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Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases

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Abstract

Tea consumption is varying its status from a mere ancient beverage and a lifestyle habit, to a nutrient endowed with possible prospective neurobiological–pharmacological actions beneficial to human health. Accumulating evidence suggest that oxidative stress resulting in reactive oxygen species generation and inflammation play a pivotal role in neurodegenerative diseases, supporting the implementation of radical scavengers, transition metal (e.g., iron and copper) chelators, and nonvitamin natural antioxidant polyphenols in the clinic. These observations are in line with the current view that polyphenolic dietary supplementation may have an impact on cognitive deficits in individuals of advanced age. As a consequence, green tea polyphenols are now being considered as therapeutic agents in well controlled epidemiological studies, aimed to alter brain aging processes and to serve as possible neuroprotective agents in progressive neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. In particular, literature on the putative novel neuroprotective mechanism of the major green tea polyphenol, (−)-epigallocatechin-3-gallate, are examined and discussed in this review.

Keywords: Green tea; (−)-Epigallocatechin-3-gallate; Neuroprotection; Antioxidation; Iron chelating; Neurodegenerative diseases

1. Introduction

Polyphenols are natural substances that are present in beverages obtained from plants, fruits, and vegetables, such as olive oil, red wine, and tea. Flavonoids are the largest group of polyphenols, a group that is mainly divided into anthocyanins, glycosylated derivative of anthocyanidin, present in colorful flowers and fruits and anthoxantins, colorless compounds further divided into several categories including flavones, isoflavones, falvanols flavans, and flavonols [1] (Fig. 1). Flavonoids are consisted of an aromatic ring that is condensed to a heterocyclic ring and attached to a second aromatic ring. The abundant phenolic hydroxyl groups on the aromatic ring confer the antioxidant activity, and the 3-OH is essential for the iron chelating activity of these compounds [2].

The importance of polyphenolic flavonoids in enhancing cell resistance to oxidative stress goes beyond simple scavenging activity and is of most interest in pathologies in which oxidative stress plays an important role. Numerous studies in the past 10 years have shown that polyphenols have in vitro and in vivo activity in preventing or reducing the deleterious effects of oxygen derived free radicals associated with several chronic and stress related human and animal diseases. Several lines of evidence suggest that oxidative stress resulting in reactive oxygen species (ROS) generation and inflammation play a pivotal role in clinical disorders such as arteriosclerosis, ischemia-reperfusion injury, cancer, stroke, and neurodegenerative disorders [3,4]. Special interest has been assigned to the therapeutic role of antioxidants in neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD) [5,6]. Oxidative damage to neuronal biomolecules and increased accumulation of iron in specific brain areas are considered major pathological aspects of PD and AD [7]. Although the etiology of both disorders and their respective dopaminergic or cholinergic neuron degeneration remains elusive, the chemical pathology of PD shows many similarities to AD, involving increase in iron concentration, release of cytochrome *c*, alpha-synuclein aggregation, oxidative stress, loss of tissue reduced glutathione (GSH), reduction in mitochondrial complex I activity and increased lipid peroxidation [8–10].

