Redox Chemistry of Green Tea Polyphenols: Therapeutic Benefits in Neurodegenerative Diseases

H.M. Hügel* and N. Jackson

Health Innovations Research Institute & School of Applied Sciences RMIT University, Melbourne, 3001 Australia

Abstract: Evidence for the medicinal and health benefits of polyphenols in green tea for the prevention of chronic diseases such as heart disease, various types of cancer and neurodegenerative diseases is advancing. Their *in vivo* effectiveness and molecular mechanisms are difficult to elucidate and remain a challenging task. We review the redox responsiveness and amyloid protein perturbation biophysical properties of the major green tea polyphenol constituent (-)-epigallocatechin-3-gallate [EGCG].

Keywords: EGCG, green tea antioxidants, prooxidants, anti-cancer, neuroprotection, Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is an age related and incurable neurodegenerative disease characterized by the progressive loss of neurons of the hippocampus and the cortex, leading to impairment of memory and a progressive cognitive decline [1]. A characteristic feature of the pathological hallmarks of AD is the presence of highly stable misfolded amyloid protein aggregates localized in neurites (axons or dendrites) and in cell bodies of neurons termed neurofibrillary tangles, protein aggregates mainly composed of hyper-phosphorylated tau, and of senile plaques, protein aggregates mainly composed of beta-amyloid. In tissue culture experiments, the accumulation of beta-amyloid induces cell death of primary hippocampal neurons and neuronal cell lines (human neuroblastoma SH-SY5Y cells). Amyloid precursor protein (APP), the precursor protein of beta-amyloid, can be cleaved by processing enzymes α, β, or γ secretases to form either the soluble (mediated by α secretase) or the insoluble, toxic form of amyloid protein (mediated by β and γ secretase). Thus the aggregated form of beta-amyloid is believed to contribute to neurodegeneration in AD patients.

DIETARY SUPPLEMENTATION

The role of human nutrition in the evolution of longevity and aging diseases is of paramount importance [2]. Emerging research has shown that the effects of the diet on the brain are linked with lifestyle factors such as exercise and sleep that are translated into the activation of molecular mechanisms involved in synaptic plasticity which has significant impact on human health and on therapeutic interventions. Next to water, tea is the most popular beverage consumed. We are not aware of any reports of any toxic effects linked to tea drinking, so the attraction of using green tea extract as green therapeutic agents is enormous. Tea plants biosynthesize secondary metabolites as a defense mechanism to combat invading pathogens. The major polyphenol constituents of green tea are catechins or flavan-3-ols such as (-)-epigallocatechin-3-gallate [EGCG], (-)-epigallocatechin [EGC], (-)-epicatechin-3-gallate [ECG], (-)-epicatechin [EC], as illustrated in Fig. (**1**). The most abundant and active compound in green tea (*Camellia sinensis*) is (EGCG), which is extensively studied for its cancer-preventive and anti-cancer activities [3,4] as well as its cellular targets. Epidemiological studies have indicated that green tea consumption is associated with the reduced risk of cancers, especially associated with the reduced risk of late stage of cancers. This risk reduction may be attributed not only to proteasome inhibition but also to numerous other intracellular molecules [4] targeted by EGCG that are involved in cell cycle regulation, apoptosis, angiogenesis, and metastasis. Nevertheless the molecular mechanisms concerning cancer prevention in animals and humans by tea catechins have not been proven.

OXIDATIVE STRESS IN AD

There is now considerable scientific evidence indicating that oxidative damage to the brain is an early event in the pathogenesis of AD since mild cognitive impairment has been linked with elevated levels of lipid, protein, DNA and RNA oxidations [5,6]. The continuous and steady accumulation of oxidative damage to biomolecules leads to age-related reduction in human function and lifespan. Oxidative metabolic pathways that generate adenosine triphosphate (ATP), the biological currency of energy, require that the mitochondrial electron transport chain redox-reduces oxygen to water. In the event of electron leak, superoxide radicals [$\cdot O_2$] escape from the mitochondrial matrix as reactive oxygen species [ROS] causing cellular oxidative damage. Levels of mitochondrial oxidized DNA bases [7] have been found around 10-fold higher than their nuclear counterparts. Also mitochondrial dysfunction, aging, leads to ROS by increased levels of hydrogen peroxide [H_2O_2] and ultimately the generation of highly toxic hydroxyl radicals [·HO] and oxidative damage. High concentrations of ROS can result in oxidative damage to

*Address correspondence to this author at the RMIT University, School Applied Sciences, Building 3, Bowen Street Melbourne VIC 3001, Australia; Tel: +61 3 9925 2626; Fax: +61 3 9925 3747;
E-mail: helmut.hugel@rmit.edu.au

1875-5607/12 $58.00+.00

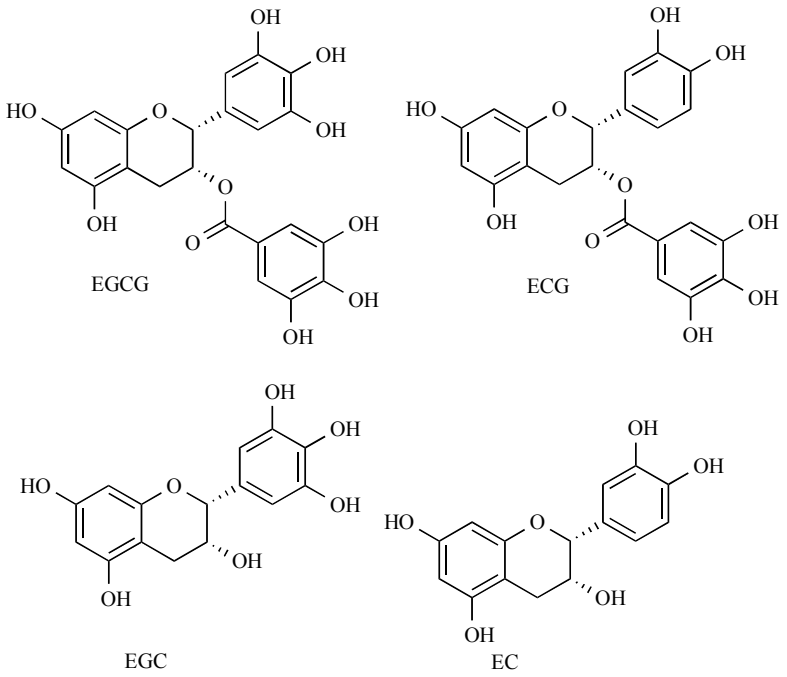


Fig. (1). The chemical structure of the main polyphenols found in green tea.

brain lipids, proteins and nucleic acids which contribute to pathologic conditions associated with aging and age–related neurodegenerative diseases such as AD. Brain lipid peroxidation yields malondialdehyde, 4-hydroxynonenal (HNE), F_2-isoprostanes and acrolein. Levels of isoprostanes derived from free radical oxidation of docosahexaenoic acid are increased in brain cortex [8,9,10]; increased concentrations of free radical-catalyzed peroxidation products of arachidonic acid are also detected in plasma, urine and cerebrospinal fluid of patients with AD [11]. Apolipoprotein E4 increases Aβ production and also reduces its clearance [12,13] with the extent of lipid peroxidation in Alzheimer's disease linked to the apoE genotype [10,11,14,15]. Elevated levels of the known neurotoxic substances acrolein and HNE were found in the hippocampus/parahippocampal gyrus (HPG), superior and middle temporal gyrus (SMTG) and cerebellum (CER) of subjects with mild cognitive impairment relative to control subjects [16,17,18]. The utilization of redox proteomics has identified the specific elevation of oxidatively modified proteins including α-enolase, heat shock cognate 71 (HSC 71), creatine kinase BB (CK BB), glutamine synthase (GS) and ubiquitin carboxy-terminal hydrolase l-1 (UCHL-1) in the hippocampus as well as the partietal lobe in AD patients [19].

