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Protective action of green tea catechins in neuronal mitochondria during aging

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1. ABSTRACT

Mitochondria are central players in the regulation of cell homeostasis. They are essential for energy production but at the same time, reactive oxygen species accumulate as byproducts of the electron transport chain causing mitochondrial damage. In the central nervous system, senescence and neurodegeneration occur as a consequence of mitochondrial oxidative insults and impaired electron transfer. The accumulation of several oxidation products in neurons during aging prompts the idea that consumption of antioxidant compounds may delay neurodegenerative processes. Tea, one of the most consumed beverages in the world, presents benefits to human health that have been associated to its abundance in polyphenols, mainly catechins, that possess powerful antioxidant properties *in vivo* and *in vitro*. In this review, the focus will be placed on the effects of green tea catechins in neuronal mitochondria. Although these compounds reach the brain in small quantities, there are several possible targets, signaling pathways and molecular machinery impinging in the mitochondria that will be highlighted. Accumulated evidence thus far seems to indicate that catechins help prevent neurodegeneration and delay brain function decline.

2. INTRODUCTION

Biological aging is associated with progressive loss of structural organization, diminishing functional capacity, decreasing adaptability and increasing likelihood of disease and death. Available data shows that age-related changes chiefly result from the accumulation of macromolecular damage by physiologically produced reactive species (1). The different types of cells of living organisms do not age at the same time. Age-associated modifications are most noticeable in long-lived postmitotic cells, such as neurons, whereas in the majority of proliferating cell populations, alterations that occur during aging are less pronounced (2, 3). Neurons possess an extensive cytoplasmic membrane where the active transport of molecules and maintenance of ionic gradients requires a high energy expenditure (4). Indeed, because the brain uses about 20 percent of the baseline inhaled oxygen, intense use of oxidative phosphorylation and electron transport to obtain energy leads to the formation of larger quantity of reactive oxygen species (5). Furthermore, there are additional factors for the increased vulnerability of the central nervous system (CNS) to oxidative stress: (i) high content of polyunsaturated fatty acids on neuronal membranes which serve as a preferred substrates for lipid peroxidation, (ii) moderate amounts of enzymatic antioxidant defenses, (iii) abundance of iron and ascorbic acid which catalyze reactions involved in the formation of reactive pro-oxidant species, (iv) increased production of hydrogen peroxide during enzymatic inactivation of neurotransmitters, and (v) a large

amount of excitatory amino acids like glutamate, capable of inducing neuronal death by overstimulation of receptors and increased intracellular Ca^{2+} concentration, resulting in progressive accumulation of oxidative damage (5, 6). Finally, although it has been postulated that neurons may be replaced due to differentiation of stem cells, the rate of such possible replacement might be too low to substantially prevent the accumulation of damage with time (7, 8). In face of such vulnerability, aging is associated with alterations of number and structure of cellular organelles, atrophy of dendritic arborization accompanied with reduction of dendritic spine number and density in some neuronal populations, reduction in brain volume and decrease of some cognitive and motor abilities (4, 9). Likewise, DNA damage with decreased expression of several genes related with synaptic function including plasticity and synaptic vesicular trafficking are commonly reported features of aging (9, 10). Previous reports also found a decrease in nuclear transcription factors such as cyclic AMP response element-binding (CREB) as well as genes responsible for neurotrophins synthesis (11). Oxidative stress leads to damage in proteins (12) that may become more susceptible to degradation, formation of glycation products and deposition of amyloid bodies in neurons, resulting in the hindrance of their function (4, 13). Oxidative stress equally damages polyunsaturated fatty acids present in cellular membranes altering permeability and fluidity (6).

