Proceedings of the Third International Scientific Symposium on Tea and Human Health: Role of Flavonoids in the Diet

Antioxidant Activity of Tea Polyphenols In Vivo: Evidence from Animal Studies[1]

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ABSTRACT Tea is particularly rich in polyphenols, including catechins, theaflavins and thearubigins, which are thought to contribute to the health benefits of tea. Tea polyphenols act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. They may also function indirectly as antioxidants through *1*) inhibition of the redox-sensitive transcription factors, nuclear factor-κB and activator protein-1; *2*) inhibition of "pro-oxidant" enzymes, such as inducible nitric oxide synthase, lipoxygenases, cyclooxygenases and xanthine oxidase; and *3*) induction of phase II and antioxidant enzymes, such as glutathione S-transferases and superoxide dismutases. The fact that catechins are rapidly and extensively metabolized emphasizes the importance of demonstrating their antioxidant activity in vivo. Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of tea and tea polyphenols to the physiological effects of tea administration in different models of oxidative stress. Most promising are the consistent findings in animal models of skin, lung, colon, liver and pancreatic cancer that tea and tea polyphenol administration inhibit carcinogen-induced increases in the oxidized DNA base, 8-hydroxy-2′-deoxyguanosine. In animal models of atherosclerosis, green and black tea administration has resulted in modest improvements in the resistance of lipoproteins to ex vivo oxidation, although limited data suggest that green tea or green tea catechins inhibit atherogenesis. To determine whether tea polyphenols act as effective antioxidants in vivo, future studies in animals and humans should employ sensitive and specific biomarkers of oxidative damage to lipids, proteins and DNA. J. Nutr. 133: 3275S–3284S, 2003.

KEY WORDS: • *tea* • *polyphenol* • *antioxidant* • *biomarker* • *oxidative damage*

The potential for the consumption of tea or tea polyphenols to prevent or ameliorate chronic disease is currently the subject of considerable scientific investigation (1). Although a number of mechanisms have been proposed for the beneficial effects of tea in different models of chronic disease, the radical scavenging and antioxidant properties of tea polyphenols are frequently cited as important contributors (2). Much of the evidence supporting an antioxidant function for tea polyphenols is derived from assays of their antioxidant activity in vitro. However, evidence that tea polyphenols are acting directly or indirectly as antioxidants in vivo is more limited. Animal studies offer a unique opportunity to assess the contribution of the antioxidant properties of tea polyphenols to the physiological effects of tea administration in different models of oxidative stress. The purpose of this article is to review the experimental evidence from animal studies thus far that tea polyphenols function as effective antioxidants in vivo.

Tea polyphenol content

Fresh tea leaves are rich in flavanol monomers known as catechins. The principal catechins found in tea are (-)-epicatechin (EC)[3] (3), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG) and (-)-epigallocatechin gallate (EGCG). EGCG is the most abundant catechin in tea (3). Tea leaves also contain polyphenol oxidase enzymes in separate layers of the leaf. When tea leaves are rolled or broken during industry manufacture, catechins come in contact with polyphenol ox-

[1] Presented as part of "The Third International Scientific Symposium on Tea and Human Health: Role of Flavonoids in the Diet," given at the United States Department of Agriculture, September 23, 2002. This conference was sponsored by the American Cancer Society, American College of Nutrition, American Health Foundation, American Society for Nutritional Sciences, Food and Agriculture Organization, and the Linus Pauling Institute at Oregon State University and was supported by a grant from the Tea Council of the U.S.A. Guest editor for this symposium was Jeffrey Blumberg, PhD, Jean Mayer USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA 02111.

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[3] Abbreviations used: 4-HNE, 4-hydroxynonenal; 4-POBN, α-(-4-pyridyl-1-oxide)-*N-tert*-butylnitrone; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; AP-1, activator protein-1; ApoE, apolipoprotein E; ARE, antioxidant response element; COX, cyclooxygenase; $E^{\circ\prime}$, standard one electron reduction potential; EC, (-)-epicatechin; ECG, (-)-epicatechin gallate; EGC, (-)-epigallocatechin; EGCG, (-)-epigallocatechin gallate; FRAP, ferric reducing antioxidant potential; GSH, glutathione; GST, glutathione-S-transferase; GPX, glutathione peroxidase; iNOS, inducible nitric oxide synthase; LOOH, lipid hydroperoxide; MDA, malondialdehyde; NF-κB, nuclear factor-κB; NO, nitric oxide; $O_2^{\cdot-}$, superoxide; ODS, Osteogenic Disorder Shinogi; $ONOO^-$, peroxynitrite; ORAC, oxygen radical absorbance capacity; SOD, superoxide dismutase; TBARS, thiobarbituric acid reacting substances; TEAC, trolox-equivalent antioxidant capacity.

idase, resulting in their oxidation and the formation of flavanol dimers and polymers known as theaflavins and thearubigins, respectively (4). Tea leaves destined to become black tea are rolled and allowed to ferment (oxidize), resulting in relatively high concentrations of theaflavins and thearubigins and relatively low concentrations of catechins. Green tea is withered and then steamed to inactivate polyphenol oxidase. Consequently, green tea contains relatively high concentrations of catechins and low concentrations of theaflavins and thearubigins. Tea also contains small amounts of flavonols (kaempferol, quercetin and myricitin) in the form of glycosides. The flavonol content is less affected by processing, and flavonols are present in comparable amounts in green and black teas (5).

