Targeting Multiple Neurodegenerative Diseases Etiologies with Multimodal-Acting Green Tea Catechins

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Abstract

Green tea is currently considered a source of dietary constituents endowed with biological and pharmacological activities relevant to human health. Human epidemiological and new animal data suggest that the pharmacological benefits of tea drinking may help protect the brain as we age. Indeed, tea consumption is inversely correlated with the incidence of dementia and Alzheimer’s and Parkinson’s diseases. In particular, its main catechin polyphenol constituent (−)-epigallocatechin-3-gallate has been shown to exert neuroprotective/neurorescue activities in a wide array of cellular and animal models of neurological disorders. The intense efforts dedicated in recent years to shed light on the molecular mechanisms participating in the brain protective action of green tea indicate that in addition to the known antioxidant activity of catechins, the modulation of signal transduction pathways, cell survival/death genes, and mitochondrial function all contribute significantly to the induction of neuron viability. Because of the multitietiological character of neurodegenerative disease pathology, these natural compounds are receiving significant attention as therapeutic cytoprotective agents that simultaneously manipulate multiple desired targets in the central nervous system. This article elaborates on the multimodal activities of green tea polyphenols with emphasis on their recently described neurorescue/neuroregenerative and mitochondrial stabilization actions. J. Nutr. 138: 1578S–1583S, 2008.

Introduction

Despite the lack of well-controlled clinical trials with tea polyphenols in neurodegenerative diseases, human epidemiological and new animal data suggest that the pharmacological benefits of tea drinking may help protect the brain as we age. Indeed, tea consumption is inversely correlated with the incidence of dementia, Alzheimer’s disease (AD), and Parkinson’s disease (PD), which may explain why there are significantly lower rates of age-related neurological disorders among Asians than in Europeans or Americans (1). In a cross-sectional study conducted in Japan aimed at investigating the association between consumption of green tea and cognitive function in elderly Japanese subjects, it was found that consumption of 2 or more cups/d (100 mL/cup) of green tea is associated with lower prevalence of cognitive impairment (2). In a case-control study in the United States, it was found that people who consumed 2 cups/d or more of tea presented a decreased risk of PD (3). In support of this finding, a recent prospective cohort study of nearly 30,000 Finnish adults aged 25–74 y followed for 13 y found that drinking 3 or more cups (200 mL/cup) of tea is associated with a reduced risk of PD (4). These findings emphasize the importance of well-designed controlled studies to assess risk reduction for PD and AD in consumers of green and black tea. The Michael J. Fox Foundation has awarded a grant to the team of Piu Chan from Xuanwu Hospital, Beijing, China, to carry out the first-ever...
multicenter, double-blind, randomized, placebo-controlled study to investigate the safety, tolerability, and potential neuroprotective effects of green tea polyphenols in patients with early PD.

Intense efforts have been dedicated during the past 5 y to shed light on the molecular mechanisms and cell-signaling pathways participating in the neuroprotective/neuroregenerative action of green tea. The emerging data indicate that the antioxidant/metal-chelating attributes of the catechin polyphenols are unlikely to serve as the sole explanation for their neuroprotective and neurorescue capacity. This article presents the state of the art in the molecular mechanisms and cell-signaling pathways implicated in the neuroprotective action of green tea catechins, with emphasis on their recently described neurorescue effect and mitochondrial stabilization potency.

Etiopathology of neurodegenerative diseases

Neurodegenerative disorders are progressive diseases of the nervous system involving damage or loss of neurons in the brain and/or spinal cord, which can occur at any time of life. Neurodegeneration in PD or AD or other neurodegenerative diseases, such as Huntington disease and amyotrophic lateral sclerosis, appears to be multifactorial, where a complex set of toxic reactions lead to the demise of neurons (5,6). Common features involve impairment of protein handling and aggregation associated with dysfunction of the ubiquitin-proteasome system, depletion of endogenous antioxidants, reduced expression of trophic factors, inflammation, glutamate excitotoxicity, expression of proapoptotic proteins, and increases of iron and nitric oxide leading to oxidative-stress (OS) damage (7–9). An unresolved question, however, is to determine which of these factors constitute the primary event, the sequence in which they act, and where the point of convergence is or the final pathway by which the predisposed neuronal cell types die in the affected brain areas. Because of the multietiological character of the pathologies, novel therapeutic neuroprotective strategies support the idea that simultaneous manipulation of multiple desired targets in the central nervous system will exert higher therapeutic effectiveness (10,11). Thus, it is not surprising, that green tea catechins have attracted increasing interest as therapeutic agents for the treatment of neurological disorders because of their broad spectrum of biological/pharmacological activities, including cardiovascular, antiinflammatory, and anti-carcinogenesis effects (12–14) and, more recently recognized, anti-diabetic (15,16), antiobesity (17), and neuroprotective/neurorestorative properties (18).