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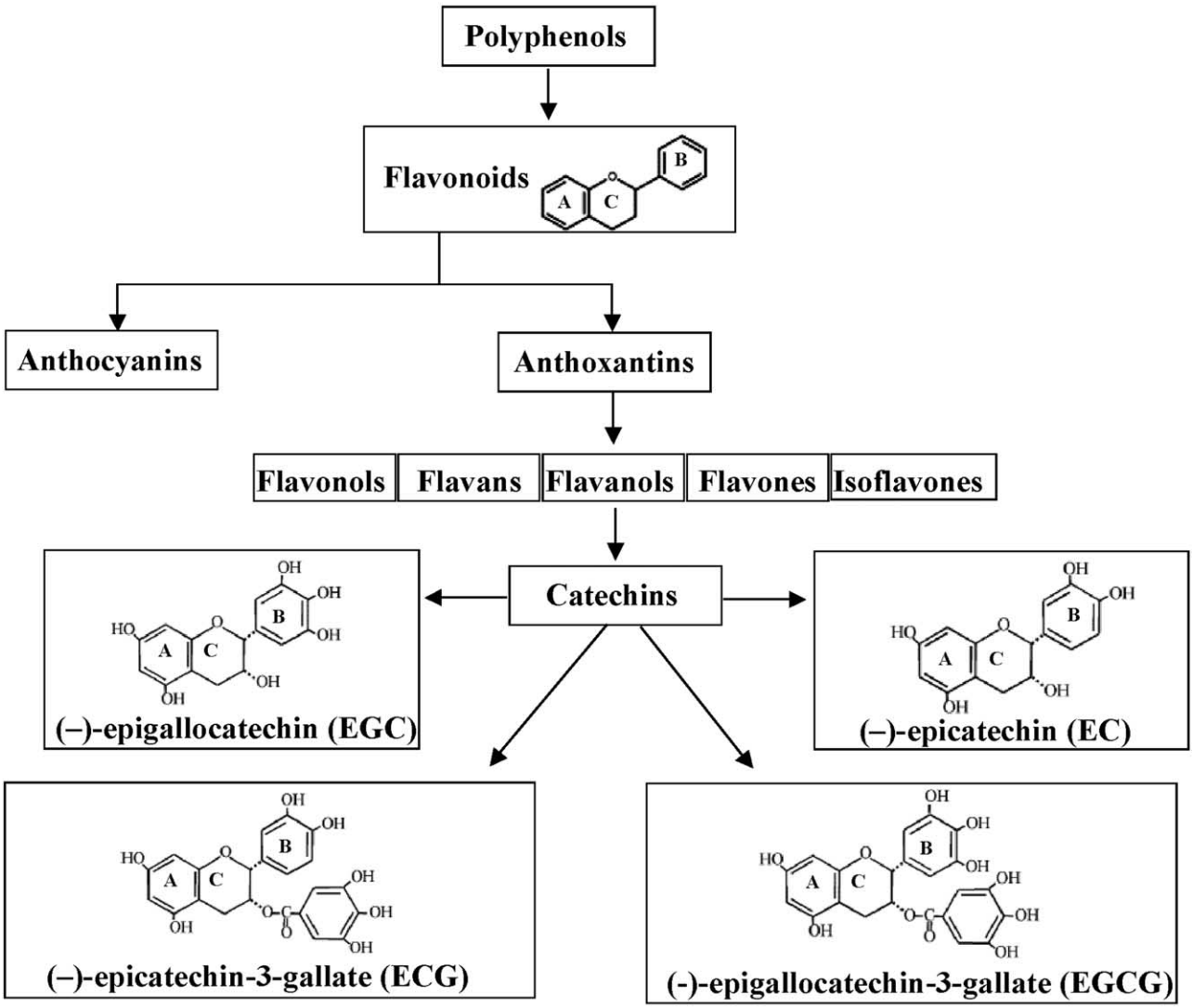

Fig. 1. Diagram of the natural polyphenol classification and the chemical structure of green tea catechins.

A large study investigating PD found a moderate risk reduction of tea consumers compared to non–tea drinkers [11]. The favorable properties ascribed to tea consumption are believed to rely on its bioactive components, catechins and their derivatives, which have been shown to act directly as radical scavengers and exert indirect antioxidant effects through activation of transcription factors and antioxidant enzymes (for reviews see [12,13]. In line with this evidence, particular attention has been placed on studying the neuroprotective action of antioxidants, iron chelating and anti-inflammatory agents, tea flavonoids, and especially the major component of green tea, (−)-epigallocatechin-3-gallate (EGCG) [14–16]. The revelation of novel molecular targets possibly implicated in their neuroprotective action include calcium homeostasis [17], the extracellular mitogen-activated protein kinases (MAPK) [18], protein kinase C (PKC) [19], antioxidant enzymes [20], antioxidant regulatory element (ARE) [21] survival genes [22], and processing of the amyloid precursor protein (APP) pathway [19].

The specific mechanisms by which green tea polyphenols exert their neuroprotective action are not clearly defined. However, recent evidence indicates that besides their anti-oxidant and iron chelating properties, polyphenols have a profound effect on cell survival/death genes and signal transduction. This review will focus on the most recent studies relating to the mechanism underlying the biochemical and molecular effects of EGCG, the major green tea polyphenol component, with particular relevance to PD and AD.

2. Green tea polyphenols

Green tea is a drink made from the steamed and dried leaves of the *Camellia sinesis* plant, a shrub native to Asia. It is a beverage that is widely consumed in Japan, China, and other Asian nations and that is becoming more popular in Western countries. Recently, green tea has attracted attention for its health benefits, particularly with respect to its

potential for preventing and treating cancer, cardiovascular diseases, inflammatory diseases, and neurodegenerative diseases in humans [23–26].

Green tea contains a number of bioactive chemicals; it is particularly rich in flavonoids (30% of the dry weight of a leaf), including catechins and their derivatives (Fig. 1). The most abundant polyphenolic compound is EGCG, which is thought to contribute to the beneficial effects attributed to green tea such as its anticancer and antioxidant properties. Catechins have been found to be more efficient radical scavengers than vitamin E and C [27,16]. Relative antioxidant activities among tea catechins (Fig. 1) has been found to be EGCG = (−)-epicatechin-3-gallate (ECG) > (−)-epigallocatechin (EGC) > (−)-epicatechin (EC) [28]. EGCG accounts for more than 10% of the extract dry weight, 20–35 mg per cup of green tea, followed by EGC > EC ≥ ECG [29].