Albeit aging might affect many cellular components, perhaps the most remarkable modifications occur in mitochondria of postmitotic cells (14). The most apparent reason for the high susceptibility of mitochondria is their direct exposure to self-generated reactive oxygen species (ROS) (15). Indeed, this organelle uses oxygen at a high rate, hence releasing oxygen radicals that exceed the various defense mechanisms including antioxidant molecules such as reduced glutathione (GSH) or vitamin E and enzymes namely superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) or glutathione reductase (16, 17). The simultaneous enhancement of lipid peroxidation and oxidative modification of proteins in mitochondria further increases mutations and oxidative damage to mitochondrial DNA (mtDNA) in the aging process (18). In recent years, a great deal of research has been devoted to the development of strategies that can delay or even reverse age-related neuronal impairments, particularly those related to mitochondria. Regarding this subject, antioxidant compounds, due to their pivotal role in the modulation of oxidative stress-associated cellular mechanisms, are gathering attention of researchers in the field of mitochondria brain aging (19-21). It has been reported that oral treatment with certain antioxidants, such as sulphur-containing antioxidants (e.g. GSH and thiazolidine carboxylate derivatives), vitamins C and E or *Ginkgo biloba* protects against age-associated oxidative damage to mtDNA and oxidation of mitochondrial glutathione in brain tissue from mice and rats (22, 23). *Ginkgo biloba* extracts have also been shown to prevent changes in neuronal mitochondrial function and morphology induced by aging (23). Green tea (GT) has also been in focus, as it is a rich source of brain-accessible antioxidants known as polyphenols (24, 25). Many of these compounds are monomeric catechins, which have been shown to exert antioxidant effects acting directly as radical scavengers or metal-chelators and also indirectly through modulation of transcription factors, signaling regulators or antioxidant enzymes (26-28). Furthermore, favorable effects of GT catechins on brain age-related degenerative alterations and cognitive impairments have been reported in aged mice with accelerated senescence (29, 30). In the current article, we review the most relevant data concerning the protective effects of GT catechins in brain aging, focusing on their interference on changes that occur in neuronal mitochondria with advanced age.

3. NEURONAL MITOCHONDRIA LOCATION AND FUNCTIONS

In neurons, mitochondria are not confined to cell bodies but are distributed throughout the length of growth cones, axons and presynaptic terminals and, in dendrites, are located mainly in the dendritic shafts and occasionally found associated with spines (31). Nevertheless, they tend to accumulate near high-energy requiring regions, such as presynaptic terminals, suggesting directed motility and clustering according to energy needs (32). Mitochondria interact with cytoskeletal components (microtubules, actin filaments and intermediate filaments) allowing their anchoring, transport and motility inside neurons, offering the unique possibility to adjust their subcellular spatial distribution to the neuron's metabolic demands, particularly in terms of growth and development (33, 34). The mechanisms that control mitochondrial movements and determine their subcellular distribution are not entirely understood but it is believed that they are regulated by local signals such as neurotrophic factors and Ca^{2+} influx (35, 36). Additionally, it has

been shown that mitochondria move rapidly within and between subcellular compartments (37), undergo fission and fusion (38), respond to electrical activity and activation of neurotransmitter and growth factor receptors (e.g. move, change their energy output, take up or release Ca^{2+}) (39) and function as signaling outposts that contain kinases, deacetylases and other signal transduction enzymes (40). In line with this, it was suggested that mitochondria emit molecular signals namely ROS, proteins and lipid mediators that can act locally or travel to distant targets, including the nucleus (41).

