Molecular Neurobiology

ISSN0893-7648/05/31(1–3): 283–293/$30.00

Natural Antioxidants for Neurodegenerative Diseases

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Abstract

The author reviews the studies on the preventing effects of natural antioxidants, such as vitamins E and C, flavonoids, and polyphenols on neurodegenerative diseases, especially summarizing the results on the protective effect of ginkgo biloba extract on neuron cells, preventing effects of green tea polyphenols on apoptosis of PC12 cells (Parkison's disease model), preventing effects of genestien on amyloid-β-induced apoptosis of hippocampal neuronal cells (Alzhemer's disease model), and preventing effect of Crataegus flavonoids on ischemic–reperfusion damage to the brain of the Mongolian gerbil (stroke model) in the laboratory.

Index Entries: Oxidative stress; natural antioxidants; neurodegenerative diseases; AD; PD; stroke; free radicals.

Introduction

There is a common characteristic of neurodegenerative diseases: all (such as Parkinson's disease [PD], Alzheimer's disease [AD], and stroke) are connected with oxidative-stress-induced neuron cellular apoptosis. It is well known that it is difficult for the patients to be cured and there is no effective drug for these diseases until now. Is there any way to prevent these diseases? From this review, we might find a positive answer: antioxidants should have preventing and even curing effects on the diseases.

Received 6/6/04, Accepted 11/15/04.

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Oxidative Stress and Neurodegenerative Diseases

It undoubtedly, extra free radical results in biological body injury. Free radicals peroxidize membrane lipids *(1)* and oxidize proteins *(2)*, resulting in damage of the plasma membrane and crosslinking of cytoeskeletal proteins. In addition, free radicals damage RNA *(3)*, nuclear DNA *(4)*, and DNA *(5)*. In the brain, the high metabolic rate, the low concentration of glutathione and antioxidant enzyme catalase, and

the high proportion of polyunsaturated fatty acids make the brain tissue particularly susceptible to oxidative damage *(6,7)*. Oxidative stress, an imbalance toward the pro-oxidant side of the pro-oxidant/antioxidant homeostasis, occurs in several brain neurodegenerative disorders. Among these neurodegenerative brain disorders are those in which protein aggregation is observed, including AD, PD, and stroke.

Oxidative Stress and AD

Accumulated data demonstrated that oxidative damage occurs in the AD brain *(5)*. Amyloid-β (Aβ) peptide has been proven to produce hydrogen peroxide (H_2O_2) through metal ion reduction, with concomitant release of thiobarbituric acid-reactive substances (TBARS), a process probably mediated by the formation of hydroxyl radicals *(8,9)*. The cytotoxicity of Aβ fibrils is also implicated as an oxidative mechanism. Aβ-Fibrils-induced H_2O_2 was detected by several laboratories *(10–12)*. There is considerable evidence consistent with the importance of oxidative stress in the pathology of AD (for recent reviews, *see* refs. *13–15*). Evidence supporting the notion of free-radical oxidative stress in the AD brain includes increased redox-active metal ions in the AD brain, increased lipid peroxidation detected by decreased levels of polyunsaturated fatty acids and increased levels of the lipid peroxidation products, acrolein, TBARS, isoprostanes, and neuroprostanes, increased protein oxidation, increased oxidation of DNA and RNA, and decreased activity of oxidatively prone enzymes, such as glutamine synthetase (GS).

Oxidation of proteins normally is caused by free radicals, and this process, from a chemical thermodynamics standpoint, is an exothermic event. Oxidative reactions of peptides are mediated mainly by the hydroxyl radical (OH). There are two possible oxidative pathways that can occur: (1) backbone oxidation and (2) side-chain oxidation *(1)*. Backbone oxidation is initiated by carbon abstraction of hydrogen by the free radical, leading to the formation of a carbon-centered radical. In the presence of oxygen, this radical is converted to a peroxyl radical. This can lead to the formation of an alkoxyl radical and subsequent hydroxylation of the peptide backbone. The oxidation of amino acid side chains greatly depends on their structure. An important oxidative process with profound functional and structural consequences involves the irreversible nitration of tyrosine residues by peroxynitrite ($ONOO^-$) *(16)*. The levels of protein oxidation in membrane systems can be indirectly monitored by the use of electron paramagnetic resonance (EPR) spin-labeling techniques *(17)*. The changes in protein conformation resulting from protein oxidation are deduced from the relevant EPR parameter.

