Simultaneous Manipulation of Multiple Brain Targets by Green Tea Catechins: A Potential Neuroprotective Strategy for Alzheimer and Parkinson Diseases

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Current therapeutic approaches for Alzheimer and Parkinson disease (AD and PD, respectively) are merely symptomatic, intended for the treatment of symptoms, but offer only partial benefit, without any disease-modifying activity. Novel promising strategies suggest the use of antiinflammatory drugs, antioxidants, iron-complexing molecules, neurotrophic factor delivery, inhibitors of the amyloid precursor protein (APP)-processing secretases, gamma and beta (that generate the amyloid-beta peptides, Aβ), anti-Aβ aggregation molecules, the interference with lipid cholesterol metabolism and naturally occurring plant flavonoids to potentially reverse the course of the diseases. Human epidemiological and new animal data suggest that tea drinking may decrease the incidence of dementia, AD, and PD. In particular, its main catechin polyphenol constituent (-)-epigallocatechin-3-gallate (EGCG) has been shown to exert neuroprotective/neurorescue activities in a wide array of cellular and animal models of neurological disorders. In the current article, we review the literature on the impact of the multimodal activities of green tea polyphenols and their neuroprotective effect on AD and PD.

The consumption of tea dates backs to almost 50 centuries in China and India [1]. Standing after water, tea signifies the second most frequently consumed beverage worldwide, which varies its status from a simple ancient drink and a cultural tradition to a nutrient endowed with possible neurobiological-pharmacological actions beneficial to human health. In general, tea is consumed in the form of green tea, oolong tea, or black tea, all brewed from *Camellia sinensis*, a small plant grown mainly in China, Japan, and Southeast Asia. Preservation of the intact green leaf is of highest importance in the preparation of green tea. The favorable medicinal properties of green tea extract had been ascribed to its high content of polyphenolic flavonoids known as catechins. Catechins are especially concentrated in green tea, which account for 30–40% of the dry weight of the leaves [2,3]. Green tea is much richer in catechins than other beverages, and compared with black tea, it contains around four times more of the catechin fraction [4]. Among the tea catechins, (-)-epigallocatechin-3-gallate

(EGCG) is the major constituent, accounting for more than 10% of the extract dry weight; several other polyphenolic compounds found in lower abundance in green tea include (-)-epigallocatechin, (EGC) > (-)-epicatechin and (EC) $\geq$ (-)-epicatechin-3-gallate (ECG). All four tea catechins have been demonstrated to be potent antioxidants, resulting from their direct oxygen and nitrogen species scavenging properties, induction of endogenous antioxidant enzymes, and the capacity to bind and chelate excess of divalent metals, such as iron and copper (for reviews, see [5,6]).

Multiple lines of evidence, mostly from preclinical and epidemiological studies, suggest that green tea consumption is associated with a reduced risk of severe human malignancies such as cancer, cardiovascular diseases, and diabetes, which have been linked to the antioxidant/prooxidant properties of its polyphenol constituents [5,7–11]. More recently, either the green tea extract or its isolated catechin constituents have been reported to display neuroprotective/neurorestorative properties [6].
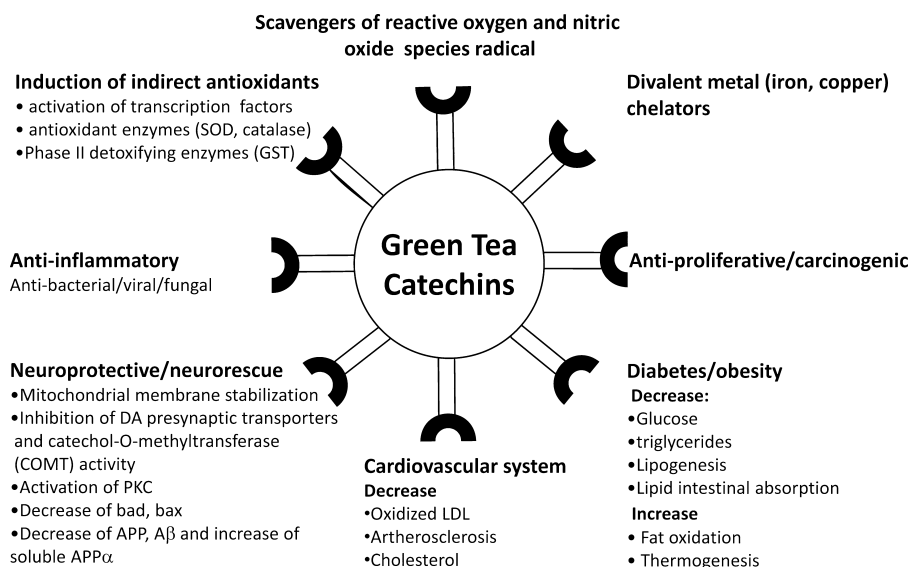
Figure 1. Multifunctional activities of green tea catechins. The diverse pharmacological activities of green tea polyphenols may account for their antioxidant, antiinflammatory, anticarcinogenic, and neuroprotective actions and possible benefits on diabetes and cardiovascular system.

In view of the diverse pharmacological activities of green tea catechins, there is a foundation to consider them as naturally occurring, multifunctional compounds for the treatment of different diseases, as depicted in figure 1.

