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REVIEWS: CURRENT TOPICS

Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration

Brad A. Sutherland, Rosanna M.A. Rahman, Ian Appleton*

Department of Pharmacology and Toxicology, University of Otago, Dunedin, P.O. Box 913, Dunedin, New Zealand

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Abstract

Catechins are dietary polyphenolic compounds associated with a wide variety of beneficial health effects in vitro, in vivo and clinically. These therapeutic properties have long been attributed to the catechins' antioxidant and free radical scavenging effects. Emerging evidence has shown that catechins and their metabolites have many additional mechanisms of action by affecting numerous sites, potentiating endogenous antioxidants and eliciting dual actions during oxidative stress, ischemia and inflammation. Catechins have proven to modulate apoptosis at various points in the sequence, including altering expression of anti- and proapoptotic genes. Their anti-inflammatory effects are activated through a variety of different mechanisms, including modulation of nitric oxide synthase isoforms. Catechins' actions of attenuating oxidative stress and the inflammatory response may, in part, account for their confirmed neuroprotective capabilities following cerebral ischemia. The versatility of the mechanisms of action of catechins increases their therapeutic potential as interventions for numerous clinical disorders. However, more epidemiological and clinical studies need to be undertaken for their efficacy to be fully elucidated.

Keywords: Catechins; Cerebral ischemia; Inflammation; Nitric oxide synthase; Antioxidant

1. Introduction

Chinese mythology purports that the emperor Shen Nung discovered tea in 2737 BC (see Ref. [1]). This is evidenced by the fact that the first reports on the beneficial effects of green tea also date back to 2700 BC. The first scientific publication was in 1211 AD, when the Japanese monk Eisai wrote a book entitled *Kissa-yojoki*, which loosely translates into, "How to keep healthy drinking tea." The medicinal properties of green tea were further recognized in the 16th century by European explorers who used tea extracts to fight fever, headache, stomachache and articulation pains (see Ref. [2] for review).

Green tea belongs to the Theacease family and comes from two main varieties [3]: *Camellia sinensis* var. *sinensis* and *C. sinensis* var. *assamica* [4]. Catechins are the main bioactive constituents of green tea leaves and account for 25% to 35% of their dry weight. The main catechin group consists of eight polyphenolic flavonoid-type compounds, namely, (+)-catechin (C), (−)-epicatechin (EC), (+)-gallocatechin (GC), (−)-epigallocatechin (EGC), (+)-catechin gallate (CG), (−)-epicatechin gallate (ECG), (+)-gallocatechin gallate (GCG) and (−)-epigallocatechin gallate (EGCG, Fig. 1). (−)-Epigallocatechin gallate is the most abundant [5] of the tea catechins and thought to be responsible for the majority of the biological activity of green tea extracts. Black tea also contains small amounts of

Abbreviations: 3-HK, 3-hydroxykynurenine; 6-OHDA, 6-hydroxydopamine; AA, arachidonic acid; AAPH, 2,2′ -azobis (2-amidinopropane) hydrochloride; AD, Alzheimer's disease; ALT, alanine aminotransferase; ARE, antioxidant response element; BBB, blood–brain barrier; C, (+)-catechin; CCA, common carotid artery; CD, cluster of differentiation; CG, (+)-catechin gallate; CHD, coronary heart disease; COX, cyclooxygenase; EC, (−)-epicatechin; ECG, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCG, (−)-epigallocatechin gallate; eNOS, endothelial nitric oxide synthase; GC, (+)-gallocatechin; GCG, (+)-gallocatechin gallate; HI, hypoxia–ischemia; ICH, intracerebral hemorrhage; IFN γ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; LDL, low-density lipoprotein; LPS, lipopolysaccharide; MCAO, middle cerebral artery occlusion; MIP, macrophage inflammatory protein; MS, multiple sclerosis; NF-κB, nuclear factor κB; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; NOS, nitric oxide synthase; PD, Parkinson's disease; PI3K, phosphatidyl inositol-3 kinase; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF α, tumor necrosis factor α; TXA_2, thromboxane A_2; VSCC, voltage-sensitive calcium channels; XO, xanthine oxidase.

* Corresponding author. Tel.: +64 3 479 5746; fax: +64 3 479 9140.
E-mail address: ian.appleton@stonebow.otago.ac.nz (I. Appleton).

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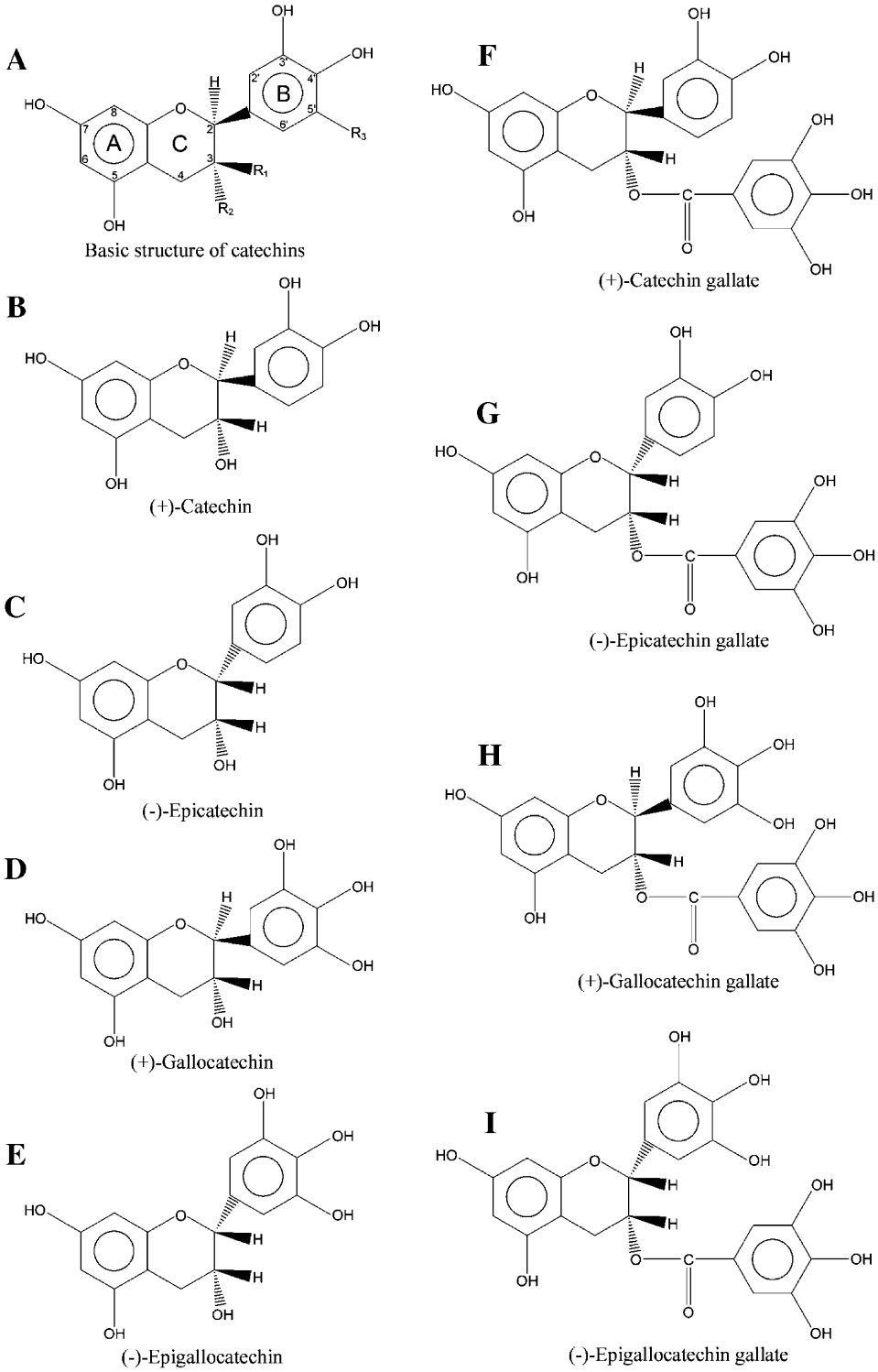

