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Physiological levels of tea catechins increase cellular lipid antioxidant activity of vitamin C and vitamin E in human intestinal Caco-2 cells

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Abstract

Oxidative stress has been linked to the development of various chronic diseases. Vegetables and fruits, which contain polyphenols, were shown to have protective effects. (-)-Epigallocatechin-3-gallate (EGCG), a polyphenol abundant in tea, has been shown to have antioxidant activities in cell-free conditions and this study focused on the effect of cellular EGCG. Using an intestinal cell model to examine the oxidative stress induced by hydroxyl radicals, we report here that physiological concentrations (0.1–1 μ M) of EGCG have dose- and incubation duration-dependent cell-associated lipid antioxidant activity (measuring malondialdehyde production). Vitamin E and vitamin C at 10–40 μ M also showed cell-associated lipid antioxidant activities under shorter incubation durations. When EGCG was included in the incubation with vitamin E or C, more antioxidant activities were consistently observed than when vitamins were added alone. Catechin (widely present in fruits and vegetables) at 1 μ M also significantly increased the antioxidant activity of vitamin E and C. Previous studies examining cell-associated activity of EGCG mainly focused on the 10–100 μ M concentration range. Our results suggest that although the physiological level (0.1–1 μ M) of dietary catechins is much lower than that of vitamins, they further contribute to the total antioxidant capacity even in the presence of vitamins.

Keywords

(-)-epigallocatechin-3-gallate; vitamin C; vitamin E; malondialdehyde; Caco-2 cells

1. Introduction

Reactive oxygen species can cause damages of macromolecules in the cell, which in turn contribute to the development of various aging-related chronic diseases. In the presence of metal, H_2O_2 is the direct source of hydroxyl radical ($\cdot OH$), a short-lived radical with broad-spectrum reactivities. One consequence of hydroxyl radical attack is lipid peroxidation [1]. The reaction leads to damages in cell membrane structure and the major product, malondialdehyde (MDA), can further react with cellular protein and DNA [2,3]. Cytotoxicity and carcinogenesis were both reported as a result of MDA exposure [4,5]. The link between hydroxyl radical attack and aging is further supported by animal study. An extension of life

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span was observed in mice overexpressing catalase, an enzyme assisting the removal of H_2O_2 [6].

Tea (-)-epigallocatechin-3-gallate (EGCG) was shown to possess antioxidant activity in cell-free systems. For example, it can protect LDL [7] as well as aqueous and lipid compartments of human plasma [8] from oxidative damages. EGCG spares α -tocopherol in the lipid compartment of plasma [8,9]. In vivo effects of EGCG were also reported after injections [10-13]. At 10-100 μM EGCG, a concentration range that is not physiologically achievable through oral ingestion [14], pro-oxidant activity of EGCG in cell-free system [15,16], and effects of EGCG on gene transcription and regulation were reported [17-19] and reviewed [20]. The effect of EGCG was also reported in cell studies with concentrations greater than 100 μM [21,22]. The emphasis of this paper is on the biological effect of EGCG at concentrations observed after dietary or supplemental intake, 0.1-1 μM [14,23]. Catechins were shown to be absorbed and secreted by intestinal epithelial cell model, Caco-2 cells [24-26]. Although net absorption is observed [24], this bi-directional movement could explain the low oral bioavailability of this group of compounds.

In a large prospective study, greater adherence to the vegetable/fruit-rich Mediterranean diet was associated with a significant reduction in total mortality especially that of coronary heart diseases [27]. Although phytochemical interaction has been examined in the cell-free system, little cell or animal information is available on the biological interaction between phytochemicals which is expected when humans ingest vegetables and fruits. Here, we tested the antioxidant interaction in cultured human intestinal cells.

2. Materials and methods

2.1. Materials

EGCG was purchased from Calbiochem (La Jolla, CA) with purity greater than 95%. (-)-epigallocatechin (EGC), α -tocopherol acetate (VEA), α -tocopherol succinate (VES) and L-ascorbic acid 2-phosphate were from Sigma-Aldrich (St. Louis, MO) with greater than 98% purity. ($\pm$)-catechin was from Fluka (>98% purity). Hydrogen peroxide and ferrous sulfate were obtained from Fisher Scientific (Pittsburgh, PA) with purity greater than 99%. 1-methyl-2-phenylindole and malondialdehyde bis(dimethyl acetal) used for MDA analysis were from Sigma-Aldrich with 99% purity. All cell culture reagents and fetal bovine serum were from Invitrogen (Carlsbad, CA). Distilled deionized water or 100% ethanol were used for solution preparation. All other chemicals used in the study were of reagent grade with greater than 99% purity.