Potential mechanisms for the antioxidant effects of tea

Radical and oxidant scavenging. Numerous studies have demonstrated that tea catechins and polyphenols are effective scavengers of physiologically relevant reactive oxygen and nitrogen species in vitro, including superoxide [$O_2^{\cdot-}$ (6,7)], peroxyl radicals, singlet oxygen (8), peroxynitrite [$ONOO^-$ (9,10)], and hypochlorous acid (11). Several structures appear to be important for these antioxidant activities of tea polyphenols (**Fig. 1**), including an *ortho*-3′4′-dihydroxyl (catechol) group or 3′4′5′-trihydroxyl (gallate) group in the B ring, a gallate group esterified at the 3 position of the C ring, and hydroxyl groups at the 5 and 7 positions of the A ring (12).

The ability of a compound to act as a free radical scavenger is partly related to its standard one-electron reduction potential ($E°'$), a measure of the reactivity of an antioxidant as hydrogen or electron donor under standardized conditions. A lower $E°'$ indicates that less energy is required for hydrogen or electron donation and is one factor in determining antioxidant activity. Tea catechins and theaflavins have $E°'$ values comparable to that of α-tocopherol (vitamin E), but higher than ascorbate (vitamin C) (**Table 1**), which is a superior hydrogen donor (antioxidant) to tea polyphenols (13,14).

Under nonstandard conditions, such as those encountered in vivo, the actual concentrations of the reactants (oxidants and antioxidants) are also important. The Nernst equation can be used to correct $\Delta E°'$ of a redox reaction for the actual concentrations encountered in vivo (15):

$$\Delta E = \Delta E°' - 60\text{mV} \log_{10} \frac{[\text{products}]}{[\text{reactants}]}$$

Even with very high intakes of tea or tea extracts, plasma and intracellular concentrations of tea catechins and polyphenols in humans are likely to be 100 to 1000 times lower than those of other physiological antioxidants, such as ascorbate, urate and glutathione (**Table 2**). Thus, the relative importance of tea catechins and polyphenols as radical and oxidant scavengers in vivo may be minor, based on their reduction potentials and concentrations achieved in plasma and tissues.

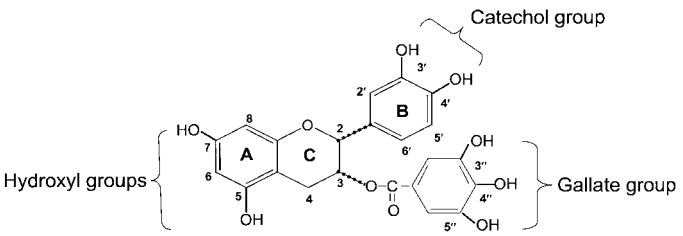

FIGURE 1 Functional groups important to the antioxidant activity of catechin monomers, dimers (theaflavins) and polymers (thearubigins): example, epicatechin gallate.

TABLE 1

Standard one-electron reduction potentials for tea catechins, tea polyphenols and other physiological antioxidants (13,14)

Antioxidant	Reduction potential[1]
	mV
Ascorbate	280
α-Tocopherol	480
Uric acid	590
Glutathione (Cysteine)	920
(−)-Epigallocatechin gallate	430
(−)-Epigallocatechin	430
(−)-Epicatechin	570
(−)-Epicatechin gallate	550
Theaflavin	510
Theaflavin digallate	540

[1] Standard reduction potential at pH 7.0, 20°C.

Metal chelation. The ability of tea polyphenols to chelate metal ions, such as iron and copper, may contribute to their antioxidant activity by preventing redox-active transition metals from catalyzing free radical formation (16). These metal-chelating properties likely explain the ability of tea polyphenols to inhibit copper-mediated LDL oxidation and other transition metal-catalyzed oxidations in vitro (17). However, it is not clear whether metal chelation is a physiologically relevant antioxidant activity, because most transition metal ions are bound to proteins in vivo where they cannot participate in metal-catalyzed free radical formation.

Inhibition of redox-sensitive transcription factors. Green and black tea, as well as individual catechins and tea polyphenols, can inhibit the activation of the redox-sensitive transcription factors, nuclear factor-κB (NF-κB) and activator protein-1 (AP-1), in cultured cells. Although other antioxidants also can inhibit these redox-sensitive transcription factors, recent research indicates that tea catechins and polyphenols are acting as kinase inhibitors in complex signaling pathways. Interestingly, the kinase inhibiting activities of tea polyphenols may not be directly related to their ability to function as hydrogen donators or antioxidants (18).

Inhibition of "pro-oxidant" enzymes. Stimulation of inflammatory cells such as macrophages by bacterial endotoxins or inflammatory cytokines results in increased expression of inducible nitric oxide synthase (iNOS) and subsequent production of large amounts of nitric oxide ($NO^{\cdot}$). Nitric oxide

TABLE 2

Plasma and intracellular concentrations of selected water-soluble antioxidants in humans, unless noted otherwise (13,15)

Antioxidant	Plasma concentrations	Intracellular concentrations
Ascorbate	30–110 μmol/L	1–5 mmol/L
Uric acid	120–420 μmol/L	<200 μmol/L
Glutathione	<2 μmol/L	3–7 mmol/L
(−)-Epigallocatechin gallate	<2 μmol/L	<1 μmol/L (rat)

reacts very rapidly with O_2^- to form $ONOO^-$ and other NO-derived oxidants capable of damaging DNA and proteins (19). Green tea and black tea (10,20), as well as individual catechins (21,22) and theaflavins (23), can inhibit lipopolysaccharide-induced iNOS gene expression and iNOS activity in cultured macrophages. Green tea catechins and black tea theaflavins appear to downregulate iNOS by inhibiting NF-κB activation (22,23).