Oxidative Stress and PD

Parkinson's disease (PD) is a progressive neurodegenerative disorder and the hallmark of this disease is selective loss of dopaminergic neurons in the substantia nigra pars compacta *(18)*. Recently, the death of dopaminergic neurons has been reported to occur by apoptosis *(19–21)*. Oxidative stress has been widely believed to be an important pathogenetic mechanism of neuronal apoptosis in PD *(22)*. Overproduction of reactive oxygen species (ROS) can lead to oxidative damage in the brain of PD, as shown by increased lipid peroxidation and DNA damage in the substantia nigra. Increased protein oxidation is also apparent in many areas of the brain, whereas substantia nigra is particularly vulnerable *(23)*. Under physiological conditions, 6-hydroxydopamine (6-OHDA) is rapidly and nonenzymatically oxidized by molecular oxygen to form hydrogen peroxide (H_2O_2) and the corresponding *p*-quinone *(24)*. The former can react with iron(II) to form the reactive and damaging hydroxyl free radical. The latter then undergoes an intramolecular cyclization, followed by a cascade of oxidative reactions resulting in the formation of an insoluble polymeric pigment related to neuromelanin *(25)*.

Oxidative Stress and Stroke

Stroke is a major cause of death and disability in the world; it is the third leading cause of death and the primary cause of long-term disability in

adults. ROS generated from the respiratory chain in mitochondria, ischemia-activated xanthine/hypoxanthine oxidase, and lipid fatty acid metabolism play an important role in the brain ischemia–reperfusion process *(26,27).* Because of the high rate of oxidative metabolic activity, high content of polyunsaturated fatty acids, relatively low antioxidant capacity, low repair activity, and nonreplicating nature of the neuronal cells, the brain is very susceptible to the damage caused by oxygen radicals generated during ischemia–reperfusion *(28).* In the cerebral circulation system, the burst in the production of ROS damages the endothelium cell and smooth muscle cell, induces blood platelet aggregation and vascular permeability changes, and results in edema *(29,30).*

Natural Antioxidants in Neurodegenerative Diseases

Nerve cell death from oxidative stress has been implicated in a variety of pathologies, including stroke, trauma, and neurodegenerative diseases such as AD and PD. Efforts have been made to investigate the effects of antioxidants on preventing the above-mentioned diseases, which involve oxidative stress. Here, we discuss the effects of natural antioxidants, including vitamins E and C, polyphenols, and flavonoids in preventing neurodegenerative diseases.

Vitamins E and C

Vitamins C and E are well-known antioxidants and widely used to treat patients. Both animal and clinical studies suggest that vitamin E deficiency contributes to nigral neurodegeneration and to the onset or progress of PD *(31).* It was found that pretreatment with large doses of vitamin E (1000 mg/kg) or carotene (200 mg/kg), prevented loss of glutathione (GSH) and partially protected the dopaminergic nigrostriatal neurons from mice receiving one injection of 40 mg/kg *N*-methyl-1,2,3,6 tetrahydropyridine (MPTP) *(32,33).*

The hypothesis that oxidative stress is implicated in the pathogenesis of AD prompted a large double-blind, placebo-controlled, randomized multicenter clinical trial with 2000 IU/d of vitamin E in 341 AD patients. The treatment was found to delay functional deterioration in moderately impaired AD patients *(34).* In another perspective study, 91 disease-free persons over the age of 65 yr were selected. After an average follow-up period of 4.3 yr, none of the 27 vitamin E users had AD, compared with the 2.5 predicted on the basis of age, sex and years of education (p = 0.03). None of the 23 vitamin C users had AD compared with 3.2 predicted (p = 0.04). These data suggest that the intake of high doses of vitamin E and C supplements could reduce the risk of AD *(35).*