Fig. 1. Chemical structures of the green tea catechins. Catechins have a three-ring structure (A) but have differing hydroxyl groups in the B ring and/or a gallate group at the 3 position of the C ring. The eight different types of catechins are (B) (+)-catechin, (C) EC, (D) GC, (E) EGC, (F) CG, (G) ECG, (H) GCG and (I) EGCG.

catechins. However, due to the fermentation process of black tea, its primary antioxidant polyphenols are theaflavins [6]. The polyphenolic tea catechins are also found in many commonly consumed fruits and beverages (Table 1).

Catechin intake has been associated with a wide variety of beneficial health effects in vitro, in vivo and clinically (see Table 2). The wide therapeutic potential of catechins, factored with their inexpensive production, makes this

Table 1
Concentration of catechins in common dietary products

Food	Catechin content[a]	Ref.
Apples (16 varieties)	1000–7000 mg/kg of fresh cortex-mainly EC	[7]
Apples (Jonagold)	17 mg/kg C + 129 mg/kg EC	[8]
Beer	0.1–5.0 mg/L	[9]
Black, red and white currants	up to 30 mg/kg	[10]
Blueberries	up to 30 mg/kg	[10]
Cacao liquor	63 mg/L C + 577 mg/L EC	[11]
Chocolate (baking-SRM)[b]	245 mg/kg C + 1220 mg/kg EC	[12]
Chocolate (black)	610 mg/kg C + EC	[13]
Chocolate (dark)	535 mg/kg	[14]
Chocolate (milk)	159 mg/kg C + EC	[14]
Cocoa	78 mg/L C + 132 mg/L EC	[11]
Gooseberries	up to 30 mg/kg	[10]
Grape seeds (*Vitis vinifera*)	1892 mg/kg C + 988 mg/kg EC + 353 mg/kg ECG	[15]
Kiwi fruit	4.5 mg/kg C + EC	[13]
Strawberry	10–70 mg/kg C + 1 mg/kg EC	[16]
Tea (black)	20 mg/L C + 37 mg/L EC + 73 mg/L ECG + 42 mg/L EGC + 128 mg/L EGCG	[17]
Tea (green)	21 mg/L C + 98 mg/L EC + 90 mg/L ECG + 411 mg/L EGC + 444 mg/L EGCG	[17]
Wine (red)	27–96 mg/L	[18]
Wine grape (red)	800–4000 mg/kg	[19]

[a] Concentrations include all catechins unless otherwise specified.
[b] These results are country and variety specific and may be underestimated because current tests only look at monomeric catechins and neglect oligomers found in chocolate (low concentrations found in tea) [20].

group of dietary polyphenols attractive candidates to treat many terminal and lifestyle-related health concerns such as cancer, vascular disease and obesity. The ability of each individual catechin to replicate these findings in clinical practice, to be administered safely at effective doses that are nontoxic to humans, has yet to be determined. Epidemiological and clinical studies have unfortunately been inconsistent regarding several of these aforementioned health benefits (see Ref. [62] for review). Plausible explanations as to why these irregularities have occurred will be discussed later on in this review.

Tea is the most widely consumed beverage in the world, next to water [63,64]. Although green tea constitutes about 20% of the tea manufactured worldwide (black and oolong make up the rest), its consumption in vast quantities is not assurance for nontoxic results when administered in purified form for therapeutic interventions. Experimental research with Swiss Webster mice has shown that EGCG causes severe hepatic necrosis and 67% mortality when given daily at 50 mg/kg ip [65]. In fact, clinical preparations of tea extracts have also met with cases of hepatic attacks, leading to a suspension of the marketing authorization of these products in France and Spain (reviewed in Ref. [64]). In contrast, there is ample evidence that purified green tea extracts in vivo are hepatoprotective against ischemia-reperfusion injury [66,67]. These results suggest that the route and method of administration may determine whether catechins induce hepatotoxicity or have hepatoprotective effects. This review will provide an in depth analysis of the green tea catechins' mechanisms of action and their potential role in inflammatory-based neurodegenerative diseases (with a focus on cerebral ischemia), followed by their present and future clinical therapeutic potential.

2. Clinical and epidemiological studies of catechins

To date, the majority of clinical and epidemiological studies involving catechins have examined the potential relationship between these dietary compounds and many types of cancer (which has been reviewed in Refs. [68–71] and will not be covered here) or vascular disease prevention. Between 1986 and 1998, Arts et al. [72] studied 34,492 participants for incidence of coronary heart disease (CHD). They found an inverse association between the intake of C and EC, and death due to CHD. Sources of apples and wine were best correlated with a decreased risk of CHD, but there was no association between tea catechins and CHD. This finding has been partly substantiated by Tabak et al. [73] who found that solid fruit, but not tea intake, was associated with reduced incidence of chronic obstructive pulmonary disease. In a related study, Japanese men who drank four or more cups of green tea a day exhibited an inverse association with coronary atherosclerosis [74]. The association between catechins and vascular diseases has been attributed, in part, to the catechins' antioxidant abilities that prevent low-density lipoprotein (LDL) oxidation and may therefore reduce the occurrence of CHD or related vascular diseases [75].