The metabolism of green tea catechins has been studied in various animals and in human subjects [30,31]. Catechin orally administration to humans is absorbed, metabolized, and excreted within 24 hours [32].A study involving healthy consumers of green tea revealed levels of EGCG, EGC, and EC in the plasma in dose-dependent concentration varying between 0.2% and 2% of the ingested amount, with maximal concentration 1.4–2.4 hours after ingestion [33]. In addition, after ingestion of 1.2 g of decaffeinated green tea solids (dissolved in 2 cups of warm water), the plasma samples collected at 1 hour from human volunteers contain 46–268 ng/mL [31]. The half-life for EGCG is about 5 hours, and for EGC and EC it varies between 2.4 and 3.4 hours [34]. Several reports indicated that tea polyphenols can be attained in the brain and exert neuroprotective effect simply by drinking [35]. Recently, it was reported that EC metabolites (epicatechin glucuronide and 3′-O-methylated epicatechin glucuronide), formed after oral ingestion of EC by rats, had gained entry to the brain [36]. Furthermore, a study with labeled EGCG demonstrated a wide distribution of radioactivity in organs in mice, including the brain, after oral administration, as well as small amounts of [^{3}H] EGCG excretion in the urine after direct administration to the stomach [37].

3. EGCG neuroprotective activities in relation to PD and AD

Because of the lack of evidence on the capacity of tea polyphenols in the human brain and the lack of well controlled clinical trials, the effect of these compounds in the progression of neurodegenerative disorders has not been studied on a large scale. Recent epidemiological studies have shown reduced risk of PD associated with consumption of 2 or more cups of tea daily [38], as well as a significant reduction of PD risk in tea drinkers in a Chinese population [39]. Despite the fact that the prevalence of PD is much lower in the Chinese population than in western individuals of white ethnicity [40,41], the association of green tea drinking and risk of PD has not well examined. No case-control study on AD reported any beneficial effect associated with tea consumption, although treatment with the extract EGb761 of other an source of natural flavonoids that does not contain catechins, Gingko biloba leaves, improved cognitive performance of AD patients [42]. Moreover, a study on a French community of individuals aged 65 and over showed an inverse relationship between moderate wine drinking and incidence of AD [43].

3.1. Neuroprotection in vitro and in animal models

Neuroprotective in vivo studies using *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have shown that both green tea extract and EGCG possess highly potent activities in preventing striatal dopamine depletion in mice as well as substantia nigra dopaminergic neuron loss induced by the parkinsonism-inducing neurotoxin [20]. One possible mechanism underlying the effectiveness of green tea and EGCG against MPTP neurotoxicity may involve its catechol-like structure, since it is known that catechol-containing compounds are potent radical anti-oxidants and chelators of ferric ion [44]. In agreement, the iron chelators, radical scavengers, and catechol derivative compounds R-apomorphine (R-APO), dopamine (DA) receptor agonist, and its S-isomer induced neuroprotection in animal models of PD [45,46]. The catechol structural resemblance may account for a recently reported inhibitory effect of green tea polyphenols on [^{3}H] DA uptake by presynaptic transporters. This inhibition was suggested to block the metabolic product of MPTP, the neurotoxin 1-methyl-4-phenylpyridinium (MPP^+) uptake, because of competition for the vesicular transporter, thereby protecting dopamine-containing neurons against MPP^+-induced injury [47]. In-vitro studies also demonstrated inhibition of $MPP^+$and 6-hydroxydopamine (6-OHDA)–induced neurotoxicity by EGCG [22]. Furthermore, EGCG inhibited the activity of the enzyme catechol-O-methyltransferase (COMT) in rat liver cytosol homogenates at a low IC_{50} concentration (0.2 μmol/L) [48]. DA and related catecholamines are physiological substrates of COMT. The COMT inhibitors entacapone and tolcapone, clinically prescribed to PD-affected individuals, dose-dependently inhibit the formation of the major metabolite of levodopa, 3-O-methyldopa, thereby improving its bioavailability in the brain [49]. The implication of the pivotal role of EGCG in neuroprotection as an iron chelator has been strengthened by the observations that both MPTP and 6-OHDA significantly increased iron in substantia nigra pars compacta (SNPC) of mice, rats, and monkeys treated with these neurotoxins [50–53]. Iron accumulation has been implicated in a range of neurodegenerative disorders [54] and iron has been reported to accumulate in the neurons in SN of patients with PD.

A considerable evidence point to an amyloid cascade of events in the pathogenesis of AD, in which APP is processed to amyloid β-peptide (Aβ), which spontaneously self-aggregates in the presence of divalent metals (Fe^{2+}, Cu^{2+}) into neurotoxic amyloid fibrils in the neocortex [55].

APP can be processed via two pathways: 1) a nonamyloidogenic secretary pathway, which involves cleavage of APP to soluble APP (sAPP) by a putative α–secretase within the sequence of Aβ peptide, thus precluding the formation of Aβ and 2) a formation of amyloidogenic Aβ peptides, which is regulated by the sequential action of β- and γ-secretases [55–57]. As the proportion of APP processed by β-secretase versus α-secretase may affect the amount of Aβ produced, the regulation of these two pathways might be critically important to the pathogenesis of AD.