Exposure of neuronal cells to NO donor, *S*-nitrosoglutathione (GSNO, 250 μ*M*) or sodium nitroprusside (SNP, 500 μ*M*) induced apoptosis in immature cultures of the cerebellar granule cell, which was characterized by chromtin condensation, nuclear fragmentation, and DNA laddering. Exposure of neuronal cells to a NO donor led to a decrease in the mitochondrial transmembrane potential and intracellular ATP content, which suggested that NO treatment caused mitochondrial dysfunction. However, pretreatment with free-radical scavengers L-ascobic acid {2-[3,4-dihydro-2,5,7,8- tetramethyl-2-(4,8,12-trimethyltridecy)-2H-1-benzopyran-6yl-hydrogen phosphete]} potassium salt (EPC-K1), a combination of vitamin E and vitamin C, attenuated NO induced mitochondrial dysfunction and oxidative stress and protected the cells from apoptosis effectively. This result suggests that superoxide/peroxynitrite-mediated oxidative stress might be an important pathway leading to NO-associated neuronal damage and antioxidant EPC-K1-attenuated NO-induced neurotoxicity by scavenging the ROS and its breakdown byproducts *(36,37).* In other study, we found that EPC-K1 could scavenge hydroxyl radicals, alkyl radicals, and lipid radicals. In a comparison with Trolox and vitamin C, EPC-K1 showed better overall antioxidative capacity in vitro and in

vivo. EPC-K1 was a moderate scavenger on hydroxyl radicals and alkyl radicals, a potent scavenger on lipid radicals, and an effective inhibitor on lipid peroxidation. EPC-K1 could react with hydroxyl radicals with a rate constant of 7.1×10^8 $dm^3/mol/s$ and could react with linoleic acid radicals, with a rate constant of $2.8 \times 10^6 dm^3/mol/s$. After administration of EPC-K1, the ability of the rat brain to scavenge superoxide radicals was significantly increased. The potent scavenging effects of EPC-K1 on both hydrophilic and hydrophobic radicals were relevant in its molecular structure *(38).*

Flavonoids

Flavonoids isolated from plants are well investigated as natural antioxidants and widely used to treat patients as effective components of many medical drugs in clinics. To determine the potential protective mechanisms of flavonoids in cell death, the mouse hippocampal cell line HT-22 was used as a model. It was found that exogenous glutamate inhibits cystine uptake and depletes intracellular GSH, leading to the accumulation of ROS and an increase in Ca^{2+} influx, which ultimately causes neuronal death. Many, but not all, flavonoids protect HT-22 cells and rat primary neurons from glutamate toxicity as well as from five other oxidative injuries. Three structural requirements of flavonoids for protection from glutamate are the hydroxylated C3, an unsaturated C ring, and hydrophobicity. Three distinct mechanisms of protection were also found. These include increasing intracellular GSH, directly lowering levels of ROS, and preventing the influx of Ca^{2+} despite high levels of ROS. These data show that the mechanism of protection from oxidative insults by flavonoids is highly specific for each compound *(39).*

Ginkgo biloba extract (EGb), which contains flavonoids and biolobalids, has been reported to protect the brain against hypoxic damage and inhibit ROS formation in cerebellar neurons. We investigated the protective effects of EGb on dissociated cortex neurons from damage caused by ROS using spin label and molecular techniques. It was found that the order parameter *(S),* rotational correlation time (τ) and the ratio of strong immobilized component to weak immobilized component (S/W) of the cell membrane treated with hydroxyl radical were higher than those of control ($p < 0.05$), indicating that the membrane fluidity attacked by the free radical was lower than that of the control. With an increase of EGb concentration (5–50 μg/mL), a dose-dependent membrane fluidity increased in hydrophobic areas of the membrane. EGb also protected the change of the protein conformation on the membrane caused by the free radical. The lactic dehydrogenase activity and cell apoptosis attacked by the hydroxyl radical were higher than that of the control ($p < 0.05$). EGb (50 μg/mL) was found to have a protective effect on the cells attacked by free radicals *(40).*

