 761. These treatments significantly inhibited the ischemia-induced reductions in COX III mRNA in the CA1 region, suggesting the involvement of mitochondrial mechanisms in the protective effect *(66).* NV-31, a bilobalide-derived compound, significantly reduced the infarct area in focal ischemia in mice *(67).*

Curcuma longa Linn

Turmeric, the powdered rhizome of the medicinal plant *Curcuma longa* Linn is widely used as a food flavoring and coloring in Asian diets. Its yellow color is imparted by curcumin (1,7-*bis* [4-hydroxy 3-methoxy phenyl]-1,6-heptadiene-3,5-dione), a polyphenolic pigment *(68).* Curcumin has a long history of use in India and Southeast Asia and proves to have a low toxicity *(69).* One of the most disputed arguments common to the phenols, as well as curcumin, include the questionable bioavailability of the drug, despite the potent biological activity at sites distant from the locus of absorption *(70).*

One of the most remarkable features of curcumin is its ability to scavenge reactive oxygen and nitrogen free radicals *(71,72).* Curcumin is a diferuloyl methane having two *o*-methoxy phenolic OH groups attached to the α,β-unsaturated β-diketone moiety. Recent studies have shown that it is the phenolic OH that is essential for both antioxidant activity and free-radical kinetics *(73).* Additionally, curcumin suppresses activation of nuclear factor (NF)-kB and elevates the activities of detoxification enzymes of xenobiotic metabolism, such as glutathione transferases and NADPH *(74,75).* It is through these antioxidant activities that curcumin can act as a potential therapy following brain ischemic damage. Indeed, curcumin is proposed to have "antiaging" or "memory-enhancing" effects and is, therefore, proposed to be a viable option in the treatment of dementia and Alzheimer's disease *(76).*

Three studies have looked specifically at the use of curcumin following cerebral ischemia. The first study demonstrated that a single curcumin injection (200 mg/kg intraperitoneally) given 30 min after CCA occlusion in rat attenuated ischemia/reperfusion injury by decreasing xanthine oxidase activity, lipid peroxidation, and superoxide production *(77).* The second study showed that curcumin treatment at 100 to 300 mg/kg intraperitoneally following middle cerebral artery occlusion reduced infarct volume and edema volume, as well as reductions in ipsilateral lipid peroxidation, superoxide dismutase and glutathione peroxidase activity, and peroxynitrite formation *(78).* More recently, curcumin has been shown to protect against delayed neuronal death induced by global ischemia/ reperfusion in gerbils *(79).* In this study, curcumin administration (30 mg/kg intraperitoneally) twice following common carotid artery occlusion significantly decreased delayed neuronal death as well as glial cell activation (Fig. 1), and substantial decreases in apoptosis measurements were also noted *(79).*