Neuroprotection/neurorescue by green tea polyphenols

There is a growing recognition that polyphenolic catechins exert a protective role in neurodegeneration. The neuroprotective effect has been long established in animal models of neurological disorders: (−)-epigallocatechin-3-gallate (EGCG), the major polyphenol component of green tea, has been shown to improve age-related cognitive decline and to protect against cerebral ischemia/reperfusion injuries (19,20) and brain inflammation and neuronal damage in experimental autoimmune encephalomyelitis (21). Furthermore, the treatment of EGCG significantly prolonged the symptom onset and life span and attenuated death signals in a mouse amyotrophic lateral sclerosis model with the human G93A-mutated Cu/Zn-superoxide dismutase (SOD) gene (SOD1) (22). Similarly, a green tea polyphenol extract or isolated EGCG prevented striatal dopamine (DA) depletion and substantia nigra dopaminergic neuron loss when given chronically to mice treated with the parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (23). More recently, long-term administration of a prepare of green tea catechins (polyphenol E) or EGGC has been demonstrated to improve spatial cognition and learning ability in rats (24) and to reduce cerebral amyloidosis in Alzheimer’s transgenic mice, respectively (25).

In line with the in vivo findings, cell culture studies have demonstrated that green tea catechins prevented neuronal cell death caused by the neurotoxins 6-hydroxodopamine (6-OHDA), 1-methyl-4-phenylpyridinium and amyloid β-peptide (Aβ) (26–29). More recently, EGGC was reported to exert a neurorescue activity in long-term serum-deprived rat pheochromocytoma (PC12) cells and to promote neurite outgrowth (30). It remains to be established whether there is any mechanistic relation between survival and differentiation induced by EGCG and also to what extent the in vitro findings could be replicated in vivo. To test this assumption, we have examined the possible neurorescue/neurorestorative activity in a post-MPTP-induced nigrostriatal DA neurodegeneration model of PD in mice. MPTP (20 mg/kg, i.p., per d) was administered for 4 d, followed by a further 4-d resting period, and, at d 8, EGCG (5 mg/kg or water) was administered orally, over a total treatment period of 22 d. MPTP caused a significant reduction in viability of tyrosine-hydroxylase-positive cells, whereas oral EGCG administration post-MPTP resulted in a substantial recovery of the neurons. Current experiments are in progress to determine effective doses and duration of treatment. Thus, the neurorescue action of EGCG may suggest a potential disease-modifying effect for the drug, similar to what has been recently described for the novel anti-Parkinson drug rasagiline (N-propargyl-L-(R)-aminoindan), a second-generation selective inhibitor of monoamine oxidase-B (31).

Molecular mechanisms of neuroprotective/neurorescue action of EGCG

The protein kinase C pathway. Emerging evidence suggests that the biological actions of green tea catechins relate not simply to their antioxidant/radical-scavenging potential but also to the modulation of various protein kinase signaling pathways. Our recent in vitro cell-signaling studies on the neuroprotective mechanistic action of EGCG revealed a specific involvement of protein kinase C (PKC) (26,32), a family of serine/threonine kinases consisting of 11 isoforms, which are divided into 3 subclasses: conventional (α, β, γ), novel (δ, ε, θ, η, μ), and atypical [i (mouse)/λ (human), γ] (33). PKC has a fundamental role in the regulation of cell survival, programmed cell death, long-term potentiation (34), and consolidation of different types of memory (35,36). Indeed, it has been suggested that pharmacological interventions aimed at modulating specific PKC isozymes or PKC-mediated signal transduction pathways may constitute a potential therapeutic tool for senescence or age-related pathologies that are responsible for memory disturbances (37). The induction of PKC activity in neurons is thought to be a prerequisite for neuroprotection against several exogenous insults. Indeed, PKCe activation after ischemic preconditioning or pharmacological preconditioning (with either PKCe, NMDA, or A1AR agonists) was shown to be essential for neuroprotection against oxygen/glucose deprivation in organotypic slice cultures (38). In accordance, activation of PKC by estrogen or by the grape flavonoid resveratrol protected rat cortical or hippocampal neurons against Aβ toxicity, respectively (39,40).