Through their peroxidase activity, lipoxygenases and cyclooxygenases are capable of co-oxidizing molecules other than their regular substrates, with the potential for increasing oxidative damage in some tissues (24). Green and black tea polyphenols were found to inhibit cyclooxygenase (COX)-2 and 5-, 12-, and 15-lipoxygenase activities in human colon mucosa cells and human colon cancer cells (25). Feeding green tea polyphenols to mice inhibited ultraviolet light-induced increases in epidermal COX activity (26), whereas topical application of green tea (27) and black tea polyphenols (28) inhibited phorbol ester-induced increases in epidermal COX and lipoxygenase activities. Precancerous lesions of colon mucosa (aberrant crypts) and COX-2 activity were lower in azoxymethane-treated rats given 2% green tea extract in their drinking water compared with controls (29).

Tea polyphenols may also inhibit the formation of reactive oxygen species by inhibiting the enzyme, xanthine oxidase. Xanthine oxidase catalyzes the oxidation of both hypoxanthine and xanthine to uric acid, while reducing O_2 to O_2^- and H_2O_2. Green tea catechins can inhibit the activity of xanthine oxidase in vitro, with EGCG exerting the most potent effect (30). In cultured human leukemia cells, EGCG from green tea and theaflavin gallates from black tea also inhibited xanthine oxidase activity (31).

Induction of phase II enzymes. Phase II detoxification enzymes promote the excretion of potentially toxic or carcinogenic chemicals. Most phase II enzymes contain *cis*-acting regulatory elements called antioxidant response elements (ARE). Glutathione S-transferases (GST) are a family of phase II enzymes that catalyze the conjugation of glutathione to electrophiles, thereby reducing their ability to react with and damage nucleic acids and proteins (24). Green tea polyphenol extract (32) as well as individual green tea catechins (33) have been found to increase ARE-mediated reporter gene activity in transfected HepG2 cells. Feeding rats green tea leaves significantly increased liver GST activity (34), and providing mice with green tea polyphenols in their drinking water also significantly increased GST activity in the liver and small intestine (35).

Limitations of in vitro research on antioxidant activity of tea polyphenols. The bioavailability of tea catechins appears to be relatively low. When healthy volunteers were given a single serving of 4.5 g of green tea solids dissolved in 500 mL of water, peak plasma concentrations of individual catechins (conjugated and unconjugated) were <2 μmol/L (36). Average peak plasma catechin concentrations (conjugated and unconjugated) in healthy volunteers given a single dose of 1.5 mmol of pure EGC, ECG or EGCG were 5.0, 3.1 and 1.3 μmol/L, respectively (37). These values represent peak plasma levels after high doses of green tea or pure catechins. Average plasma catechin concentrations are likely to be considerably lower. Because theaflavins and thearubigins are difficult to detect in blood or urine, there is little information regarding the biotransformation or bioavailability of black tea polyphenols in humans or animals.

Upon ingestion, tea catechins are rapidly and extensively metabolized in the intestines, liver and kidneys. The major biotransformation reactions of tea catechins are glucuronidation, sulfation and methylation (18). Following tea ingestion 4′-*O*-methyl-EGC and its glucuronidated and sulfated metabolites were found in human plasma at concentrations 4–6 times higher than unconjugated EGC (38). Tea catechins are also metabolized by intestinal microflora. Bacterial ring fusion metabolites of EGC and EC have been detected in human urine and plasma in amounts several times higher than their precursors (39). Studies in cultured cells indicate that catechin metabolites have different antioxidant and biological activities than their precursors (40).

A great deal of research has evaluated the antioxidant and biological activities of green and black tea as well as their individual catechins and polyphenols in vitro. Until recently, relatively little of the in vitro research published employed physiologically relevant concentrations of catechins. Evidence that catechins are extensively metabolized in vivo and that the antioxidant and biological activities of catechin metabolites may differ from those of their parent compounds emphasizes the importance of demonstrating the antioxidant effects of tea and tea polyphenols in vivo.

Antioxidant activity of tea and tea polyphenols in animal models of oxidative stress

Endogenous antioxidants and antioxidant enzymes. The addition of green tea catechins to plasma (41) or LDL (42) resulted in sparing of endogenous α-tocopherol during in vitro oxidation. In hypercholesterolemic rabbits, green and black tea administration increased plasma α-tocopherol concentrations after 8 and 17 wk of tea administration but not after 21 wk (43). The total plasma antioxidant capacity was not affected by green or black tea administration over the 21-wk study period. In rats, administration of green tea catechins prevented decreases in plasma and erythrocyte α-tocopherol concentrations resulting from a diet high in PUFA (6), but green tea flavonoid administration to marginally vitamin C-deficient Osteogenic Disorder Shionogi (ODS) rats did not increase plasma α-tocopherol concentrations (44).

Tea administration prevented decreases in tissue glutathione (GSH) concentrations in three out of four animal studies. Consumption of black tea leaves prevented carbon tetrachloride-induced liver depletion of GSH in male rats (45) but not in female rats (46). Similarly, providing green tea extract in the drinking water of male rats prevented decreases in liver GSH concentrations induced by ethanol administration (47). In mice infected with *Mycobacterium tuberculosis*, oral administration of green tea extract attenuated decreases in erythrocyte GSH concentrations caused by the infection (48).