EGCG REDOX ACTIVITY

Phenolic compounds found in green tea can act as powerful antioxidants, possess free iron scavenging activities and may provide therapeutic intervention in the prevention of oxidative damage in the pathogenesis of cancer [20] and potentially AD. The application of density functional theory for the calculation of the gas-phase bond dissociation enthalpy (BDE) of the O-H bond in green tea catechins predicted the order of antioxidant activity [21] as EC < ECG < EGC < EGCG. The antioxidant activity of EGCG by the quenching of ROS and or intercept free radicals is most likely by a H-atom transfer [HAT] reaction whereby the resultant phenoxy radical is stabilized by intramolecular hydrogen bonding as illustrated in Scheme **1**. This occurs in acidic solution/cellular environment. The pyrogallyl moiety of the 3-gallate-group can also potentially undergo HAT and contribute to the antioxidant activity. However in heterogeneous systems such as in water-lipid environments in membranes and in the brain, the antioxidant capacity of phenolic groups is related not only by the intrinsic properties such as O-H BDE or shape/steric hindrance but also to physiochemical features such as phase partitioning, diffusion, solubility, micelle-formation, all of which are impacted by hydrophobicity. The influence of the physiochemical hydrophobic-hydrophilic nature of polyphenolic groups on their antioxidant activity and function is not well understood. The HAT - Variational transitional state calculations [22] related to the antioxidant-lipid peroxy radical have revealed that the effective antioxidant mechanism of the catechol/pyrogallyl groups can be attributed to a very compact reactant complex. This complex is illustrated in Scheme **1** and is composed of hydrogen bonds between two OH groups of the catechol group and two oxygen atoms of lipid peroxyl radical resulting in an extremely narrow adiabatic potential-energy profile facilitating hydrogen abstraction by the peroxyl radical with a significant quantum-mechanical tunneling effect increasing the reaction rate of peroxyl radical quenching [22]. The EGCG oxidized α-hydroxy-*ortho*-quinone product may react with nucleophiles, electrophiles and undergo dimerisation and this needs further investigation. Furthermore, molecular dynamics simulations [23] to study the binding interactions of green tea catechins compounds with lipid bilayers also supported the suggestion

that the biological effects of catechins parallel the degree of hydrogen bonding effects with cell membranes and the implication that the gallate ester side chain enhances the biological efficacy of catechins.

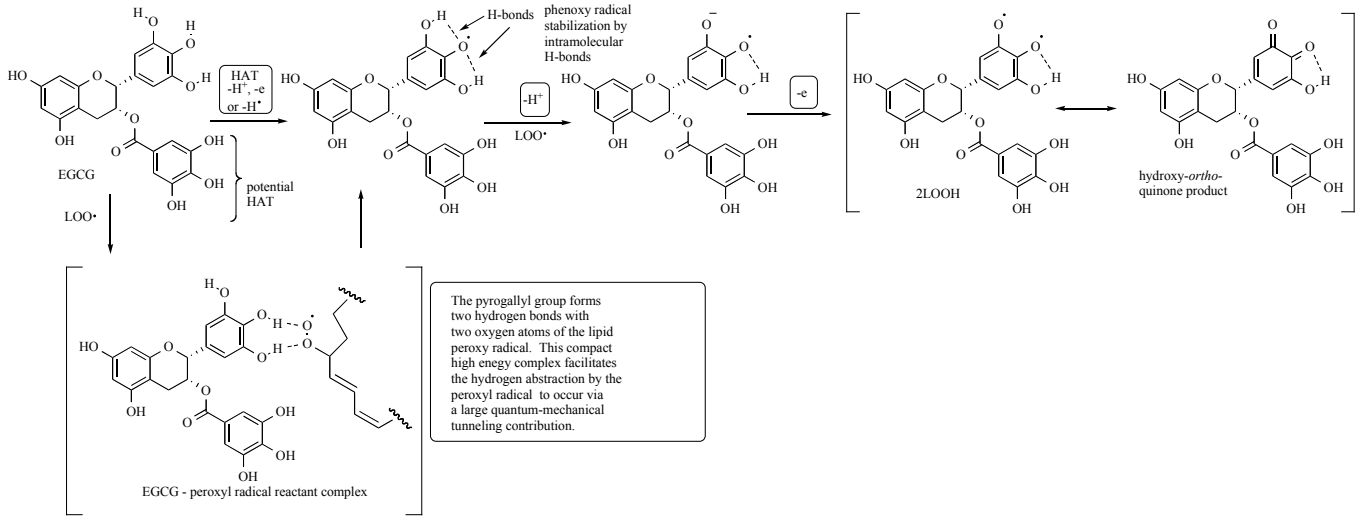
ANTIOXIDANT ACTIVITY OF GREEN TEA POLYPHENOLS [GTP]

In *in vitro* models of cancer, green tea polyphenols have been shown to act as antioxidants as Jurkat T cells subjected to oxidative damage by addition of iron II (Fenton) were protected by a green tea extract (10 mg/mL), containing high amounts of EGCG. The protective effect was attributed to the antioxidant capacity of the extract [24], and likely due to HAT by green tea polyphenols [25]. Green tea extract also prevented H_2O_2-induced cell death in bladder cancer and normal urothelium cells [26]. The Folin–Ciocalteu assay for total phenolic content has been used to show that green tea contains roughly 300 mg/g (expressed as EGCG) of dried leaves [27]. The beneficial effects of green tea polyphenols [28,29] are attributed to their redox responsiveness in OS environments, antioxidant/prooxidant activity, HAT, IP, EGCG-protein adduct formation, EGCG/amyloid bonding interactions. The galloylated catechins are more lipophilic, are more potent antioxidants [30] due to their higher efficacy for divalent chelation of metal ions Cu(II) and Fe(II) and inhibit their redox generation of free radicals [31,59]. GTP plasma levels in humans increased after 42 days of the consumption of 2 cups of GT containing about 250 mg polyphenols; plasma peroxide levels decreased [24]. Green tea polyphenols have been reported to have antioxidant effects *in vivo*. A 4% increase in human plasma antioxidant capacity, was found 40 min after drinking 400mL of green tea, and peak values were found in urine samples after 1 h [32]. The antioxidant capacity of plasma, measured by the trolox equivalent anti-oxidant capacity (TEAC) assay, after consuming 150, 300, and 450mL of green tea (2.5, 5.0, and 7.5g of dried green tea leaves, respectively) increased in a dose-dependent fashion [33].