In spite of the lack of systematic clinical trials with tea polyphenols in neurodegenerative diseases, human epidemiological and new animal data suggest that tea consumption inversely correlates with incidence of dementia, Alzheimer disease (AD), and Parkinson disease (PD). In elderly Japanese subjects, it was found that higher consumption of green tea is associated with a lower prevalence of cognitive impairment [12], and in the United States, people that consumed 2 cups/day or more of tea presented a decreased risk of PD [13]. In consensus, a recent prospective 13-year study of nearly 30,000 Finnish adults demonstrated that drinking three or more cups of tea is associated with a reduced risk of PD [14]. These findings emphasize the importance of well-designed controlled studies to assess risk reduction of PD and AD in consumers of green and black tea. Mechanistic studies intended to shed light on the multiple cell signaling pathways involved in the neuroprotective properties of green tea indicate that the antioxidant/metal-chelating attributes of catechin polyphenols alone are unlikely to be an adequate explanation for their neuroprotective and neurorescue capacity. This review discusses a scenario concerning the potential of natural, nontoxic green tea catechins to simultaneously manipulate multiple CNS targets as a novel neuroprotective strategy for AD and PD.

Histopathology of Neurodegeneration

Neurodegeneration in PD and AD or other neurodegenerative diseases, such as Huntington disease and amyotrophic lateral sclerosis (ALS), appears to be multifactorial, whereby several mechanisms are implicated in a cascade of events involving many biochemical and signaling pathways [15,16]. Common features involve impairment of protein handling and aggregation associated with dysfunction of the ubiquitin–proteasome system (UPS), depletion of endogenous antioxidants, reduced expression of trophic factors, inflammation, glutamatergic excitotoxicity, and induced expression of proapoptotic proteins and increase of iron and nitric oxide levels, leading to oxidative stress (OS) damage, [17–20]. The main neuropathological features of brains from AD sufferers are the amyloid or "senile" plaques consisting of extracellular deposition of insoluble amyloid-β (Aβ) peptide and intracellular neurofibrillary tangles (NFT), composed of hyperphosphorylated microtubule-associated protein-tau (PHFτ). PD is pathologically characterized by severe loss of substantia nigra (SN) dopaminergic neurons, observable as depigmentation of the SN in the midbrain. It is estimated that at the time of clinical diagnosis, approximately 60–70% of the SN dopamine (DA) cells are lost. Histologically, the main characteristic of PD is the Lewy body (LB), a cytoplasmic inclusion composed of several aggregated and/or phosphorylated proteins, including alpha-synuclein (α-synuclein), neurofilaments, and ubiquitinated proteins [21].

Iron content alteration has been described in brains of PD and AD patients, which may be caused, to a large degree, by endogenous dysregulation of iron uptake, transport, distribution, and storage [17,22–24]. Iron is one of the most essential transition metals involved in the formation of oxygen-free radicals, owing to its interaction with hydrogen peroxide through Fenton chemistry and generation of the aggressively reactive hydroxyl radical. Free radical-related OS causes molecular damage that can lead to a critical failure of biological functions and ultimately cell death [25,26]. Accumulation of iron, specifically in the SN pars compacta (SNpc) is one cardinal feature of PD [18] and is considered to be a major contributor to OS. Transcranial sonography has shown detection of increased iron and decreased neuromelanin levels at the SN, even before the clinical manifestation of PD [27]. Analysis of AD brains indicates iron accumulation within specific brain regions, displaying selective vulnerability to neurodegeneration, such as the hippocampus and cerebral cortex [28,29] in association with both NFT- and Aβ-containing senile plaques.

Neuroprotection by Green Tea Polyphenols

Considering the diverse etiological nature of AD and PD, drugs directed against single functional components of the different disease pathologies, such as cognition or movement disorder, will be limited in efficacy. A novel therapeutic approach gaining large acceptance focuses on the implementation of cocktail of drugs, or a single molecule, possessing two or more active neuroprotective moieties that simultaneously target different disease mechanisms [30]. Accumulating new data suggests that green tea catechins may well fulfill the requirement for a putative neuroprotective drug because of their diverse pharmacological activities. The following sections will present an array of animal and cellular studies describing neuronal protection by green tea extract/EGCG and a collection of mechanistic studies aimed to illuminate the cellular processes and signaling pathways involved in their neuroprotective/neurorescue action.

Preclinical *In Vivo* Studies

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: EGCG has been shown to improve age-related cognitive decline and protect against cerebral ischemia/reperfusion injuries [31,32] and brain inflammation and neuronal damage in ex-

perimental autoimmune encephalomyelitis (EAE) [33]. Also, the treatment of EGCG significantly prolonged the symptom onset and life span and attenuated death signals in ALS mice model with the human G93A-mutated Cu/Zn-superoxide dismutase (*SOD1*) gene [34]. Similarly, a green tea polyphenol extract or individual EGCG prevented striatal DA depletion and SNpc dopaminergic neurons loss when given chronically to mice treated with the parkinsonism-inducing neurotoxin, *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) [35]. More recently, long-term administration of a preparate of green tea catechins (polyphenol E) or EGCG was demonstrated to improve spatial cognition learning ability in rats [36,37] and reduce cerebral amyloidosis in Alzheimer's transgenic mice, respectively [38].