Administration of tea and tea polyphenols has been reported to prevent or attenuate decreases in antioxidant enzyme activities in a number of animal models of oxidative stress. Providing hairless mice with green tea polyphenols in their drinking water significantly inhibited UVB-induced decreases in epidermal catalase and glutathione reductase activities (26). Oral administration of green tea extract to mice infected with *M. tuberculosis* attenuated infection-associated decreases in erythrocyte superoxide dismutase (SOD) activity (48), while oral administration of either black or green tea extract resulted in increased serum SOD activity in mice exposed to the carcinogen, 3-methylcolanthrene (49). Providing rats with green tea extract in their drinking water attenuated ethanol-associated decreases in serum and liver SOD as well as liver glutathione peroxidase (GPX) and catalase activities (50). An electrical muscle stimulation protocol that elicited oxidative damage to muscle proteins in rats did not result

in significant changes in muscle SOD and GPX activities, hence it is not surprising that the activity of these enzymes did not differ between rats given a diet containing 0.1% EGCG and those given a control diet (51).

In contrast to studies in animal models of oxidative stress, studies in healthy humans have not found tea or tea polyphenol consumption to result in significant changes in plasma antioxidant concentrations or antioxidant enzyme activities (2). Although consumption of tea or tea polyphenols by humans frequently results in modest transient increases in the total antioxidant capacity of plasma, as measured by the ferric-reducing antioxidant potential (FRAP), oxygen radical absorbance capacity (ORAC), or Trolox-equivalent antioxidant capacity (TEAC) assays, recent research suggests that concomitant increases in plasma urate accounts for much, if not all, of the increased plasma antioxidant capacity (37,52).

Ex vivo lipoprotein oxidation. In animal models of atherosclerosis, the majority of studies suggests that tea administration increases the resistance of lipoproteins to ex vivo oxidation, usually by prolonging the lag phase of copper-mediated lipid peroxidation (**Table 3**). In hamsters fed normal or high cholesterol diets, the lag phase of copper-mediated LDL + VLDL oxidation was significantly increased in those animals given green or black tea in their drinking water (53). Feeding green tea flavonoids (8 g/kg of diet) to marginally vitamin C-deficient ODS rats resulted in a significantly increased lag phase of copper-mediated LDL oxidation (44). Feeding green tea polyphenols dose-dependently decreased the concentration of thiobarbituric acid-reacting substances (TBARS) in LDL of cholesterol-fed rats after 4 h of copper-mediated oxidation, with the highest dose (2.5%) conferring similar resistance to that of the antioxidant, probucol (54).

TABLE 3

Effects of tea and tea polyphenol administration on ex vivo lipoprotein oxidation and atherosclerotic lesion formation in animal models of atherosclerosis

Reference	Species	Treatment (*n*)	Lipoprotein oxidation	Atherosclerosis
Yamaguchi et al., 1991 (59)	Mice	Control Green tea extract 50 mg (kg · d) 100 mg/(kg · d) 200 mg/(kg · d)		Aortic cholesterol: dose-dependent ↓ with green tea extract
Tijburg et al., 1997 (43)	New Zealand white rabbits	Control (20) Green tea (20) Black tea (20) Vitamin E (20) β-carotene (20)	Lag phase: ↔ with green tea ↑ with black tea ↑ ↑ with vitamin E Oxidation rate: ↓ with green tea, black tea, and vitamin E	Aortic atherosclerotic lesion area: NS,[1] 31% ↓ with green tea (P = 0.11) ↔ with black tea and vitamin E
Hayek et al., 1997 (61)	ApoE-deficient mice	Ethanol (control) (10) Red wine (10) Quercetin (10) Catechin (10)	TBARS after Cu^{+}-, AAPH, or macrophage-mediated oxidation: ↓ with red wine and quercetin ↔ with catechin	Aortic atherosclerotic lesion area: ↓ with red wine, quercetin and catechin
Vinson and Dabbagh, 1998 (53)	Syrian golden hamsters	NC diet (6) NC + green tea (6) NC + black tea (6) HC diet (6) HC + green tea (6) HC + black tea (6)	Lag phase ↑ with NC + green tea, HC + green tea, and NC + black tea	
Crawford et al., 1998 (56)	LDL receptor-deficient mice	Control (17) Black tea (19) Antioxidant (18)	Lag phase: ↑ with antioxidant vs. black tea and control	Aortic fatty streak lesion area: ↓ with antioxidant vs. black tea and control
Anderson et al., 1998 (55)	Sprague-Dawley rats	Control (10) Green tea (10) Vitamin E (10) Soy protein, high-genistein (10) Soy protein, low-genistein (10) β-carotene (10)	Lag phase: ↑ with green tea, vitamin E, and soy protein (high and low genistein)	
Miura et al., 2001 (60)	ApoE-deficient mice	Control (17) Green tea (16)		Aortic atheromatous area, aortic cholesterol, and aortic triacylglycerol: ↓ with green tea
Kasaoka et al., 2002 (44)	ODS Rats (cannot synthesize ascorbate)	Ascorbate 300 mg/kg (8) Ascorbate 25 mg/kg (8) Ascorbate 25 mg/kg + green tea (8)	Lag phase: ↑ with ascorbate + green tea vs. both ascorbate groups	
Yokozawa et al., 2002 (54)	Wistar rats	Control diet High cholesterol diet (HC) HC + 0.1% green tea HC + 0.5% green tea HC + 2.5% green tea HC + 0.1% probucol	TBARS after copper-mediated oxidation: Dose-dependent ↓ with green tea; comparable to ↓ with probucol	

[1] Abbreviations used: ApoE, apolipoprotein E; HC, high cholesterol; NC, normal cholesterol; NS, nonsignificant; ODS, Ostogenic Disorder Shinogi; TBARS, thiobarbituric acid reacting substances; ↓, significant decrease unless otherwise noted; ↑, significant increase; ↔, unchanged.