Although AD case-control studies did not report any significant outcome relative to tea consumptions, in vitro observations show that EGCG inhibits induced oxidative stress and neurotoxicity, [19,58], and EC reduces the formation of Aβ-fibril formation [59]. In addition, EGCG is able to regulate the proteolytic processing of APP both in vivo and in vitro [19], suggesting that green tea polyphenols might be potentially promising therapeutic agents not only for PD but also for AD. EGCG promoted the nonamyloidogenic α-secretase pathway of APP neuronal cell cultures. The increase was dose-dependent, and the stimulatory effect of EGCG on sAPPα secretion was inhibited by the hydroxamic acid–based metalloprotease inhibitor Ro31-9790, indicating that this effect was mediated via α-secretase processing. Also, long term treatment of mice with EGCG resulted in decreases in cell-associated, full length APP levels, as well as increases in sAPP levels in the hippocampus. In addition, inhibition of PKC activity, whose involvement in sAPP release is well established [56,60], prevented EGCG-induced sAPPα release, indicating a key role of PKC in mediating EGCG effect. Although it is not known which isoenzyme of PKC plays a major role in modulating APP processing, several lines of evidence suggest the involvement of PKCα and PKCϵ in APP processing [61,62]. Previous studies in brains of AD patients demonstrated reduction of PKCϵ activity in the membrane fraction [63]. In agreement with these findings, repeated administration of EGCG for 1 or 2 weeks caused significant increases in the protein expression of PKC isoenzymes α and ϵ in the hippocampus of mice [19].

4. EGCG antioxidative and iron chelating mechanism of action

The protective effect of EGCG against neuronal diseases may involve its radical scavenging and iron chelating activity and/or regulation of antioxidant protective enzymes.

Tea polyphenols have been found to be potent scavengers of free radicals such as singlet oxygen, superoxide anions, hydroxyl radicals, and peroxy radicals in a number of in vitro systems [15,16,64]. 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 may be attributed to the presence of the trihydroxyl group on the B ring (Fig. 1) and the gallate moiety at the 3′ position in the C ring [16]. 3-Hydroxykynurenine (3-HK) is an endogenous metabolite of tryptophan in the kynurenine pathway and is a potential neurotoxin in several neurodegenerative disorders. EGCG attenuated 3-HK–induced cell viability reduction and increase in the concentration of ROS and caspase-3 activity in neuronal culture, presumably via its antioxidant activity [65]. In rat brain tissue, green tea and black tea extracts were shown to inhibit lipid peroxidation promoted by iron ascorbate in homogenates of brain mitochondrial membranes (IC_{50}: 2.44 and 1.40 μmol/L, respectively) [66]. A similar effect was also reported using brain synaptosomes, in which the four major polyphenol catechins of green tea were shown to inhibit iron-induced lipid peroxidation [44]. In this regard, it has been shown that EGCG attenuated paraquat-induced microsomal lipid peroxidation and increased the survival rate of paraquat-poisoned mice [67]. The herbicide paraquat is a strong redox agent that contributes to the formation of ROS and induces toxicity of the nigrostriatal dopaminergic system; and it is therefore used as a model for Parkinsonism in vivo [68]. EGCG inhibited paraquate-induced malondialdehyde production in rat liver microsome system containing $FeSO_4$ by two possible mechanisms. One may be scavenging of superoxide radicals, which are responsible for the reduction of ferric to ferrous catalyzed by the Fenton reaction. The other is iron chelating activity, given that the inhibition disappeared when excessive amount of $FeSO_4$were added to the reaction, which indicates that EGCG inhibits iron driven lipid peroxidation by pulling out available iron in the mixture.

The ability of green tea polyphenols and catechins, in particular, to chelate metal ions such as iron and copper may contribute to their antioxidant/neuroprotective activity by inhibiting transition metal-catalyzed free radical formation. The two points of attachment of transition metal ions to the flavonoids molecule are: the *o*-diphenolic groups in the 3′,4′-dihydroxy positions in the B ring, and the keto structure *4-keto*,3-hyroxy or *4-keto* and 5-hydroxy in the C ring of the flavonols [2,69]. The ability of green tea polyphenols to act as relatively potent metal chelators [44,70] may be of major significance for the treatment of neurodegenerative diseases, in which accumulation of iron has been found in areas of the brain where neurodegeneration occurs. The localization of iron and ferritin in PD patients is restricted to specific brain areas [7,71,72], in the SNPC and not the reticulata, even though the latter region has higher iron content than that of the SNPC [71]. Similarly, AD pathogenesis is also associated with iron accumulation and is linked to the characteristic neocortical Aβ deposition, phosphorylation of tau, and tangle formation, which may be mediated by abnormal interaction with excess of free-chelatable iron. Ionic iron can, in turn, participate in the Fenton reaction with subsequent generation of ROS, which is thought to initiate the processes of oxidative stress and inflammatory cascade resulting in production of cytotoxic cytokines (tumor necrosis factor–α (TNF-α), interleukin-1 and -6) in the microglia and in the surrounding neurons [73–76], and activation of transcription factors and nuclear

