# Implications of the Mechanisms of Action of Tea Polyphenols as Antioxidants *in vitro* for Chemoprevention in Humans (44377)

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Considerable evidence points to the health benefits of the phytochemical constituents of fruit, vegetables, beverages, and grains in protection against cardiovascular disease and certain cancers. This may be accounted for through contributions from their constituent antioxidants (vitamin E, vitamin C, β-carotene, other carotenoids and the non-nutrient flavonoids, ubiquitous phytochemical components of the diet), as well as through other anticarcinogenic and cardioprotective effects independently of their antioxidant effects. Indeed, the epidemiological evidence for a risk-reducing role of vitamin C for cancer is not as strong as that for fruit and vegetables (1).

Dietary components may contribute to antioxidant function in several ways that relate to their structural chemistry (2): i) by directly scavenging free radicals through hydrogen/electron donation, depending on their reducing properties, or reduction potentials; ii) by intercepting the propagatory chain reactions of lipid peroxidation and scavenging peroxyl radicals, also dependent on their reducing properties. Their effectiveness as inhibitors of lipid peroxidation will also relate to their accessibility to the site of action, defined by their partition coefficients; iii) by scavenging reactive nitrogen species and competitively inhibiting tyrosine nitration and DNA deamination; and iv) as preventative antioxidants by chelating transition metal ions and inhibiting the formation of iron-induced free radicals, and the iron-mediated propagation of free radical reactions.

Many of the polyphenols, especially those as constituents of tea (Table I), exhibit all these antioxidant properties.

This review will focus on the polyphenolic constituents of tea and their biological effects in inhibiting damage induced by reactive oxygen and nitrogen species *in vitro*, processes that have implications for chemoprevention in humans.

## Hydrogen-Donating Properties of Tea Polyphenols

The reducing properties of polyphenols are defined by the number and the structural arrangement of their phenolic hydroxyl groups. The structures of the tea catechins are shown in Figure 1. The reduction potential of o-dihydroxy catechol structure in the B ring is lower than that of the m-hydroxy structure of the A ring, and the prime feature contributing to the antioxidant activity of catechin, epicatechin, and epigallocatechin is the phenolic arrangement of the B ring. The presence of the gallate moiety attached to the 3-hydroxyl group in the C ring enhances its potential for hydrogen donation. Thus screening of the tea polyphenols for their relative abilities to scavenge directly a model free radical cation formed from 2,2′-azinobis-(3-ethyl benzothiazoline) 6-sulphonic acid (ABTS) (3–6, 2) shows the hierarchy of antioxidant potential as: Epigallocatechin gallate (EGCG) ≈ Epicatechin gallate (ECG) > Epigallocatechin (EGC) > Gallic acid (GA) > Epicatechin (EC) = Catechin (C). As shown in Table II, the green tea polyphenols are more powerful antioxidants *in vitro* on a molar basis than vitamin C and vitamin E. The antioxidant activity of green tea can be accounted for, in the main, by the contributions from the catechin/gallate constituents.

Recent studies have demonstrated the antioxidant activity of one glass (150 ml) of green or black tea (0.25% as consumed in the UK) relative to one serving of other beverages (same volume) or fruit/vegetable extracts (150 g portion) (Fig. 2) (7).

## Protective Effects of Green Tea Polyphenols Against Reactive Oxygen Species

Many researchers have investigated the protective effects of the catechin/gallate family of flavonoids against oxidation of lipids and low-density lipoproteins (LDL) (3, 8–12). In assigning chain-breaking antioxidant properties to

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**Table I.** Phenolic Components of Green Tea (% Dry Weight)

| Phenolic components of green tea (% dry weight) | | | | |
|---|---|---|---|---|
| Catechins | 30–42 | ⇒ | Epigallocatechin gallate | 11.2 |
| Flavonols | 2 | | Epigallocatechin | 10.3 |
| Simple polyphenols | 2 | | Epicatechin gallate | 2.3 |
| | | | Epicatechin | 2.5 |
| | | | Catechin | 0.5 |