The protective effect of EGb761 and its active constitutes against apoptosis were also examined and the results showed that hydroxyl radicals generated by the Fenton reaction induced apoptosis in cerebellar granule cells, which was associated with the decrease in Bcl-2 mRNA level and the increase in the protein levels and caused the changes in the sulfhydryl group binding sites on the membrane proteins, and its different constitutes showed different effects. The total flavonoid components of EGb761 and a mixture of flavonoids and terpens protected the cerebellar granule cell from oxidative damage and apoptosis induced by hydroxyl radicals. The total terpens of EGb761 did not protect against apoptosis. Flavonoids and terpens did show a synergic effect in this regard *(41,42).*

Green Tea Polyphenols

Green tea polyphenols and their major constituents, such as (–)-epigallocatechin-3-gallate (EGCG), have diverse pharmacological activities, such as antimutagenic and anticarcinogenic effects. It is believed that these beneficial effects of green tea polyphenols are the result of their potent antioxidative properties. In fact,

it was demonstrated that green tea polyphenols serve as powerful antioxidants against free radicals such as 1,1-diphenyl-2-picryl-hydrazyl (DPPH) radicals *(43),* superoxide anion *(44–46),* lipid free radicals, and hydroxyl radicals *(47,48).* In the central nervous system (CNS), there is also some evidence to show that oral administration of green tea polyphenols and flavonoid-related compounds has preventive effects on iron-induced lipid peroxide accumulation and age-related accumulation of neurotoxic lipid peroxides in the rat brain *(49,50).*

Tea catechins (TCs) are usually expected to be scavengers of free radicals, but different components have different functions. Here, we studied the effect of TCs on the PC12 cells exposed to 6-OHDA and it was demonstrated that TC could protect PC12 cells against apoptosis caused by 6-OHDP. We investigated the effects of exposure of PC12 cells to 6-OHDA alone or associated with pretreatment with TC. Exposure of PC12 cells to 6-OHDA induced a concentration-dependent decrease in cell viability determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl (MTT) assay and apoptosis of PC12 cells observed by flow cytometry, fluorescence microscopy, and DNA fragmentation technique. TCs displayed significantly inhibitory effects against PC12 cell death. EGCG and (-)-epicatechin gallate (ECG) were more effective than TC, but (-)-epigallocatechin (EGC), (-)-epicatechin (EC), and (+)-C were less effective *(51).* 6-OHDA-induced apoptosis was greatly inhibited by green tea polyphenols at 200 to 400 μ*M.* From 50 to 400 μ*M,* the protective effects increased with the concentrations and EGCG was better than green tea polyphenols at the same concentrations. From the flow cytometry data, the apoptotic cells were inhibited substantially by 200 to 400 μ*M* of green tea polyphenols and EGCG (the inhibitory ratios of green tea polyphenols were 83.1% and 84.8%; of EGCG, they were 88.3% and 90.3%, respectively). The nuclear changes characteristic of apoptosis disappeared, especially in the EGCG-protected PC12 cells. The DNA ladder also disappeared in the channels of 200 to 400 μ*M* of green tea polyphenols and EGCG *(52).*

In an animal study, Mandel's group demonstrated the neuroprotective property of green tea extract and ECG in the MPTP-treated mice model of PD. MPTP neurotoxin caused dopamine neuron loss in substantia nigra concomitant with a depletion in striatal dopamine and tyrosine hydroxylase protein levels. Pretreatment with either green tea extract (0.5 and 1 mg/kg) or ECG (2 and 10 mg/kg) prevented these effects. In addition, the neurotoxin caused an elevation in striatal antioxidant enzymes superoxide dismutase (SOD) (240%) and catalase (165%) activities, both effects being prevented by ECG. ECG also increased the activities of both enzymes in the brain. The neuroprotective effects are not likely to be caused by the inhibition of MPTP conversion to its active metabolite 1-methyl-4-phenylpyridinium by monoamine oxidase-B, as both green tea and ECG are very poor inhibitors of this enzyme in vitro (770 mg/mL and 660 m*M,* respectively). The brain-penetrating property of polyphenols, as well as their antioxidant and iron-chelating properties, might make such compounds an important class of drugs to be developed for treatment of neurodegenerative diseases where oxidative stress has been implicated *(53).*