factor–κB (NF-κB) [77,78]. Indeed, a 70-fold increase in NF-κB immunoreactivity was found in the nucleus of melanized dopaminergic neurons of Parkinsonian SNPC, as compared to normal brains [79]. EGCG was found to inhibit the nuclear translocation of NF-κB in in vitro systems: immunofluorescence and electromobility shift assays showed that introduction of green tea extract before 6-OHDA–induced oxidative stress inhibited both NF-κB nuclear translocation and binding activity in neuroblastoma SH-SY5Y cells [66]. Furthermore, the reduced activity of NF-κB by EGCG and the theaflavin-3,3′-digallate polyphenol from black tea was associated with inhibition of lipopolysaccharid (LPS)–induced TNF-α production [24] and the enzyme inducible nitric oxide synthase (iNOS) [77,80] in activated macrophages. This enzyme is an inducible form of the nitric oxide synthase (NOS), which is responsible for the production of the short-lived free radical nitric oxide, functioning as a signaling molecule. The inhibition of enzymes, whose activity may promote oxidative stress and an increase in antioxidant enzyme activities, might have a beneficial significance to neuroprotection. EGCG was found to elevate the activity of two major antioxidant enzymes, superoxide dismutase (SOD) and catalase in mice striatum [20].

5. The mechanisms underlying EGCG-induced cell survival/cell death

5.1. Modulation of cell signaling pathways

Emerging evidence suggests that the antioxidant activity of green tea polyphenols cannot be the sole mechanism responsible for their neuroprotective action; rather, their ability to alter signaling pathways may significantly contribute to the cell survival effect. The modulation of cellular survival and signal transduction pathways has significant biological consequences that are important in understanding the various pharmacological and toxicological responses of antioxidant drugs. A number of intracellular signaling pathways have been described to play central functions in EGCG-promoted neuronal protection against a variety of extracellular insults such as the MAPK [21,81,82], PKC [22,83], and phosphatidylinositol-3-kinase (PI-3 kinase)-Akt [84–86] pathways, as described in Fig. 2. Given the critical role of MAPK pathways in regulating cellular processes that are affected in neurodegenerative diseases, the importance of MAPKs as transducers of extracellular stimuli into a series of intracellular phosphorylation cascades disease pathogenesis is being increasingly recognized. Oxidative stress seems to be a major stimulus for MAPK cascade, which might lead to cell survival/cell death. Among the MAPKs the extracellular signal-regulated kinases (ERK1/2) are mainly activated by mitogen and growth factors [87], whereas p38 and c-jun-N-terminal kinase (JNK) respond to stress stimuli [88,89]. Previous in vitro studies [21] demonstrated the potency of EGCG to induce ARE-mediated defensive genes and MAPK pathways including ERK, JNK, and p38 MAPK [90], which enhanced cell survival and beneficial homeostatic response. The role of ERK1/2 signaling seems to be connected to attenuation of neuronal death and cellular injury by oxidative stress [91]. In addition, EGCG counteracted the decline in ERK1/2 induced by 6-OHDA in neuroblastoma cells, whereas neither EGCG nor catechin at their neuroprotective concentrations (1–10 μmol/L) affected the levels of ERK1/2 phosphorylation by themselves, in the absence of any exogenous damage, in neuronal cell line and primary neuron cultures [22,92].

EGCG neuroprotective activity also involves the intracellular signaling mediator PKC [19], which is thought to have an essential role in the regulation of cell survival and programmed cell death [93,94]. PKCs are a family of serine/threonine kinases consisting of 11 isoforms: conventional (α, β_I, β_{II}, γ), novel (δ, ϵ, θ, η, μ), and atypical (ι/λ, ζ). A rapid loss of neuronal PKC activity is a common consequence of several forms of brain damage [95,96]. A previous study [97] demonstrated a significantly reduction of phorbol ester binding, a direct activator of PKC, in the substantia nigra of PD patients compared to controls. The induction of PKC activity in neurons by EGCG (1–10 μmol/L) is thought to be a prerequisite for neuroprotection against several neurotoxins, such as Aβ [19], serum withdrawal [86], and 6-OHDA [22]. Inhibition of PKC phosphorylation abolished completely the protection induced by EGCG and by the PKC activator phorbol 12-myristate 13-acetate (PMA). These in vitro results were supported by a recent study [19] showing that EGCG (2 mg/kg) administered orally to mice caused significant increase of the PKC isoenzymes α and ϵ protein levels in the membrane and cytosolic fractions of hippocampus. These isoforms play a crucial role in cell survival and differentiation pathways [98,99] and may be involved in APP processing that elevated pathogenesis of AD [61,62].