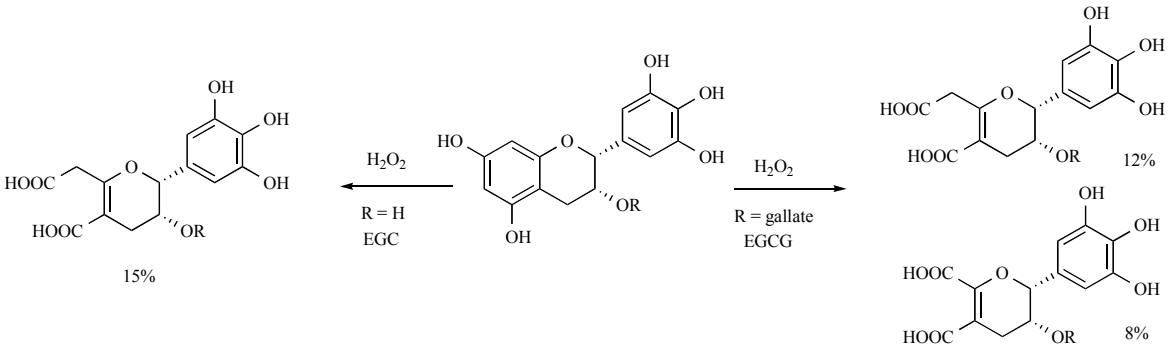
Enhanced plasma antioxidant capacity by 15.6%, measured by a fluorescence-based assay, was found in humans that consume a green tea extract (18.6 mg/ day), and the effect was greatest in smokers [20]. Antioxidant capacity of human plasma, measured by the trolox equivalent antioxidant capacity assay, was enhanced to a greater extent (1.4%) by consumption of green tea poly-phenols (461.9 mg/day) in tablet form compared to a green tea beverage (697.1 mg/day) [34], suggesting tablets may be an effective way to enhance plasma antioxidant capacity by green tea polyphenols. This difference may be due to pharmacokinetic differences in delivery of polyphenols by tablet compared to beverage. Polyphenols in beverage may be ingested and absorbed more slowly than in tablet. The elevation in antioxidant power of human plasma could be the basis for a role of green tea in cancer preventive effects, yet the effects are likely to be due mainly to metabolites of green tea polyphenols [35,36,37].

PRO-OXIDANT EFFECTS OF GREEN TEA *IN VITRO* EFFECTS

When EGCG and EGC are mixed with dilute H_2O_2 for two days, conditions that simulate potential cellular oxidative stress environments, ring A cleaved oxidation products most likely formed *via* hydroxylation/oxidation/ decarboxylation pathways were isolated and characterized as shown in Scheme **2** [38]. EGCG also undergoes other oxidative reactions leading to dimerization products that occur at physiological pH (7.4) [39]. Green tea polyphenols can accelerate *in vitro* pro-oxidant effects at high pH. EPR analysis has shown that EGCG and ECG readily form free radicals during autoxidation in alkaline (pH 13) conditions by radical oxidation of the B and D rings [40] as outlined in Scheme **3**. Surprisingly, oxidation of EGCG by superoxide radical anion formed the unstable D-ring radical followed by degradation to the more stable gallic radical. No radical-degradation mechanism was proposed for the cleavage or substitution of the galloyl group at the C-3 flavanol position and this needs to be investigated. The O_2-alkaline autoxidation produced neutral radicals with one deprotonated hydroxyl group whereas the superoxide radical anion scavenging effect on the D ring of EGCG resulted mainly in formation of a monoanion radical with two deprotonated



Scheme 1. Variational Transition-State Theory accounts for $LOO^{\cdot}$ - EGCG antioxidant mechanism [22].



Scheme 2. EGCG and ECG reaction products with H_2O_2

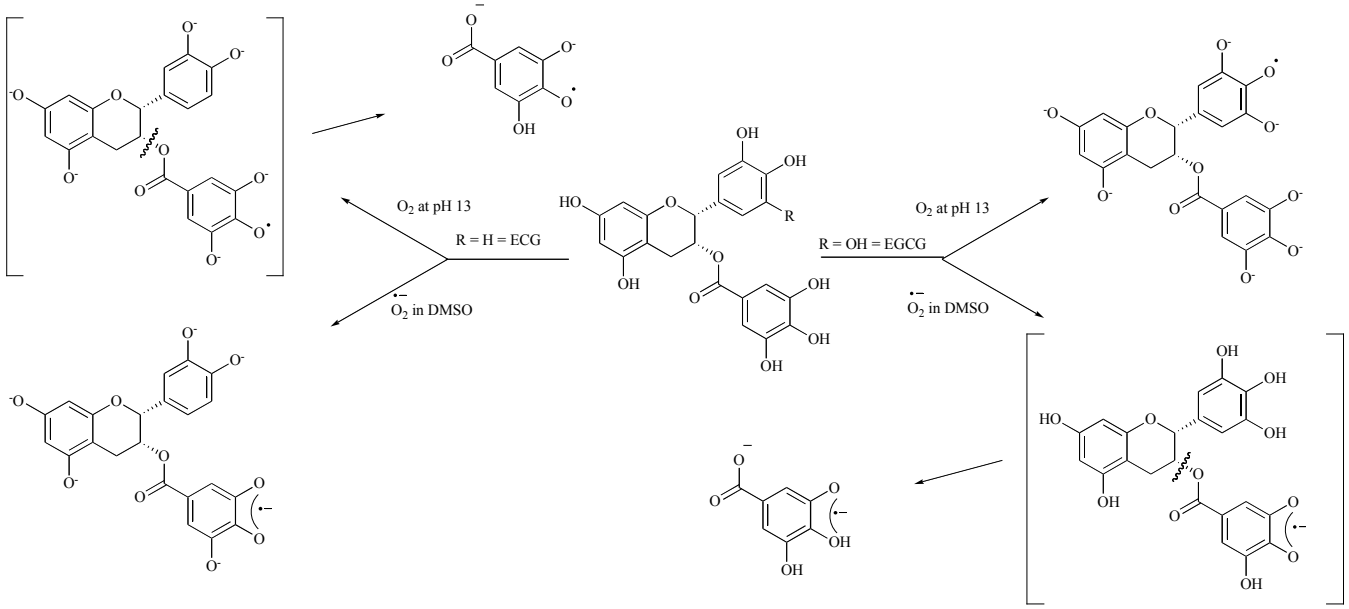
hydroxyl groups as shown in Scheme **3**. Interestingly only pyrogallol priority free radical oxidized polyphenol products were observed although such autoxidation of catechol groups have been observed [41, 42]. This underscores the types of pro-oxidant reactions green tea catechins may affect in complex cellular oxidative environments in the presence of oxygen, superoxide anion, hydroxide radicals, hydrogen peroxide, copper and iron. Indeed the pro-oxidant capacity of green tea catechins is considered an integral part of their cancer inhibition and induction of apoptosis mechanism of action [42,43,44].