2.2. Cell culture, treatment and oxidative stress induction

Human colon adenocarcinoma-derived Caco-2 cells were purchased from ATCC. These cells were cultured as described previously in our publication [28]. Under the seeding condition of 4×10^4 cells/cm² in 6-well plates, Caco-2 cells reached confluence on d⁵ after seeding and were used for the experiment on d⁸. All catechins (EGCG, EGC and ($\pm$)-catechin), and VES were prepared as 10 mM sterile ethanol stock solutions and kept at -20° C as described before [28]. Aqueous sterile stock solution (10 mM) of L-ascorbic acid 2-phosphate was also stored in -20° C and was found to be stable. Ethanol solution of VEA (10 mM) was made freshly for each experiment. Ethanol treatment at 0.4% (the highest level of ethanol vehicle used in our study) for 2-24 hours did not lead to more MDA production (results not shown). Hydrogen peroxide and ferrous sulfate solutions were also prepared freshly in 37°C Hanks' balanced salt solution (HBSS) and mixed together by vigorous shaking. The mix was used immediately at a final reaction concentration of 30 μM each. We previously reported that at physiologically relevant concentrations, H_2O_2 and FeSO_4 ($\text{H}_2\text{O}_2/\text{Fe}^{2+}$) induced treatment time-dependent

increase in cellular MDA production [28]. As a result, 3 hour 30 μM $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ treatment was used to induce oxidative stress in this study.

All incubations were carried out inside the 37° C, 5% CO_2 cell culture incubator. In the co-incubation experiment, EGCG, $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ were included in the HBSS together during the 3-hour oxidative stress. EGCG was first diluted from the stock solution to the desirable concentrations in HBSS and immediately added to cell monolayers. Freshly prepared $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ solution was added to the above cell monolayers 30 minutes later at a final concentration of 30 μM each to initiate the oxidation reaction. The cells were incubated for an additional 3 hours in the presence of EGCG and $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ mixture before MDA assay. In the pre-incubation experiment, individual catechin, vitamin or their combination at the concentrations indicated was added to the normal growth medium for 2-24 hours. Media and phytochemicals were then removed and monolayers rinsed twice with HBSS. Cells were then incubated with antioxidant-free $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ solution (30 μM each) for another 3 hours for oxidative stress. Each treatment in an experiment was repeated three times with three different wells of cells. Control experiment with cells not treated with $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ was routinely carried out in each set to validate the oxidative stress. Concentrations of catechins, vitamin C and vitamin E used were all within the range observed after oral ingestion [29].

2.3. MDA measurement

The extent of lipid peroxidation was determined by measuring cellular content of MDA. We used a commercially available (#437634, Calbiochem) MDA-specific colorimetric assay kit following the instruction provided with minor modifications [28]. This assay does not use thiobarbituric acid (TBA) and measures predominantly free MDA. Briefly, cell monolayers were lysed in 20 mM Tris buffer (pH 7.4) containing 5 mM butylated hydroxytoluene (BHT) through repeated freeze-thawing and a brief sonication. The fresh cell lysate was then allowed to react with N-methyl-2-phenylindole for 30 minutes at 65° C in the presence of 1.8N HCl. MDA content in the cell lysate was determined based on OD_{586} and the MDA standard curve. MDA in each cell lysate sample was determined by duplicate measurements and the mean of the duplicate measurements represented an independent sample. Results from three independent samples were used to compute the mean and standard deviation of a treatment group. Standard curve of MDA was determined in every experiment. The level of MDA was further normalized by the protein content of each sample as determined by the Lowry assay.

2.4. Statistical analysis

All results shown in the figures are mean $\pm$ SD of three different wells of cells. To compare the malondialdehyde production between 2 treatments, Student's *t*-test was used. To examine the differences between the untreated control and samples that were treated with EGCG or vitamins for various durations, One-way ANOVA was used followed by post-hoc Bonferroni-Dunn tests. All other statistical analyses were performed with Univariate Analysis of Variance (UNIANOVA). Post-hoc custom hypothesis tests were performed comparing different treatments as indicated in the figures. Malondialdehyde productions by two treatment groups were considered significantly different from each other if $P < 0.05$.

3. Results

3.1. Lipid antioxidant activities of EGCG, vitamin E and vitamin C

The lipid antioxidant activity of EGCG is shown in Fig. 1. We first determined the ability of EGCG to reduce the level of basal malondialdehyde (MDA), an indicator of lipid oxidation [5]. Well-differentiated Caco-2 cells that have been incubated with 1 μM EGCG in the medium for 24 hours had a significantly lower basal level of cell-associated MDA (Fig. 1A). This observation is consistent to that in our previous publication [28] where including 1 μM EGCG

in the buffer was shown to significantly reduce the MDA production due to $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ exposure, indicating a protection of cells from oxidative stress.