PKC activation by EGCG prevents apoptosis and mitochondrial membrane potential collapse. A rapid phosphorylative activation of PKC by EGCG is thought to be the main
mechanism accounting for its neuroprotective activity against several neurotoxins such as Aβ (28), serum withdrawal (30,41), and 6-OHDA (26) and for its neurorescue effect against long-term growth factor withdrawal (30). In addition, EGCG induced a rapid translocation of the isoform PKCa to the membrane compartment in response to EGCG in human astroglia or rat PC12 cells (30,42). This isoform is particularly important in neuronal growth and differentiation in the brain. These findings are supported by animal studies showing that a 2-wk oral consumption of EGCG prevented the extensive depletion of PKCa isoform and counteracted the robust increase of Bax protein in the striatum and the dopaminergic neurons of the substantia nigra pars compacta of mice intoxicated with MPTP, respectively (43).

Recently, we identified a novel pathway in the neuroprotective mechanism of action of EGCG that involves a rapid PKC-mediated degradation of the Bad protein by the ubiquitin-proteasome system and a more pronounced reduction after 24 h in cell culture (32). Bad may directly contribute to the opening of the mitochondrial megachannel permeability transition pore by its heterodimerization with the mitochondrial death suppressor proteins Bcl-2 and/or BclXL, thus neutralizing their antiapoptotic function (44). Indeed, we have recently found that the administration of EGCG for 30 min prevented the dissipation of the mitochondrial membrane potential, ΔΨm, induced by short-term (4 h) exposure to 6-OHDA (data not presented). This involved activation of the PKC signaling pathway because pretreatment with the pharmacological general PKC inhibitor GF109203X blunted the protective effect of EGCG on ΔΨm.

**PKC activation by EGCG is beneficial for AD and PD.** Neuronal amyloidosis in AD is characterized by extracellular deposition of Aβ peptide, derived from proteolytic cleavage of amyloid precursor protein (APP), a type I integral membrane protein. APP can be processed via alternative pathways: a nonamyloidogenic secretory pathway includes cleavage of APP to sAPPα by a putative α-secretase within the sequence of the amyloidogenic Aβ peptide, thus precluding the formation of Aβ, whereas the formation of Aβ is regulated by the sequential action of β- and γ-secretases (45). Our pioneer studies have demonstrated that either short- or long-term incubation with EGCG promotes the generation of the nontoxic sAPPα via PKC-dependent activation of α-secretase (28,46). New supportive data came from a study conducted in an Alzheimer’s transgenic mouse model, showing that EGCG promotes sAPPα generation through activation of α-secretase cleavage (25). This was accompanied by a significant reduction in cerebral Aβ levels and β-amyloid plaques.

Another potential beneficial effect of PKC activation in AD is related to the recent finding that neuronal overexpression of PKCζ in transgenic mice expressing familial AD mutant forms of the human APP decreases Aβ levels and plaque burden, and this is accompanied by increased activity of endothelin-converting enzyme, which degrades Aβ (47). Because EGCG has been shown to increase the levels of PKC isoforms α and ε in mouse hippocampus and striatum (28,43), it can be hypothesized that in AD pathology, EGCG may reduce Aβ levels, both via concomitant stimulation of sAPPα secretion and promotion of Aβ clearance through increased endothelin-converting enzyme activity.

In PD, a possible beneficial effect of green tea polyphenols maybe related to the increased internalization of the DA presynaptic transporters (DAT) by EGCG, eventually resulting in a rise in the synaptic DA level. This effect was mimicked by phorbol 12-myristate 13-acetate, a potent activator of PKC, and abolished by blockade of the PKC pathway (48), suggestive of a potential therapeutic target of PKC in the brain as a result of green tea intake. This observation, together with the finding that EGCG inhibited catechol-O-methyltransferase (COMT) activity at a low IC50 concentration (0.2 μmol/L) in rat liver cytosol homogenates (49), may be of particular significance for PD patients given that DA and related catecholamines are physiologically substrates of COMT. Indeed, COMT inhibitors entacapone and tolcapone, clinically prescribed to PD-affected individuals, dose-dependently inhibit the formation of the major metabolite of levodopa, 3-O-methylidopa, thereby improving its bioavailability in the brain (50).