Most of adenosine triphosphate (ATP) in the brain (greater than 95 percent) is produced by oxidative phosphorylation in the mitochondria, whereas glycolysis alone in the cytoplasm contributes to only 1–5 percent of ATP production. This means that the concentration of ATP is maintained under steady-state conditions solely in the presence of an adequate supply of oxygen and substrates (32). It has been shown that impairment of mitochondrial ATP generation clearly threatens the viability of both neurons and glial cells, the activity of neuronal networks and consequently normal brain function (32, 42). However, mitochondria are not exclusively an important source of cellular energy. They also maintain intracellular Ca^{2+} levels within closely defined ranges for the mediation of signaling and control ROS metabolism (43). These ROS have been implicated in several physiologic processes such as phagocytosis, proliferation, differentiation, apoptosis and cell signaling. Indeed, a new concept is now emerging that mitochondrial ROS production is likely to be highly regulated as a part of physiological mitochondrial functions and the underlying molecular mechanisms are being gradually uncovered (44). For instance, while microM levels of nitric oxide ($NO^{\bullet}$) acutely inhibit cell respiration by binding to cytochrome c oxidase, nanoM levels of this endogenous free radical trigger mitochondrial biogenesis in diverse cell types through a cGMP-dependent manner (45). By generating energy and regulating subcellular Ca^{2+} and redox homeostasis, mitochondria play critical roles in the control of fundamental processes in neuroplasticity including neural differentiation, neurite outgrowth, neurotransmitter release and dendritic remodeling (41). Failure of mitochondrial Ca^{2+} buffering and/or release of sequestered Ca^{2+} present within mitochondria contributes to the severe damage of brain tissue (32).

Mitochondrial function modulates the cytosolic pH of the host cell, thereby potentially altering cell function and neuronal excitability, due to proton pumping required for energy generation (32). Among the ion channels modulated by changes in pH are voltage-gated Ca^{2+} channels which can be activated by alkalosis and blocked by acidosis (46). Mitochondria are also key mediators of apoptosis because mitochondrial permeability transition (MPT), an increase in the permeability of the mitochondrial membranes, is a critical step in apoptosis (47). The opening of MPT pores causes release of apoptogenic factors such as cytochrome c, procaspases 2, 3, and 9 and the apoptosis-inducing factor from the intermembrane space (47, 48). Mitochondria-mediated cytosolic pH changes have also been reported to be involved in mitochondria-associated apoptosis, with cytosolic acidosis promoting activation of caspases by cytochrome c (49).

In view of these several mitochondrial functions and their integration into various cellular signaling pathways it is not surprising that alterations in mitochondrial physiology are currently being considered as pivotal events in neurodegeneration associated to the aging process.

4. MITOCHONDRIA IN THE AGING PROCESS

One of the most relevant theories raised to explain aging, was proposed by Denham Harman in 1956. According to this theory, the aging process occurs due to the progressive accumulation of molecular lesions caused by lifelong reactions between free radicals and cellular components (50). In 1972, Harman revisited his free radical theory of aging proposing the mitochondria as the main source of free radicals and, simultaneously, the main target of free radical action during aging (51). Since then, the *Free Radical Theory of Aging* has become the *Mitochondrial Free Radical Theory of Aging*, which is the most famous version of Harman's theory. Thereafter, the role of mitochondria in the process of the age-dependent deterioration of tissues has become the focus of many studies with the gradually accepted idea that mitochondrial decay is a major contributor to aging. Despite Harman's theory having focused on free radicals such as superoxide anion ($O_2^{\bullet -}$), hydroxyl ($HO^{\bullet}$) and $NO^{\bullet}$, it is now known that other non-radical pro-oxidant species are involved, namely hydrogen peroxide (H_2O_2), singlet oxygen and peroxynitrite, also endowed with enormous chemical instability (1, 6). Altogether these radical and non-radical compounds are grouped as