Prevention of cerebrovascular diseases by catechins has been evidenced by Sato et al. [76] who conducted a 4-year follow-up study and found that the incidence of cerebral hemorrhage and stroke, and mortality from stroke were twofold or higher in those who were ≥60 years and took less green tea (less than five cups a day) than in those who took five cups or more daily. Interestingly, green tea intake had no association with the incidence of hypertension [76]. Keli et al. [77] were able to virtually replicate this result

Table 2
Studies that demonstrate the catechins' vast array of beneficial health effects

Beneficial health effects	Ref.
Anticarcinogenic	[21–26]
Antitumorigenic	[27–32]
Antimutagenic	[33,34]
Chemopreventative	[35,36]
Antiproliferative	[37]
Anti-inflammatory	[38,39]
Antioxidant	[40–45]
Antidiabetic	[46]
Antiallergic	[47–49]
Antihypertensive	[50]
Antiplatelet	[51–53]
Antiobesity	[54–58]
Hypocholesterolemic	[59]
Protects against ulcerative colitis	[60,61]
Neuroprotective	See Table 3

with the Zutphen Study, where 552 men aged between 50 and 69 were followed up for 15 years. They found an inverse association between flavonoid intake (particularly with black tea) and the incidence of stroke. Men with a high intake of flavonoids had a 73% lower risk of stroke. Despite the previous positive findings, Arts et al. [78] found no relationship between catechin intake and the incidence of stroke in a Netherlands-based study. They showed that catechin intake, mainly due to black tea, in elderly Dutch men was inversely associated with ischemic heart disease mortality but had no association with stroke incidence or mortality.

The conflicting reports on whether catechins are effective and which is the most effective source of catechins, that is, tea or fruit, have made conclusions concerning a "preventative" catechin diet difficult to establish. Despite the differential results, there exists ample evidence that tea catechins may be sufficient in themselves to prevent many debilitating disorders such as chronic inflammatory diseases [rheumatoid arthritis and multiple sclerosis (MS)] [79] and lifestyle-related diseases (including cardiovascular disease and cancer, reviewed in Refs. [1,33,80,81]). The discrepancy between experimental and clinical results and between similar clinical studies could be due to a number of naturally occurring confounding factors. These factors are only applicable to humans and would therefore only be exposed in clinical practice, influencing whether or not statistical significance is obtained. For example, many studies rely on personal reporting of catechin intake (whereas experimental intake is strictly regimented), and each individual in turn relies on product descriptions. In the case of green tea products, Manning and Roberts [82] demonstrated that actual catechin content from commercially available products ranged from 9% to 48% of label claims, and all values were significantly lower than those claims. This practice would increase the likelihood of a nonsignificant result as individuals ingest lower amounts of catechins than reported. This also demonstrates the general problem that exists with quality control in the dietary supplement and herbal medicine industry.

An additional slew of confounding factors that are exclusive to clinical studies include the fact that unhealthy (healthy) behavior tends to cluster. For example, catechin intake has been shown to be lower in smokers and increased with socioeconomic status [83]. There also exists a positive correlation between tea catechin intake and fruits and vegetables intake. This is significant because many fruits and vegetables also have catechin and flavonol components (see Table 1) [62]. These factors may have contributed to the inconclusive epidemiological studies involving catechin intake and cerebrovascular disorders.

3. Mechanisms of catechin action

All of the catechins have a wide array of biological actions pertaining particularly to their chemical structure (see Fig. 1 and Refs. [84,85] for reviews). At present, many of the catechins' actions have been identified, but the mechanisms behind these actions have not been fully elucidated. Their most famous and widely renowned biological action is their antioxidant and free radical scavenging properties. However, it is the emerging evidence of diverse actions on alternate cellular pathways that has attracted so much attention recently (see Ref. [86]). In addition, the catechins' glucuronide metabolite that forms B-ring *O*-methylated catechins has little antioxidant activity due to its reduced H-donating properties [87] but can protect against cytotoxicity as effectively as its parent compound [88]. This suggests that the catechins protect against cytotoxicity independently of their free radical scavenging properties. Therefore, catechins and their active metabolites may act at different sites and so contain diverse actions, which will subsequently increase the versatility of catechins as potential therapeutic interventions. This section investigates many of the multiple actions of the catechins, with a particular focus on their free radical scavenging capabilities, inhibition of oxidative stress, influence on apoptosis, prooxidant properties, anti-inflammatory actions and anticholesterolemia effects. The anticarcinogenic and antiproliferative properties of the catechins have been well documented (see Refs. [17,89,90] for reviews) and are beyond the scope of this review.

3.1. Free radical scavenging/antioxidant actions of catechins

Reactive oxygen species (ROS) such as superoxide and hydroxyl free radicals, and reactive nitrogen species such as nitric oxide (NO) and peroxynitrite are formed when a cell undergoes oxidative stress or inflammation and assists in the host defense system against pathogens (see Ref. [91] for review). Free radicals can have damaging effects directly on the cell, particularly on DNA, proteins and lipids, causing lipid peroxidation, ultimately leading to apoptotic cell death [92]. It is well established that the catechins contain free radical scavenging properties and act as biological antioxidants. It has been demonstrated that they can scavenge both superoxide and hydroxyl radicals [40–44], as well as the 1,1-diphenyl-3-picrylhydrazyl radical [43,44,93], peroxyl radicals [94], NO [95], carbon-center free radicals, singlet oxygen and lipid free radicals [41,44], and peroxynitrite by preventing the nitration of tyrosine [96]. Catechins chelate metal ions such as copper(II) and iron(III) to form inactive complexes and prevent the generation of potentially damaging free radicals [41,42,97,98]. Another mechanism by which the catechins exert their antioxidant effects is through the ultrarapid electron transfer from catechins to ROS-induced radical sites on DNA [99]. A third possible mechanism by which catechins scavenge free radicals is by forming stable semiquinone free radicals, thus, preventing the deaminating ability of free radicals [41]. In addition, after the oxidation of catechins, due to their reaction with free radicals, a

dimerized product is formed, which has been shown to have increased superoxide scavenging and iron-chelating potential [100].

The prevention of damage by catechins against free radicals is effective because catechins can inhibit the ROS-induced damage from a wide array of initiators. These include 2,2′ -azobis (2-amidinopropane) hydrochloride (AAPH) [101,102], primaquine [97], hydrogen peroxide [40,97], iron [97,98], paraquat [103], azo-bisisobutyrylnitrile [94] and radiolysis [99]. Furthermore, the catechins have shown increased antioxidant effects compared to other antioxidants, such as α-tocopherol [101,104] and vitamins C and E [105].