Cell Culture Studies

In line with the *in vivo* findings, cell culture studies have demonstrated that EGCG prevented neuronal cell death caused by the neurotoxins 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenylpyridinium (MPP$^+$) in human neuroblastoma SH-SY5Y cells [39] and protected primary hippocampal neurons [40] and rat pheochromocytoma (PC12) cells [41,42] from Aβ-induced toxicity. More recently, both catechin and epicatechin were shown to protect cultured rat cortical neurons against Aβ (25–35)-induced neurotoxicity through inhibition of cytosolic calcium elevation [43].

In addition to the reported preventive action of green tea catechins, recent studies from our laboratory have demonstrated that EGCG is able to rescue and reduce mortality of neuroblastoma cells when given up to 3 days after a long-term serum starvation, a progressive model of apoptotic damage [44,45]. For a more comprehensive elucidation of the cellular pathways and individual proteins involved in the neurorescue effect of EGCG, we have applied a large-scale proteomic analysis. Table 1 demonstrates that the differentially expressed proteins clustered into three major functional categories: (1) cytoskeletal and structural proteins (e.g., beta-tubulin IV and tropomyosin 3), (2) binding proteins and heat shock proteins (e.g., 14–3-3 gamma, heat shock protein gp96), and (3) proteins involved in metabolic energy balance processes (e.g., ATP synthase, H$^+$-transporting, mitochondrial F1 complex-beta, glucosidase II-beta, and nerve vascular growth factor [VGF]-inducible precursor) [44,46,47]. These findings receive further support from our recent animal studies, where EGCG was shown to restore nigrostriatal DA neuron degeneration when administered to mice post-MPTP (unpublished results).

Table 1 Summary of proteins with significant differential expression, initially screened and identified by mass spectrometry in serum-deprived human SH-SY5Y neuroblastoma cells cultured with or without EGCG (0.1–1 μM)

SSP	Gi-accession #	Identified protein	Peptide match identified	%Coverage match peptide	Regulation
Cytoskeletal and structural proteins					
3502	55665782	Tropomyosin 3	8	31	Up
3306	1297274	Beta-tubulin IV	8	22	Up
6202	9863668	Histone H1e	5	22	Up
7105	1568557	Histone H2b	5	33	Up
Binding and heat shock proteins					
2201	48428721	14-3-3 protein gamma	6	23	Up
2205	58530887	Ubiquitin-conjugating enzyme E2R 2 (UBE2R2)	4	15	Down
7307	39545947	Heterogeneous nuclear ribonucleoprotein G (HNR G)	3	12	Down
4701	15010550	Heat shock protein gp96	17	29	Down
3405	34304590	Heat shock 90 kDa protein-beta	16	25	Down
4705	6470150	Heat shock 70 kDa protein 5, BiP protein (glucose-regulated, 78 kDa)	13	24	Down
Metabolism					
4609	48735337	Procollagen-proline, 2-oxoglutarate 4-dioxygenase; Prolyl 4-hydroxylase, beta subunit	2	10	Down
4301	49457530	Creatine kinase-B	3	54	Up
3305	16741373	ATP synthase, H^{+-}transporting, mitochondrial F1 complex, beta	3	12	Up
3704	48255891	Glucosidase II, beta (Protein kinase C substrate 80K-H)	5	14	Up
7306	39645299	VGF nerve growth factor inducible precursor	8	20	Up

A peptide was considered as high quality in Pep-Miner 80 and the Sequest Xcore identification, if the score was greater than 1.5 for singly charged peptides, 2.5 for doubly charged peptides, and 3 for triply charged peptides. SSP, the identification number of the selected spot assigned by the image analysis software; Gi accession #, Gi accession number of proteins; peptide match identified, the number of peptide match; % coverage match peptide, sequence coverage of matched peptide (%).

Mechanism of Neuroprotective Action of Green Tea Polyphenol EGCG

Antioxidant Activity

Tea catechins are phenolic compounds, and as such, they possess the ability to chelate transition metal ions, thereby preventing the formation of iron-induced free radicals and acting as powerful hydrogen-donating radical scavengers of reactive oxygen and nitrogen species (ROS and RNS, respectively) in *in vitro* and cell/tissue systems [48,49]. In brain tissue, green tea and black tea extracts were shown to strongly inhibit propagatory chain reaction of lipid peroxidation promoted by iron-ascorbate in homogenates of brain mitochondrial membranes (IC50: 2.44 and 1.40 μmol/L polyphenols, respectively) [50]. A similar effect was also reported using brain synaptosomes, in which the four major polyphenol catechines of green tea were shown to inhibit iron-induced lipid peroxidation [51]. In the majority of these studies, EGCG was shown to be more efficient as a radical scavenger than its counterparts ECG, EC, and EGC, which might be attributed to the presence of the trihydroxyl group on the B ring and the gallate moiety at the 3′ position in the C ring. [48].