reactive oxygen and nitrogen species and, while they may be generated in lysosomes, peroxisomes and smooth and rough endoplasmic reticulum, a large part of their formation occurs in mitochondria as metabolic intermediates of oxidation reactions in which oxygen is the final electron acceptor in oxidative phosphorylation (6, 52). For example, the steady state concentration of $O_2^{\bullet -}$ in the mitochondrial matrix is about 5- to 10-fold higher than in the cytosolic and nuclear spaces. This $O_2^{\bullet -}$ undergoes dismutation originating H_2O_2, which can further react to form $HO^{\bullet}$ (53). Pro-oxidant species generated by mitochondria, or from other sites within or outside the cell, increase with age and cause mitochondrial dysfunction inactivating enzymes, altering transmembrane transport and oxidizing macromolecules. These deleterious events may play major roles in various age-related degenerative processes (52, 54). A usual finding in aging is the increased content of oxidation products of phospholipids, proteins and nucleic acids that correspond to the ROS- mediated oxidation of cellular and mitochondrial constituents (55). Concerning mtDNA, as it is in close proximity to the sites of ROS production and because it lacks protective histones or effective repair systems, it is a sensitive target for ROS attack: as a logical corollary, the level of oxidatively modified bases in mtDNA is 10- to 20-fold higher than that in nuclear DNA (53). A progressive accumulation of oxidative lesions, deletions, point mutations and aberrant forms have been found in mtDNA of postmitotic tissues upon aging, often involving the sites coding for respiratory chain proteins (23, 56). As a result, mitochondria of aged postmitotic cells have decreased activity of the Krebs cycle, beta-oxidation and oxidative phosphorylation enzymes and consequently produce less ATP than the mitochondria of young cells (14, 56). In addition, respiratory enzymes containing the defective mtDNA-encoded protein subunits may increase the production of ROS, which in turn would aggravate the oxidative damage to mitochondria (47), rapidly extending mitochondrial dysfunction to disturbance of cell homeostasis.

5. AGE-RELATED NEURONAL MITOCHONDRIAL DYSFUNCTION

An age-dependent decline of mitochondrial oxidative phosphorylation function may be related to decreased electron transfer activity, increased H^+ permeability of the inner membrane and diminished H^+-driven ATP synthesis (57). Enzyme activities of complexes I and IV and nitric oxide synthase (NOS) were significantly reduced in inner mitochondrial membranes from whole brain, cortex and hippocampus of aged and senescent rats compared to those observed in young animals (58). Moreover, the observed decrease of electron transfer activity in aged mammalian brain mitochondria was found to be simultaneous with the development of a mitochondrial subpopulation with increased fragility and swelling (23, 59). Usually, mitochondrial size varies more in old cells, as compared to corresponding young cells, with a high proportion of large, sometimes extremely large “giant mitochondria” (14, 60). Moreover, mitochondria undergo other structural modifications during aging such as loss or shortening of cristae, matrix vacuolization and inner membrane or cytoplasmic lamellae deterioration, which may be associated with age-related impairment in mitochondrial membrane potential (14, 61). Consequently, the number of defective mitochondria within long-lived postmitotic cells progressively increases with age.

In addition, memory loss in old rats was associated with brain mitochondrial decay and RNA/DNA oxidation in the hippocampus, an important region implicated in spatial memory (62). Protein carbonyls, TBARS, organic hydroperoxides and 8-hydroxy-2’-deoxyguanine are important biomarkers of oxidative damage and have been reported to be augmented in brain mitochondria of aged mammals (57). For instance, Navarro and colleagues found that TBARS and protein carbonyls were significantly increased in mitochondria isolated from whole brain, cortex and hippocampus of aged and senescent rats when compared with young animals. They also observed an inverse relationship between the content of oxidation products and the enzyme activities in neuronal mitochondria indicating that brain regions age with simultaneous oxidative damage and mitochondrial enzyme dysfunction (58). The hippocampus and other brain regions accumulate dysfunctional mitochondria during aging, which can lead to apoptosis and tissue atrophy (58). Neurons with dysfunctional mitochondria need only small disturbances of cell environment to initiate apoptosis, starting by increasing cytosolic Ca^{2+} concentration. This activates NOS, mainly mitochondrial NOS, raising intracellular $NO^{\bullet}$ and H_2O_2 which increase lipid peroxidation, mitochondrial dysfunction and cytochrome c release, ultimately leading to apotosome assembly, caspase activation and DNA fragmentation (63).