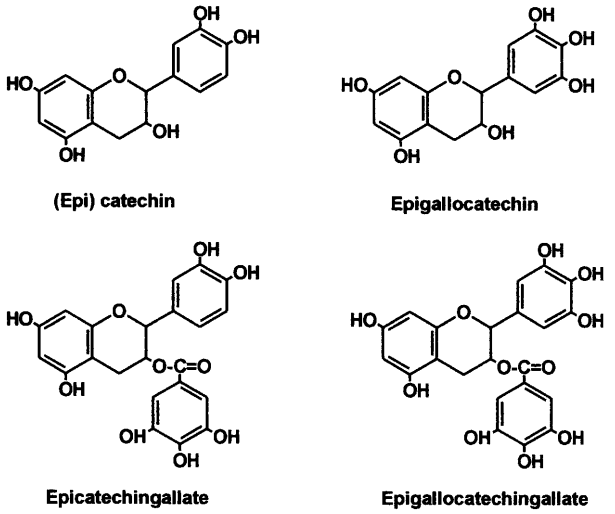


**Figure 1.** Structures of green tea polyphenols.

the catechin polyphenols, it is important to interpret the effects in systems in which other contributing antioxidant properties cannot be accorded to the components in question. Investigation of the preventative effects of the catechins against LDL oxidation (avoiding the potential copper-polyphenol interactions and thus using haem protein-mediated oxidation), the sequence of reactivities in scavenging the lipid peroxyl radical is: ECG = EGCG = EC = C > EGC (3). This, with the exception of EGC, might be predicted, matching the sequence of reducing activity as presented previously. However, the accessibility of the antioxidant to the radicals in question is an essential consideration. The partition coefficient of EGC (as assessed in octanol/water mixtures) is 0.12 compared to 1.2, 1.14, 11.8 for EC, EGCG, and ECG respectively,[1] and thus the greater solubility of EGC in the aqueous phase explains its lesser efficacy in inhibiting lipid peroxidation. Furthermore, monitoring the sparing of the endogenous LDL α-tocopherol in the presence of the added catechin polyphenols during the oxidation of LDL in *in vitro* systems, EGC has no effect compared with the efficacy of the less hydrophilic green tea components.

[1] Rice-Evans *et al.*, unpublished data.

**Table II.** Relative Antioxidant Properties of Tea Catechins and Green Tea (3, 5)

| Component | Trolox equivalent antioxidant capacity[a] |
|---|---|
| Epigallocatechin gallate | 4.8 ± 0.06 |
| Epigallocatechin | 3.8 ± 0.06 |
| Epicatechin gallate | 4.9 ± 0.02 |
| Epicatechin | 2.4 ± 0.02 |
| Green tea (1000 ppm) | 3.8 ± 0.03 |
| Black tea (1000 ppm) | 3.5 ± 0.03 |
| Vitamin C | 1.0 ± 0.02 |
| Vitamin E | 1.0 ± 0.03 |

[a] TEAC is the millimolar concentration of Trolox (reference standard) having the equivalent antioxidant activity to a 1 m*M* concentration of the antioxidant compound or defined concentration or volume of food extract/beverage under investigation.

## Inhibition of Reactive Nitrogen Species by Green Tea Polyphenols

Overproduction of nitric oxide associated with chronic inflammation has been postulated to be a likely candidate associated with the aetiology of atherosclerosis and cancer. Peroxynitrite is a toxic oxidizing and nitrating species that can be produced by rapid interaction of superoxide radical and nitric oxide (13) and is implicated in the mechanism of LDL modification in the arterial wall. Peroxynitrite at physiological pH is protonated to form peroxynitrous acid, which is highly unstable and might react *via* a vibrationally excited intermediate, by homolytic dissociation to form nitrogen dioxide radical and hydroxyl radical, *via* heterolytic dissociation to form nitronium ion (Fig. 3), or rapid decay to form a mixture of products (14).