The neuroprotective effect of green tea polyphenols *in vivo* may also involve the regulation of antioxidant protective enzymes. EGCG was found to elevate the activity of two major oxygen radical species-metabolizing enzymes, SOD and catalase in mice striatum [35]. Furthermore, in peripheral tissue, it has been shown that a number of flavonoids and phenolic antioxidants at low concentrations activate the expression of some stress response genes, such as phase II drug-metabolizing enzymes, glutathione-s-transferase, and heme oxygenase-1 (HO-1), in correlation with an increase in the activity and nuclear binding of the transcription factors nuclear factor erythroid 2-related factor (Nrf)1 and Nrf2 to the antioxidant regulatory element (ARE) sequences contained in their promoters [52,53].

Activation of the Protein Kinase C (PKC) Pathway

Emerging evidence suggests that the antioxidant activity cannot be the sole mechanism responsible for their

neuroprotective action but rather, that their ability to alter kinase signaling pathways may significantly contribute to the cell survival effect. Our pioneer *in vitro* cell signaling studies revealed a specific involvement of the PKC pathway in the neuroprotective mechanistic action of EGCG [39,54]. PKC has a fundamental role in the regulation of cell survival, programmed cell death, long-term potentiation (LTP) [55], and consolidation of different types of memory [56,57]. Additionally, the induction of PKC activity in neurons has been shown to protect against various exogenous insults, such as oxygen/glucose deprivation in organotypic slice cultures [58] or Aβ toxicity in rat cortical or hippocampal neurons, respectively [59,60].

Mechanistic studies aimed at investigating the neuroprotective potential of physiological relevant concentrations of EGCG revealed that low micromolar concentrations are responsible for the antiapoptotic/neuroprotective actions, whereas high doses account for the antiproliferative, antiangiogenic, and proapoptotic actions, having implications in cancer management [39,61]. This biphasic mode of action was shown to be mutual to that of other antioxidants and iron chelators [62]. A rapid phosphorylative activation of PKC by low micromolar concentrations of EGCG is thought to be the main mechanism accounting for its neuroprotective activity against several neurotoxins, such as Aβ [42], serum withdrawal [41,44], and 6-OHDA [39], and neurorescue effect against long-term growth factors withdrawal. The neuroprotective effect involved reduction of the apoptotic markers, cleaved caspase 3, its downstream cleaved substrate poly-ADP-ribose-polymerase (PARP), a nuclear zinc finger DNA-binding protein that detects and binds to DNA strand breaks, and Bad, a member of a group of "BH3 domain only" proteins of the Bcl-2 family [44,46]. This is supported by the observation that EGCG could not overcome neuronal death under PKC pathway blockade, suggesting that this cascade is essential for the neuroprotection and neurorescue effects of EGCG [44]. Recently, we have identified a novel pathway in the neuroprotective mechanism of action of EGCG, which involves a rapid PKC-mediated degradation of Bad protein by the UPS in NB SH-SY5Y [54]. Bad has been suggested to link survival signals to the mitochondrial cell death machinery. Thus, the newly described role of Bad during the initial response to EGCG-induced cell signaling may potentially contribute to the illumination of the EGCG mechanism of neuroprotection/neurorescue action.

In addition, EGCG was shown to induce a rapid translocation of the isoform PKCα to the membrane compartment in human astroglioma or rat PC12 cells [44,63]. This isozyme is particularly important in neuronal growth
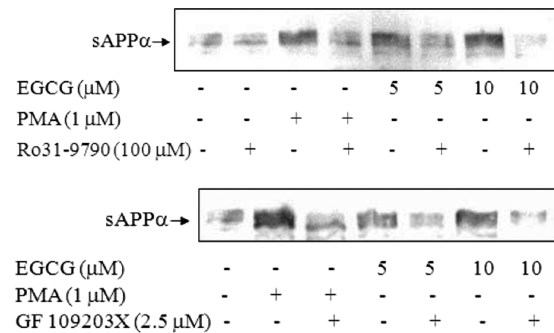

Figure 2. Effect of EGCG on sAPPα release in PC12 cells. PC12 cells were preincubated for 30 min with vehicle alone, or with the general PKC inhibitor, GF109203X (upper panel) or the hydroxamic acid-based metalloprotease inhibitor of α-secretase, Ro31–9790 (lower panel), followed by a 2-h exposure to EGCG or the PKC activator, phorbol 12-myristate 13-acetate (PMA). Proteins released into the conditioned media were collected and analyzed for sAPPα. Treatment with either EGCG or PMA resulted in an increased release of sAPPα. The observation that both inhibitors attenuated the release of sAPPα induced by EGCG or PMA, suggests the involvement of a PKC- and an α-secretase-dependent pathway in EGCG effect. Adapted from [39].

and differentiation in the brain. These findings are supported by animal studies showing that 2 weeks of oral consumption of EGCG prevented the extensive depletion of PKCα and counteracted the robust increase of Bax protein in the striatum and SNpc of mice intoxicated with MPTP [64].