**Other signaling pathways.** In addition to PKC, other cell-signaling pathways have been implicated in the action of green tea catechins, such as the mitogen-activated protein kinases (MAPK), phosphatidylinositol 3’-OH kinase/akt and protein kinase A signaling cascades, and cell calcium influx regulation [for review see Mandel et al. (18)]. These cascades have been shown to play central functions in neuronal protection against a variety of extracellular insults and to be essential for neuronal differentiation and survival (51,52). In general, flavonoids can activate MAPK signaling cascades in both neuronal and extraneuronal tissues and neutralize the decline in the mitogen and growth factor-induced extracellular signal-regulated kinase (ERK1/2) activity caused by exogenous OS-inducing agents (26,53). Low doses of (−)-epicatechin were recently shown to stimulate phosphorylation of the cAMP-response element binding protein, a regulator of neuronal viability and synaptic plasticity activity through both ERK1/2 and AKT in primary cortical neurons (54). Using the same cell culture conditions, this group of researchers demonstrated that activation/phosphorylation of both kinases was also involved in the antiapoptotic action of submicromolar concentrations of the flavanone hesperetin and its metabolite, 5-nitro-hesperetin (55). A number of flavonoids and phenolic antioxidants, at their respective low protective concentrations, were demonstrated to activate the expression of some stress-response genes, such as the phase II drug-metabolizing enzymes glutathione-S-transferase and heme-oxygenase-1, likely via activation of the MAPK pathway (56). Although EGCG had no effect on ERK1/2 phosphorylative levels in the absence of any exogenous damage, it was able to counteract the decline in ERK1/2 induced by 6-OHDA in neuroblastoma cells (26).

**Antioxidant and iron chelating activity of green tea polyphenols.** Tea catechins are powerful hydrogen-donating antioxidants and free radical scavengers of reactive oxygen and nitrogen species in in vitro systems (57–59). The neuroprotective effect of green tea polyphenols may also involve the regulation of antioxidant protective enzymes such as SOD and catalase in mouse striatum (23). In peripheral tissue, it has been shown that a number of flavonoids and phenolic antioxidants activate the expression of some stress-response genes such as the phase II drug-metabolizing enzymes glutathione-S-transferase and heme-oxygenase-1 in correlation with an increase in the activity and nuclear binding of the transcription factors Nrf1 and Nrf2 to the antioxidant regulatory element sequences contained in their promoters (60).

It is well established that iron progressively accumulates in the brain with age, as well as in those brain areas affected by neurodegenerative diseases, and is considered to be a major contributor to OS (7,61). Transcranial sonography has detected increased iron and decreased neuromelanin levels at the substantia nigra, even before the clinical manifestation of PD (62),...
Similarly, analysis of AD brains indicates iron accumulation within specific brain regions displaying selective vulnerability to neurodegeneration, such as the hippocampus and cerebral cortex (63,64), in particular in association with neurofibrillary tangles and Alzheimer’s Aβ-containing senile plaques, both considered central pathological hallmarks of AD.

These observations have formed the basis for the implementation of iron-complexing molecules that can cross the blood-brain barrier and possess neuroprotective/neurorestorative activities as a new therapeutic approach in neurologic disorders. Examples include the novel nontoxic lipophilic, brain-permeable multifunctional iron chelators HLA20 and M30, in which the N-propargylamine neuroprotective moiety of the antiparkinsonian drug rasagiline was incorporated into the skeleton of the prototype iron chelator 8-hydroxyquinoline derivative VK28 (Varinel, West Chester, PA) [for review see Youdim and Baccacuoso (65)]. Recent lines of research reported that several metal-binding natural antioxidants, including polyphenols of wine (e.g., resveratrol, myricetin, quercetin, kaemferol), curcumin, (+)-catechin, (−)-epicatechin, nordihydroguaiaretic acid, and rosmarinic acid inhibit formation of nascent Aβ and α-synuclein fibrils, elongation of the fibrils, and destabilization of the formed assemblies (66,67), suggesting a promising therapeutic approach of naturally occurring polyphenols for the treatment of neurodegenerative diseases.

In light of the multitietiological character of neurodegenerative disease pathology, novel pharmacological approaches suggest the use of antioxidant metal-chelating molecules possessing 2 or more active neuroprotective moieties that simultaneously manipulate multiple desired targets. A wealth of new data suggests that green tea catechins may well fulfill the requirements for a putative neuroprotective/neurotherapeutical prototype iron chelator 8-hydroxyquinoline derivative VK28-epicatechin, nordihydroguaiaretic acid, and rosmarinic acid might be responsible for the acute decrease in Bad protein (32) and regulation of DAT transporters (48) and elevation of sAPPα secretion (28). A proposed schematic model for the neuroprotective/neurorestorative effect by EGCG is illustrated in Fig. 1.

Collectively, the accumulated data support and extend the emerging view that green tea catechins are multimodal-acting, brain-permeable natural iron chelators-antioxidants endowed with polypharmacological activities and acting at multiple brain targets to prevent or delay neuronal death in the degenerating brain. The intervention with these compounds is assumed to have a profound impact on neuron preservation and disease progression. The presently described neurorescue/neurorestorative action of EGCG may well fulfill the requirements for a putative polyphenol.

Other articles in this supplement include references (68–77).

**Literature Cited**


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