Tyrosine is especially susceptible to peroxynitrite-dependent reactions, becoming nitrated and forming 3-nitrotyrosine. Nitrated products are immunogenic, and nitration can alter their function and stability, thus interfering with cell signaling pathways, cytoskeletal structure, and repair mechanisms. 3-Nitrotyrosine has been postulated to be a fingerprint for peroxynitrite reactions in the tissues, and the presence of nitrated proteins is associated with a wide range of pathologies, including atherosclerosis, rheumatoid arthritis, inflammatory bowel disease, lung disorders, septic shock, and gastritis. Investigations on the protective effects of green tea catechins against peroxynitrite-dependent nitration reactions have been undertaken through competitively inhibiting nitration of tyrosine (Fig. 4). The results

1 x red wine ⇔ 2 x green or black tea ⇔

3.5 x blackcurrant juice ⇔ 4 x apple ⇔

5 x onion ⇔ 7 x orange juice ⇔ 12 x white wine

**Figure 2.** Relative antioxidant activities of servings of tea compared with other beverages (150 ml) and fruit (150 g) (7).

$$NO^{\cdot} + O_2^{\cdot-} \rightarrow OONO^-$$

$$OONO^- + H^+ \rightarrow HOONO$$

$$HOONO \nearrow NO_2^{\cdot} + {}^{\cdot}OH$$

$$HOONO \searrow NO_2^+ + OH^-$$

**Figure 3.** Peroxynitrite and its reactive metabolites.

show that the ability of the catechin polyphenols at 10 $\mu M$ concentration to minimize tyrosine nitration induced by peroxynitrite is in the sequence EGC (38%) ≈ EGCG (32%) ≈ gallic acid ≈ (32%) > catechin (24%) ≈ epicatechin (23%) ≈ EGC (20%), all having more efficacy than the equivalent concentration of Trolox (the water-soluble vitamin E analog). The antioxidant protection imparted by these phenolics is mediated by the direct competition with tyrosine for nitration. The findings are that gallic acid and the gallate esters of the tea flavonoids are more effective than the tea polyphenols themselves, suggesting that the gallate moiety is an important center, for interaction with peroxynitrite as well as the polyphenol itself (15). Furthermore, our recent studies suggest that peroxynitrite scavenging by dietary phenolic compounds can lead to nitration or oxidation, depending on the phenolic in question (16).

## Acidic Nitrite-Derived Reactive Nitrogen Species

Exposure of humans to excess nitrate/nitrite from the diet or arising from overproduction of endogenous nitric oxide (e.g., at sites of chronic inflammation) may play a role in the etiology of cancer of the GI tract, particularly of the stomach. Dietary nitrate is principally derived from leafy green vegetables and nitrite from processed meats and other foods, where it is used as a preservative. Nitrate/nitrite are absorbed by the atomach and rapidly by the small intestine into the bloodstream. Nitrate is concentrated into the salivary glands increasing its concentration to many times that of plasma. Salivary nitrate is reduced to nitrite in the oral cavity which, when in the acidic environment of the stomach, forms nitrous acid. A large proportion of the nitrite and components formed from nitrite in the stomach are thought to be absorbed into the stomach and upper intestine and converted into nitrate (17). Nitrate is subsequently recirculated in the systemic circulation and concentrated in the saliva (18, 19). Nitrite/nitrate are subsequently excreted in the urine (Fig. 5). A major concern arises from the conversion of dietary nitrate and nitrite to reactive nitrogen species in the acidic environment of the stomach. Nitrous acid is a strong oxidizing agent and can be converted to potent nitrosating ($NO^+$) and nitrating agents (Fig. 6). Overproduction of RNS from dietary nitrate or endogenous sources can lead to damage in several ways. RNS (especially $NO^+$, $NO_2$ and $N_2O_3$) are potent nitrating and nitrosating agents and may react to form potentially mutagenic nitrosamines by N-nitrosation of amines. Formation of RNS