There is a wealth of literature that suggests that the potency of the catechins' free radical scavenging abilities relates directly to the chemical structure of each compound, namely, the gallate moiety esterified at the 3 position of the C ring, the catechol group (3,4-dihydroxyl groups) on the B ring and the hydroxyl groups at the 5 and 7 positions on the A ring (see Fig. 1). In many studies that have compared the free radical scavenging effects of primarily EGCG, ECG, EGC and EC, the results have been generally consistent. They showed that EGCG and ECG were the most potent free radical scavengers compared to the other catechins [41,43,44,96,102,104,106]. This was attributed to the presence of the C ring gallate group. The galloylated catechins were more active antioxidants due to their higher phospholipid/water partition coefficients and so affected the properties of the phospholipid bilayers of membranes and hence increased solubilization [107]. However, the differences between the antioxidant activity of ECG and EGCG were slight (their only difference in structure is the orthohydroxyl group at position 5 of the B ring, see Fig. 1) and depended on the free radical involved and the model used. The observation was also made that the more hydroxyl groups the catechin possesses, such as ECG and EGCG, the more effective free radical scavenger the catechin becomes [44]. The A ring of the catechins' structures are also important for their antioxidant activity. The A ring becomes oxidized and decarboxylated after reaction with hydrogen peroxide, which, for EGC and EGCG, produces different reaction products [108].

In addition to the catechins' direct antioxidant effect, they can also indirectly increase the body's endogenous antioxidants to reduce oxidative damage. Rats given green tea extract orally exhibited increased levels of endogenous antioxidants such as glutathione peroxidase and reductase, superoxide dismutase (SOD) and catalase [109]. Furthermore, catechins can directly prevent the levels of endogenous antioxidants, such as α-tocopherol and β-carotene, from being depleted by lipid oxidation through AAPH [102]. These animal studies were backed up by clinical evidence that showed that green tea administration increased endogenous antioxidants. In a study by Erba et al. [110], subjects drank two cups of green tea daily for 42 days and their endogenous plasma total antioxidant activity was increased while their plasma peroxides and oxidative stress-induced damage was decreased. In a crossover clinical trial by Young et al. [111], where green tea extract was administered, plasma antioxidant activity increased, which subsequently decreased oxidative damage.

In addition to directly scavenging free radicals, tea catechins may inhibit ROS accumulation by inhibiting xanthine oxidase (XO). Xanthine oxidase is an enzyme that catabolizes purines to produce uric acid and ROS [112], where up-regulation can lead to gout and oxidative stress. (−)-Epigallocatechin gallate inhibited XO with the same potency as the drug of choice (allopurinol) for gout, suggesting that inhibition of XO is an effective mechanism to prevent free radical formation.

3.2. Preventing lipid peroxidation due to oxidative stress with catechins

Catechins can protect different cells from lipid peroxidation and DNA deamination induced by oxidative stress. This is evidenced by the fact that green tea extract can decrease lipid peroxidation markers in the liver, serum and brain, including lipid hydroperoxides, 4-hydroxynonenal and malondialdehyde in rats [109]. Catechins have proven, in isolation, to protect against lipid peroxidation by initiators of oxidative stress such as *t*-butylhydroperoxide [113], 6-hydroxydopamine (6-OHDA) [106,114–117], hydrogen peroxide [118,119], 3-hydroxykynurenine (3-HK) [120], lead [121,122], ultraviolet B radiation [123] and iron(II/III) [41,119].

3.3. Catechins and apoptosis

Epigallocatechin gallate has shown evidence of modulating apoptotic pathways to protect against oxidative stress. Koh et al. [118] showed that after hydrogen peroxide exposure in PC12 cells, EGCG inhibited many points of the apoptotic sequence, including caspase 3, cytochrome *c* release, poly(ADP-ribose) polymerase cleavage, the glycogen synthase kinase-3 pathway and modulated cell signaling by activating the phosphatidyl inositol-3 kinase (PI3K)/Akt pathway (which promotes cell survival). Further studies have confirmed this by showing that after 3-HK exposure in SH-SY5Y human neuroblastoma cells, apoptosis and caspase 3 activity were inhibited by EGCG [120]. Furthermore, after hepatocyte exposure to cytotoxins, such as rubratoxin B and bromobenzene, EGCG and its methylated metabolite EGCG-3″-OMe protected against necrosis and apoptosis (by suppressing caspase 3 activity) [124].

Catechins can also modulate apoptosis by altering the expression of antiapoptotic and proapoptotic genes. (−)-Epigallocatechin gallate prevented the expression of proapoptotic genes Bax, Bad and Mdm2 while inducing the antiapoptotic genes Bcl-2, Bcl-w and Bcl-x_L to protect SH-SY5Y cells from 6-OHDA-induced apoptosis [117]. In this study, EGCG also promoted cell survival by restoring the protein kinase C and extracellular signal-related kinases 1/2 pathways. Weinreb et al. [125], in part, confirmed this

by revealing that low doses of EGCG (1–10 μM) showed antiapoptotic properties by decreasing Bax, Bad and caspase-6. However, they also found that high doses of EGCG (50–500 μM) demonstrated proapoptotic properties by increasing Bax, Bad, caspase-6, fas and gadd45 and decreasing Bcl-x_L and Bcl-2 (see Ref. [86] for review). Therefore, the effects of catechins on apoptotic pathways may in fact be divergent: low concentrations of catechins may exhibit antiapoptotic effects, whereas high doses promote apoptosis. Hence, this effect may help explain the divergent toxicological findings with catechins in vivo (see Section 6).

3.4. Prooxidant properties

In addition to the plethora of evidence that catechins are cytoprotective via antioxidant and antiapoptotic effects, recent observations suggest that the catechins may also contain prooxidant properties, particularly at high concentrations. Thus, at low concentrations in vitro (1–50 μM), they are antioxidant and antiapoptotic, whereas at higher concentrations (100–500 μM), the reverse is true. DNA isolated from humans were exposed to 200 μM of EGC and EGCG, which induced oxidative damage due to the production of hydrogen peroxide [126]. Green tea extract (10–200 μg/ml) and EGCG (20–200 μM) exacerbated oxidant activity, oxidative stress, genotoxicity and cytotoxicity induced by hydrogen peroxide in RAW 264.7 macrophages [127]. Catechins, particularly EGCG (100 μM), have also been shown to increase the oxidative damage incurred after exposure of DNA to 8-oxo-7,8-dihydro-2′-deoxyguanosine [128,129]. This was due to the generation of the hydroxyl radical and hydrogen peroxide in the presence of copper(II) and iron(III). Interestingly, copper-oxidized catechins were more efficient prooxidants than unoxidized catechins [130], suggesting that the antioxidant mechanism of scavenging metals by catechins to stop the formation of free radicals may lead to prooxidant actions on DNA. In contrast, the prooxidant effects of high-dose catechins may induce an up-regulation of endogenous antioxidants such as SOD, catalase and glutathione, which may account for some of the cytoprotective actions of catechins (see Ref. [131] for review).