6. GREEN TEA CATECHINS AND NEUROPROTECTION

Tea, one of the most commonly consumed beverages in the world, is prepared by infusion of young leaves of the plant *Camellia sinensis* and, depending on the type of treatment they are subjected to, can be classified into green, oolong or black (64, 65). In GT, polyphenol oxidase is inactivated after harvesting by brief exposure to heat in order to prevent fermentation and, as a consequence, the obtained beverage has a polyphenolic composition very similar to plant leaves (64, 66). Many of the benefits to human health ascribed to GT consumption have been associated to its abundance in polyphenols (66). These compounds are grouped into different classes according to their chemical structure, one of the main classes being flavonoids to which belong hundreds of molecules distributed into subclasses such as flavanols or flavan-3-ols. The simplest compounds in this subclass are the monomers of (+)-catechin and (-)-epicatechin (EC) and, unlike other flavonoids, are in the free state or esterified with gallic acid, a type of phenolic acid (67, 68). Catechins, their derivatives and gallic acid have been implicated in the majority of favorable properties of GT consumption (66). The main flavanols present in GT include catechins which can represent 30 to 42 percent of the dry weight of GT leaves. The major catechins are (+)-catechin, EC, (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG) and (-)-epigallocatechin-3-gallate (EGCG), the latter being 50 to 80 percent of total catechins (64). It was estimated that a cup of GT (2.5. g of GT leaves brewed in 200 ml of water) may contain 90 mg of EGCG (69) which is thought to be the main contributor to the beneficial health effects attributed to GT intake.

It is recognized that only a small fraction of the ingested catechins is detectable in plasma or urine, suggesting that these compounds are poorly absorbed or are modified after absorption (70, 71). The uptake of these phytochemicals depends on factors such as the molecular structure, amount ingested, food matrix, nutritional status as well as genetic factors (67, 72). Furthermore, some authors highlight the importance of the intestinal microflora in the biotransformation of GT catechins and, consequently, in their bioavailability and biological effects (73, 74). In this respect, the polymeric catechins that remain in the gut may favor proliferation of commensal bacteria (75). Given the recognition of the involvement of intestinal microflora in bidirectional interactions between the CNS and the digestive system (76), it is proposed that they may influence different brain functions, including behavior (77, 78). Anyhow, the absorbed fraction, after hepatic first-pass metabolism, reaches systemic circulation either modified or unaltered, being distributed to organs and tissues and exerting their biological effect (71, 79).

The access of these compounds to the brain may still be hampered by the blood-brain barrier (BBB) which, due to its high selectivity, hinders the passage of hydrophilic, polar substances or compounds with high molecular weight to the CNS (80). The ability of these compounds to cross the BBB depends on their lipophilicity and on the activity of transporters such as P-glycoprotein efflux pump expressed in the apical surface of endothelial cells of brain capillaries limiting bioavailability (25). In this regard, Youdim and colleagues showed that tea flavonoids and their conjugates with physiological relevance cross the BBB in *in vitro* and *in situ* models (81, 82). In a more recent paper, (+)-catechin was able to cross RBE-4 cells, an immortalized cell line of the rat cerebral capillary endothelial cells, in a time-dependent manner (83). The same authors also found that (+)-catechin, EC and their metabolites (4'-O-methylcatechin, 3'-O-methylepicatechin and 4'-O-methylepicatechin) are transported through a human BBB model, hCMEC/D3 cells, in a time-dependent manner with the metabolites showing higher transport efficiency than the native catechins (84). EC metabolites (epicatechin glucuronide and 3'-O-methylepicatechin glucuronide) have also been detected in brain tissue of rats that ingested EC (85). Moreover, oral administration of (^{3}H)EGCG allowed detection of radioactivity in various organs including the brain and a small amount of (^{3}H)EGCG was excreted in the urine of male and female mice (86). The absorption and pharmacokinetics of EGCG has also been evaluated in conscious and freely moving rats in various brain regions after oral administration of EGCG (100 mg/kg). These authors found that oral bioavailability of EGCG was about 4.9.5 percent (87).