In contrast, most other animal studies suggest that tea or tea polyphenol consumption is not as effective as supplementation with other antioxidants in improving the resistance of isolated lipoproteins to ex vivo oxidation. When hypercholesterolemic rabbits were given tea in their drinking water for 13 wk, the lag phase of copper-mediated LDL oxidation was significantly increased by 15% in those animals given black tea, and non-significantly increased by 13% in those given green tea (43). However, significant differences between tea and control groups were no longer found after 21 wk. In the same study, 21 wk of green tea or black tea administration significantly decreased the rate of LDL oxidation ex vivo by 21%, but 21 wk of vitamin E supplementation (200 mg/kg diet) increased the lag phase by 45% and decreased the rate of LDL oxidation by 32%. In rats fed green tea powder (20 g/kg diet) for 3 wk, the lag phase of copper-mediated LDL + VLDL oxidation was significantly prolonged by 33%, although a diet enriched in vitamin E (1,000 IU/kg diet) resulted in more substantial increases in all parameters of LDL + VLDL resistance to oxidation (55). Black tea in the drinking water of LDL receptor-deficient mice fed a high cholesterol diet did not significantly alter the lag phase of copper-mediated LDL oxidation, although antioxidant supplementation with vitamin C, vitamin E and β-carotene resulted in a significant increase in the lag phase compared with animals given tea or controls (56). In contrast to data from animal models of atherosclerosis, only two out of six controlled trials in humans found small but significant increases in the lag phase of ex vivo LDL oxidation (57,58).

Atherosclerosis. Although LDL oxidation is thought to represent an early event in the development of atherosclerosis, the physiological relevance of assays of ex vivo lipoprotein oxidation has been questioned. Indeed, in animal models of atherosclerosis, the effect of tea or tea polyphenol administration on atherosclerotic lesion formation does not always reflect the results of ex vivo lipoprotein oxidation (table 3). Providing green tea extract in the drinking water of mice fed an atherogenic diet dose-dependently decreased the accumulation of aortic cholesterol compared with control mice (59). In apolipoprotein E (ApoE)-deficient mice, aortic atherosclerotic lesion area and aortic cholesterol accumulation were also lower in those animals given green tea catechins in their drinking water (60). Administration of red wine, quercetin or catechin resulted in significantly smaller atherosclerotic lesion areas in the aortas of ApoE-deficient mice when compared with mice administered an ethanol-containing placebo, despite the fact that only red wine and quercetin administration significantly increased the resistance of LDL to ex vivo oxidation (61). In hypercholesterolemic rabbits given green tea, black tea, vitamin E or β-carotene for 21 wk, aortic atherosclerotic lesion areas were 31% smaller in animals given green tea than in control animals, although this difference was not statistically significant ($P = 0.11$) (43). Black tea administration to LDL-receptor deficient mice did not affect aortic fatty streak lesion area, although fatty streak lesion areas in animals supplemented with antioxidants (vitamin C, vitamin E and β-carotene) were 60% smaller than those of control animals (56). Thus, limited data suggest that green tea or catechin administration inhibits atherogenesis in some animal models of atherosclerosis.

Biomarkers of lipid peroxidation. Assessment of TBARS is often used to measure plasma and tissue concentrations of malondialdehyde (MDA), a decomposition product of oxidized lipids, and as an index of plasma and tissue lipid peroxidation. Most of the numerous animal studies that have measured plasma or tissue TBARS have reported significant decreases with tea or tea polyphenol administration. However, the utility of the TBARS assay as a measure of lipid peroxidation in vivo is questionable due to its lack of specificity for MDA in biological samples and its susceptibility to artifactual ex vivo oxidation (62). Consequently, studies using only TBARS to assess lipid peroxidation are not further considered in this review of biomarkers of in vivo lipid peroxidation.

Animal studies employing relevant measures of lipid peroxidation are limited (**Table 4**). Basal levels of lipid hydroperoxides (LOOH) measured iodometrically in LDL were decreased in ApoE-deficient mice fed red wine, quercetin or catechin in their drinking water compared with an ethanol-containing placebo (61). In contrast, there were no differences in the basal levels of LOOH in LDL from hypercholesterolemic rabbits given green or black tea in their drinking water compared with controls (43). In rats injected with the colon carcinogen 1,2-dimethylhydrazine, phosphatidylcholine hydroperoxides were significantly lower in the colonic mucosa of those rats receiving green tea extract in their drinking water (**Fig. 2**) (63). Lipid hydroperoxides may decompose to form aldehydes such as MDA and 4-hydroxynonenal (4-HNE). In rats on a high fat diet, green tea administration prevented ethanol-induced increases in 4-HNE adducts to liver proteins and significantly decreased ethanol-induced liver necrosis (64).

TABLE 4

Biomarkers of in vivo lipid peroxidation in animal models of oxidative stress

Reference	Species	Oxidative stress	Treatment (*n*)	Results
Matsumoto et al., 1996 (63)	Sprague-Dawley rats	DMH[1]	Tap water + saline (19) Green tea + saline (19) Tap water + DMH (19) Green tea + DMH (19)	Intestinal mucosal PCOOH: ↓ with green tea + DMH vs. tap water + DMH
Tijburg et al., 1997 (43)	New Zealand white rabbits	Atherogenic diet	Control (20) Green tea (20) Black tea (20) Vitamin E (20) β-carotene (20)	Basal LDL LOOH: ↔
Hayek et al., 1997 (61)	ApoE-deficient mice	Atherogenic diet	Ethanol (Control) (10) Red wine (10) Quercetin (10) Catechin (10)	Basal LDL LOOH: ↓ with red wine, quercetin, and catechin LDL uptake by macrophages: ↓ with red wine, quercetin, and catechin

[1] Abbreviations used: ApoE, apolipoprotein E; DMH, 1,2-dimethyl-hydrazine; LOOH, lipid hydroperoxides; PCOOH, phosphatidylcholine; ↓, significant decrease; ↑, significant increase; ↔, unchanged..