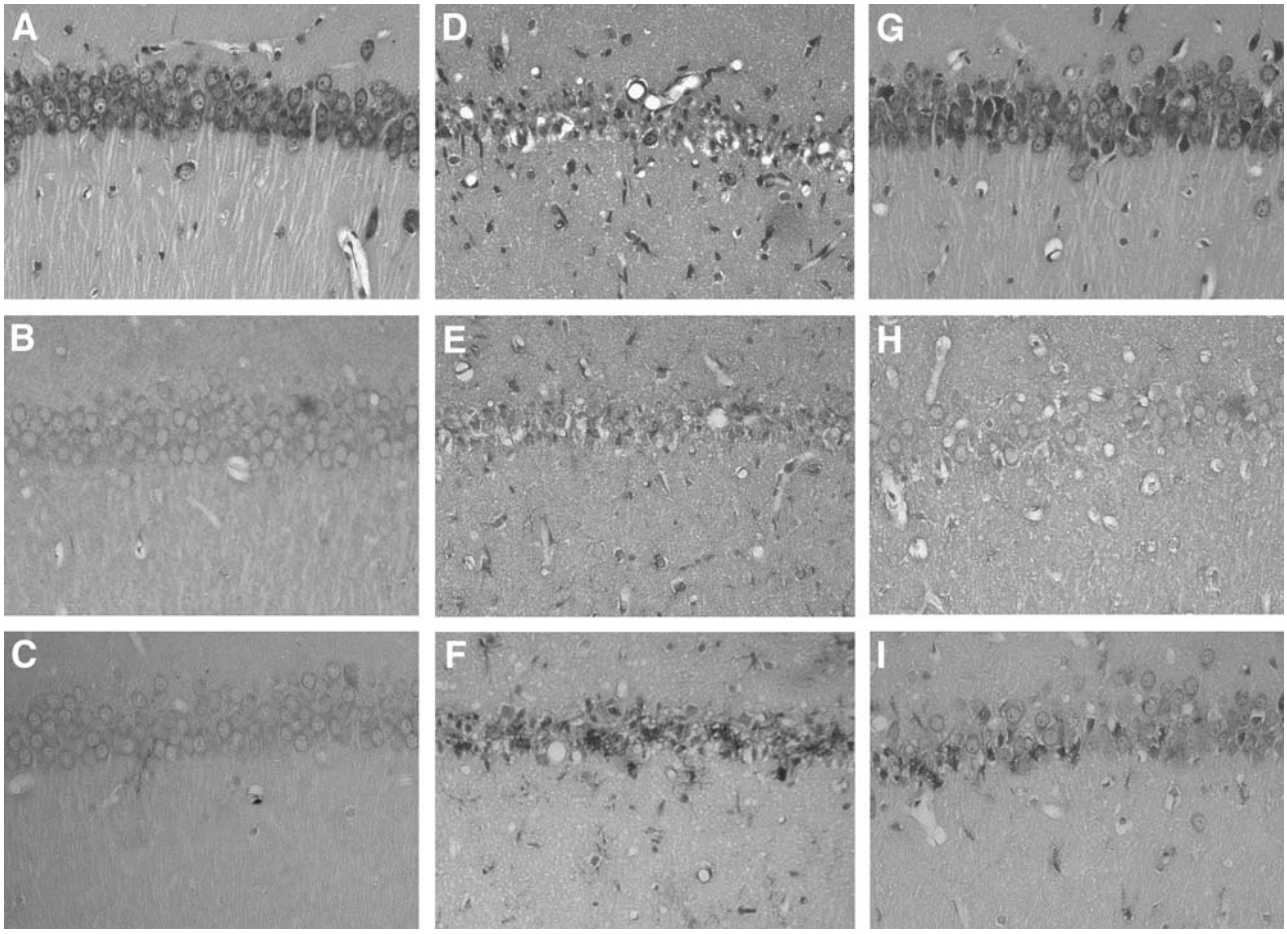

Fig. 1. Representative photomicrographs showing neurons, astrocytes, and microglial cells stained with cresyl violet, GFAP, and isolectin B4 in the hippocampal CA1 area of gerbil 4 d after a 5-min CCA occlusion. **(A,D,G)** Cresyl violet staining; **(B,E,H)** GFAP staining; **(C,F,I)** isolectin B4 staining. **(A,B,C)** Sham-operated control; **(D,E,F)** ischemia/reperfusion; **(G,H,I)** ischemia + curcumin. Magnification: ×400.

Other Plant Extracts/Polyphenols

More and more studies have started evaluating the antioxidant property of polyphenols from diverse natural products, with the focus on investigating their therapeutic actions as neuroprotectants against cerebral ischemia. Biological products from ancient herbal medicines to common fruits like blueberries have been considered. The followings are examples from the literature.

Wogonin (5,7-dihydroxy-8-methoxyflavone), a plant flavon obtained from *Scutellaria baicalensis* Georgi, is known to have neuroprotective effects and it has been traditionally used in ancient medicine. Laboratory studies on wogonin have showed that it inhibits NO production by suppressing inducible nitric oxide synthase (iNOS) *(80).* It is also a direct inhibitor of cyclo-oxygenase 2 *(81).* In a study using the transient global ischemia four-vessel occlusion model of rat, it was demonstrated that wogonin (10 mg/kg intraperitoneally) given immediately after ischemia attenuates hippocampal neuronal cell death and its presence reduces the inflammatory mediators,

iNOS and tumor necrosis factor (TNF)-α in the hippocampus *(82).*

Genistein is an isoflavon from soy extracts. It is a phytoestrogen, a widely used antioxidant and a protein-tyrosine kinase inhibitor. A mouse model of singlet oxygen-induced cerebral stroke was used to study the in vivo antioxidant effect of genistein. Multiple injection of genistein was administered prior to and after irradiation. The size of the cerebral lesion in the genistein-treated mice was significantly smaller than that of control mice *(83).* Another in vivo study used the gerbil transient global ischemia model and found that genistein (local injection into the hippocampus before ischemia) blocked tyrosine phosphorylation and prevented delayed neuronal cell death in the CA1 region of the hippocampus *(84).*