3.5. Anti-inflammatory effects of catechins through the NO synthase pathway

Catechins appear to have anti-inflammatory effects as evidenced by their inhibition of carrageenin-induced edema [132], but it remains unclear as to the mechanisms of action of this effect. There is substantial evidence that the catechins' anti-inflammatory effects may be due, in part, to their scavenging of NO and reduction of NO synthase (NOS) activity [133–137]. Furthermore, NO and peroxynitrite can be directly scavenged by catechins and green tea extract with EGCG being the most effective [138]. However, catechins have varying effects on the three different isoforms of NOS.

The neuronal NOS (nNOS) isoform of NOS produces toxic effects through NO, and so catechin inhibition of nNOS may be a mechanism through which catechins are anti-inflammatory. Stevens et al. [139] showed that EGCG and oligomeric proanthocyanidins (which are made up of esterified catechins) inhibited nNOS activity in BL21(DE3) *Escherichia coli* cells. In addition, in mouse peritoneal cells, nNOS activity was inhibited by EGCG after stimulation with lipopolysaccharide (LPS) and interferon γ (IFN γ) [140].

Evidence also exists that the inhibition of inducible NOS (iNOS) may also be a mechanism behind the anti-inflammatory effects of catechins. (−)-Epigallocatechin gallate and other catechins have inhibited the induction of iNOS mRNA and activity after treatment with LPS, IFN γ [138,140,141], interleukin (IL)-1 and tumor necrosis factor α (TNF α) [135] in vitro. Inhibition of iNOS by catechins appears not to be through a direct mechanism but by preventing inhibitor κB disappearance, which inhibits nuclear factor κB (NF-κB) from binding to the promoter of the iNOS gene thereby inactivating it [141]. However, Tedeschi et al. [135] showed that green tea extract did not inhibit iNOS by reducing NF-κB but down-regulated DNA binding activity of the transcription factor signal transducer and activator of transcription-1. In contrast, we have shown in a model of wound healing that ECG improved the quality of scarring by inducing iNOS and cyclooxygenase (COX)-2, which were originally thought to be exclusively proinflammatory enzymes [38]. However, this can be explained by the fact that NO derived from iNOS is vital to the wound healing process (an angiogenic-dependent process) and can enhance angiogenesis by inducing vascular endothelial growth factor [142].

The third isoform of NOS, endothelial NOS (eNOS), is a vasodilator-inducing enzyme, and its modulation may directly contribute to the anti-inflammatory effects of catechins. When EGCG was administered to rat aortic rings, dose-dependent vasorelaxation occurred simultaneously with eNOS activity induction [143]. The mechanism of action was proposed to be that EGCG induced eNOS to produce NO, which in turn activated guanylate cyclase to produce cyclic guanosine monophosphate and caused vasorelaxation by PI3K, protein kinase A and Akt-dependent signaling pathways. However, another mechanism may be that eNOS contains an antioxidant response element (ARE) on its promoter and green tea polyphenols can bind to the ARE and activate eNOS [144].

3.6. Other anti-inflammatory effects of catechins

Catechins modulate a vast array of other mediators involved in inflammation. Topical administration of EGCG to rats that had been exposed to ultraviolet B prevented immunosuppression, infiltration of cluster of differentiation (CD)11b+ leukocytes, including neutrophils and lymphocytes, and depletion of antigen-presenting cells such as macrophages and dendritic cells [123]. Furthermore, CD11b

expression in isolated CD8+ T lymphocytes was decreased by both EGCG and ECG [145]. This resulted in a decreased ability of CD8+ T cells to adhere to the intracellular adhesion molecule-1, thereby decreasing the migration of CD8+ T cells to the site of inflammation in response to the chemokines, macrophage inflammatory protein (MIP)-1α and MIP-1β. (−)-Epigallocatechin gallate also prevented the induction of vascular adhesion molecule-1 by TNF α and IL-1, which subsequently reduced monocyte adhesion [146]. Polymorphonuclear leukocytes exposed to LPS promoted the proinflammatory cytokine IL-1β production, which was prevented by many catechins [147]. Furthermore, catechins attenuated IL-8 production by decreasing the expression of adhesion molecules CD11b and CD18 on isolated polymorphonuclear leukocytes that had been exposed to IL-1β [148]. Catechins also enhanced the production of anti-inflammatory cytokines such as IL-10 [147]. The inflammation associated with arthritis was inhibited by catechins, particularly EGCG and ECG, due to prevention of the breakdown of proteoglycan and Type II collagen in bovine nasal and metacarpophalangeal cartilage, suggesting that drinking green tea may be a useful prophylactic against arthritis-induced cartilage degradation [149].

3.7. Anticholesterolemia properties

High plasma lipid levels and plaque formation can lead to an increased risk of CHD or ischemic stroke. Catechins have well-established anticholesterolemic properties that may in fact prevent the occurrence of cardiovascular disease (see Ref. [150] for review). Green tea consumption reduced serum cholesterol and cholesterol absorption in ovariectomized rats that are well established to have a greater risk of CHD [151]. Interestingly, the epimers CG and GCG of common catechins ECG and EGCG were more effective at inhibiting cholesterol absorption [152]. However, another study showed that in atherosclerosis-susceptible apoprotein-E-deficient mice, green tea extract did not lower plasma lipid levels directly but prevented the development of atherosclerosis by reducing lipid peroxides, aortic cholesterol and aortic atheromatous areas [153]. The oxidation of the LDL form of cholesterol is also a major factor that leads to the pathogenesis of atherosclerosis. (−)-Epicatechin gallate and EGCG inhibited this oxidative modification of LDL in porcine serum [154]. Furthermore, catechins inhibited LDL-induced human vascular smooth muscle proliferation, which is associated with atherogenesis [155].

4. Chronic neurodegenerative disorders and catechins

The catechins' proven efficacy as prophylactive and neuroprotective agents against neurodegenerative/neuroinflammatory diseases such as Parkinson's disease (PD) [156–162], Alzheimer's disease (AD) [156,157,159–161,163,164] and MS [157,165] has been well documented and therefore will not be discussed here. Briefly, catechins' efficacy in the treatment of PD, AD and MS have been primarily attributed to their antioxidant and anti-inflammatory capabilities [156,157] as reviewed in Sections 3.1, 3.2, 3.5 and 3.6. Recent evidence suggests that catechins may also have a role in other neurodegenerative disorders. For example, catechins have been shown to protect, in vivo, against white matter oxidative damage in childhood-onset hydrocephalus in rats [166] and liver damage in chronically ethanol-intoxicated rats [167], and have been found to be effective for improving learning and memory in senescence-accelerated mice (SAMP10) [168].