The mechanism by which PKC activation leads to neuroprotection and cell survival has not clearly been defined. Studies with extra-neuronal tissues support a role for PKCα as a kinase of the antiapoptotic Bcl-2, probably through direct or indirect phosphorylation of this cell survival protein [100]. In addition, over-expression of PKCϵ in a hematopoietic cell line resulted in increased expression of the mitochondrial protein Bcl-2 [98]. A recent study in human epidermal keratinocytes taken from EGCG-treated skin of healthy subjects indicated that EGCG promoted cell survival by increasing the ratio of Bcl-2 to proapoptotic Bax and phosphorylation of another proapoptotic Bcl-2 family member, Bad, through ERK and Akt signaling pathways [101]. Using mitogen-activated protein kinase 1 (MEK1) inhibitor (PD98059), EGCG induced only the phosphorylation of Ser136 of Bad; and when the investigators used PI-3 kinase inhibitor (LY294002), EGCG induced the phosphorylation of Ser112 only. These results indicated that the effect of EGCG (0.5 μmol/L) on ERK pathway involved the phosphorylation of Ser112 and its effect on PI-3 Kinase/Akt pathway involved the phosphorylation of Ser136 of Bad. Nonetheless, a study

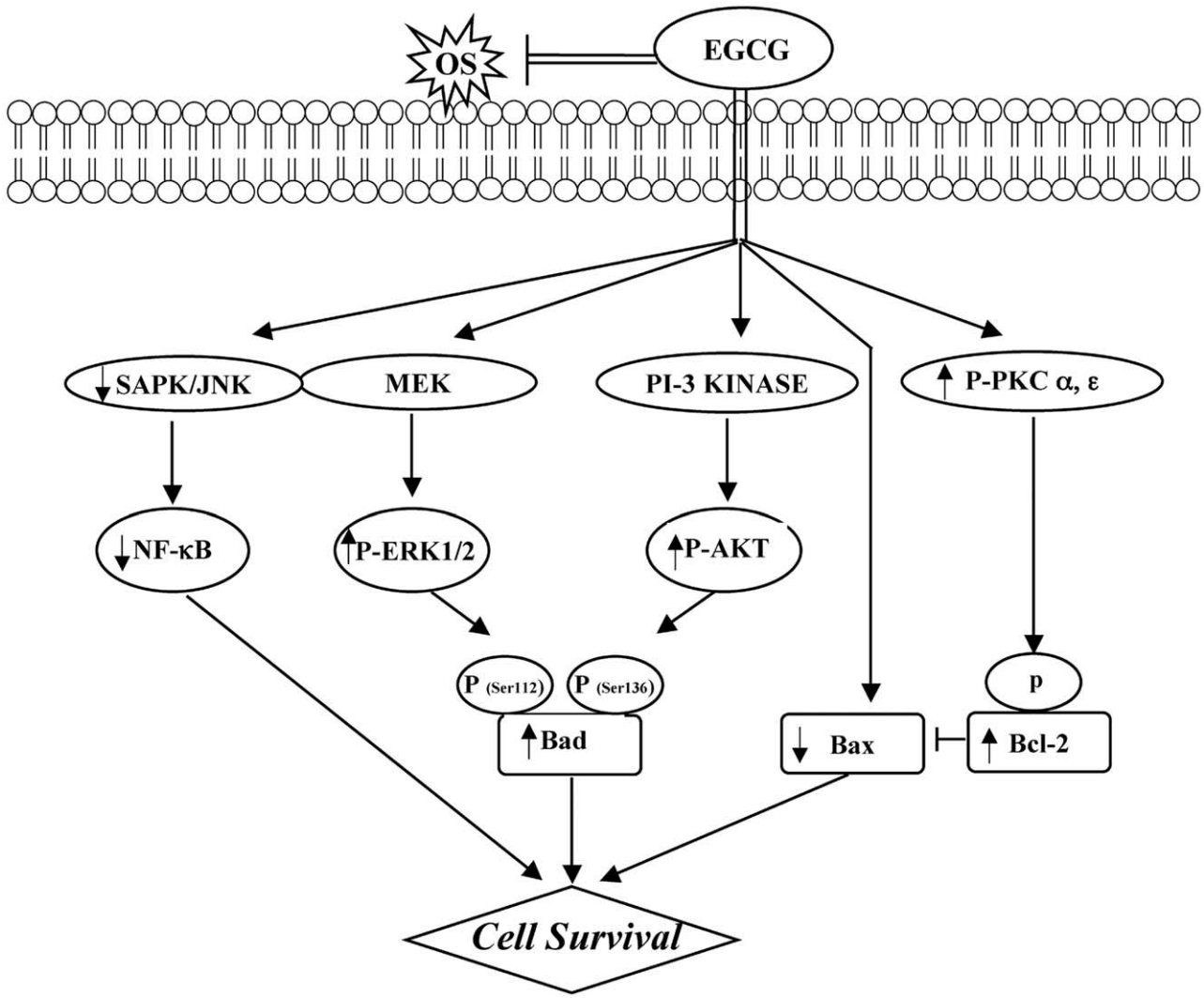

Fig. 2. Model for the protective mechanism of action of (−)-epigallocatechin-3-gallate (EGCG), indicating potential pathways of action with respect to its proposed modulative effect in neuronal and extra-neuronal tissues.

