Possible Controversy over Dietary Polyphenols: Benefits vs Risks

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Introduction

Dietary phytochemicals including flavonoids, polyunsaturated fatty acids, tocopherols, and others have been the subject of increasing amounts of research for their potential beneficial effects as both disease preventive and therapeutic agents (*1*, *2*). For example, the major polyphenolic compounds in green tea (*Camellia sinensis*, Theaceae), the catechins, have been extensively studied for their cancer preventive activity and have shown activity in a number of animal models of carcinogenesis at organ sites including the lung, prostate, oral cavity, intestine, colon, liver, and skin (*3*). A number of mechanisms have been proposed to account for the disease preventive effects of dietary polyphenols; antioxidative and free radical scavenging activities are often cited (*4*, *5*). Indeed, these compounds have strong antioxidant activities in vitro. Such effects, however, have been harder to demonstrate in vivo and to correlate with disease prevention in vivo.

Whereas diet-derived compounds are generally regarded as safe based on their long history of use in the diet and/or as traditional medicines, it is becoming increasingly apparent that these compounds could have deleterious effects (i) at pharmacological concentrations, (ii) in certain vulnerable populations, and (iii) in certain disease or polypharmaceutical contexts (*6*). Herein, we will review the available data on the toxic potential of polyphenols as a prototypical class of dietary phytochemicals. As specific examples, we will discuss the prooxidative vs antioxidant potentials of tea catechins, the hepatic and intestinal toxicities of high doses of tea catechins, and the potential DNA damaging effects and leukemiogenic activities of flavonoids. We interpret the results of these studies with a consideration of the factors governing the bioavailability of the test compounds. We feel that the bioavailability will ultimately govern the occurrence of toxic events in vivo. More in-depth studies on the potential adverse effects of dietary phytochemicals are required in order to assess the potential toxicities and to determine their potential usefulness as disease preventive and treatment agents. Ongoing human intervention studies should include protocols to assess potential adverse effects including hepatotoxicity.

Dietary Polyphenols: Antioxidant or Pro-oxidant

Dietary polyphenols have been widely touted as antioxidants, and numerous studies have attributed the potential health beneficial effects of these compounds to their antioxidative activities (*7*, *8*). Whereas the antioxidant activities of these compounds have been demonstrated in vitro, it has been much harder to demonstrate a significant antioxidative activity in vivo (*9*−*11*). By contrast, there is an increasing body of evidence to suggest the pro-oxidative effects of polyphenols. Our laboratory and others have demonstrated that the tea catechins, including (−)-epigallocatechin-3-gallate (EGCG),[1] are unstable under cell culture conditions and undergo oxidative polymerization with cogeneration of H_2O_2 (*12*, *13*). This oxidative stress is responsible for some of the observed anticancer activities of EGCG. For example, exogenous catalase completely inhibited EGCG-induced apoptosis in H661 human lung cancer cells (*14*). Treatment of human lung and esophageal cancer cell lines with EGCG resulted in decreased levels of phosphorylated epidermal growth factor receptor (EGFR) and total EGFR protein. These effects were diminished by the addition of superoxide dismutase (SOD) and catalase, which stabilize EGCG (*15*).

These prooxidative activities may have implications regarding potential toxicity. For example, Galanti et al. have reported that treatment of rat hepatocytes with 200 μM EGCG reduced cell viability (*16*). Cell death was associated with increased production of reactive oxygen species and depletion of reduced glutathione (GSH). Treatment with two inhibitors of catechol-*o*-methyltransferase (COMT) enhanced EGCG-mediated cell death, suggesting that COMT is a key enzyme in protecting cells from EGCG-mediated oxidative stress and hepatotoxicity (*16*). Mechanistically, this is due to the fact that methylation occurs primarily at the 4′- and 4″-hydroxyl groups (Figure 1), both of which are possible sites for quinone formation and redox cycling (*5*).

Our laboratory has found two lines of evidence that suggest that EGCG can induce oxidative stress in vivo. First, intraperitoneal administration of EGCG resulted in the formation of two cysteine conjugates of EGCG (EGCG-2′-cysteine and EGCG-2″-cysteine) (*17*). These compounds were only formed at toxic doses of EGCG (200 and 400 mg/kg ip), and we propose that they arise from the formation of an EGCG quinone (Figure 1), which then reacts with the sulfhydryl group on cysteine and likely other cysteine-containing molecules such as glutathione. Second, treatment of lung tumor-bearing nude mice with either daily injections of 40 mg/kg ip EGCG for 40 days resulted in increased expression of phosphorylated histone 2AX (a marker of DNA damage) and metallothionein (a marker of response to oxidative stress) in the liver and tumors relative to vehicle-treated mice (Hou et al., unpublished results). Treatment of tumor-bearing mice with 0.2% EGCG in the diet increased expression of these markers in the tumors but not the liver. Such differences in response may be the result of differential sensitivity and antioxidant capacity between the xenograft tumor and the liver.