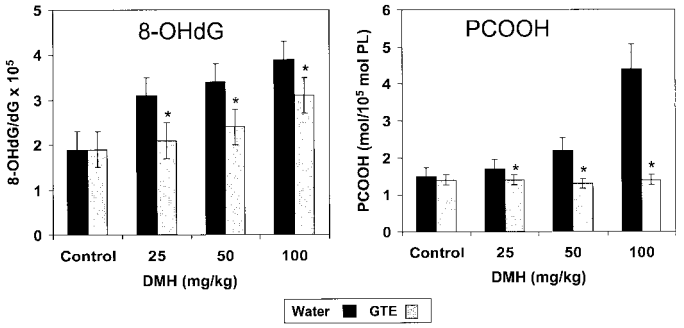

FIGURE 2 Providing green tea extract (GTE) in the drinking water of rats for 10 d prior to subcutaneous injection with the colon carcinogen, 1,2-dimethylhydrazine (DMH) significantly inhibited increases in 8-hydroxy 2′ -deoxyguanosine [8-OHdG (72)] and phosphatidylcholine hydroperoxides [PCOOH (63)] in colon mucosa. Black bars represent mean values in animals given water and gray bars represent mean values in animals given GTE. Mean values represent eight independent cases for 8-OHdG and five independent cases for PCOOH. Bars marked with an asterisk (*) represent values that are significantly different from those for animals given water ($P < 0.05$). Reproduced with permission from Elsevier Science and Masao Inagake.

Plasma and urinary F_2-isoprostanes, nonenzymatic oxidation products of arachidonic acid, have been shown to be sensitive and specific markers of in vivo lipid peroxidation in animals and humans. Surprisingly, assays of F_2-isoprostanes have not yet been used to assess the effect of tea administration on in vivo lipid peroxidation in animal models of oxidative stress. However, in several small placebo-controlled trials in humans, green or black tea consumption did not significantly change plasma or urinary F_2-isoprostane concentrations in healthy, hypertensive or hypercholesterolemic volunteers (65–67).

Biomarkers of oxidative damage to proteins. Oxidative damage to proteins may result in chemical modification of amino acids, aggregation or crosslinking of proteins or protein fragmentation. Of three different animal studies that assessed the effects of oral tea administration on oxidative damage to proteins in vivo, each used a different model of oxidative stress and a different measure of oxidative protein damage (**Table 5**). Supplementing the diets of rats with 1% EGCG significantly inhibited increases in muscle protein carbonyl content induced by electrical muscle stimulation (51). Protein glycation results from the reactions between primary amino groups of proteins and reducing sugars, such as glucose. Oxidation and structural rearrangement of glycated proteins results in the formation of advanced glycation end products, such as N^{ϵ}-(carboxymethyl)lysine and pentosidine. Old rats (up to 22 mo-of-age) given green tea extract in their drinking water starting at 6 mo-of-age were found to have decreased aortic collagen-linked Maillard-type fluorescence, a marker for advanced glycation endproducts (68). As mentioned above, oral administration of green tea prevented ethanol-induced increases in 4-HNE adducts to liver proteins (64). The only controlled study to examine the effect of tea polyphenol consumption on oxidative damage to proteins in humans compared a low flavonoid diet with the same diet fortified with green tea extract over a 3-wk period (69). Levels of oxidatively modified plasma and hemoglobin proteins were not significantly different between the two diets.

Biomarkers of oxidative DNA damage. The anticarcinogenic effects of tea and tea polyphenols have been amply demonstrated in a number of animal models involving tumors of the lung, digestive tract, prostate, bladder, mammary glands and skin (18). Data from animal studies also support a role for tea in the prevention of oxidative damage to DNA bases induced by chemical carcinogens (**Table 6**). Although enzymes present in mammalian cells can recognize and excise oxidatively damaged DNA bases, mutations may occur if excision and repair processes cannot keep pace with the rate of oxidative damage. Topical EGCG inhibited the epidermal formation of the oxidized DNA bases, thymidine glycol, 5-hydroxymethyl-2′-deoxyuridine and 8-hydroxy 2′-deoxyguanosine (8-OHdG) in mice treated with phorbol ester-type tumor promoters (70).

The most commonly measured oxidized DNA base in animal studies of tea administration is 8-OHdG. In addition to decreasing lung adenomas, providing green tea or EGCG to mice in their drinking water significantly inhibited increases in lung DNA levels of 8-OHdG induced by the tobacco carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (71). Providing green tea extract to rats in their drinking water (72) and black tea polyphenols by gavage (73) significantly inhibited 8-OHdG increases in colon mucosa induced by the colon carcinogen, 1,2-dimethylhydrazine (Fig. 2). In hamsters, providing green tea catechins in the drinking water significantly inhibited 8-OHdG increases in the pancreas induced by the pancreatic carcinogen, *N*-nitrobis(2-oxopropyl)amine (74).