The experiment in Fig. 1A cannot distinguish the contribution of cell-associated and medium EGCG. We thus used the following preincubation studies to focus on the cell-associated lipid antioxidant activity of EGCG. In Fig. 1B, Caco-2 cells were incubated with 0.1 or 1 μM EGCG for 0 to 24 hours. Oxidative stress was introduced after EGCG had been removed from the medium. The MDA level was then measured after the 3-hour oxidative stress. Compared to cells that had no EGCG exposure (preincubation time=0), a trend of time-dependent lower MDA production was observed for both concentrations of EGCG (Fig. 1B). For 0.1 μM EGCG, 24-hour preincubation was needed to significantly reduce the MDA level upon oxidative stress. For 1 μM EGCG, a significant reduction of MDA level was observed after as short as 2-hour preincubation.

Vitamin E and vitamin C are known antioxidants. We used the same preincubation method to determine their time- and concentration-dependent cell-associated antioxidant activities as a comparison to EGCG. We chose to use α -tocopherol acetate (VEA), α -tocopherol succinate (VES) and L-ascorbic acid 2-phosphate because these are the three commonly used forms of pro-vitamins in supplement. Including these pro-vitamins in the growth medium has been shown to lead to the cellular accumulation of their corresponding vitamin, α -tocopherol or L-ascorbate [30,31]. Using these pro-vitamins in the incubation also avoids the chemical reaction between vitamins and other medium components as well as EGCG. To test the biological effect of cell-associated vitamins, oxidative stress was initiated after vitamin-supplemented growth medium was removed.

Preincubation time- and concentration-dependent antioxidant activity of VES was observed. At 10 μM , 24-hour VES preincubation was needed for a significant antioxidant effect to be observed (Fig. 2A). A higher concentration of VES (40 μM) led to more effective reduction of MDA production upon oxidative stress. The effect can be observed at as short as 2-hour preincubation. VEA was equally effective in reducing the oxidative stress at 10 and 40 μM after 2-hour preincubation (Fig. 2B). Longer preincubation with VEA did not further reduce the MDA production. After 24-hour incubation of both 10 and 40 μM VEA, we actually observed a significantly higher MDA production upon oxidative stress compared to cells not treated with VEA (time=0). Although vitamin C is better known as a water-soluble antioxidant, its ability to protect LDL and HDL in the plasma has been shown [32,33]. Here, in our cultured cell experiment, the ability of vitamin C to protect lipids in cells was examined. Vitamin C preincubation led to a dose- dependent lower MDA production upon oxidative stress (Fig. 2C). At 40 μM , a significant reduction of MDA production could be observed after 2- and 12- hour preincubation but 12-hour preincubation was needed for 10 μM vitamin C to be effective (Fig. 2C). The antioxidant effect of vitamin C diminished after 24 hour preincubation. The production of MDA upon oxidative stress was not different between cells not treated with vitamin C (time=0), and cells treated with vitamin C for 24 hours.

3.2. Antioxidant activities of vitamin E and vitamin C in the presence of EGCG

We then examined the interaction between vitamins and EGCG in a series of preincubation experiment. In Fig. 3A and 3B, 40 μM and 10 μM VES, respectively, was preincubated with and without 0.1-1 μM EGCG. Consistent with Fig. 1B, EGCG by itself had concentration-dependent lipid antioxidant activity (comparison among the white bars). Consistent with Fig. 2A, VES by itself had antioxidant activities (comparison of the left first set of white and grey bars in the absence of EGCG). Although EGCG in the incubation was at a much lower concentration compared to VES (0.1-1 μM vs. 10-40 μM), under all concentration conditions, EGCG further significantly decreased MDA production in cells incubated with VES

(comparison among gray bars, increasing concentrations of EGCG from left to right at a fixed VES concentration).

In Fig. 3C, the antioxidant activities of EGCG and VEA were studied. Consistent with Fig. 1B, EGCG by itself (comparison among the white bars) showed concentration-dependent antioxidant activities. EGCG at 1 μM also decreased the MDA production of cells treated with 10 μM VEA (Fig. 3C, comparison between the gray bar of no EGCG and the gray bar at 1 μM EGCG). The total lipid antioxidant activity of EGCG with 10 μM VEA, though, was less compared to EGCG alone (comparison between the white and grey bar in each pair).