with high concentrations with EGCG reported cell proliferation arrest of tumor cells and inhibition of ERK1/2 and Akt phosphorylation, which was associated with reduced phosphorylation of Bad [102]. In line with these results, a study in human prostate carcinoma LNCaP cells [103] reported that high concentrations of EGCG induced apoptosis via negative regulation of the activity of the transcription factor NF-κB, thereby decreasing the expression of Bcl-2 and up-regulation of the transcriptional activity of p53, resulting in activation of its downstream targets, cyclin-dependent kinase inhibitor p21/WAF1, and Bax. The latter alterations caused a change in the ratio of Bax/Bcl-2 in favor of cell death, followed by activation of caspases 9, 8, and 3. This biphasic mode of biological activity of EGCG relies on its concentration-dependent window of pharmacological action: EGCG exhibits pro-oxidant and pro-apoptotic activity at high concentrations that are responsible for the anti-cancer cell death effect, whereas at low doses it is neuroprotective against a wide spectrum of neurotoxic compounds. A biphasic mode of action has been described for most of the typical radical scavengers and antioxidants such as ascorbic acid (vitamin C) [104], iron chelators such as DA receptor agonist R-APO [105], and also for green tea polyphenols [66].

5.2. Effect on cell survival and apoptotic gene expression

Studies based on customized cDNA and quantitative real-time reverse transcriptase–polymerase chain reaction (RT-PCR) were recently conducted to verify the molecular mechanisms involved in the cell survival and cell death action of EGCG [22,106,107]. In these studies, the gene expression profile of EGCG was compared to that of three other neuroprotective/antioxidant drugs, namely, DA, R-APO (both catechol derivatives), and the pineal indoleamine hormone melatonin, at low and high concentrations [108]. EGCG (1 μmol/L), DA (10 μmol/L), and R-APO (1 μmol/L), behaved as potent neuroprotective agents, de-