TABLE 5

Biomarkers of in vivo oxidative damage to proteins in animal models of oxidative stress

Reference	Species	Oxidative stress	Treatment (*n*)	Results
Nagasawa et al., 2000 (51)	Sprague-Dawley rats	Electrical stimulation to hindlimb	Control (6) EGCG[1] (6)	Muscle protein carbonyl content: ↓ with EGCG
Song et al., 2002 (68)	Sprague-Dawley rats	Aging	Young (6) Old (12) Old + green tea (12)	Collagen-linked Maillard-type fluorescence: ↓ with green tea in aorta ↔ with green tea in skin Collagen carbonyl content: ↔ with green tea in aorta or skin
Arteel et al., 2002 (64)	Wistar rats	High-fat diet + ethanol	HF (6) HF + ethanol (6) HF + green tea (6) HF + ethanol + green tea (6)	4-HNE adducts to liver proteins: ↓ with green tea Ethanol-induced liver necrosis: ↓ with green tea

[1] Abbreviations used: 4-HNE, 4-hydroxynonenal; EGCG, (−)-epigallocatechin gallate; HF, high-fat diet; ↓, significant decrease; ↑, significant increase; ↔, unchanged.

TABLE 6

Biomarkers of in vivo oxidative damage to DNA in animal models of oxidative stress

Reference	Species	Oxidative stress	Treatment (*n*)	Results
Xu et al., 1992 (71)	A/J mice	NNK[1]	Water (11) Green tea (11) EGCG (10) NNK (10) NNK + green tea (11) NNK + EGCG (12)	Lung 8-OHdG: ↓ with green tea and EGCG Liver 8-OhdG: ↔ with green tea or EGCG Lung adenomas: ↓ with green tea and EGCG
Wei and Frenkel, 1993 (70)	SENCAR mice	Topical phorbol ester	Acetone + acetone (4) Phorbol ester + acetone (4) Phorbol ester + EGCG (8)	Epidermal dTG, HMdU, and 8-OHdG: ↓ with topical EGCG
Inagake et al., 1995 (72)	Sprague-Dawley rats	DMH	Water (8) Water + 25 mg DMH (8) Water + 50 mg DMH (8) Water + 100 mg DMH (8) Green tea (8) Green tea + 25 mg DMH (8) Green tea + 50 mg DMH (8) Green tea + 100 mg DMH (8)	Colon 8-OHdG: ↓ with green tea at all DMH doses Liver 8-OhdG: ↔ with green tea at all DMH dose levels
Lodovici et al., 2000 (73)	Fisher 344 rats	DMH	Control (11) DMH (26) Thearubigin + DMH (11) Theafulvin + DMH (10)	Colon mucosa 8-OHdG: ↓ with thearubigin ↔ with theafulvin
Hasegawa et al., 1995 (75)	Fisher 344 rats	2NP	Control (10) 2NP (10) 2NP + green tea (10) 2NP + green tea extract (10)	Liver 8-OHdG: ↓ with green tea and green tea extract
Sai et al., 1998 (76)	Fisher 344 rats	2NP	Control (5) Low-dose 2NP (5) High-dose 2NP (5) Low 2NP + green tea (5) High 2NP + green tea (5)	Liver 8-OHdG: ↓ with green tea in low-dose and high-dose 2NP-treated animals
Sai-Kato et al., 1995 (78)	B6C3F$_1$ mice	PCP	Control (5) PCP (5) PCP + EGCG (5) PCP + Vitamin E (5) PCP + Ellagic acid (5)	Liver 8-OHdG: ↓ with vitamin E ↔ with EGCG and ellagic acid
Tamura et al., 1997 (77)	Fisher 344 Rats	DEN or CDD	Control DEN DEN + green tea CDD CDD + green tea	Liver 8-OHdG: ↓ with DEN + green tea ↓ with CDD + green tea Preneoplastic lesions: ↓ with DEN + green tea
Takabayashi et al., 1997 (74)	Syrian golden hamsters	N-nitrobis(2-oxopropyl)amine	Green tea (15) Control (15)	Peak pancreatic 8-OHdG: ↓ with green tea
Hong et al., 2000 (81)	Wistar rats	Brain IR	Sham operated (6) IR (6) IR + green tea (6)	Brain 8-OHdG: ↔ with green tea
Hong et al., 2001b (80)	Mongolian gerbils	Brain IR	Sham operated (6) IR (6) IR + 0.5% green tea (6) IR + 2% green tea (6)	Brain 8-OHdG: ↓ with 2% green tea
Giovannelli et al., 2000 (83)	Fisher 344 Rats	High fat diet	Water (15) WCPT (9) Thearubigins (14)	Single strand breaks (comet): ↔ with WPCT and thearubigins Oxidized pyrimidines: ↓ with WCPT ↔ with thearubigins Oxidized purines: ↓ with WCPT Thearubigins not tested

[1] Abbreviations used: 2NP, 2-nitropropane; 4-HNE, 4-hydroxynonenal; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CDD, choline-deficient diet; DEN, diethylnitrosamine; DMH, 1,2-dimethyl-hydrazine; dTG, dithymidine glycol; EGCG, (−)-epigallocatechin gallate; HMdU, 5-hydroxymethyl-2′deoxyuridine; IR, ischemia-reperfusion; NNK, 4-methyl-nitrosamino-1-3-pyridyl-1-butanone; PCP, pentachlorophenol; WCPT, wine complex polyphenols and tannins; ↓, significant decrease; ↑, significant increase; ↔, unchanged.

Administering green tea to rats in their drinking water inhibited increases in liver 8OHdG induced by the hepatic carcinogen, 2-nitropropane, in two separate studies (75,76). Green tea administration to rats also inhibited increases in liver 8-OHdG resulting from diethylnitrosamine exposure or cirrhosis induced by a choline-deficient diet (77). Although pentachlorophenol-induced increases in liver 8-OHdG were significantly inhibited by supplementing the diets of mice with vitamin E, supplementation with EGCG did not significantly inhibit liver 8-OHdG formation (78). Thus, with a few ex-

ceptions, tea and tea polyphenols have consistently been found to inhibit increases in 8-OHdG, a biomarker of oxidative DNA damage, induced by a number of different chemical carcinogens in different species and different target tissues.