In Fig. 4, the lipid antioxidant activities of EGCG and vitamin C were studied. Consistent with Fig. 2C, vitamin C by itself had antioxidant activities (comparison of the left first set of white and grey bars in the absence of EGCG). EGCG at 1 μM enhanced the antioxidant activity of 40 μM vitamin C significantly by further reducing the MDA production (Fig. 4A, comparison among the gray bars, increasing concentrations of EGCG from left to right at a fixed 40 μM vitamin C). This is also observed at 10 μM EGCG (Fig. 4B, comparison between the gray bars).

3.3. Antioxidant activities of vitamin E and vitamin C in the presence of other catechins

While EGCG is mainly found in the tea especially green tea, other members of the catechin family were also found in fruits and vegetables [34]. We then compared the interaction of vitamin C and VES with two other forms of catechins, (-)-epigallocatechin (EGC) and ($\pm$)-catechin (Cat), using the cell preincubation experimental model. EGCG, EGC and Cat are absorbed by Caco-2 cells with similar efficiency [26]. Consistent with the observation in Fig. 3 and 4, additional EGCG increased the lipid antioxidant activity of VES and vitamin C (Fig. 5A and 5B, comparison between the gray bars of vitamin-treated cells in the absence and presence of EGCG). EGC did not affect the antioxidant activity of VES or vitamin C (Fig. 5A, 5B, comparison between the gray bar of vitamin-treated cells in the absence and presence of EGC). Although ($\pm$)-catechin by itself does not have significant antioxidant activity, it decreased the MDA production of cells treated simultaneously with VES or vitamin C compared to the vitamin alone (Fig. 5A, 5B, comparison between the gray bar of vitamin-treated cells in the absence and presence of catechin).

4. Discussion

Antioxidant effect of EGCG have been demonstrated in vivo after direct injection [10-13]. We demonstrated here and in our previous publication that at physiological achievable concentrations after oral ingestion, EGCG protected cells from the basal oxidative stress (Fig. 1A) as well as $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ -induced oxidative stress (Fig. 1B,3-5) [28]. This is consistent with the known broad-spectrum antioxidant activity of EGCG. EGCG was found to scavenge superoxide anion as well as hydroxyl and peroxy radicals in vitro [35-37]. Cell preincubated with EGCG at 0.3 μM were shown to have less tert-butyl hydroperoxide-induced chromosomal damage [38]. Using the cell viability assay, EGCG at 1 μM was shown to prevent glutamate-induced oxidative stress in hippocampal neuron cells [39]. Our cell observation is also consistent with the in vitro spin-trapping study. Chemical interaction between EGCG and Cu^{+2} [40], which could happen in the cell culture medium in our basal oxidative stress experiment (Fig. 1A), do not induce free radical damage [41].

EGCG is highly hydrophilic and was proposed to act as antioxidant in the aqueous compartment [8]. In vitro antioxidant activity of EGCG was reported in the lipid compartments of plasma [8,9]. Cellular lipid antioxidant activity of EGCG under physiologically achievable concentrations (0.1-1 μM) as we reported here, in our previous publication [28] further underline the interdependency between the scavenging of radicals in the hydrophilic and hydrophobic environments. This interdependency in radical scavenging is also evident based

on the cellular lipid antioxidant activity that we observed for vitamin C, a known water-soluble antioxidant (Fig. 2C,4 and 5B). Although vitamin C was shown to protect LDL and HDL [42], this study further demonstrated its ability to protect the lipid compartment of cells. The effect of vitamin C could also be due to its ability to recycle endogenous vitamin E [43,44]. Compared to vitamins, EGCG is a more potent antioxidant in our cell model. VES, VEA and vitamin C did not show lipid antioxidant activities at 0.1-1 μM range (results not shown). Another polyphenol, quercetin, was concluded to be a more potent antioxidant than vitamin C and E in delaying LDL oxidation in the cell-free experiment [42].

The consistently observed cell-associated antioxidant activity of EGCG was incubation concentration- and time-dependent (Fig. 1B). This is expected based on the known limited bioavailability of EGCG [14,24-26]. At the concentrations we used, 0.1-1 μM , EGCG was not known to affect gene expression at 24-hour incubation [18-20] so the antioxidant activity we observed was likely a direct chemical chelation. Pro-oxidant activity of EGCG was mentioned in previous publications at higher EGCG concentrations [15,16], and may represent a general biphasic response of many antioxidants. The steady cell-associated antioxidant activity of EGCG is in contrast to the biphasic activity we observed after 10-40 μM VEA and vitamin C incubation (Fig. 2B, 2C). Short preincubation of cells with lipid-soluble VEA can prevent $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ -induced lipid peroxidation (less MDA production) but 24 hours of preincubation of VEA enhanced $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ -induced lipid peroxidation (Fig. 2B). Prolonged preincubation with vitamin C also led to the loss of its ability to prevent $\text{H}_2\text{O}_2/\text{Fe}^{2+}$ -induced lipid peroxidation (Fig. 2C). There are several possible explanations for the biphasic response but cellular ascorbate concentrations were similar after 12- and 24-hour incubation with vitamin C phosphate [45] (results not shown). Pro-oxidant effect of high-dose or prolonged in vivo vitamin supplement has been reported [46,47]. Our observation here can serve as a model for further examination of the potentially significant cellular event.