They demonstrated highly potent antioxidant-radical scavenging activities of green tea (GT) and black tea (BT) extracts on brain mitochondrial membrane fraction, against iron (2.5 μ*M*)-induced lipid peroxidation. Both extracts (0.6–3 μ*M* total polyphenols) were shown to attenuate the neurotoxic action of 6-OHDA-induced neuronal death. 6-OHDA (350 and 50 μ*M*) activated the iron-dependent inflammatory redox-sensitive nuclear factor (NF)-κB in PC12 and SH-SY5Y cells, respectively. Immunofluorescence and electromobility shift assays showed increased nuclear translocation and binding activity of NF-κB after exposure to 6-OHDA in SH-SY5Y cells, with a concomitant disappearance from the cytoplasm. Introduction of GT extract (0.6 and 3 μ*M* total polyphenols) before 6-OHDA inhibited both NF-κB nuclear translocation and binding activity induced by this toxin in SH-SY5Y cells.

Neuroprotection was attributed to the potent antioxidant and iron-chelating actions of the polyphenolic constituents of tea extracts, preventing nuclear translocation and activation of cell death promoting NF-κB. *(54)*

Mandel's group also demonstrated that EGCG restored the reduced protein kinase C (PKC) and extracellular signal-regulated kinases (ERK1/2) activities caused by 6-OHDA toxicity. However, the neuroprotective effect of EGCG on cell survival was abolished by pretreatment with PKC inhibitor GF 109203X (1 μ*M*). Because EGCG increased phosphorylated PKC, they suggest that PKC isoenzymes are involved in the neuroprotective action of EGCG against 6-OHDA. In addition, gene expression analysis revealed that EGCG prevented both the 6-OHDA-induced expression of several mRNAs, such as Bax, Bad, and Mdm2, and the decrease in Bcl-2, Bcl-w, and Bcl-xL. These results suggest that the neuroprotective mechanism of EGCG against oxidative-stress-induced cell death includes stimulation of PKC and modulation of cell survival/cell cycle genes *(55).*

Green tea polyphenols (GTPs) are usually expected to be a potent chemopreventive agent because of their scavenging free radicals and chelating metal ions ability. However, not all of the actions of GTPs are necessarily beneficial. We demonstrated that a high concentration of GTPs significantly enhanced the neurotoxicity by treatment of sodium nitroprusside (SNP), a nitric oxide donor. SNP induced apoptosis in human neuroblastoma SH-SY5Y cells in a concentration- and time-dependant manner as estimated by cell viability assessment, FACScan analysis, and DNA fragmentation assay, whereas treatment with GTPs alone had no effect on cell viability. Pretreatment with low-dose GTPs (50 and 100 μ*M*) had only a slightly deleterious effect in the presence of SNP, whereas high-dose GTPs (200 and 500 μ*M*) synergistically damaged the cells severely. Further research showed that coincubation of GTPs and SNP caused loss of mitochondrial membrane potential, depletion of intracellular GSH, and accumulation of reactive oxygen species and exacerbated NO-induced neuronal apoptosis via a Bcl-2-sensitive pathway *(56).*

Genistein

Genistein, the most active component of soy isoflavone, has been investigated to have a affinity to estrogen receptor *(57),* antioxidation *(58,59),* increased cellular reduced glutathione *(60),* inhibiting protein tyrosine kinase (PTK) *(61)* and has other physiological functions *(62).* The existing data strongly suggest that the soy isoflavones have a protective action against several chronic diseases such as atherosclerosis *(63),* the diseases associated with postmenopausal estrogen deficiency, and hormone-dependent breast and prostate cancers *(64).* Recently, some researchers found that genistein showed neuroprotection. Andersen et al. *(65)* reported that genitein protected rat brain synatosome from insult induced by Aβ25–35. Genistein, a phytoestrogen capable of crossing the blood–brain barrier *(66),* has been reported to have antioxidantion from insult from ultraviolet light *(67)* and chemicals *(59).*