5. The effects of catechins on cerebral ischemia

Cerebral ischemia or stroke is a highly prevalent disease, but unfortunately, there are few efficacious treatments available (see Ref. [169] for review). Many experimental therapeutics that have shown promise in in vivo models of stroke have failed to show efficacy in clinical trials (see Refs. [169–171] for reviews). Recently, the field view on neurodegeneration poststroke has changed from being one of a necrotic and apoptotic process to a progressive disorder with a latent but large and long-term neuroinflammatory component (see Ref. [172] for review). As catechins elicit diverse biological effects by acting at multiple sites relating to vascular disorders, oxidative stress and neuroinflammation (see Sections 3.1 Sections 3.2 Sections 3.3 and Sections 3.5 Sections3.6 Sections 3.7), they may prove to be potent agents in preventing the neuronal damage that occurs after a stroke.

5.1. Neuroprotective effects of catechins

The neuroprotective properties of the catechins are rapidly becoming established. In 1995, Uchida et al. [173] demonstrated that long-term EGCG administration in the drinking water of stroke-prone spontaneously hypertensive rats reduced stroke incidence and increased lifespan. Since then, catechins have repeatedly been shown to reduce infarct volume in a variety of cerebral ischemia models (see Table 3). In contrast, Dajas et al. [174] and Rivera et al. [175] have shown that after permanent middle cerebral artery occlusion (MCAO), C administration did not reduce the ischemic lesion. These negative results may be attributed to the fact that C has less potent biological effects than other catechins such as EGCG and ECG (see Section 3). It was also suggested that in these cases, the aqueous preparation of C that was injected intraperitoneal did not cross the blood–brain barrier (BBB) and thus did not reach the brain to elicit an effect. Of all the catechins, ECG has the greatest capacity to pass through biological membranes [107]. However, due to economical factors, ECG does not tend to be used for in vivo studies. [^{3}H]-EGCG has been found in the brain 24 h after an intraperitoneal injection [185], suggesting that EGCG does in fact cross the BBB, which may account for its neurological/neuroprotective activities.

The neuroprotective effects of the catechins appear to be dose dependent and linear. Doses of 10, 25 and 50 mg/kg ip

Table 3
Studies that have investigated the neuroprotective effects of individual catechins or green tea extracts using experimental models of cerebral ischemia

Model	Catechin	Dose	Protective?	Ref.
Rat permanent MCAO	C	Intraperitoneal 30 min preischemia	No	[174]
Rat permanent MCAO	C	Intraperitoneal	No	[175]
Rat transient MCAO	Green tea extract	0.5% (2% caused toxicity) in drinking water for 3 weeks prior to MCAO	Yes	[176]
Rat transient MCAO	EGCG	25 or 50 mg/kg ip immediately postischemia	Yes (only 50 mg/kg)	[177]
Rat transient MCAO	Green tea extract	0.25% or 0.5% in drinking water for 5 days prior to MCAO	Yes (0.25% and 0.5% dose dependently)	[136]
Rat transient MCAO	EGCG	50 mg/kg ip daily beginning immediately pre-MCAO	Yes	[178]
Gerbil transient right CCA ischemia	EGCG	25 or 50 mg/kg ip 30 min pre- and immediately postischemia	Yes (only 50 mg/kg)	[179]
Gerbil transient bilateral CCA ischemia	Green tea extract	0.5% or 2% in drinking water for 3 weeks preischemia	Yes	[180]
Mouse permanent bilateral CCA ischemia	C and EC	100 mg/kg iv immediately preischemia	Yes	[132]
Gerbil transient bilateral CCA ischemia	C	0.1 and 1 mg/ml in drinking water 2 weeks pre- and 1 week postischemia	Yes (only with 1 mg/ml)	[181]
Gerbil transient bilateral CCA ischemia	EGCG	10, 25 or 50 mg/kg ip immediately postischemia	Yes (25 and 50 mg/kg dose dependently)	[182]
Gerbil transient bilateral CCA ischemia	EGCG	50 mg/kg ip 1 or 3 h postischemia	Yes	[183]
Rat transient bilateral CCA ischemia	EGCG	50 mg/kg ip NB not stated when EGCG was administered	Yes	[134]
Rat transient global hypoxia (4 h)	EGCG	10, 25 or 50 mg/kg ip immediately prehypoxia	Yes (25 and 50 mg/kg dose dependently)	[184]
Rat permanent left CCA ischemia + transient global hypoxia	EGCG	50 mg/kg ip daily beginning 24 h pre-HI	Yes	[137]

CCA — common carotid artery.

have been examined for neuroprotective effects in a model of global ischemia in the gerbil. It was found that EGCG had a dose-dependent response with 25 and 50 mg/kg eliciting significant neuroprotection [182]. These doses had similar effects at reducing hypoxic damage in rats [184]. Since then, most studies have used the effective dose of 50 mg/kg [134,137,177–179,183] with one study using 100 mg/kg to show efficacy [132].

5.2. Possible mechanisms behind the neuroprotective effects of catechins

There exists a wealth of knowledge about the role catechins play in tumor cells and their mechanisms of action in vitro, but little as to the role catechins play in the neurodegeneration and inflammatory response initiated after an ischemic event. Due to the fact that catechin metabolites are biologically active [87], in fact, more so than their parent compounds, it would be meaningless to compare the observed effects of a catechin in vitro with the effects in vivo. We have therefore highlighted key areas of the neurodegenerative and neuroinflammatory cascade that the catechins may be targeting to produce a neuroprotective effect after cerebral ischemia, with an emphasis on in vivo mechanisms (see Fig. 2).

The neuronal damage following cerebral ischemia appears to be largely due to free radical damage induced by the stimulation of apoptosis and exacerbated by inflammation. Catechins have been identified as potent free radical scavengers that can inhibit lipid peroxidation (see Sections 3.1 and 3.2) and have subsequently been shown to inhibit neuronal loss after a stroke (see Section 5.1). It was demonstrated by Inanami et al. [181] that C reduced cell death postischemia by increasing the brain's ability to scavenge superoxides. Prior administration of green tea extract decreased hydrogen peroxide, lipid peroxidation and apoptotic cells after ischemia in rats and gerbils [176,180]. Furthermore, malondialdehyde levels (indicative of oxidative stress) were reduced by EGCG administration after ischemia [177,179]. Ishige et al. [186] have proposed that catechins prevent oxidative stress by inhibiting the influx of calcium ions into the cell despite high levels of ROS, which has been shown in vitro [122]. The increase in intracellular calcium concentration after ischemia is pivotal to the neurodegenerative cascade (Fig. 2) as it triggers calcium-dependent restriction endonucleases initiating apoptosis. The inhibition of calcium influx by catechins may therefore be an important mechanism by which catechins prevent neuronal damage.