Reactive oxygen and nitrogen species generated upon reperfusion of ischemic tissue appear to play a critical role in ischemia-reperfusion injury. By trapping free radicals with the spin-trapping reagent, α-(-4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN), and measuring the resulting adduct with electron spin resonance spectroscopy, Zhong and colleagues demonstrated that ischemia-reperfusion of the liver in rats resulted in a twofold increase of 4-POBN/radical adducts detected in bile (79). Feeding rats 0.1% green tea extract or 0.085% EC significantly decreased bile 4-POBN/ radical adducts to levels comparable to those of sham-operated rats. Brain injury due to ischemia-reperfusion is also thought to result, at least in part, from oxidative damage. Providing gerbils with 0.5% or 2% solutions of green tea extract in their drinking water for 3 wk dose-dependently inhibited increases in brain 8-OHdG levels following ischemia-reperfusion (80). Although a similar trend in brain 8-OHdG levels was observed when rats were provided with a 0.5% solution of green tea extract 3 wk prior to the induction of ischemia-reperfusion, the inhibition was not statistically significant (81).

Single cell alkaline gel electrophoresis, also known as the comet assay, is a sensitive assay of oxidative and nonoxidative DNA damage. The comet assay may be adapted to measure oxidative damage to DNA bases by measuring DNA strand breaks induced by treatment with relevant repair enzymes, e.g., FapyGua glycosylase for oxidized purine lesions and endonuclease III for oxidized pyrimidine lesions (82). Only one published animal study has used the comet assay to assess the effect of tea polyphenols on oxidative DNA damage. Rats consuming a high fat diet were given a red wine polyphenol preparation, thearubigins extracted from black tea or water by gavage for 10 d prior to killing (83). Single-strand breaks and oxidized pyrimidine bases in the DNA of colonic mucosal cells did not differ between thearubigin-treated animals and those treated with water. Oxidized purine and oxidized pyrimidine bases were significantly lower in the colonic mucosa cells of animals treated with red wine polyphenols than those treated with water, although single-strand breaks did not differ between the two groups. Thus, research on the role of tea or tea polyphenol administration in the prevention of oxidative damage to DNA in animal models of oxidative stress other than that induced by chemical carcinogens is limited and the protective effects of tea are less consistent. Evidence from controlled human trials that tea or tea polyphenol consumption inhibits oxidative DNA damage is lacking (69).

The addition of milk to tea

In a number of countries, tea is commonly consumed with milk. Interactions between tea polyphenols and proteins found in milk have been found to diminish total antioxidant capacity in vitro (84), but it is presently unclear whether consuming tea with milk substantially alters the biological activities of tea flavonoids in vivo. The addition of milk to black tea did not significantly alter areas under the curve for plasma catechins (85) or flavonols (86) in human volunteers, suggesting that adding milk to tea does not substantially affect the bioavailability of tea catechins or flavonols. Two studies in humans found that the addition of milk decreased (87) or eliminated (88) increases in plasma antioxidant capacity induced by tea consumption, whereas another found no effect (89). Few studies have examined the effects of adding milk to tea in animal models. Although at least two studies in animal models found that adding milk to black tea did not diminish its inhibitory effect on tumorigenesis induced by chemical carcinogens (90,91), it is not known whether these effects were related to the antioxidant activity of tea polyphenols.

SUMMARY

Data from animal studies provide some support for the notion that tea polyphenols act as antioxidants in vivo. Administration of green tea extract and, in one case, black tea extract attenuated decreases in SOD activity caused by infection, ethanol or the carcinogen, 3-methylcolanthrene. Although green and black tea administration improved the resistance of lipoproteins to ex vivo oxidation in several animal models, the improvement was generally much less than that conferred by supplementation with other antioxidants. Animal studies examining the effect of tea administration on biomarkers of in vivo lipid peroxidation other than TBARS are limited. However, there is some evidence from mouse models of atherosclerosis that green tea catechin consumption is antiatherogenic. Very limited data suggest that green tea or EGCG administration may protect proteins from oxidative damage. Most promising are the consistent findings that tea or tea polyphenol administration prevented carcinogen-induced increases in the oxidized DNA base, 8-OHdG, in animal models of skin, lung, colon, liver and pancreatic cancer.

Why are the results of animal and human studies different?

Although the consumption of tea or tea polyphenols results in modest transient increases in plasma antioxidant capacity in humans, limited research has not generally revealed significant decreases in biomarkers of in vivo oxidative damage (2). Tea concentrations used in animal and human studies are often similar, but animals generally receive much higher doses relative to body weight than humans. Findings that tea or tea polyphenol administration inhibits increases in 8-OHdG induced by chemical carcinogens provide the most consistent evidence that tea and tea polyphenols have antioxidant effects in vivo. In contrast, observational studies in humans do not generally support a significant cancer chemoprotective effect of black or green tea consumption (2). Although observational studies in humans have a number of limitations, it is also possible that animal models employing chemical carcinogens may not be entirely relevant to the causes of oxidative stress and cancer in humans. While genetic variability is limited in animal models, wide genetic variations in the response of humans to oxidative stress may obscure small changes in biomarkers induced by tea polyphenols. To determine whether increased consumption of tea or tea polyphenols prevents oxidative damage to biomolecules and associated pathology in humans, research in humans and animals should employ sensitive and specific markers of oxidative damage to lipids, proteins and DNA, such as F_2-isoprostanes, protein carbonyls and the comet assay. To increase the applicability of data from animal studies, animal models selected to assess the antioxidant activity of tea polyphenols should be relevant to likely sources of oxidative stress and associated diseases in humans.

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