The amount of administered/consumed green tea polyphenol may be the crucial factor that controls/determines whether green tea polyphenols act as antioxidants or pro-oxidants *in vitro*. EGC and EGCG, both generate hydrogen peroxide at concentrations greater than 10 millimolar [mM] [45].

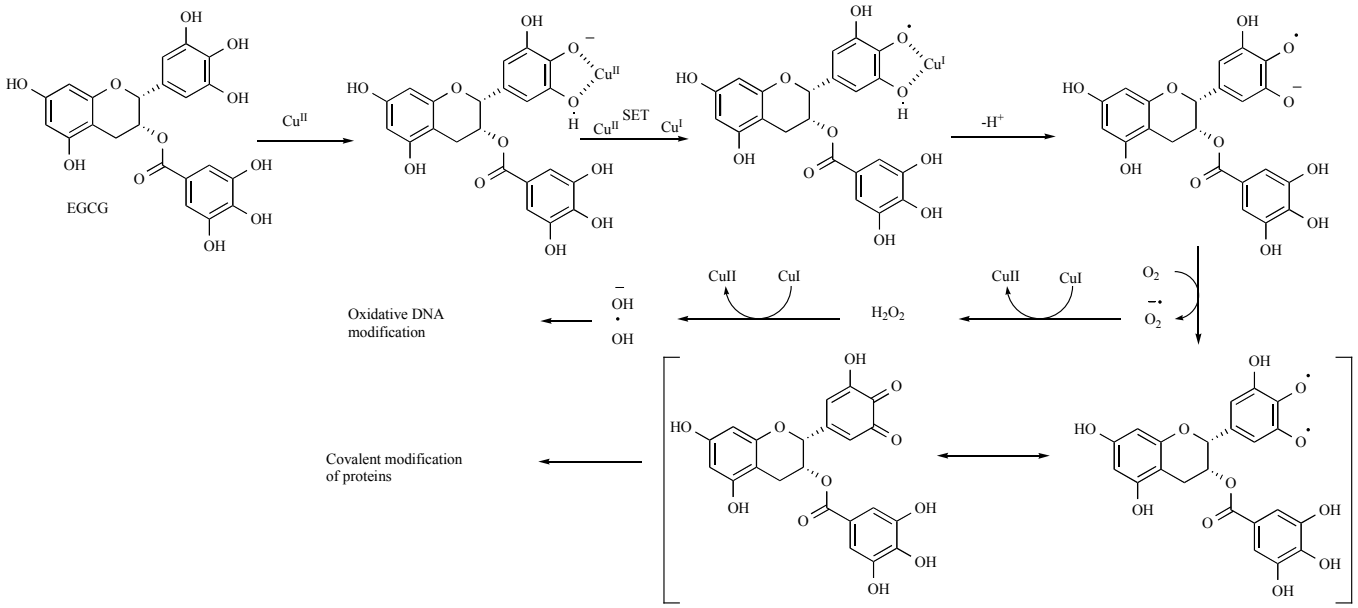
This was shown in lymphoblastoid cell lines, where both EGCG and ascorbic acid at levels of 1–10 mM offered DNA protection against bleomycin, yet the protective effects were lost at 100 mM [46]. Green tea polyphenols do not appear to induce ROS-mediated DNA damage below concentrations of 10 mM, but rather prevent hydrogen peroxide-mediated DNA damage in a dose-dependent manner at lower concentrations. In general, concentrations of green tea polyphenols in excess of 10 mM upset redox balance when added to cells, creating a pro-oxidant environment. This may also be true *in vivo*, as the total concentration of green tea polyphenols can exceed 10 mM in human biological samples [47].

EGCG β-AMYLOID DETOXIFICATION

EGCG protein binding is non-specific and this may explain its diverse activities. From a screening of a library of over 5000 natural substances using a membrane filter retardation assay identified EGCG [IC_{50} ~2μM] as the most potent green tea polyphenol inhibitor of mutant hungtingtin exon 1 [48]. The amyloid aggregation assays indicated that the presence of the gallate esters rather than the antioxidative properties of polyphenols are crucial for the inhibitory effect *in vitro*. EGCG also diverts amyloid-β and α-synclein into alternative and benign folding trajectories avoiding the deleterious conformations frequented during amyloido-genesis resulting in the assembly of large spherical EGCG-stabilized oligomers unable to seed fibrillogenesis and importantly exert neuroprotection by the modulation of the



Scheme 3. EGCG and ECG autoxidation and superoxide reactions.



Scheme 4. EGCG pro-oxidant reactions [1].

protein kinase C pathway (PKC) [49,50]. Other dietary flavonoids [51,52,53] have been found to possess neuroprotective effects in cell cultures, *in vitro* and *in vivo* suggesting that flavonoids may function by a similar mechanism. Herbal medicines containing flavonoids have been reported to have beneficial effects in treating cognitive decline and dementia [54, 55]. Recent research indicated that baicalin protects rat cortical neurons from amyloid β-induced toxcicity by its inhibition of lipoxygenase [56]. Interestingly, baicalein and its quinone oxidized derivative that forms a Schiff base with a lysine in α-synuclein prevented fibrillization in addition to their ability to disaggregate exisiting fibrils by stabilizing a soluble oligomeric form [57].

NEUROPROTECTIVE EFFECTS OF EGCG

EGCG

- was found to elevate the activity of SOD and catalase, the two major oxygen-radical species metabolizing enzymes in mice striatum [58, 59]
- possesses neuroprotective effects against a variety of toxic insults and inflammatory neuronal injuries [51,60,61,62,]
- inhibited inflammatory stress-induced neuronal cell death in the case of ischemia/reperfusion-induced brain injury [63,64]
- has anti-β-secretase activity *in vitro* [65]
- enhances the release of soluble APP (a neuron protective form of amyloid precursor protein) in human SH-SY5Y neuroblastoma and pheochromocytoma (PC12) cells as well as in the hippocampus of a mouse brain [31,66,]
- elevated N-terminal APP cleavage producing soluble APP-α through α-secretase activity in murine neuron-like cells transfected with Swedish mutant form of APP (SweAPP N2a cells) as well as transgenic mice carrying Swedish mutant APP (Tg APPsw line 2576) [67]
- prevents LPS-induced Aβ production by the inhibition of secretase activity, and improved effects on the memory deficiency in LPS-induced AD mice models [68]
- directly binds/interacts/transforms mature α-synclein and amyloid-β fibrils into nontoxic smaller, amorphous protein aggregates [69]
- pre-treatment of neurons prevented Aβ-induced mitochondrial dysfunction, impairment of NMDA, Ca^{2+} influx and ROS production [70]

Isothermal titration calorimetry (ITC) of *in vitro* studies of the interactions between EGCG and Aβ under different experimental conditions of EGCG and Aβ concentrations, pH, temperatures, salt concentrations provided an analysis of the thermodynamic parameters which significantly influence the EGCG-Aβ interactions [71]. The stoichiometry *(N)* is linearly related to the EGCG/Aβ ratio at pH 7.4 assuming EGCG stability. As indicated previously and shown in Table **1**. below, not only the nature of the redox activity of EGCG but also the binding interactions is affected by the applied dose of EGCG. The EGCG - Aβ binding was enhanced by increasing temperature, salt concentration and at pH values from pI of Aβ.