Clinical Significance of PKC Activation by EGCG to AD

Our pioneer studies have demonstrated that either short- or long-term incubation with EGCG promotes the generation of a soluble form of APP, sAPPα, via PKC-dependent activation of α-secretase [42,65] (Fig. 2). Cleavage of APP to sAPPα involves an alternative nonamyloidogenic secretory pathway executed by a putative α-secretase, which cleaves APP within the sequence of the amyloidogenic Aβ peptide, thus precluding the formation of Aβ; the latter is regulated by the sequential action of β- and γ- secretases [66]. In contrast to Aβ, sAPPα possesses neuroprotective activities against excitotoxic and oxidative insults in various cellular models [67]. It also promotes neurite outgrowth [68], and synaptogenesis and exerts trophic effects on cerebral neurons in culture [69]. Since sAPPα and Aβ are formed by two mutually exclusive mechanisms, it can be assumed that stimulation of the secretory processing of sAPPα might prevent the formation of the amyloidogenic Aβ. New supportive data came from a study conducted in an Alzheimer's transgenic mice model, showing that EGCG promotes sAPPα generation through activation of α-secretase cleavage

[38]. This was accompanied by a significant reduction in cerebral Aβ levels and β-amyloid plaques.

Other potential beneficial effect of PKC activation in AD is related to the recent finding showing that neuronal overexpression of PKC in transgenic mice expressing familial AD mutant forms of the human APP decreases Aβ levels and plaque burden, and this is accompanied by an increased activity of endothelin-converting enzyme (ECE), which degrades Aβ [70]. Since EGCG has been shown to increase the levels of PKC isoforms α and ε in mice hippocampus and striatum [42,64], it can be hypothesized that, in AD pathology, EGCG may reduce Aβ levels, both via a concomitant stimulation of sAPPα secretion and promotion of Aβ clearance through increased ECE activity. Although there is a general support for the amyloid hypothesis as a key contributor to neuronal death and dementia in AD, a direct connection between Aβ deposits in senile plaques and neurodegeneration has not been yet established. In this respect, Alzheimer's transgenic mice with Aβ deposits do not exhibit significant neuronal loss in hippocampus and association cortex [71]. Therefore, the ultimate proof for the effectiveness of drugs, capable of lowering or eliminating brain Aβ aggregation/accumulation, will be their clinical benefit.

Iron Chelation

The observations that iron induces aggregation of inert α-synuclein and Aβ peptides to toxic aggregates have reinforced the critical role of iron in OS-induced pathogenesis of neurodegeneration, supporting the notion that a combination of iron chelation and antioxidant therapy may be one significant approach to neuroprotection. In this respect, treatment with desferal/desferrioxamine (DFO) as a prototype iron chelator or with the antibiotic iron and copper chelator, 5-chloro-7-iodo-8-hydroxyquinoline (clioquinol), was shown to be neuroprotective against 6-OHDA and MPTP-induced neurotoxicity in rats and mice, respectively [72,73]. Similarly, a study examining the effect of transgenic expression of the iron-binding protein ferritin, on the susceptibility to MPTP, has shown to be a protective strategy against the toxin [73]. Clioquinol has undergone a phase II clinical trial for its impact of moderately severe AD patients, but further studies are needed to evaluate the potential of clioquinol for AD treatment [74]. Interestingly, chronic administration of clioquinol to transgenic mice bearing the APP "Swedish" mutation (Tg2576) lead to a significant reduction in brain Aβ burden and slowed the rate of cognitive decline [75]. A major limitation of DFO resides in its hydrophilic nature and large molecular size, limiting its absorption across the gastrointestinal tract and preventing it from penetrating the blood brain barrier (BBB) [76]. Recent studies have clearly shown that our novel hybrid, lipophylic iron-complexing molecule M-30 (5-[N-methyl-N-propargylaminomethyl]-8-hydroxyquinoline), amalgamating the active neuroprotective moieties of the iron chelator VK-28 and the anti-PD drug rasagiline [77], prevented and restored lactacystin-induced nigrostriatal DA neuron damage [78]. Green tea polyphenols have been shown to penetrate the brain [79,80] and possess relatively potent metal-chelating properties [51,81], which have been attributed to the gallate moiety present in the C-ring of both EGCG and ECG [82]. Thus, the ability of polyphenols to act as radical scavengers and chelate transitional metals such as iron and copper may be of major significance for treatment of PD and AD, where accumulation of iron at brain areas associated with neurodegeneration has been shown [83].

Attenuation of Aβ and PHFτ Aggregation

In AD, iron may operate targets central to pathogenesis, including the induction of extraneuronal Aβs 39–43 amino acid length, Aβ protofibrils aggregation/fibrillization [84], and the promotion of PHFτ aggregation, resulting in the formation of intraneuronal NFTs [85]. Partial aggregated and oligomerized intracellular Aβ was documented to be cytotoxic and synaptotoxic in cell culture and *in vivo* owing to the generation of ROS-induced lipid membrane peroxidation, DNA breakdown, and protein oxidation [84,86,87], which can be attenuated by a number of antioxidants and metal chelators [84,88]. *In vitro* studies have shown that both the prototype iron chelator, DFO, and clioquinol prevented the formation of β-pleated sheets of Aβ (1–42) and effectively dissolved synthetically preformed or AD brain-derived Aβ [89]. Similar to Aβ, α-synuclein associated with presynaptic membranes is not toxic, while in the presence of iron, it forms toxic aggregates that are considered to contribute to the formation of LBs via OS [90,91]. Thus, the radical scavenging and free-iron-complexing activities of green tea polyphenols may directly influence aggregation and deposition of either Aβ or α-synuclein in brains of AD and PD patients, respectively. Indeed, it was shown that several metal-binding natural antioxidants, including polyphenols of green tea and wine (e.g., resveratrol, myricetin, (+)-catechin, (-)-epicatechin) inhibit formation of nascent Aβ and α-synuclein fibrils and elongation of the fibrils, and promote destabilization of the formed assemblies [92,93]. In support, it has been recently shown that EGCG interferes with an early step in the amyloid formation cascade; it directly binds to the natively unfolded