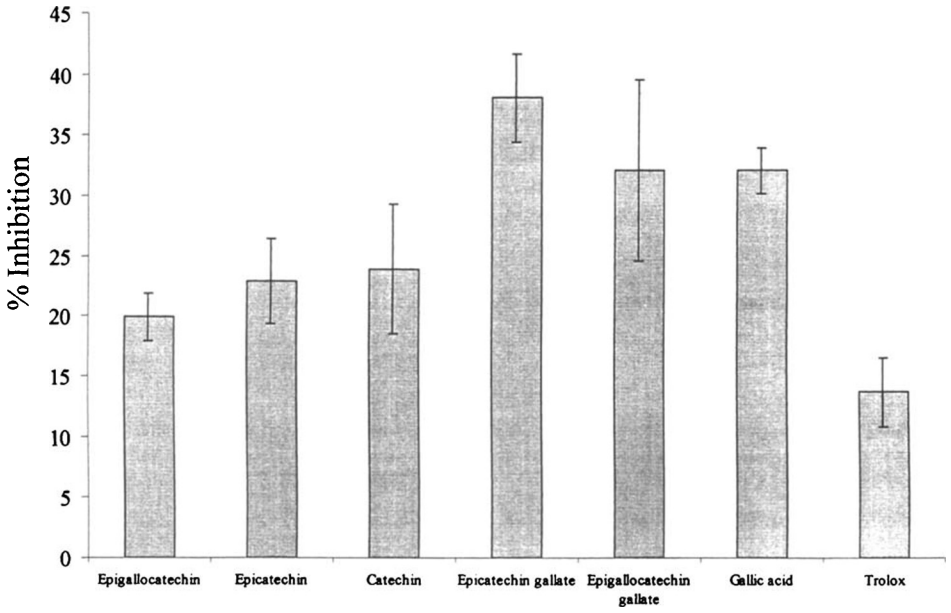


**Figure 4.** Inhibition of 3-nitrotyrosine formation by green tea polyphenols (16).

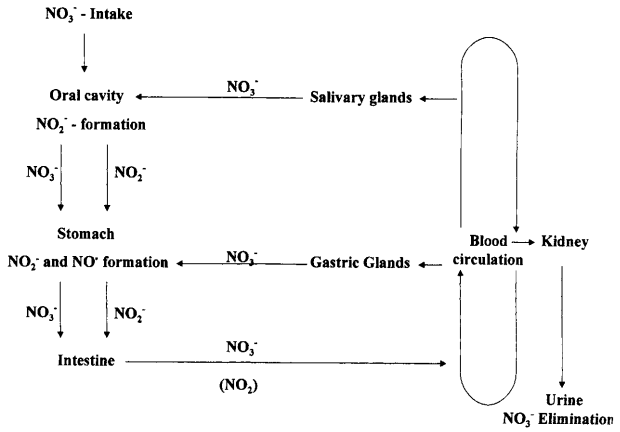


**Figure 5.** Intake and *in vivo* handling of nitrate and nitrite.

$$NO_2^- + H^+ \Leftrightarrow HNO_2$$

$$HNO_2 + H^+ \Leftrightarrow H_2NO_2^+ \Leftrightarrow H_2O + NO^+$$

$$2HNO2 \Leftrightarrow N_2O_3 + H_2O$$

$$N_2O_3 \Leftrightarrow NO^+:NO_2^-$$

**Figure 6.** Reactive nitrogen species formed from acidic nitrite.

from nitrite in the acidic environment of the stomach could chemically alter DNA bases causing deamination (20), important consequences for a variety of cytotoxic and pathological mechanisms. Nitration of aromatic compounds including tyrosine is caused by acidic nitrite (21). Such mechanisms are implicated as contributory causative factors in the occurrence of cancers of the GI tract. Thus, it is essential to minimize the nitrating and nitrosating potential of the nitrate- and nitrite-containing constituents of the diet.

In model stomach conditions of acidic nitrite, green tea polyphenols have been investigated for the ability to inhibit tyrosine nitration and deamination of DNA bases mediated by RNS derived from acidic nitrite. Table III demonstrates the relative abilities of the green tea polyphenols to inhibit tyrosine nitration induced by acidic nitrite, in terms of the concentration of compounds giving 50% inhibition of