GREEN TEA POLYPHENOL/METABOLITES AND BIOAVAILABILITY

The considerable challenge of correlating the biological activities of plant products like green tea polyphenols from *in vitro* to possible benefits *in vivo* is its bioavailability, [72,73,74,75,76,77,78,79]. The low bioavailability of EGCG can be related to such factors as: (a) the instability of EGCG in neutral and alkaline conditions [39] (b) poor cellular

Table 1. ITC Analysis of How the Concentration of [EGCG]/[Aβ] Relates to the Thermodynamics of their Binding

Concentration ratios M = [EGCG]/[Aβ]	Predominant binding interaction
M < 16	Hydrogen bonding, enthalpy driven
M>46	Hydrophobic interactions, entropy driven

uptake because of its hydrophilicity and poor hydrophobicity to be absorbed by cells and (c) metabolic transformations such as methylation, glucuronidation and sulfation after absorption [80,81]. There is *in vitro* evidence that formulation factors may potentially enhance the tea polyphenol profile in the small intestine [82]. Moreover, unraveling the role of colonic microbiota in the analysis of the bioconversion of polyphenols into their metabolites [83] is complex and requires further investigation. It is also unclear the extent green tea polyphenols can penetrate the blood brain barrier in sufficient concentrations for effective neuroprotection however peracetylated- [84] or nanolipidic-EGCG particles [85] improved *in vitro* efficacy and more than doubled its oral bioavailability *in vivo* and has potential therapeutic applications.

CONCLUSION

Biomedical research is investigating the mechanisms whereby green tea promotes good health and lowers the risk of major chronic diseases such as heart disease, various types of cancer and neurodegenerative diseases. Epigallocatechin gallate is the major active component of the green tea polyphenols and evidence suggests the polyphenols have multiple bioactivities: 1) Their concentration determines its antioxidant or oxidant function. They decrease the oxidation of LDL cholesterol and lower the risk of heart disease, and may inhibit action of ROS mediating the oxidation of DNA associated with carcinogenesis. 2) They induce detoxifying enzymes, glucuronosyl transferases, eliminating active forms of carcinogens and other toxicants, accounting for the lower cancer risk. 3) They lower the duplication rates of cancer cells and the growth of cancer inhibited, with increased apoptosis and lower angiogenesis. 4) Alter intestinal bacterial flora, suppressing undesirable bacteria and favoring growth of beneficial bacteria. 5) Result in inhibition of aging phenomena, and diseases associated with the formation of ROS. 6) Antagonize amyloidogenesis characteristic of Alzheimer's and Parkinson's diseases. Their mechanism of action *in vivo* may differ according to the cellular redox status, bioavailability at proposed site of action and nature of binding interaction. There is no doubt that more surprises, good and bad, are certainly ahead. Still, we should keep high our expectation that the green tea polyphenols and related compounds have yet to yield all their secrets and therapeutic opportunities for neuroprotection.

REFERENCES

[1] Jakob-Rotetne, R.; Jacobsen, H. Alzheimer's Disease: From Pathology to Therapeutic Approaches. *Angew. Chem., Int. Ed.* **2009,** *48,* 3030-3059.

[2] Finch, C.E. Evolution of the human lifespan and diseases of aging: Roles of infection, inflammation, and nutrition. *PNAS,* **2010,** *107,* 1718-1724.

[3] Cabrera, C.; Artacho, R.; Gimenez, R. Beneficial effects of green tea – a review. *J. Am. Coll. Nutr*. **2006**, *25,* 79-99.

[4] Yang, C.S.; Wang, H. Mechanistic issues concerning cancer prevention by tea catechins. *Mol. Nutr. Food Res.* **2011,** *55,* 819-831.

[5] Singh, M.; Arseneault, M.; Sanderson, T.; Murthy, V.; Ramassamy, C. Challenges for Research on Polyphenols from Foods in Alzheimer's Disease: Bioavailability, Metabolism, and Cellular and Molecular Mechanisms. *J. Agric. Food Chem.* **2008,** *56,* 4855-4873

[6] Lovell, M.A.; Markesbery, W.R. Oxidatively modified RNA in mild cognitive impairment. *Neurobiol. Dis.* **2008,** *29,* 169-175.

[7] Wang, J.; Xiong, S.; Xie, C.; Markesbery, W.R.; Lovell, M.A. Increased oxidative damage in nuclear and mitochondrial DNA in Alzheimer's disease. *J. Neurochem.* **2005,***93,* 953-962.

[8] Nourooz-Zadeh, J.; Liu, E.H.; Yhlen, B.; Anggard, E.E.; Halliwell, B. F4-isoprostanes as specific marker of docosahexaenoic acid peroxidation in Alzheimer's disease. *J. Neurochem.* **1999,** *72,* 734-740.

[9] Ramassamy, C.; Averill, D.; Beffert, U.; Bastianetto, S.; Theroux, L.; Lussier-Cacan, S.; Cohn, J.S.; Christen, Y.; Davignon, J.; Quirion, R.; Poitier, J. Oxidative damage and protection by antioxidants in the frontal cortex of Alzheimer's disease related to the apolipoprotein E genotype. *Free Radical Biol. Med.* **1999,** *27,* 544-553.

[10] Ramassamy, C.; Averill, D.; Beffert, U.; Theroux, L.; Lussier-Cacan, S.; Cohn, J.S.; Christen, Y.; Schoofs, A.; Davignon, J.; Poitier, J. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. *Neurobiol. Dis*. **2000,** *7,* 23-37.

[11] Montine, K.S.; Reich, E.; Neely, M.D.; Sidell, K.R.; Olson, S.J.; Markesbery, W.R.; Montine, T.J. Distribution of reducible 4-hydroxynonenal adduct immunoreactivity in Alzheimer's disease is associated with inheritance of APOE4. *J. Neuropathol. Exp. Neurol.* **1998,** *57,* 415-425.

[12] Beffert, U.; Aumont, N.; Dea, D.;Lussier-Cacan, S.; Davignon, J.; Poitier, J. Apolipoprotein E isoform-specific reduction of extracellular amyloid in neuronal cultures. *Brain Res. Mol. Brain Res.* **1999,** *68,* 181-185.

[13] Stadtman, E.R.; Protein oxidation and aging. *Science*, **1992,** *257,* 1220-1224.

[14] Pratico, D.; Clark, C.M.; Lee,, V.M.; Trojanowski, J.Q.; Rokach, J.; Fitzgerald, G.A. Increased 8,12-iso-iPF2α-VI in Alzheimer's disease: Correlation of a noninvasive index of lipid peroxidation with disease severity. *Ann. Neurol.***2000,** *48,* 809-812.

[15] Montine, K.S.; Olson, S.J.; Amarnath, V.; Whetsell, W.O., Jr.; Graham, D.G.; Montine, T.J. Immunohistochemical detection of 4-hydroxy-2-nonenal adducts in Alzheimer's disease is associated with the inheritance of APOE genotype. *Am. J. Pathol.* **1997,***150,* 437-443.