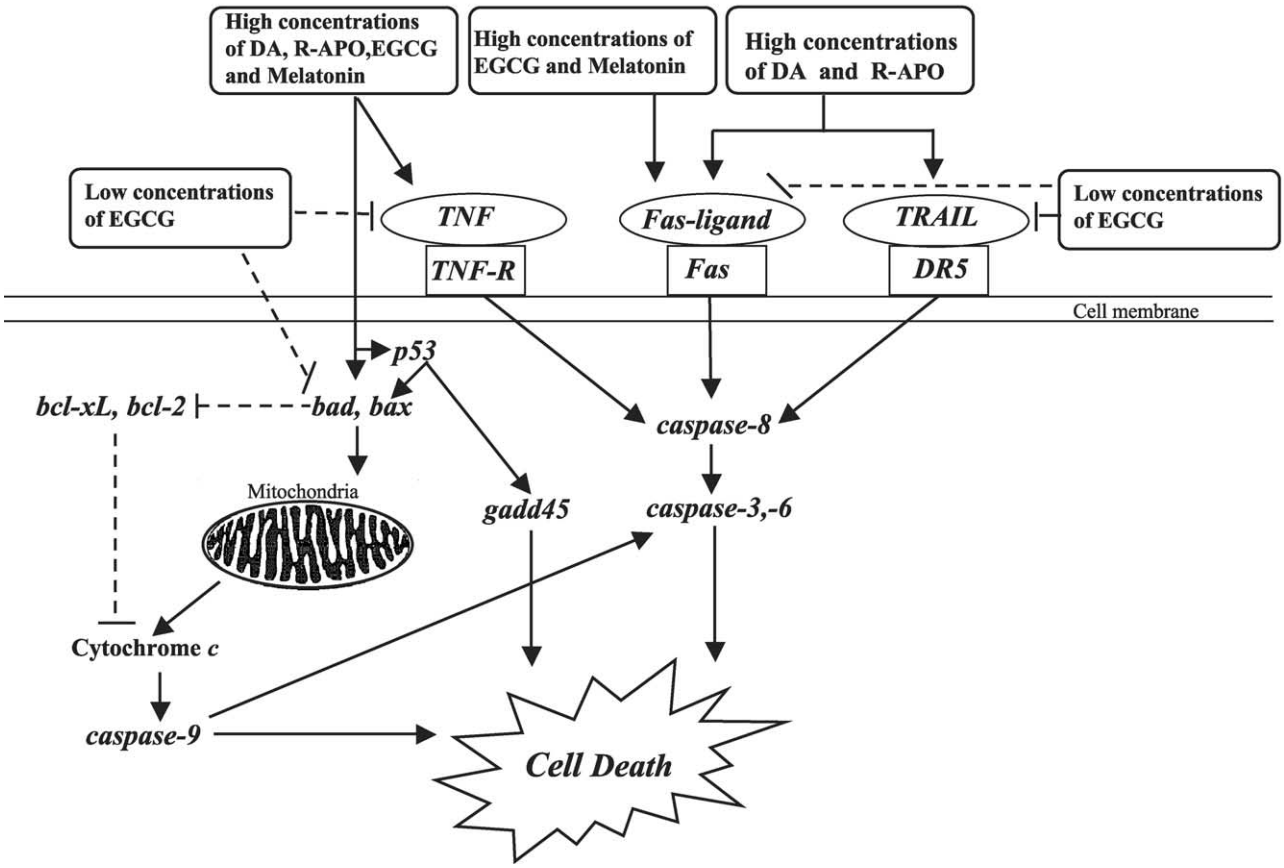

Fig. 3. Schematic overview demonstrating possible gene targets involved in the anti-apoptotic and pro-apoptotic actions of low and high concentrations of (−)-epigallocatechin-3-gallate (EGCG), compared with other antioxidants. Solid arrows and dotted lines indicate induction and inhibition of gene expression, respectively. (See text for full explanation.)

creasing the expression of the pro-apoptotic genes bax, bad, gadd45, and fas ligand. However, EGCG did not affect the expression of the anti-apoptotic bcl-w, bcl-2, and bcl-xL, although the other three compounds increased them. In addition, when cell viability of neuroblastoma SH-SY5Y cells was challenged with 6-OHDA, low micromolar concentrations of EGCG abolished the induction of proapoptotic-related mRNAs and the decrease in bcl-2, bcl-w, and bcl-xL. The EGCG neuroprotective effect is thought to be mediated through down-regulation of pro-apoptotic genes, as shown for mdm2, caspase-1, cyclin-dependent kinase inhibitor p21, and TNF-related apoptosis-inducing ligand (TRAIL), rather than up-regulation of anti-apoptotic genes. These findings support the assumption that complementary mechanisms, in addition to radical scavenging, are involved in their neuronal survival effect. In contrast to the anti-apoptotic effect observed with low concentrations (<10 μmol/L), a pro-apoptotic pattern of gene expression is observed with high concentrations (>20 μmol/L) of EGCG, DA, R-APO, and melatonin. It includes the expression of bax, gadd45, caspase family members (caspase 3, 6, and 10), and TNF receptor family member fas and fas-ligand mRNAs. The results revealed a significant functional group homology between these drugs in genes coding for signal transducers, transcriptional repressors, and growth factors, which may account for their mechanism of action, as illustrated in Fig. 3. This might be predictable, given that EGCG, DA, and R-APO share similar attributes, being catechol-like derivatives, iron chelators, and protective agents against neurotoxicity induced by 6-OHDA or MPTP [109,110].

6. Conclusions and perspectives for the future

To date, accumulated evidence on the health properties of green tea polyphenols on neurodegenerative diseases, in particular AD and PD, is associated with oxidative damage and iron accretion. However, the effect of green tea and its constituents have not been sufficiently evaluated in these disorders. Most studies are carried out in animal models and cell culture to evaluate the effect of acute and chronic administration of the compounds. EGCG appears to affect the mortality of neuronal cells. Given the central role that mitochondria play in oxidative stress–induced apoptosis, it may be speculated that EGCG-mediated inhibition of apoptosis might implicate mitochondrial targets. This may be a consequence of the blockade of mitochondrial permeability transition pore opening, since EGCG has an effect on the

mitochondrial protein expression, the Bcl-2 family members, such as Bax and Bad. Similarly, the anti-Parkinson neuroprotective anti-apoptotic drug rasagiline was recently shown to prevent the fall in mitochondrial membrane potential and the opening of mitochondrial voltage dependent anion channel via the increase in Bcl-2 and Bcl-xl mRNAs and their proteins [111,112]. In addition, the toxicity of the metabolic product of MPTP, MPP+, which is also a mitochondrial complex I inhibitor [113], induces oxidative stress, iron signaling, and α-synuclein expression [114], is attenuated by EGCG probably via its metal chelating ability. Consistent with this idea, a recent study reported a novel iron-responsive element (IRE-type II) within the 5′-untranslated region of the Alzheimer's APP transcript that can be regulated by metal chelators [115] as EGCG. In light of this aggregate of information, we strongly advocate the use of brain-permeable iron chelators as neuroprotective drugs to reduce iron concentrations in those brain areas in which it preferentially accumulates in neurodegenerative diseases.

Future efforts in the understanding of the protective effect mechanism of action of green tea polyphenols must concentrate on deciphering the cell targets affected by these compounds and other neuroprotectants. Furthermore, in vivo studies are needed to clarify whether EGCG and its metabolites, at sufficient concentrations, can reach the brain and can alter cell signaling pathways after a peripheral injection and can affect the progression of neurodegenerative disorders. This will allow a more specific therapy design, especially when a multi–pharmacological action drug cocktail is considered for formulation, which can be more effective when used in the clinical treatment of AD and PD.

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