Additional investigation into the properties of catechins have revealed that they have further mechanisms of actions, apart from free radical scavenging and inhibition of calcium influx, to attenuate ischemic damage such as modulation of NOS. The activity of the differing isoforms of NOS, which produce NO at varying times after ischemia, has been shown to be important to the functional outcome following an ischemic episode (see Ref. [187] for review). The modulation of the various NOS isoforms can explain the neuroprotective effects of catechins due to the dual role of NO in inflammation. It has been shown that EGCG inhibited nNOS in vitro [140] and in vivo in hypoxic rats,

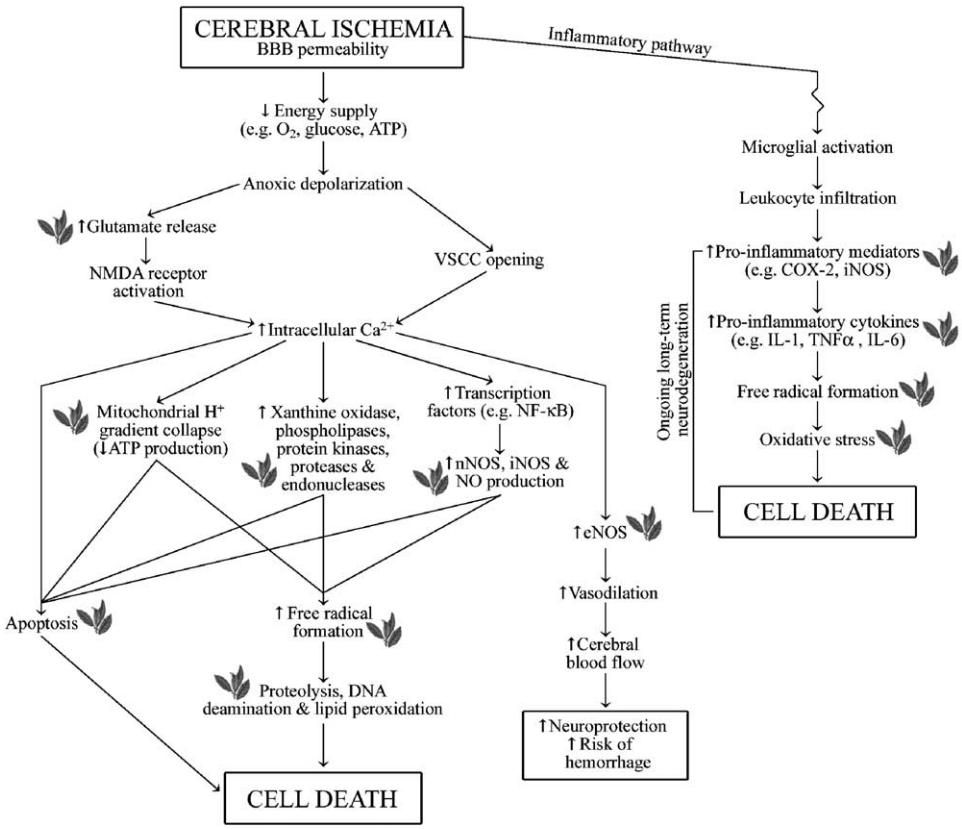

Fig. 2. Mechanisms of action of catechins in the neurodegenerative and neuroinflammatory cascades after cerebral ischemia. Catechins (symbolized by) inhibit many aspects of the neurodegenerative cascade leading to the prevention of cell death. In addition, catechins induce eNOS production, which acts as a neuroprotectant. Over time, however, a neuroinflammatory pathway becomes induced, which can remain activated for months postischemia. Again, the catechins are able to neuroprotect by attenuating multiple mechanisms in this response. NMDA — *N*-methyl-D-aspartate; VSCC — voltage-sensitive calcium channels.

reducing oxidative stress [184]. In one of our previous studies, nNOS protein expression was induced by EGCG after hypoxia–ischemia (HI) in juvenile rats, but the local NO produced from the increased nNOS expression may not have been sufficient to cause neurotoxicity [137]. There is conclusive evidence that part of the anti-inflammatory action of catechins is due to the inhibition of nNOS, but this mechanism of action alone is not sufficient to explain such an extensive effect in vivo.

In addition to the catechins' effects on nNOS, it has been well established in vitro and in vivo that catechins inhibit iNOS activity and expression, thereby attenuating the inflammatory response [135,137,138,140,141]. In a juvenile rat model of HI, we found that EGCG prevented the induction of iNOS protein expression and activity associated with the inflammatory response caused by HI [137]. Therefore, the inhibition of a substantial amount of NO production by iNOS by EGCG may directly contribute to the neuroprotective properties demonstrated by EGCG.

In contrast, because eNOS-derived NO directly induces vasodilation (hence, its original term *endothelial-derived relaxing factor*) and improves cerebral blood flow, pharmacological inducers of eNOS, such as statins and steroid hormones, reduced neuronal cell death caused by ischemia [188,189]. We have shown that 50 mg/kg EGCG treatment prior to and after HI in rats induced eNOS protein expression compared to controls, which actions may, in part, explain the neuroprotective effects of EGCG [137]. Therefore, the combination of eNOS induction to increase cerebral blood flow and the inhibition of neurotoxic iNOS and nNOS by EGCG suggested that EGCG may provide potent neuroprotection following cerebral ischemia through modulation of NOS isoforms.

The catechins have also been shown to act at other sites in the inflammatory cascade in vivo. The production of eicosanoids through catalysis of arachidonic acid (AA) catabolism by COX is a major pathway leading to the endpoints of inflammation and is increased postcerebral ischemia (see Refs. [190,191] for reviews). Cyclooxygenase is the enzyme that nonsteroidal anti-inflammatory drugs, such as aspirin and ibuprofen, inhibit [192]. It has been shown that green tea extract reduced eicosanoid formation significantly postischemia [176], suggesting that this pathway may represent another site of action where catechins provide neuroprotection.

Cerebral ischemia induces damage to cell-signaling pathways throughout the brain and impairs the ATP-producing ability by neuronal mitochondria, which leads

to cell apoptosis (see Ref. [193] for review). Mitochondrial respiratory chain complexes that produce ATP for cellular functioning have been shown both in vivo and in vitro to be damaged after ischemic episodes [194–196]. We have repeatedly shown that after an HI insult, mitochondrial complexes I, II–III, IV and V sustained deficits [137,197], whereas administration of EGCG prevented the damage to the complexes induced by HI [137]. Furthermore, citrate synthase activity (a measure of mitochondrial membrane integrity), which was diminished due to HI, was significantly preserved by EGCG treatment [137]. Therefore, EGCG protected mitochondrial complex activity as well as prevented the leakage of important mitochondrial matrix components into the extracellular space, thus, preserving normal ATP function and preventing cell death.