**Table III.** Green Tea Catechins and the Inhibition of Acidic Nitrate-Induced Tyrosine Nitration (21)

| Compound | $IC_{50}$ [a] |
|---|---|
| Epigallocatechin gallate | 35.8 ± 9.0 |
| Epicatechin gallate | 38.4 ± 7.3 |
| Catechin | 41.6 ± 3.7 |
| Epicatechin | 50.2 ± 8.2 |
| Epigallocatechin | 64.8 ± 12.8 |
| Ferulic acid | 73.0 ± 15.1 |
| Rutin | 75.8 ± 23.6 |

[a] $IC_{50}$ = the concentration (μ*M*) of compound giving 50% inhibition of nitrotyrosine formation. [Tyrosine] and [Nitrate] = 400 μ*M*

**Table IV.** Green Tea Catechins and the Inhibition of DNA Damage-Induced by Acidic Nitrate (21)

| Compounds | $IC_{50}$ μ*M* | |
|---|---|---|
| | Hypoxanthine | Xanthine |
| Epigallocatechin gallate | 44.7 ± 10.6 | 31.2 ± 7.8 |
| Caffeic acid | 53.0 ± 2.1 | 62.9 ± 14.6 |
| Catechin | 67.2 ± 7.6 | 67.9 ± 2.8 |
| Epicatechin | 69.9 ± 6.2 | 64.9 ± 9.2 |
| Epigallocatechin | 74.0 ± 1.2 | 63.9 ± 2.5 |
| Quercetin | 85.3 ± 2.5 | 75.7 ± 5.7 |
| Gallic acid | 150.5 ± 19.6 | 226.2 ± 4.3 |
| 3,4-Dihydroxyphenyl acetic acid | 171.9 ± 33.6 | 247.3 ± 1.5 |

nitrotyrosine formation, relative to rutin (queretin-3-rutinoside), a major flavonol constituent of many fruits and vegetables and a minor component of green tea, and ferulic acid, a major phenolic in grains and a minor component of many fruits. Results show that order of effectiveness is EGCG ≈ ECG ≈ catechin > epicatechin > EGC ≈ ferulic acid ≈ rutin. At 50 μ*M* concentration, EGCG and ECG exert a 2-fold greater inhibitory effect than rutin and ferulic acid (21). Acidic nitrite also causes the deamination of DNA bases, adenine and guanine to hypoxanthine and xanthine, respectively. All the catechin polyphenols are effective in suppressing such potentially mutagenic effects (Table IV) (21).

The findings in these model systems have important

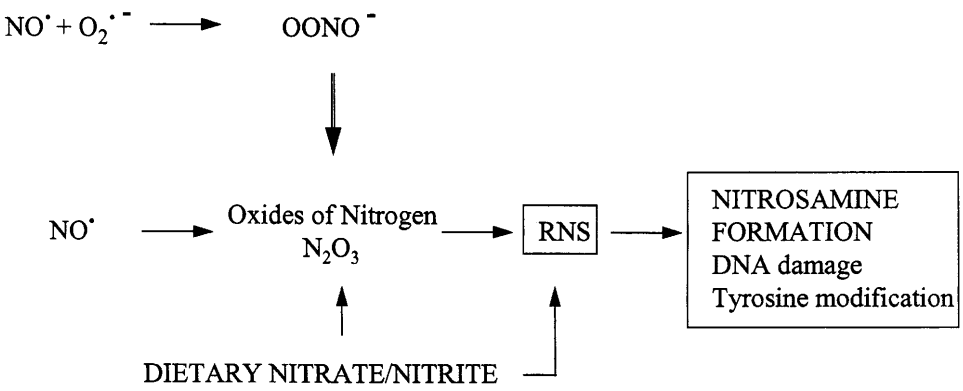


**Figure 7.** Scheme of the potential toxicity of RNS derived from endogenous NO production and dietary nitrate and nitrite.

implications for the efficacy of green tea polyphenols *in vivo* in the acidic conditions of the stomach. The antioxidant effects of tea catechins against nitration and deamination induced by the RNS under these conditions have implications for arresting the damaging effects schematized in Figure 7 and thus for potential chemoprevention in humans. Further studies are required on target cells of the gastrointestinal system and at the *in vivo* level. The ability of phenolics to scavenge RNS derived from acidic nitrite may contribute to the protective effects of tea polyphenols against gastric cancer.

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