α-synuclein and Aβ polypeptides, thus inhibiting their fibrillogenesis and redirecting them into an alternative "off pathway" before they become toxic [94]. Similarly, the radical scavenging and free-iron-complexing activities of green tea polyphenols or other metal-chelating polyphenols such as curcumin [95] may alleviate the brain from free-reactive iron overload and directly influence aggregation and deposition of Aβ in brains of AD patients.

Inhibition of APP Translation

Recent studies have identified a novel link between iron and AD associated with an enhancement of endogenous APP translation and subsequent Aβ formation, via activation of an iron responsive element (IRE-type II) in the 5$'$ untranslated region (UTR) of APP mRNA [96]. This finding opened a new potential therapeutic avenue aimed at reducing amyloidosis with iron-complexing drugs that modulate APP mRNA translation. Potential candidates include DFO (Fe^{3+} chelator), tetrathiomolybdate (Cu^{2+} chelator), and dimercaptopropanol (Pb^{2+} and Hg^{2+} chelator), which were found to suppress APP holoprotein expression via APP 5$'$-UTR-modulation and lower Aβ peptide secretion [97,98]. Interestingly, a recent *in vitro* study has demonstrated that EGCG reduced full-length APP in SH-SY5Y cells, without altering APP mRNA levels, while exogenous iron supplementation reversed its effect [65] (Fig. 3A, B); this suggests a posttranscriptional action, presumably by the mechanism of chelating intracellular iron pools. This is further supported by the observation that EGCG suppressed translation of a luciferase reporter gene driven by the IRE-type II-containing sequences of APP [65] (Fig. 3C). In mouse hippocampus, both EGCG and the multifunctional iron chelator M-30 were shown by us to induce a significant downregulation of membrane-associated holo-APP level [6]. Furthermore, it was found that EGCG markedly reduced secreted Aβ levels in the conditioned medium of Chinese hamster ovary cells overexpressing "Swedish" mutated APP (CHO/ΔNL) [65] and in primary neuronal cells derived from transgenic mice bearing the APP "Swedish" mutation [38].

Interestingly, Friedlich et al. [99] have recently described a putative IRE in the 5$'$-UTR of PD-related α-synuclein mRNA and predicted that this RNA structure may have the potential to function as a posttranscriptional regulator of α-synuclein protein synthesis in response to iron and redox events, in a pattern that resembles that of APP and the iron-associated protein ferritin [83,96]. This finding can explain, in part, our previous results demonstrating that the iron-chelating compounds R-apomorphine and EGCG, prevented iron-dependent upregulation of α-synuclein in the SNpc of MPTP-treated mice, resulting in neuroprotection of nigro-striatal DA neurons [64].

Induction of Hypoxia-Inducible Factor-1 (HIF-1)

An emerging target for neuroprotection associated with iron chelation implicates the activation of a hypoxia signal transduction pathway that culminates in the stabilization of the transcriptional activator HIF-1 and increased transcription of genes mediating compensatory survival processes in response to OS. The presence of HIF-1 within the cells is under the strict control of a class of iron-dependent and oxygen sensor enzymes named the HIF prolyl-4-hydroxylases [100]. This family of enzymes hydroxylates critical proline and asparagine residues in HIF upon high oxygen levels and iron overload, targeting it for degradation by the UPS. This may explain the decrease in HIF-dependent cell survival genes described in neurodegenerative diseases, such as phosphofructokinase and the angiogenic vascular endothelial growth factor (VEGF) [101]. In this scheme, iron chelators would stabilize HIF-1α, which in turn would heterodimerize with its partner HIF-1β in the nucleus, bind to an hypoxia-responsive element in regulatory genes, and transactivate the expression of established protective genes, including VEGF, erythropoietin, p21$^{waf1/cip1}$, glucose transporter-1 (GLUT-1), and the glycolytic enzymes aldolase and enolase-1 [102,103]. Indeed, EGCG and ECG were shown to induce HIF-1α protein and HIF-1 activity and increase the mRNA expression levels of GLUT-1, VEGF, and p21$^{waf1/cip1}$, whereas this effect was blocked by iron and ascorbate, indicating that these catechins may activate HIF-1 through the chelation of iron [104,105]. Applying a neurorescue paradigm in neuronal culture, we have recently found that EGCG decreased mRNA transcript and protein levels of the beta-subunit of prolyl-4-hydroxylase and the protein levels of two molecular chaperones, which are associated with HIF-regulation, the immunoglobulin-heavy chain-binding protein, BiP, and the heat shock protein 90 (Table 1) [44,46]. Thus, it is possible that the protective effect of EGCG under OS/hypoxic condition may combine the suppression of hydroxyl radical formation via Fenton chemistry as well as inhibition of iron-dependent prolyl hydroxylase .