[16] Williams, T.L.; Lynn, B.C.; Markesbery, W.R.; Lovell, M.A. Increased levels of 4-hydroxynonenal and acrolein, neurotoxic markers of lipid peroxidation, in the brain in mild cognitive impairment and elderly Alzheimer's disease. *Neurobiol. Aging* **2006,** *27,* 1094-1099.

[17] Lovell, M.A.; Xie, C.; Markesbery, W.R.; Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures. *Neurobiol. Aging* **2001,** *22,* 187-194.

[18] Qin, Z,; Hu, D.; Han, S.; Reany, S.H.; di Monte, D.A.; Fink, A.L. effect of 4-hydroxy-2-nonenal modification on α-synuclein aggregation. *J. Biol. Chem.* **2007,***282,* 5862-5870.

[19] Castegna, A.; Aksenov, M.; Thongboonkerd, V.; Klein, J.B.; Pierce, W.M.; Booze, R.; Markesbery, W.R.; Butterfield, D.A. Proteomic identification of oxidatively modified proteins in Alzheimer's disease brain. Part II: dihydropyrimidinase-related

protein 2, α-enolase and heat shock cognate 71. *J. Neurochem.* **2002,** *82,* 1524-1532.

[20] Forester, S.C.; Lambert, J.D. The role of antioxidant versus pro-oxidant effects of green tea polyphenols in cancer prevention. *Mol. Nutr. Food Res.* **2011,** *55,* 844-854.

[21] Wright, J.S.; Johnson, E.R.; DilAbio, G.A. Predicting the Activity of Phenolic Antioxidants: Theoretical Method, Analysis of Substituent Effects, and Application to Major Families of Antioxidants. *J. Am. Chem. Soc.* **2001,** *123,* 1173-1183.

[22] Tejero, I.; Gonzalez-Garcia, N.; Gonzalez-Lafont, A.; Lluch, J.M. Tunneling in Green Tea: Understanding the Antioxidant Activity of Catechol-Containing Compounds. A Variational Transition-State Theory Study. *J. Am. Chem. Soc.* **2007,** *129,* 5846-5854.

[23] Sirk, T.W.; Brown, E.F.; Friedman, M.; Sum, A.K. Molecular Binding of Catechins to Biomembranes: Relationship to Biological Activity. *J. Agric. Food Chem.* **2009,** *57,* 67200-6728.

[24] Erba, D., Riso, P., Colombo, A., Testolin, G., Supple- mentation of Jurkat T cells with green tea extract decreases oxidative damage due to iron treatment. *J. Nutr.* **1999**, *129,* 2130–2134.

[25] Anderson, R. F., Fisher, L. J., Hara, Y., Harris, T. *et al*., Green tea catechins partially protect DNA from hydroxy radical-induced strand breaks and base damage through fast chemical repair of DNA radicals. Carcinogenesis **2001**, *22,* 1189–1193.

[26] Coyle, C. H.; Philips, B. J.; Morrisroe, S. N.; Chancellor, M. B.; Yoshimura, N.; Antioxidant effects of green tea and its polyphenols on bladder cells. *Life Sci.* **2008**, *83*, 12–18.

[27] Lin, Y. L.; Juan, I. M.; Chen, Y. L.; Liang, Y. C.; Lin, J. K. Composition of polyphenols in fresh tea leaves and associations of their oxygen-radical-absorbing capacity with antiproliferative actions in fibroblast cells. *J. Agric. Food Chem.* **1996,** *44,* 1387–1394.

[28] Sutherland, B. A.; Rahman, R. M.; Appleton, I. Mechanisms of action of green tea catechins, with a focus on ischemia-induced neurodegeneration. *J. Nutr. Biochem.* **2006**, *17*, 291–306.

[29] Guo, Q.; Zhao, B.; Li, M.; Shen, S.; Xin, W. Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. *Biochim. Biophys. Acta* **1996**, *1304*, 210–222.

[30] Caturla, N.; Vera-Samper, E.; Villalain, J.; Mateo, C. R.; Micol, V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. *Free Radical Biol. Med.* **2003**, *34*, 648–662.

[31] Levites, Y.; Amit, T.; Mandel, S.; Youdim, M. B. Neuroprotection and neurorescue against Abeta toxicity and PKC- dependent release of nonamyloidogenic soluble precursor protein by green tea polyphenol (-)-epigallocatechin-3-gallate. *FASEB J.* **2003**, *17*, 952–954.

[32] Benzie, I. F. F.; Szeto, Y. T.; Strain, J. J.; Tomlinson, B. Consumption of green tea causes rapid increase in plasma antioxidant power in humans. *Nutr. Cancer* **1999,** *34,* 83–87.

[33] Sung, H.; Nah, J.; Chun, S.; Park, H.; Yang, S.E.; Min, W.K. *In vivo* antioxidant effect of green tea. *Eur. J. Clin. Nutr.* **2000**, *54,* 527–529.

[34] Henning, S. M.; Niu, Y. T.; Lee, N. H.; Thames, G. D.; Minutti, R.R.; Wang, H.; Go, V.L.W.; Heber, D. Bioavailability and antioxidant activity of tea flavanols after consumption of green tea, black tea, or a green tea extract supplement. *Am. J. Clin. Nutr.* **2004**, *80*, 1558–1564.

[35] Takagaki, A.; Nanjo, F.; Metabolism of (-)-epigallocatechin gallate by rat intestinal flora. *J. Agric. Food Chem.* **2010**, *58,* 1313–1321.

[36] Van't Slot, G.; Humpf, H. U.; Degradation and metabolism of catechin, epigallocatechin-3-gallate (EGCG), and related compounds by the intestinal microbiota in the pig cecum model. *J. Agric. Food Chem.* **2009,** 57, 8041–8048.

[37] Zhang, L.; Chow, M. S. S.; Zuo, Z.; Effect of the co-occurring components from green tea on the intestinal absorption and disposition of green tea polyphenols in Caco-2 monolayer model. *J. Pharm. Pharmacol.* **2006,** *58,* 37–44.

[38] Zhu, N.; Huang, T-C.; Yu, Y.; LaVoie, E.J.; Yang, C.S.; Ho, C-T. Identification of Oxidation Products of (-)-Epigallocatechin Gallate and (-)-Epicatechin with H_2O_2. *J. Agric. Food Chem.* **2000,** *48,* 971–981.

[39] Sang, S. M.; Lee, M. J.; Hou, Z.; Ho, C. T.; Yang, C. S. Stability of tea polyphenol (-)-epigallocatechin-3-gallate and formation of dimers and epimers under common experimental conditions. *J. Agric. Food Chem.* **2005,** *53,* 9478–9484.

[40] Severino, J. F.; Goodman, B. A.; Kay, C. W. M.; Stolze, K.; Tunega, D.; Reichenauer, T.G.; Pirker, K.F. Free radicals generated during oxidation of green tea polyphenols: electron paramagnetic resonance spectroscopy combined with density functional theory calculations. *Free Radic. Biol. Med.* **2009,** 46, 1076–1088.