Alternate theories exist as to how the catechins confer their neuroprotective properties. However, the majority of studies investigating the molecular actions of the catechins are in vitro studies. Because there are differences in experimental design between in vivo and in vitro studies, the results may be completely different in a whole animal compared to isolated cells. Therefore, before one can conclude as to the mechanisms of action of the catechins' neuroprotective effects, they must first be confirmed in vivo.

5.3. Risk of intracerebral hemorrhaging with catechins

Recently, we discovered that although EGCG is neuroprotective postischemia, it may also elicit more significant neurodegenerative effects. Even though we found that 50 mg/kg ip EGCG for 3 days was significantly neuroprotective, we discovered a substantial increase in the incidence of intracerebral hemorrhaging (ICH) following MCAO [178]. The increase in ICH due to EGCG administration is supported by the evidence illustrating the antiplatelet and antithrombotic activities of catechins. Kao et al. [198] showed that 82 mg/kg EGCG increased red blood cell number and hemoglobin by 20%. Both C and EC inhibited human plasma platelet aggregation but did not prolong the clotting time (did not inhibit fibrinogen or plasma protein activity), suggesting that catechins only affect primary hemostasis in human blood [51]. Moreover, green tea extract and EGCG protected mice from pulmonary thrombosis while increasing mice tail bleeding time and inhibiting platelet aggregation [199]. As neither green tea extract nor EGCG altered plasma coagulation parameters, it was concluded that catechins might not directly act on the release of thromboplastin or thrombin formation. The antiplatelet effects of green tea catechins were mediated, in part, by the inhibition of cytoplasmic calcium increase, leading to the prevention of fibrinogen–glycoprotein IIb/IIIa binding and inhibition of inositol triphosphate formation [52]. The inhibition of prothrombotic thromboxane A_2 (TXA_2) formation through the reduction of AA release and TXA_2 synthase activity also provide mechanisms of the catechins' antiplatelet effects [53]. However, EGCG may have more than one effect on platelet aggregation as it has been shown to also stimulate tyrosine phosphorylation of platelet proteins to induce platelet aggregation [200].

The induction of ICH by catechins may not only be due to their antiplatelet effects but also due to the concomitant induction of eNOS, which produces an increase in cerebral blood flow. Therefore, catechins increase the risk of ICH by acting as antiplatelets, not as anticoagulants, and may be toxic if given as a primary intervention against ischemia-induced damage. However, the antiplatelet and hypocholesterolemic effects of the catechins may make them excellent candidates as prophylactics for the pretreatment or prevention of ischemic stroke in high-risk individuals.

6. Safety of catechin administration

As described in Section 5.1, the catechins have been documented to reduce the neuronal damage that occurs after cerebral ischemia. In spite of this, efficacious doses of 50 mg/kg EGCG in female Swiss Webster mice have caused hepatic necrosis as shown by significant increases in plasma alanine aminotransferase (ALT) levels [65]. Therefore, the doses used above may have caused toxicity to the rat or gerbil, but unfortunately, very few of the studies listed in Table 3 have investigated the toxicological effects of catechin administration. Kao et al. [198] showed that ALT levels were not altered in adult rats administered 82 mg/kg EGCG. Furthermore, Rahman et al. [178] demonstrated that 50 mg/kg ip EGCG did not cause any deterioration of the animals' general health (as evidenced by the lack of organ wet weight loss) or hepatotoxicity (as measured by ALT levels), suggesting that in larger animals, higher doses of catechins may be less toxic. However, it remains to be seen whether an equivalent dose in humans will elicit toxic effects.

7. How much green tea do you need to drink to be effective?

The question is often asked, "How much green tea will I need to drink to prevent a stroke?" To even begin to answer this question, we must first look at the pharmacokinetics of the compounds in green tea and, in particular, EGCG, as it is the most abundant. All of the catechins are rapidly absorbed and widely distributed after ingesting a cup of green tea (see Ref. [201] for review), with plasma concentrations reaching their peak 1.4 to 2.4 h after ingestion [202]. Because the catechins are metabolized through methylation (EGCG) [203] or conjugated with glucuronide and/or sulfate groups (all catechins except EGCG; see Ref. [204] for review), the free catechins' ability to produce their biological actions are reduced. However, the length of time the metabolites remain active for is unknown. Just as the catechins are rapidly absorbed following ingestion of green tea, they are also rapidly eliminated with a half-life of approximately 3 h, except for EGCG, which has a half-life of approximately

5 h [202]. Even though catechins have relatively low bioavailability, in vivo studies have shown that 0.33% of EGCG administration can reach the brain and a subsequent dose 6 h later can increase EGCG levels in the brain by four to six times [185]. Average catechin intake is approximately 18 to 50 mg/day [204], and this can be raised with increased consumption of green tea. Furthermore, greater intake of green tea, such as five cups or more per day, has been associated with a reduction in the incidence and mortality of stroke [76,77]. Each 200-ml cup of green tea contains approximately 200 mg catechins, including 88 mg EGCG, which equates to 1.3 mg EGCG/kg body weight [205]. Unfortunately, the products from various brands of green tea contain inconsistent catechin values [206] and do not always manufacture the reported purity (as discussed in Section 2). This makes it difficult to pinpoint the amount of green tea needed, to be drunk, daily in order to have an effect. The duration of infusion will also confound the results and conclusion as to how much tea is required to be neuroprotective.

At present, we are not equipped with sufficient information to accurately predict how many cups of green tea are required per day to provide a neuroprotective effect, even though studies have revealed that five cups or more per day were effective [76]. Until such time that individual catechins are tested in humans, the answers to these questions will remain unknown. Furthermore, we are still unaware as to which catechins will prove the most efficacious in humans and whether a combination therapy would be even more effective. Nevertheless, the evidence from experimental studies is overwhelming, in that green tea consumption has wide, positive, beneficial health effects, which, to varying degrees, translates to clinical efficacy.

8. Conclusion

There is much evidence pertaining to the wide beneficial health effects of catechins and, in particular, their ability to protect the brain from ischemia-induced damage. In addition to their known antioxidant properties, the catechins utilize many alternate neuroprotective mechanisms of action that are widespread and elicit effects in both the primary neurodegenerative pathway postischemia and the subsequent neuroinflammatory cascade (see Fig. 2). Unfortunately, current epidemiological and clinical evidence correlating catechin intake and the incidence of stroke is inconsistent. Until more research is carried out, the full effects of the catechins will not be known. However, the outlook for the therapeutic use of catechins is promising. They will likely be used as a prophylactic intervention for the prevention of stroke rather than as an acute therapy due to their antiplatelet effects.

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