When EGCG was included in the vitamin C preincubation, significantly less MDA production was observed compared to vitamin C treatment alone indicating a higher antioxidant activity (Fig. 4, 5B comparison among gray bars of each panel, left to right with increasing EGCG concentrations in the presence a fixed concentration of vitamin C). Similar to vitamin C experiment, when EGCG was included in cell preincubation medium with vitamin E, significantly less MDA production was observed compared to cells that were treated with vitamin E alone, indicating a higher antioxidant activity (Fig. 3, 5A comparison among gray bars in each panel, left to right with increasing EGCG concentrations in the presence a fixed concentration of vitamin E). Although ($\pm$)-catechin does not show lipid antioxidant activity itself, it further decreased the MDA production in cells co-incubated with vitamin C and vitamin E (Fig. 5A and 5B, comparison between the gray bars of vitamin-treated cells in the absence and presence of catechin). Our cell observation is consistent with the prediction and observations made in the cell-free condition. Catechins and phenolic acid were shown to regenerate vitamin E effectively [48-52]. Polyphenols were found to further delay Cu^{+2} -mediated LDL oxidation when added with vitamin C and vitamin E [42]. Feeding catechins has been shown to increase plasma and liver α -tocopherol concentration in rats [52].

In conclusion, our cell studies further support the nutritional value of dietary catechins. In our cell model, EGCG appears to be a more potent lipid antioxidant than vitamin C and E. EGCG also does not have prooxidant activity in physiological achievable concentrations. Although their physiological presence was at a much lower concentration than vitamin C and vitamin E, catechins do contribute additionally to the total lipid antioxidant activity based on our cell studies. Further studies are needed to determine whether our cell culture observation based on non-selective hydroxyl radical attack of lipid can be extrapolated to other types of oxidative stress-induced damages or under in vivo condition.

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References

1. Minotti G, Aust SD. The requirement for iron (III) in the initiation of lipid peroxidation by iron (II) and hydrogen peroxide. *J Biol Chem* 1987;262:1098–104. [PubMed: 3027077]
2. Slatter DA, Avery NC, Bailey AJ. Identification of a new cross-link and unique histidine adduct from bovine serum albumin incubated with malondialdehyde. *J Biol Chem* 2004;279:61–9. [PubMed: 14570889]
3. Niedernhofer LJ, Daniels JS, Rouzer CA, Greene RE, Marnett LJ. Malondialdehyde, a product of lipid peroxidation, is mutagenic in human cells. *J Biol Chem* 2003;278:31426–33. [PubMed: 12775726]
4. Marnett L. Chemistry and biology of DNA damage by malondialdehyde. *IARC Sci Publ* 1999;150:17–27. [PubMed: 10626205]
5. Ji C, Rouzer CA, Marnett LJ, Pietenpol JA. Induction of cell cycle arrest by the endogenous product of lipid peroxidation, malondialdehyde. *Carcinogenesis* 1998;19:1275–1283. [PubMed: 9683189]
6. Schriener SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 2005;308:1909–1911. [PubMed: 15879174]
7. Ignatov S, Shishniashvili D, Ge B, Scheller FW, Lisdat F. Amperometric biosensor based on a functionalized gold electrode for the detection of antioxidants. *Biosens Bioelectron* 2002;17:191–9. [PubMed: 11839472]
8. Aldini G, Yeum KJ, Carini M, Krinsky NI, Russell R. (-)-Epigallocatechin-3-gallate prevents oxidative damage in both the aqueous and lipid compartments of human plasma. *Biochem Biophys Res Commun* 2003;302:409–14. [PubMed: 12604363]
9. Lotito SB, Fraga CG. Catechins delay lipid oxidation and alpha-tocopherol and beta-carotene depletion following ascorbate depletion in human plasma. *Proc Soc Exp Biol Med* 2000;225:32–8. [PubMed: 10998196]
10. Lee S, Suh S, Kim S. Protective effects of the green tea polyphenol (-)-epigallocatechin gallate against hippocampal neuronal damage after transient global ischemia in gerbils. *Neurosci Lett* 2000;287:191–4. [PubMed: 10863027]
11. Nagai K, Jiang M, Hada J, Nagata T, Yajima Y, Yamamoto S, Nishizaki T. (-)-Epigallocatechin gallate protects against NO stress-induced neuronal damage after ischemia by acting as an antioxidant. *Brain Res* 2002;956:319–22. [PubMed: 12445701]
12. Giakoustidis A, Giakoustidis D, Iliadis S, Papageorgiou G, Koliakou K, Kontos N, Taitzoglou I, Botsoglou E, Papanikolaou V, Atmatzidis K, Takoudas D, Antoniadis A. Attenuation of intestinal ischemia/reperfusion induced liver and lung injury by intraperitoneal administration of (-)-epigallocatechin-3-gallate. *Free Radic Res* 2006;40:103–10. [PubMed: 16298765]
13. Zhang B, Osborne N. Oxidative-induced retinal degeneration is attenuated by epigallocatechin gallate. *Brain Res* 2006;1124:176–87. [PubMed: 17084820]
14. Lambert JD, Lee MJ, Diamond L, Ju J, J H, Bose M, Newmark HL, Yang CS. Dose-dependent levels of epigallocatechin-3-gallate in human colon cancer cells and mouse plasma and tissues. *Drug Metab Dispos* 2006;34:8–11. [PubMed: 16204466]
15. Elbling L, Weiss RM, Teufelhofer O, Uhl M, Knasmueller S, Schulte-Hermann R, Berger W, Micksche M. Green tea extract and (-)-epigallocatechin-3-gallate, the major tea catechin, exert oxidant but lack antioxidant activities. *FASEB J* 2005;19:807–9. [PubMed: 15738004]
16. Nakagawa H, Hasumi K, Woo JT, Nagai K, Wachi M. Generation of hydrogen peroxide primarily contributes to the induction of Fe(II)-dependent apoptosis in Jurkat cells by (-)-epigallocatechin gallate. *Carcinogenesis* 2004;25:1567–74. [PubMed: 15090467]
17. Choi Y, Jung C, Lee S, Bae J, Baek W, Suh M, Park J, Park C, Suh S. The green tea polyphenol (-)-epigallocatechin gallate attenuates beta-amyloid-induced neurotoxicity in cultured hippocampal neurons. *Life Sci* 2001;70:603–14. [PubMed: 11811904]