[41] Pirker, K.F.; Stolze, K.; Reichenauer, T.G.; Nohl, H.; Goodman, B.A. Are the biological properties of Kaempferol determined by its oxidation products? *Free Radic. Res.* **2006,** *40,* 513-521.

[42] Pirker, K.F.; Kay, C.W.M.; Stolze, K.; Tunega, D.; Reichenauer, T.G.; Nohl, H.; Goodman, B.A. Free radical generation in rosmarinic acid investigated by electron paramagnetic resonance spectroscopy. *Free Radic. Res.* **2009,** *30,* 47-57.

[43] Ermakova, S. P.; Kang, B. S.; Choi, B. Y.; Choi, H. S.; Choi, H.S.; Schuster, T.F.; Ma, W-Y.; Bode, A.M.; Dong, Z.(-)-Epigallocatechin gallate overcomes resistance to etoposide-induced cell death by targeting the molecular chaperone glucose-regulated protein 78. *Cancer Res.* **2006,** *66,* 9260–9269.

[44] Shim, J. H.; Choi, H. S.; Pugliese, A.; Lee, S. Y.; Chae, J-I.; Choi, B.Y.; Bode, A.M.; Dong, Z. (-) - Epigallocatechin gallate regulates CD3-mediated T cell receptor signaling in leukemia through the inhibition of ZAP-70 kinase. *J. Biol. Chem.* **2008**, *283,* 28370–28379.

[45] Shim, J. H.; Su, Z. Y.; Chae, J. I.; Kim, D. J.; Zhu, F.; Ma, W-Y.; Bode, A.M.; Yang, C.S.; Dong, Z. Epigallocatechin gallate suppresses lung cancer cell growth through Ras-GTPase-activating protein SH3 domain-binding protein 1. *Cancer Prev. Res.* **2010,** *3*, 670–679.

[46] Taniguchi, S., Fujiki, H., Kobayashi, H., Go, H. *et al*., Effect of (-)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. **Cancer Lett. 1992,** *65,* 51–54.

[47] Hsu, T. C.; Zhao, Y. J.; Wang, R. Y.; Wu, Y. F.; Spitz, M.R.; Hong, W.K. Comparative efficacy as antioxidants between ascorbic acid and epigallocatechin gallate on cells of two human lymphoblastoid lines. *Cancer Genet. Cytogenet.* **2001,** *124,* 169–171.

[48] Stalmach, A.; Troufflard, S.; Serafini, M.; Crozier, A.; Absorption, metabolism and excretion of Choladi green tea flavan-3-ols by humans. *Mol. Nutr. Food Res.* **2009,** *53,* S44–S53.

[49] Ehrnhoefer, D. E.; Duennwald, M.; Markovic, P.; Wacker, J. L.; Engemann, S.; Roark, M.; Legleiter, J.; Marsh, J. L.; Thompson, L. M.; Lindquist, S.; Muchowski, P. J.; and Wanker, E. E. Green tea (-)-epigallocatechin-gallate modulates early events in huntingtin misfolding and reduces toxicity in Huntington's disease models. *Hum. Mol. Genet.* **2006,** *15,* 2743–2751.

[50] Mandel, S. A.; Amit, T.; Weinreb, O.; Reznichenko, L.; and Youdim, M. B. Simultaneous manipulation of multiple brain targets by green tea catechins: A potential neuroprotective strategy for Alzheimer and Parkinson diseases. *CNS Neurosci. Ther.* **2008,** *14,* 352–365.

[51] Ehrnhoefer, D. E.; Bieschke, J.; Boeddrich, A.; Herbst, M.; Masino, L.; Lurz, R.; Engemann, S.; Pastore, A.; and Wanker, E. E. EGCG redirects amyloidogenic polypeptides into unstructured, off-pathway oligomers. *Nat. Struct. Mol. Biol.* **2008,** *15,* 558–566.

[52] Wang, C.N.; Chi, C.W.; Lin, Y.L.; Chen, C.F.; Shiao, Y.J. The neuroprotective effects of phytoestrogens on amyloid beta protein-induced toxicity are mediated by abrogating the activation of caspase cascade in rat cortical neurons. *J. Biol. Chem.* **2001,** *276,*5287-5295.

[53] Kim, H.; Park, B-S.; Lee, K-G.; Choi, C.Y.; Jang, S.S.; Kim, Y-H.; and Lee, S-E. Effects of Naturally Occurring Compounds on Fibril Formation and Oxidative Stress of β-Amyloid. *J. Agric. Food Chem.* **2005,** *53,* 8537-8541..

[54] Datla, K. P.; Christidou, M.; Widmer, W. W., Rooprai, H. K., and Dexter, D. T. Tissue distribution and neuroprotective effects of citrus flavonoid tangeretin in a rat model of Parkinson's disease *Neuroreport* **2001,** *12,* 3871–3875.

[55] Perry, E. K.; Pickering, A. T.; Wang, W. W.; Houghton, P. J.; and Perry, N. S. (1999) J. Pharm. Pharmacol. 51, 527–534.

[56] Watanabe, C. M.; Wolffram, S.; Ader, P.; Rimbach, G.; Packer, L.; Maguire, J. J.; Schultz, P. G.; and Gohil, K. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 6577– 6580.

[57] Lebeau, A.; Esclaire, F.; Rostene, W.; and Pelaprat, D. Baicalein protects cortical neurons from [beta]-amyloid (25-35) induced toxicity *Neuroreport* **2001,** *12,* 2199 –2202.

[58] Levites Y, Weinreb O, Maor G, Youdim MBH, Mandel S . Green tea polyphenol (–)- Epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridineinduced dopaminergic neurodegeneration. J Neurochem **2001**,78, 1073-1082.

[59] Mandel, S.A.; Amit, T.; Weinreb, O.; Youdim, M.B.H. Understanding the Broad-Spectrum Neuroprotective Action Profile of Green Tea Polyphenols in Aging and Neurodegenerative Diseases. *J. Alz. Dis*. **2011,** *25,* 187-208.

[60] Zhu,M.; Rajamani, S.; Kaylor, J.; Han, S.; Zhou, F.; Fink, A.L. The Flavonoid Baicalein Inhibits Fibrillation of α-Synuclein and Disaggregates Existing Fibrils. *J. Biol. Chem.* 279, **2004,** *279,* 26846–26857.

[61] Mandel, S.; Einreb, O.; Amit, T.; Youdim, M.B. Cell signaling pathways in the neuroprotective actions of the green tea polyphenol (−)-epigallocatechin-3-gallate: implications for neurodegenerative diseases. *J. Neurochem.* **2004,** *88,* 1555–15569.

[62] Cho, H.S.; Kim, S.; Lee, S.Y.; Park, J.A.; Kim, S.J.; Chun, H.S. Protective effect of the green tea component, L-theanine on environmental toxins-induced neuronal cell death. *Neurotoxicology* **2008,** *29,* 656–662.

[63] Zhang, B.;Rusciano, D.; Osborne, N.N. Orally administered epigallocatechin gallate attenuates retinal neuronal death *in vivo* and light-induced apoptosis *in vitro*. *Brain Res.* **2008,** *1198,* 141–152.