With the improvement of methodological approaches, it is becoming clear that polyphenols do have access to the CNS and, albeit they reach brain tissue in small quantities, this supports a possible local neuroprotective activity. Indeed, several possible targets of GT catechins' action in the brain have been proposed: calcium homeostasis (88), extracellular mitogen-activated protein kinases (89) and protein

kinase C (PKC) (90), regulation of antioxidant enzymes and antioxidant response element (91, 92), cell death and cell survival genes and proteins associated with mitochondrial function (93, 94), amyloid precursor protein processing pathway and iron regulators and sensors (95, 96). Additionally, EGCG inhibits catechol-O-methyltransferase and averts depletion of dopamine in the striatum, prevents the loss of dopaminergic neurons in the substantia nigra, increases the activity of SOD and CAT in brain tissue and the levels of GSH and PKC on the hippocampal formation while reducing neurotoxicity and memory deficits induced by amyloid *beta*-peptide (97, 98). In this regard, it was also shown that both EGCG and whole GT extracts improve cholinergic function by cholinesterase inhibition and reduce the activity of *beta*-secretase, an enzyme involved in the cleavage of the protein that originates amyloid *beta*-peptide and is responsible for its extraneuronal accumulation (99, 100). In 19-month old rats consuming GT since 12 months of age this catechin-rich beverage, was able to reverse most of the impairments associated with aging to levels similar to those found in 12-month old control rats. These ameliorations were observed specifically in the levels of lipid peroxidation, protein carbonyls, antioxidant enzymes, deposition of neuronal lipofuscin in the hippocampal region and cognitive performance (101). Using the same model, GT was also shown to increase the activation of CREB and the levels of brain-derived neurotrophic factor and anti-apoptotic protein Bcl-2 in the hippocampal formation when compared to age-matched controls, establishing a molecular mechanism of action for GT in the prevention of age-dependent memory decline (102).

7. CATECHINS AND NEURONAL MITOCHONDRIAL AGING

Oxidative stress is a major player in aging and neurodegenerative disorders. Macromolecular damage occurs as a result of oxidative stress that affects the mitochondria, often culminating in cell death by apoptosis or necrosis (103). GT catechins have emerged as antioxidant nutraceuticals with neuroprotective activity, counteracting age-associated oxidative damage in brain tissue (104). It was recently reported that oral EGCG supplementation (2 mg/kg body weight/day) for a period of 30 days upregulated the antioxidant system (SOD, CAT, GPx, ascorbic acid, *alpha*-tocopherol and GSH), improved lipid peroxidation and decreased carbonyl levels in aged rat brain mitochondria when compared with age-matched controls (105). (+)-Catechin has also been shown to inhibit the nonenzymatic lipid peroxidation in rat brain mitochondria induced by either ascorbic acid or ferrous sulfate, measured as MDA levels through the TBARS test (106). Immunohistochemical analysis has revealed that EGCG supplementation decreased 4-hydroxynonenal (HNE)-protein adducts produced as a consequence of lipid peroxidation in cerebellar Purkinje cells of old rats (105). 4-HNE is a major byproduct of lipid peroxidation, thought to be the most reactive, and an important mediator of free radical damage (107). It has been shown that 4-HNE reacts with key mitochondrial enzymes leading to age-dependent loss in energy generation and enhanced susceptibility of neurons to apoptosis (108). In this regard, the reduction of 4-HNE-modified proteins supports the potentially beneficial effects of EGCG against brain mitochondria aging.

In the same line, it has been shown that flavonoids display different effects on H_2O_2 production by brain mitochondria. EC strongly inhibits H_2O_2 production by brain mitochondria, even when H_2O_2 production rate was stimulated by the mitochondrial inhibitors rotenone and antimycin A (109). Studies involving monoamine oxidase (MAO) A and MAO B activity measurements both in human brain from post-mortem samples or in rodent brain regions have shown a generalized age-related increase in MAO B activity and little or no variation in MAO A activity. The enhancement of MAO B is believed to cause oxidative stress contributing to increased susceptibility to neurodegeneration (110). Indeed, the activity of this mitochondria-bound enzyme is known to be a considerable source of H_2O_2 generated by this organelle. The H_2O_2 produced during dopamine oxidation may interact with free iron to form highly reactive hydroxyl radicals that can damage nucleic acids, proteins and membrane lipids, and lead to neuronal degeneration (6). Interestingly, EGCG has been reported to be an effective MAO B inhibitor in adult rat brain (111), although neither EC, EGC, ECG nor EGCG affect MAO A activity in mouse brain mitochondria (112). This inhibition of MAO B activity may well underlie part of the neuroprotective effects of EGCG.