Another link between hypoxia and iron is reflected by the hypoxia-mediated positive regulation of the iron regulatory proteins, IRP1 and IRP2, and the consequential transactivation of their target mRNAs, ferritin and transferrin receptor (TfR). Interestingly, the free-iron-induced proteasomal-mediated degradation of IRP2 also involves activation of a prolyl hydroxylase and is inhibited by iron chelators [106,107]. Thus, it is possible that IRP2
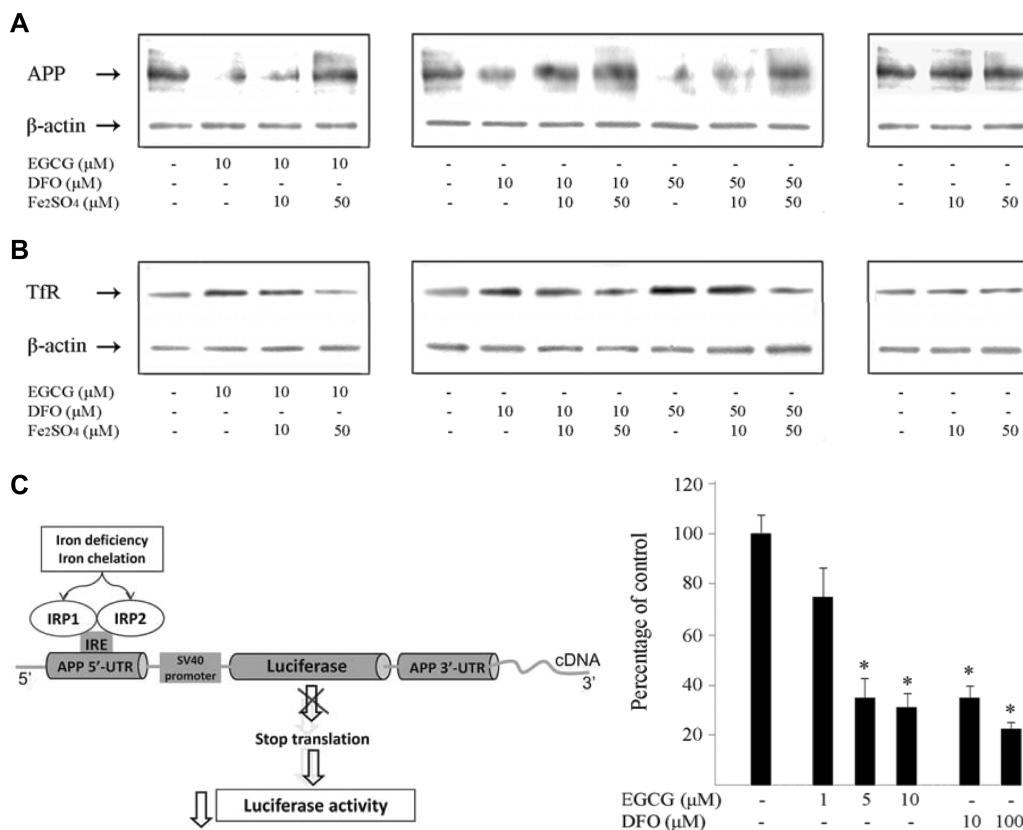
Figure 3. Effect of EGCG on the regulation of the iron metabolism-related proteins APP and TfR and suppression by iron. (**A and B**) Human neuroblastoma SH-SY5Y cells were incubated without or with EGCG (10 μM) or DFO (10 and 50 μM; positive control) for 2 h, and then treated with or without increasing concentrations of Fe$_2$SO$_4$ (10 and 50 μM) for 2 days. APP and TfR, an iron homeostasis protein negatively regulated by iron, were evaluated by Western blot analysis using 22C11 and anti-TfR antibodies, respectively. EGCG markedly reduced holo-APP protein levels, while addition of Fe(II) reversed the EGCG-suppressive effect (**A**). The positive effect of EGCG on TfR levels was also blocked by Fe(II) (**B**). Iron chelation by DFO, which, by being a pure iron chelator, served as positive control, gener-ated a similar response in both proteins. (**C**) The efficacy of EGCG as an iron chelator, to modulate the translation of a luciferase reporter gene driven by the APP 5'-UTR sequences, was tested in U-87-MG glioma cells, cotransfected with 10 μg of DNA from pGALA plasmid (APP 5'-UTR + APP 3'-UTR sequences) and 5 μg of DNA from a construct that expresses GFP, to standardize for transfection efficiency. Cell plates were grown in the absence (control) or presence of increasing concentrations of EGCG (1–10 μM) for 48 h. Values represent luciferase activity normalized to GFP (mean ± SEM, from four independent experiments, each conducted in six replicates). $*P$ < 0.01, versus untreated control. EGCG gradually suppressed APP 5'-UTR reporter gene expression in a concentration-dependent manner.

is a substrate for this enzyme, in a similar way as HIF, signaling it for protein degradation. Thus, the reduction in the chelatable iron pool by EGCG may result in the inhibition of prolyl hydroxylases and consequently in the concerted activation of both HIF and IRP2. As IRPs and HIF-1 coordinate the expression of a wide array of genes involved in cellular iron homeostasis, survival, and proliferation [108,109], their activation could be of a major importance in neurodegenerative diseases. In support, recent findings suggest the application of low molecular weight or peptide inhibitors of HIF prolyl 4-hydroxylase as novel neurological therapy for neurodegenerative diseases [102].