18. Shimizu M, Deguchi A, Hara Y, Moriwaki H, Weinstein I. EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells. *Biochem Biophys Res Commun* 2005;334:947–53. [PubMed: 16053920]
19. Shimizu M, Deguchi A, Joe A, Mckoy J, Moriwaki H, Weinstein I. EGCG inhibits activation of HER3 and expression of cyclooxygenase-2 in human colon cancer cells. *J Exp Ther Oncol* 2005;5:69–78.
20. Khan N, Afaq F, Saleem M, Ahmad N, Mukhtar H. Targeting multiple signaling pathways by green tea polyphenol (-)-epigallocatechin-3-gallate. *Cancer Res* 2006;66:2500–5. [PubMed: 16510563]
21. Monteiro R, Calhau C, Martel F, Faria A, Mateus N, Azevedo I. Modulation of MPP⁺ uptake by tea and some of its components in Caco-2 cells. *Naunyn Schmiedebergs Arch Pharmacol* 2005;372:147–52. [PubMed: 16193318]
22. Jung J, Han C, Jeong Y, Kim H, Lim H, Lee K, Park H, Oh W, Kim S, WJ K. Epigallocatechin gallate inhibits nitric oxide-induced apoptosis in rat PC12 cells. *Neurosci Lett* 2007;411:222–7. [PubMed: 17116366]
23. Chu KO, Wang CC, Chu CY, Chan KP, Rogers MS, Choy KW, Pang CP. Pharmacokinetic studies of green tea catechins in maternal plasma and fetuses in rats. *J Pharm Sci* 2006;95:1372–81. [PubMed: 16625654]
24. Vaidyanathan JB, Walle T. Cellular uptake and efflux of the tea flavonoid(-)-epicatechin-3-gallate in the human intestinal cell line Caco-2. *J Pharmacol Exp Ther* 2003;307:745–752. [PubMed: 12970388]
25. Zhang L, Zheng Y, Chow MS, Zuo Z. Investigation of intestinal absorption and disposition of green tea catechins by Caco-2 monolayer model. *Int J Pharm* 2004;287:1–12. [PubMed: 15541906]
26. Chan K, Zhang L, Zuo Z. Intestinal efflux transport kinetics of green tea catechins in Caco-2 monolayer model. *J Pharm Pharmacol* 2007;59:395–400. [PubMed: 17331343]
27. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *New England J Med* 2003;348:2599–2608. [PubMed: 12826634]
28. Peng I-W, Kuo S-M. Flavonoid structure affects the inhibition of lipid peroxidation in Caco-2 intestinal cells at physiological concentrations. *J Nutr* 2003;133:2184–2187. [PubMed: 12840176]
29. Stahl W, van den Berg H, Arthur J, Bast A, Dainty J, Faulks RM, Gartner C, Haenen G, Hollman P, Holst B, Kelly FJ, MC P, Rice-Evans C, Southon S, van Vliet T, Vina-Ribes J, Williamson G, Astley SB. Bioavailability and metabolism. *Mol Aspects Med* 2002;23:39–100. [PubMed: 12079770]
30. Fariss MW, Nicholls-Grzemeski FA, Tirmenstein MA, Zhang JG. Enhanced antioxidant and cytoprotective abilities of vitamin E succinate is associated with a rapid uptake advantage in rat hepatocytes and mitochondria. *Free Radic Biol Med* 2001;31:530–41. [PubMed: 11498286]
31. Chepda T, Cadau M, Girin P, Frey J, Chamson A. Monitoring of ascorbate at a constant rate in cell culture: effect on cell growth. *Vitro Cell Dev Biol Anim* 2001;37:26–30.
32. Hillstrom RJ, Yacapin-Ammons AK, Lynch SM. Vitamin C inhibits lipid oxidation in human HDL. *J Nutr* 2003;133:3047–51. [PubMed: 14519782]
33. Pfanzagl B. Ascorbate is particularly effective against LDL oxidation in the presence of iron(III) and homocysteine/cystine at acidic pH. *Biochim Biophys Acta* 2005;1736:237–43. [PubMed: 16169276]
34. USDA. USDA database for the flavonoid content of selected foods. US Department of Agriculture; 2003.
35. Li C, Xie B. Evaluation of the antioxidant and pro-oxidant effects of tea catechin oxypolymers. *J Agric Food Chem* 2000;48:6362–6. [PubMed: 11141289]
36. Salah N, Miller N, Paganga G, Tijburg L, Bolwell G, Rice-Evans C. Polyphenolic flavanols as scavengers of aqueous phase radicals and as chain-breaking antioxidants. *Arch Biochem Biophys* 1995;322:339–46. [PubMed: 7574706]
37. Jovanovic SV, Simic MG. Antioxidants in nutrition. *Ann N Y Acad Sci* 2000;899:326–34. [PubMed: 10863550]
38. Sugisawa A, Umegaki K. Physiological concentrations of (-)-epigallocatechin-3-o-gallate (EGCg) prevent chromosomal damage induced by reactive oxygen species in WIL2-NS cells. *J Nutr* 2002;132:1836–1839. [PubMed: 12097656]
39. Fu Y, Koo M. EGCG protects HT-22 cells against glutamate-induced oxidative stress. *Neurotox Res* 2006;10:23–30. [PubMed: 17000467]