Moreover, EGCG treatment enhanced the activities of Krebs cycle enzymes (succinate dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase, citrate synthase, aconitase and fumarase) and electron transport chain complexes I-IV in aged rat brain mitochondria

in comparison to age-matched animals (105). Furthermore, in neuroblastoma cells expressing mutant amyloid *beta*-protein precursor, *in vitro* screening of 25 natural compounds for their ability to attenuate mitochondrial dysfunction revealed that EGCG was one of the top mitochondrial restorative compounds (113). *In vivo* testing of EGCG to determine its effects on brain mitochondrial function in an amyloid *beta*-protein precursor/presenilin 1 double mutant transgenic mouse model of Alzheimer´s disease, showed that this polyphenol is able to restore mitochondrial respiratory rates, mitochondrial membrane potential, ROS production, and ATP levels by 50 to 85 percent in mitochondria isolated from the hippocampus, cortex, and striatum (113). In addition, EGCG and ECG inhibited the enzymatic activity of rat brain mitochondrial proton F0F1-ATPase/ATP synthase, the enzyme that synthesizes ATP during oxidative phosphorylation (IC_{50} 17 and 45 microM, respectively), pointing that the inhibition of this enzyme should be considered when examining the neuronal effects of GT polyphenols (114). In mitochondria isolated from rat brain, up to 100 microM EC produced only a small reduction of complex I activity in comparison with other polyphenols and had no effect on complexes II-IV, without affecting the rate of oxygen consumption. However, EC was also able to stoichiometrically reduce purified cytochrome c (109). It thus seems that different GT catechins exert distinct effects on the modulation of metabolic functions of mitochondria. Considering that aging results in decreased activity of the Krebs cycle and oxidative phosphorylation enzymes when compared to young cells (14, 56) and that EGCG is the catechin present in higher amounts, GT polyphenols may improve metabolic functions of mitochondria.

EGCG appears to affect also the survival 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. EGCG in low concentrations (0.1.-10 microM) was found to decrease the expression of proapoptotic genes *bax*, *bad*, *caspase-1* and *-6*, cyclin-dependent kinase inhibitor *p21*, cell-cycle inhibitor *gadd45*, *fas ligand* and tumor necrosis factor-related apoptosis-inducing ligand *TRAIL* in SH-SY5Y neuronal cells (93, 94). In accordance, it was shown that EGCG given orally (2 mg/kg body weight/day) for 10 days reduced Bax-positive immunoreactivity to levels lower than those of control mice in dopaminergic neurons of the substantia nigra pars compacta (115). The decline in Bax expression by EGCG may favor the increase in the ratio of Bcl-2/Bcl-xL to Bax/Bad proteins, thereby contributing to mitochondrial stability and regulation of MPT pores (116). Protection of mitochondrial integrity is of major importance, especially in the case of postmitotic cells such as neurons which are commonly not renewed. Another study of the antiapoptotic action of EGCG revealed that 90-95 percent of the catechin accumulated in the mitochondrial fraction of primary cultures of rat cerebellar granule neurons. In this experiment, EGCG displayed selective antiapoptotic effects, protecting from some, but not all, inducers of mitochondrial oxidative stress (117). The neuroprotective potential of EGCG was also evaluated in a H_2O_2-induced oxidative stress model in PC12 cells treated with H_2O_2 with or without EGCG. PC12 cells, derived from pheochromocytoma and embrionically derived from the neural crest, can easily differentiate into neuron-like cells (118). EGCG prevented the decrease in the cellular thiol concentration and the increase the protein carbonyl content induced by H_2O_2 in PC12 cells. Cell death was decreased by EGCG treatment what was paralleled by an increase in mitochondrial membrane potential and a decrease in TNF-*alpha* levels, suggesting that EGCG exerts neuroprotective actions through antioxidant, antiapoptotic and anti-inflammatory effects (103). Similarly, $NO^{\bullet}$ is associated with many pathophysiological processes of the CNS including brain ischemia, neurodegeneration and inflammation. One study evaluated the effect of EGCG on $NO^{\bullet}$-induced cell death in PC12 cells and showed that EGCG inhibited the cytotoxicity and apoptotic morphogenic changes induced by the administration of sodium nitroprusside, a $NO^{\bullet}$ donor. EGCG also decreased the generation of ROS and prevented apoptosis induced by the $NO^{\bullet}$ precursor, by changing the Bax to Bcl-2 expression ratio, avoiding the release of cytochrome c from the mitochondria into the cytosol and the upregulation of the voltage-dependent anion channel, a cytochrome c releasing channel. Furthermore, EGCG prevented the activation of caspase-9, caspase-8 and caspase-3 induced by increasing $NO^{\bullet}$ availability (119). Therefore, catechins, especially EGCG, are significant modulators of cell death, process that is amplified during aging and the anti-apoptotic, pro-survival effects they display seem to largely depend on their interaction with mitochondria.