We studied the neuroprotective effect of genistein against Aβ25–35-induced apoptosis in cultured hippocampal neurons *(68).* It was found that exposure to aged Aβ25–35 for 24 h increased the DCF fluorescence intensity twofold relative to controls. The increase in DCF fluorescence intensity was eliminated by 63% when cotreated with genistein at 40 μ*M* and aged Aβ25–35, whereas genistein at 0.1 μM decreased about 18% of the production of ROS induced by Aβ25–35. Therefore, genistein at a high concentration has stronger antioxidative activities in comparison with a low concentration in the prevention of neuronal cell death induced by Aβ25–35. Treatment with 25 μg/mL aged Aβ25 to 35 for 24 h decreased the viability of hippocampal neuronal cells about 43.3% compared to controls. Genistein at concentrations of 0.1 and 40 μ*M* rescued aged Aβ25–35-induced decrease of viability rate by 7.2 and 13.9%, respectively. The estrogen receptor (ER) antagonist, ICI182,780, significantly blocked the protec-

tive effect of genistein at a concentration of 0.1 μ*M*, whereas it had little effect on genistein at a concentration of 40 μ*M*. ICI182,780 did not affect aged Aβ25 to 35-induced decrease of cell viability. In addition, no difference was seen on the cells' viability when the cells were treated with genistein (0.1 or 40 μ*M*) alone compared to controls. Treatment with 25 μg/mL aged Aβ25 to 35 for 24 h decreased the viability of hippocampal neuronal cells about 43.3% compared to controls. It can concluded that Aβ25–35-induced apoptosis and all of these phenotypes induced by Aβ25–35 are reversed by genistein. Our results further show that at the nanomolar level, genistein protects neurons from Aβ25–35-induced damages largely via the ER-mediated pathway, and at the micromolar level, the neuroprotective effect of genistein is mainly mediated by its antioxidative properties.

Crataegus Flavonoids

Crataegus is one of the oldest medicinal plants and is described by many pharmacopoeias. Crataegus extracts (from leaves with flowers) has been used to treat the early stages of congestive heart failure and angina pectoris *(69)*. Evidences show that Crataegus extracts (from several parts of the plant, including leaves) has an antioxidant effect in vitro or in vivo, Crataegus extracts scavenges superoxide, hydroxyl, and hydrogen peroxides and inhibits lipid peroxidation *(70)*. In vivo experiments show that Crataegus extracts (from fruit) increases the concentration of α-tocopherol and inhibits the oxidation of human low-density lipoprotein (LDL) *(71)*.

We studied the preventing effect of Crataegus flavonoids (CF) from the leaves of Crataegus on the damage of ischemia–reperfusion in the Mongolian gerbil *(72)*. One hour following 5 min ischemia, ROS trapped by *N*-tert-butyl-a-phenylnitrone (PBN) in the brain homogenate significantly increased by about 36.89% in the ischemia/reperfusion (IR) group compared with the sham group. Pretreatment with CF for 15 d, compared to the IR group, significantly decreased ROS by about 17.37 and 31.14% respectively in the low-dose group (32.5 mg/kg/d) and high-dose group (167 mg/kg/d). ROS in the high-dose group were even lower than that in the sham group. Contrasted with ROS, NO trapped by diethyldithiocarbamate (DETC)–ferrous complex significantly decreased by about 19.17% in the IR group compared with the sham group. Compared with the IR group, NO significantly increased about 44.67 and 77.56% in low- and high-dose groups with pretreatment with CF for 15 d, respectively. The NO concentrations in those groups were also significantly higher than that of the sham group.

IR insult significantly increased the TBARS content in the brain homogenate by about 74.04% compared with that of sham group. Although pretreatment with CF decreased the elevation of lipid peroxidation caused by IR in a dose-dependent manner, decreases of 24.25 and 47.39% were observed in the low- and high-dose group, respectively. The TBARS content in the high-dose group was even lower than that in the sham group. The nitrite/nitrate content in the brain homogenate was determined and it was found that IR insult significantly increased the nitrite/nitrate content by about 122.21%, and pretreatment with CF significantly decreased the elevation of nitrite/nitrate content by about 20.13 and 34.61% in the low- and high-dose group, respectively.