40. Kuo S-M, Leavitt P, Lin C-P. Dietary flavonoids interact with trace metals and affect metallothionein level in human intestinal cells. *Biol Trace Element Res* 1998;62:135–153.
41. Yoshioka H, Senba Y, Saito K, Kimura T, Hayakawa F. Spin-trapping study on the hydroxyl radical formed from a tea catechin-Cu(II) system. *Biosci Biotechnol Biochem* 2001;65:1697–706. [PubMed: 11577706]
42. Yeomans VC, Linseisen J, Wolfram G. Interactive effects of polyphenols, tocopherol and ascorbic acid on the Cu+2-mediated oxidative modification of human low density lipoproteins. *Eur J Nutr* 2005;44:422–428. [PubMed: 15827683]
43. Packer J, Slater T, Willson R. Direct observation of a free radical interaction between vitamin E and vitamin C. *Nature* 1979;278:737–8. [PubMed: 431730]
44. Niki E. Interaction of ascorbate and alpha-tocopherol. *Ann N Y Acad Sci* 1987;498:186–99. [PubMed: 3304060]
45. Sugimoto M, Okugawa Y, Miwa N. Preventive effects of phosphorylated ascorbate on ultraviolet-B induced apoptotic cell death and DNA strand cleavage through enrichment of intracellular vitamin C in skin epidermal keratinocytes. *Free Radic Res* 2006;40:213–21. [PubMed: 16390831]
46. Brown KM, Morrice PC, Duthie GG. Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and nonsmokers: dose response to vitamin E supplementation. *Am J Clin Nutr* 1997;65:496–502. [PubMed: 9022535]
47. Versari D, Daghini E, Rodriguez-Porcel M, Sattler K, Galili O, Pilarczyk K, Napoli C, Lerman L, Lerman A. Chronic antioxidant supplementation impairs coronary endothelial function and myocardial perfusion in normal pigs. *Hypertension* 2006;47:475–81. [PubMed: 16446399]
48. Laranjinha J, Vieira O, Madeira V, Almeida L. Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: consumption vs regeneration. *Arch Biochem Biophys* 1995;323:373–81. [PubMed: 7487101]
49. Laranjinha J, Cadenas E. Redox cycles of caffeic acid, alpha-tocopherol, and ascorbate: implications for protection of low-density lipoproteins against oxidation. *IUBMB Life* 1999;48:57–65. [PubMed: 10791916]
50. Laranjinha J. Redox cycles of caffeic acid with alpha-tocopherol and ascorbate. *Methods Enzymol* 2001;335:282–95. [PubMed: 11400377]
51. Mukai K, Mitani S, Ohara K, Nagaoka S. Structure-activity relationship of the tocopherol-regeneration reaction by catechins. *Free Radic Biol Med* 2005;38:1243–56. [PubMed: 15808422]
52. Frank J, Budek A, Lundh T, Parker R, Swanson J, Lourenco C, Gago B, Laranjinha J, Vessby B, Kamal-Eldin A. Dietary flavonoids with a catechol structure increase alpha-tocopherol in rats and protect the vitamin from oxidation in vitro. *J Lipid Res* 2006;47:2718–25. [PubMed: 16951402]