8. CONCLUDING REMARKS

It is now becoming clear that the protective effects of GT catechins go far beyond their simple

antioxidant properties and include interactions with proteins and lipids of plasma membranes, triggering several protective transcription factors, protein kinases and growth factors. Moreover, these polyphenols are transported to various intracellular compartments, particularly the mitochondria which constitute a feasible target of GT catechins' action. However, a significant part of the investigation regarding the neuroprotective effects of GT catechins have been performed *in vitro* and, certainly, the functional effects can be different *in vivo*. Main reasons include the dramatic changes in bioavailability due to the already described complex absorption through the gut and the BBB. In addition, polyphenol concentration ranges used in most *in vitro* reports are not achievable in the plasma/serum or tissue of living experimental animals added to the known pro-oxidant properties of high non-physiological concentrations. Therefore, recommendations of a daily intake of GT catechins need to consider functional active doses and the issue of catechin bioavailability. Another important question concerns the effects of complex mixtures of catechins. Currently, it is not clear if these compounds act in independently, synergistically, additively or even in an antagonistic manner.

However, the relatively few animal studies on the subject show that GT catechins display protective effects in models of neurodegeneration. Yet, further investigations are required to understand the effects of catechins in mitochondria of old animals and to identify biomarkers that can respond to simultaneous active and physiological concentrations of catechins. Only with these lacking data, it will be possible to perform pharmacodynamics and clinical studies in man with the high levels of evidence that are still not available. Consequently, their beneficial effects in humans have not been clearly demonstrated in clinical trials. The difficulty of these compounds to penetrate the BBB is a conceivable reason for this lack of reported effects. In this regard, much effort is currently being invested in developing brain delivery systems for specific targeting, especially to mitochondria, where their pharmacological activity is mostly required to increase therapeutic efficacy in the field of neuroprotection. Furthermore, since mitochondrial dysfunction probably represents an early pathological event, human studies on the efficacy of GT catechins will likely need to be initiated early in the course of the aging process.

In conclusion, mitochondria are key regulators of cellular energy metabolism, redox homeostasis and cell fate and have been proposed to act as central organelles in the regulation of aging. Oxidative stress is the major factor underlying mitochondrial dysfunction in different brain regions and, therefore, therapeutic strategies that aim at manipulating redox metabolism represent promising options at the center stage of targeted drug development. Apart from the limitations mentioned above, available evidence is strong and it can be assumed with high level of confidence that some *in vitro* results can be extended to normal physiological conditions in the living organism. As a result, it seems safe to state that GT catechins may help coping with the increase of mitochondrial oxidative stress accompanying aging and, perhaps allied with other adjustments throughout life, can assist in the prevention of neurodegeneration and delay of brain function decline.

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