The brain homogenate-associated antioxidant level was estimated by determining the scavenging ability on superoxide and hydroxyl free radicals. The scavenging ability on superoxide anion of homogenate from the IR group was weaker by 35% compared to the sham group. Pretreatment with CF increased the scavenging ability, compared with IR group, the ability significantly increased by about 21.28 and 46.81% in the low- and high-dose group, respectively. The antioxidant level from the high-dose group was even significantly higher than that of the sham group. The group subjected to IR had weaker hydroxyl scavenging ability than that of sham group about 29% ($p < 0.05$). Compared with the IR group, pretreatment with CF increased the scavenging

ability on hydroxyl by about 9.5% for the low-dose group ($p > 0.05$) and 30% for high-dose group ($p < 0.05$). The antioxidant level of the high-dose group was even significantly higher than that of the sham group.

The CA1 region of the hippocampus is susceptible to the transient ischemic insult. We assessed cell survival in the CA1 region of the hippocampus with Nissl staining and the photographs of coronal sections containing the hippocampal CA1 region obtained 6 d after ischemia–reperfusion insult. In the CA1 region from the IR group, the pyramidal cells have almost completely disappeared. There were 12 ± 7 cells/mm in the CAI region from the IR group, whereas there were 270 ± 30 cells/mm in the CA1 region from the sham group. In contrast to the IR group, pretreatment with CF significantly increased the number of survival pyramidal cells in the CA1 region in a dose-dependent fashion (129 ± 64 cells/mm for the low-dose group, 254 ± 35 cells/mm for the high-dose group). Apoptosis in the CA1 region of the hippocampus after IR insult was detected by the TUNEL method and it was found the pyramidal cells of the CA1 region from the sham group were TUNEL-negative, whereas most of the pyramidal cells of the CA1 region from the IR group were TUNEL-positive. The neuronal dendrites of some pyramidal cells were TUNEL-positive staining, which suggested the transportation of the fragmented DNA from the nuclei to the neuronal fiber terminal. Pretreatment with CF decreased the TUNEL-positive neuron in a concentration-dependent manner.

The outcome of transient ischemic insult was also examined by transmission electron microscopy after ischemic insult for 3 d. The nuclei of the pyramidal cells in the CA1 region from the sham group are normal; DNA evenly disperses in the nucleus, whereas most of the nucleus of the corresponding region from the IR group shrunk and the DNA condensed around the nucleus membrane. Some of the pyramidal cells of the CA1 region from the low-dose group suffered the same process as the IR group. The cells from the high-dose group were similar to that of the sham group. The results suggests that pretreatment with CF protects the pyramidal cells in the CA1 region from IR damage.

The reverse transcription–polymerase chain reaction (RT-PCR) products from total RNA of iNOS from the hippocampus were analyzed by electrophoresis with ethidium bromide in agarose gel. A single band was detected in RT-PCR products from RNA of the iNOS. It was found that levels of mRNA in sham group are very weak, ischemia–reprfusion enhanced the levels, and pretreatment with CF decreased the levels in hippocampus, suggesting that the enhancement of NO free radicals detected in the ischemia–reperfusion might be generated by enhanced iNOS and the free radical decrease in the pretreated animal might come partly from the inhibition effect of CF on iNOS.

The expression of NF-κB p65 and tumor necrosis factor (TNF)-α was investigated by Western blot in the hippocampus of the animal. It was found that the expression of NF-κB p65 was enhanced by about 75% by ischemia–reperfusion and pretreatment of CF decreased the expression in a dose-dependent manner. There was a similar result for TNF-α; the enhancement of TNF-α reached about 50% and pretreatment by CF significantly decreased the expression of TNF-α especially for the higher-dose group.

Conclusion

It is difficult to cure these patients after they have these diseases, so the best way is to protect people from neurodegenerative diseases. This review suggests that some natural antioxidants can prevent neurodegenration, which might open a new way to protect against oxidative-stress-related disease. It is easy to take the natural antioxidants from drinking tea and soybean milk and eating fresh fruits and vegetables every day.

Acknowledgment

This work was supported by a grant from the National Science Foundation of China (29935080).

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