[64] Antonio, A.M.; Druse, M.J. Antioxidants prevent ethanol-associated apoptosis in fetal rhombencephalic neurons. *Brain Res.* **2008,** *1204,* 16–23.

[65] Hong, J.T.; Ryu, S.R.; Kim, H.J.; Lee, J.K.; Lee, S.H.; Kim, D.B.; Yun, Y.P.; Ryu, J.H.; Lee, B.M.; Kim, P.Y. Neuroprotective effect of green tea extract in experimental ischemia-reperfusion brain injury. *Brain Res. Bull.* **2000,** *53,* 743–749.

[66] Hong, J.T.; Ryu, S.R.; Kim, H.J.; Lee, J.K.; Lee, S.H.; Yun, Y.P.; Lee, B.M.; Kim, P.Y. Protective effect of green tea extract on ischemia/reperfusion-induced brain injury in Mongolian gerbils. *Brain Res.* **2000,** 8*8,* 11–18.

[67] Okello, E.J.; Savelev, S.U.; Perry, E.K. *In vitro* anti-beta-secretase and dual anti-cholinesterase activities of Camellia sinensis L. (tea) relevant to treatment of dementia. *Phytother. Res.* **2004,** *18,* 624–647.

[68] Storey, E.; Cappai, R. The amyloid precursor protein of Alzheimer's disease and the Abeta peptide. *Neuropathol. Appl. Neurobiol*. **1999,** *25,* 81–97.

[69] Rezai-Zadeh, K.; Shytle, D.; Sun, N.; Mori, T.; Hou, H.; Jeanniton, D.; Ehrhart, J. Townsend, K. Zeng, J., Morgan, D.; Hardy, J.; Town, T.; Tan, J. Green tea epigallocatechin-3-gallate (EGCG) modulates amyloid precursor protein cleavage and reduces cerebral amyloidosis in Alzheimer transgenic mice. *J. Neurosci.* **2005,** *25,* 8807–8814.

[70] Lee, Y.K.; Yuk, D.Y.; Lee, J.W.; Lee, S.Y.; Ha, T.Y.; Oh, K.W.; Yun, Y.P.; Hong, J.T. (-)-Epigallocatechin-3-gallate prevents lipopolysaccharide-induced elevation of beta-amyloid generation and memory deficiency. *Brain Research,* **2009,** *1250,* 164-174.

[71] Bieschke, J.; Russ, J.; Friedrich, R.P.; Ehrnhoefer, D.E.; Wobst, H.; Neugebauer, K.; Wanker, E.E. EGCG remodels mature α-synclein and amyloid-β fibrils and reduces cellular toxcicity. *PNAS,* **2010,** *107,* 7710-7715.

[72] He Y, Cui J, Lee JC-M, Ding S, Chalimoniuk M, Simonyi A, Sun AY, Gu Z, Weisman GA, Wood WG and Sun GY Prolonged exposure of cortical neurons to oligomeric amyloid-b impairs NMDA receptor function *via* NADPH oxidase-mediated ROS production: protective effect of green tea (–)-epigallocatechin-3-gallate. *ASN NEURO* **2011**, *3,* 13-24.

[73] Wang, S-H.; Liu, F-F.; Dong, X-Y.; Sun, Y. Thermodynamic Analysis of the molecular Interactions between Amyloid β-Peptide 42 and (-)-Epigallocatechin-3-gallate. *J. Phys. Chem. B* **2010,** *114,* 11576-11583.

[74] Manach C, Williamson G, Morand C, Scalbert A, Rémésy C. Bioavailability and bioefficacy of polyphenols in humans. I. Review of 97 bioavailability studies. *Am J Clin Nutr.* **2005,** *81,* 230–242.

[75] Chow HH, Hakim IA, Vining DR, Crowell JA, Ranger-Moore J, Chew WM, *et al*. Effects of dosing condition on the oral bioavailability of green tea catechins after single-dose administration of Polyphenon E in healthy individuals. *Clin Cancer Res.* **2005**, *11,* 4627–4633.

[76] Feng, W.Y. Metabolism of green tea catechins: an overview. *Curr. Drug Metab.* **2006,** *7,* 755–809.

[77] Henning, S.M.; Choo, J.J.; Heber, D. Nongallated compared with gallated flavan-3-ols in green and black tea are more bioavailable. *J. Nutr.* **2008,** *138,* 1529S–1534S.

[78] Lambert, J.D.; Lee, M.J.; Lu, H.; Meng, X.; Hong, J.J.; Seril, D.N.; Sturgill, M.G.; Yang, C.S. Epigallocatechin-3-gallate is absorbed but extensively glucuronidated following oral administration to mice. *J. Nutr.* **2003,** *133,* 4172–4177.

[79] Lambert, J.D.; Hong, J.; Kim, D.H.; Mishin, V.M.; Yang, C.S. Piperine enhances the bioavailability of the tea polyphenol (−)-epigallocatechin-3-gallate in mice. *J. Nutr.* **2004,** *134,* 1948–1952.

[80] Lambert, J.D.; Lee, M.J.; Diamond, L.; Ju, J.; Hong, J.; Bose, M.; Newmark, H.L.; Yang, C.S. Dose- dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. *Drug Metab. Dispos.* **2006,** *34,* 8–11.

[81] Lin, L.C., Wang, M.N., Tseng, T.Y., Sung, J.S., Tsai, T.H., Pharmacokinetics of (−)-epigallocatechin-3-gallate in conscious and freely moving rats and its brain regional distribution. *J. Agric. Food Chem.* **2007**, *55,* 1517–1524.

[82] Green, R.J.; Murphy, A.S.; Schulz, B.; Watkins, B.A.; Ferruzi, M.G. Common tea formulations modulate *in vitro* digestive recovery of green tea catechins. *Mol Nutr Food Res.* **2007**, *51*, 1152-1162.

[83] Van Duynhovena, J.; Vaughana, E.E.; Jacobsa, D.M.; Kempermana, R.A.; van Velzena,E.J.J.; Grossa,G.D.; Rogera, L.C.; Possemiers, S.; Smilde, A.K.; Doréb, J.; Westerhuisc, J.A.; Van de Wieled, T. Metabolic fate of polyphenols in the human superorganism *PNAS,* **2011** *108,* 4531-4538.

[84] Lambert, J.D.; Sang, S.; Hong, J.; Kwon, S.J.; Lee, M.J.; Ho, C.T.; Yang, C.S.Peracetylation as a means of enhancing *in vitro* bioactivity and bioavailability of epigallocatechin-3-gallate. *Drug Metab. Dispos.* **2006,** *34,* 2111–2116.

[85] Smith, A.; Giunta, B.; Bickford, P.C.; Fountain, M.; Tan, J.; Shytle, R.D. Nanolipidic particles improve the bioavailability and α-secretase inducing ability of epigallocatechin-3-gallate (EGCG) for the treatment of Alzheimer's disease. *International Journal of Pharmaceutics,* **2010,** *389,* 207-212.

Received: July 25, 2011 Revised: October 10, 2011 Accepted: October 11, 2011