Conclusions and Outlook

Two main aspects are significantly contributing to the raising concept viewing green tea consumption of relevance to brain health: the factors and events that influence the incidence and progression of PD and AD are becoming better defined and understood; in parallel, the experimental evidence documenting the neuroprotective properties of green tea catechins both in cell culture and animal studies is persistently increasing. It becomes evident that syndromes such as AD and PD will require multiple drug therapy to address the varied pathological aspects of the disease. Therefore, the polypharmacological activities of green tea catechins may be of
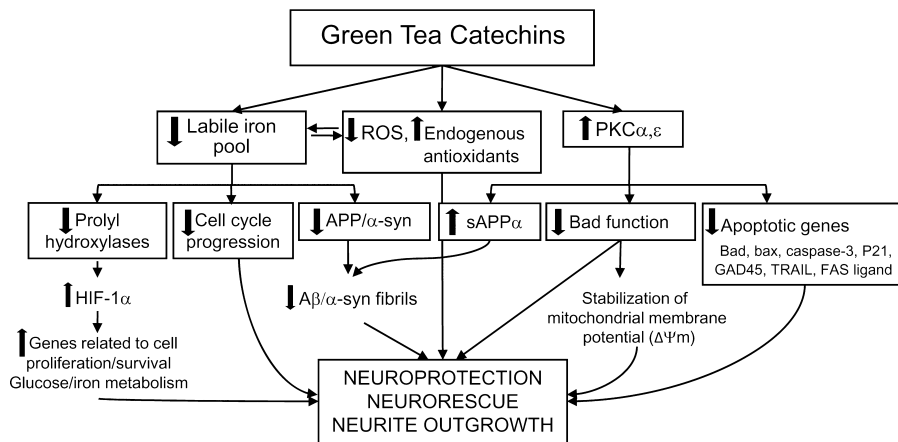
Figure 4. A schematic model illustrating the proposed mechanisms of green tea/EGCG neuroprotective/neurorescue action. This scheme summarizes suggested optional mechanisms of neuroprotective/neurodifferentiative action of EGCG: our pioneer studies have demonstrated an immediate and specific activation of PKC upon administration of EGCG, constituting one major pathway mediating its neuroprotective activity. This, in turn, may activate various survival pathways that can contribute as well to EGCG beneficial effect. One of them is related to the fast degradation of Bad in a PKC- and proteasomal-dependent manner, the maintenance of mitochondrial potential and reduced expression of apoptotic genes upon oxidative stress in response to EGCG. Other PKC-accredited beneficial effects of green tea polyphenols maybe related to activation of α-secretase to promote generation of the nontoxic, nonamyloidogenic neurotrophic, soluble amyloid precursor protein-alpha (sAPPα). In addi- tion, EGCG was shown to regulate APP protein at the translational level via reduction of the labile iron pool. The net result of the latter two processes will be the reduction of Aβ fibrils formation. Additional targets of EGCG associated with its iron-chelating effect involve the capacity of the catechin to interfere with cell cycle progression and inhibition of the iron-dependent hypoxia-inducible factor (HIF)-1 prolyl-4-hydroxylase that regulates HIF stability, resulting in selective induction of cell survival genes including VEGF, erythropoietin, and HO-1. Green tea catechins are also potent oxygen and nitrogen radical scavengers and inducers of endogenous antioxidant defenses. Aβ, amyloid beta-peptide; α-syn, alpha synuclein; HIF-1, hypoxia-inducible factor-1; PKC, protein kinase C; sAPPα, soluble amyloid precursor protein-alpha; VEGF, vascular endothelial growth factor.

significance for neuroprotection. Earlier viewed as simple radical scavengers, green tea catechin polyphenols are at present considered as multimodal acting molecules invoking a myriad of cellular neuroprotection/neurorescue mechanisms involving iron chelation, scavenging of oxygen and nitrogen radical species, and activation of PKC signaling pathway and prosurvival genes. Their nontoxic, lipophilic (and thus, brain permeable) nature is advocated for "ironing out iron" from those brain areas where it preferentially accumulates in neurodegenerative diseases [110]. The chelation of the reactive free-iron pool by EGCG and the consequent reduction in full-length APP translation would contribute to the decreased Aβ generation/fibrillization, which, together with the promotion of the nonamyloidogenic pathway and induction of neurite outgrowth, may converge in a slowdown in the process of nerve cells loss in AD. A proposed schematic model for the neuroprotective/neurorestorative effect by EGCG is illustrated in figure 4.

The brain therapeutic future of green tea active constituents relies on whether their neuroprotective/neurorestorative actions can be successfully translated into prospective human studies.

Conflict of Interest

The authors declare no conflict of interest.

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