Acori graminei rhizome (AGR), which mainly consists of α-arsarone and β-arsarone and Uncariae Ramulus et Uncus (URE), is widely used ancient herbal medicine against ischemia. To study their influence on cerebral ischemia-induced neuronal and cognitive impairments, rats were pretreated with AGR and URE (100 mg/kg/d orally) for 3 wk and focal ischemia was induced by the intraluminal filament technique. The pretreatment attenuated the ischemia-induced learning and memory deficits in Morris water maze and radial arm maze and had a protective effect against cell damage in the hippocampus, striatum, and cortex *(85).*

Lowbush blueberries (*Vaccinium angustifolium* aiton) contain substantially high levels of polyphenols and have a high antioxidant capacity. Rats were fed with blueberry diet 6 wk before and 1 wk after ischemia. Ischemic insult was induced by ligation of the left common carotid artery followed by hypoxia. The left and right hemispheres were compared and neuroprotection was observed in CA1 and CA2 areas of the hippocampus *(86).*

Buckwheat polyphenol (BWP), as the name suggests, is a polyphenol that comes from *Fagopyrum esculentum* MOENCH. Using a repeated cerebral ischemia model in the rat, the neuroprotective effect of BWP was evaluated. Glutamate release and NO production were measured. BWP (600 mg/kg, continuous for 21 d orally) inhibited the excess release of glutamate and also suppressed delayed increase in NO. In addition, an improvement in performance in an eight-arm radial maze and a decrease in hippocampal neuronal death were reported *(87).*

Kava *(Piper methysticum),* comes from the original Canone plants of ancient Hawaii. Its α-pyrone contents are kawain, dihydrokawain, methysticin, and yangonin. To test if kava extract protects against ischemic damage, focal cerebral ischemia was induced in rats and mice by microbipolar coagulation of the left MCA. The administration of kava extract (150 mg/kg, 1 h before ischemia) reduced ischemia-induced brain infract in both rats and mice *(88).*

Ginseng root has been used for more than 1000 yr as a medicinal plant in China. Using the gerbil transient global ischemia model, it was shown that oral administration of red ginseng powder before but not after ischemia decreased delayed neuronal death in the hippocampus and improved the spatial learning deficit *(89).* Ginsenoside Rb1, an active ingredient of ginseng, had the same effect and was found to be a free-radical scavenger in vitro *(90).* Another ingredient of ginseng, ginsenoside Rg1, also showed neuroprotection when given both before and after ischemia (10 mg/kg intraperitoneally) and increased cell proliferation and enhanced the surviving rate of newborn cells in the hippocampus *(91).*

Studies have also been done on herbal glycoside recipes. In such recipes, two or more herbal glycosides are mixed together in required proportions. A mice focal ischemia model of MCA occlusion was used and animals were treated with different doses of herbal glycoside (baicalein and dioscin, ratio 1 : 1). The treatment reversed spatial memory impairment and reduced infarct volume in a dose-dependent way. A microarray analysis showed an increased expression of many genes (38–46 from 1176) including nine genes related to spatial learning and memory in the hippocampus *(92).*

Concluding Remarks

The evidence presented in this article suggests the potential of polyphenols in both preventive and therapeutic usages for cerebral ischemia/reperfusion injuries. Furthermore, no toxic or other adverse side effects were reported with the dietary use of high concentration of polyphenols, although regulated clinical trials have not been performed. In addition, their bioavailability, absorption, and metabolism also require more studies, especially in humans. It would be particularly important to compare individual polyphenols with extracts of fruits, beverages, and vegetables in preclinical and clinical projects and to further investigate possible mechanisms of their effects. Numerous studies have indicated that compounds in an extract can act synergistically so it would be advantageous to use multiple polyphenols in the treatment of stroke. In particular, when stroke symptoms appear, a substantial damage has already taken place in the brain. Therefore, treatments have to start as early as possible in order to reduce further neurodegeneration and promote regeneration. However, the preventive use of plant products will be likely the most effective strategy for the treatments of stroke and other age-related neurodegenerative disorders.

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