FIGURE 1.

The antioxidant activity of (-)-epigallocatechin gallate (EGCG). (A) The basal production of malondialdehyde was compared between cells that were exposed to culture medium containing 1 μ M EGCG for 24 hours and those that were cultured in the normal growth medium (-EGCG). (B) EGCG at 0.1 or 1 μ M was included in the culture medium for the duration indicated. After medium EGCG has been removed, the production of malondialdehyde upon oxidative stress was measured. One-way ANOVA was performed for each concentrations of EGCG and was followed by the post-hoc Bonferroni-Dunn test comparing cells treated with EGCG for various durations to cells not exposed to EGCG (control). All results shown in the figures are mean $\pm$ SD. * different from no EGCG control, $P < 0.05$.

FIGURE 2.

The antioxidant activity of (A) α -tocopherol succinate (VES), (B) α -tocopherol acetate (VEA), and (C) vitamin C (VitC). Cells were preincubated with individual vitamin at 10 or 40 μ M for the duration indicated and then vitamins removed. The cellular content of malondialdehyde after oxidative stress was measured. One-way ANOVA was performed for each concentration of vitamin and was followed by the post-hoc Bonferroni-Dunn test comparing cells treated with the vitamin for various durations to cells not exposed to the vitamin (control). All results shown in the figures are mean $\pm$ SD. * different from control, $P < 0.05$.

FIGURE 3.

The antioxidant activity of EGCG in combination with (A) 40 μ M α -tocopherol succinate (VES), (B) 10 μ M VES, and (C) 10 μ M α -tocopherol acetate (VEA). Cells were preincubated with 0.1 or 1 μ M EGCG for 24 hours in the absence or presence of the indicated vitamin. Medium EGCG and vitamins were removed prior to the oxidative stress. The cellular content of malondialdehyde after oxidative stress was measured. Univariate Analysis of Variance was performed for results in each panel and then followed by post-hoc custom hypothesis tests as indicated by the brackets above the bars. All results shown in the figures are mean $\pm$ SD. * different from each other, $P < 0.05$.

FIGURE 4.

The antioxidant activity of EGCG in combination with vitamin C. Cells were preincubated with various concentrations of EGCG for 12 hours in the absence or presence of 40 μ M of L-ascorbic acid 2-phosphate. Medium EGCG and vitamin C were removed prior to the oxidative stress. The cellular content of malondialdehyde after oxidative stress was measured. Univariate Analysis of Variance was performed for results in each panel and then followed by post-hoc custom hypothesis tests as indicated by the brackets above the bars. All results shown in the figures are mean $\pm$ SD. * different from each other, $P < 0.05$.

FIGURE 5.

The antioxidant activity of various catechins in combination with (A) 10 μ M α -tocopherol succinate (VES), and (B) 40 μ M L-ascorbic acid 2-phosphate (Vit C). Cells were preincubated with 1 μ M individual catechin, EGCG, (-)-epigallocatechin (EGC), or ($\pm$)-catechin (Cat) for (A) 24 or (B) 12 hours in the absence or presence of the indicated vitamin. Medium catechins and vitamins were removed prior to the oxidative stress. The cellular content of malondialdehyde after oxidative stress was measured. Univariate Analysis of Variance was performed for results in each panel and then followed by post-hoc custom hypothesis tests as indicated by the brackets above the bars. All results shown in the figures are mean $\pm$ SD. * different from each other, $P < 0.05$.