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[1]Abbreviations: AML, acute myelogenous leukemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUC, area under the curve; CI, confidence interval; C_{max}, maximal concentration; COMT, catechol-*o*-methyltransferase; EC, epicatechin; EGCG, (−)-epigallocatechin-3-gallate; EGFR, epidermal growth factor receptor; *F*, absolute bioavailability; GCG, (−)-gallocatechin-3-gallate; GSH, reduced glutathione; ig, intragastric administration; *MLL*, mixed-lineage leukemia; OR, odds ratio; po, oral administration; SOD, superoxide dismutase.

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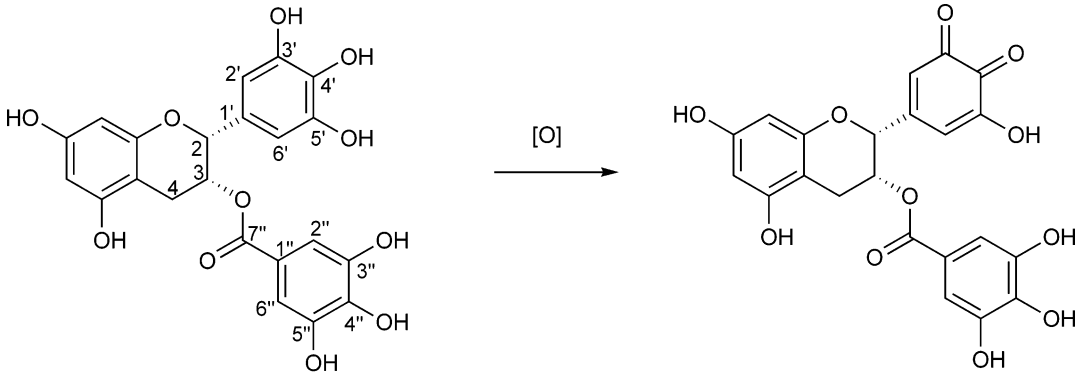

Figure 1. Formation of a reactive EGCG-*o*-quinone.

Hepatic and Gastrointestinal Toxicities of Green Tea Polyphenols

Despite several human studies that showed no toxicity of tea polyphenol preparations and that the major adverse effects associated with consumption of high doses of tea preparations are due to gastrointestinal irritation, there have been a number of recent case reports of hepatotoxicity related to the consumption of high doses of tea-based dietary supplements (10−29 mg/kg/day po) (*18*). In nearly all cases (eight out of nine), patients presented with elevated serum alanine aminotransferase (ALT) and bilirubin levels. In two of nine cases, periportal and portal inflammation were observed. All cases resolved following cessation of supplement consumption. A causative role for the green tea preparations is suggested by the fact that reinjury was observed following rechallenge with the same preparations. The authors of these reports, however, could not conclusively rule out the involvement of potentially hepatotoxic pharmaceutical agents such as acetaminophen or other dietary supplements. One intriguing possibility is that susceptible individuals have a polymorphism in a key biotransformation pathway for the tea polyphenols, such as low activity COMT, which increases exposure to the unmetabolized parent compound. Such possibilities need to be further explored.

Laboratory studies in rodents and dogs have supported the potential toxic effects of high doses of green tea-derived preparations (*16*, *19*). Oral administration (po) of Teavigo (a green tea polyphenol preparation containing 90% EGCG) or Polyphenon E for 13 or nine weeks, respectively, to Beagle dogs resulted in dose-dependent toxicity and death (*19*). Vomiting and diarrhea were observed throughout both studies. In addition, 500 mg/kg, po, Teavigo caused proximal tubule necrosis and elevated serum bilirubin in all dogs treated. Most male dogs (2/3) had elevated serum aspartate aminotransferase (AST) levels. Female dogs (2/3), but not male dogs, had liver necrosis. Although it is likely that the catechins are responsible for the toxicity in the dogs, a role for caffeine cannot be rule out since although Teavigo is caffeine-free, Polyphenon E contains 0.7% caffeine. Oral administration of 2000 mg/kg and intragastric administration (ig) Teavigo to rats resulted in lethality in 80% of animals treated (*19*). Histological analysis revealed hemorrhagic lesions in the stomach and intestine. Intraperitoneal administration of EGCG to mice resulted in dose-dependent lethality beginning at 150 mg/kg (*16*). Lethality was associated with increases in serum ALT levels, suggesting the involvement of hepatotoxicity.

These data suggest that high doses of EGCG can induce toxicity in the liver, kidneys, and intestine. Toxicity, especially in the liver and kidney, appears to be correlated with the bioavailability of EGCG. In the rat, where bioavailability is low [absolute bioavailability (F) = 1.6%], toxicity is confined to the gastrointestinal tract following po (*20*). In the dog, where bioavailability is much higher, hepatotoxicity, nephrotoxicity, and intestinal toxicity were observed. Toxicity was greater in fasted, than in prefed, dogs (*19*). The AUC_{plasma} (area under the curve) in the prefed dogs was 19.8 μg h/mL as compared to 205 μg h/mL in fasted dogs following administration of 300 mg/kg, po. Recent studies in humans have also demonstrated that fasting increases the bioavailability of EGCG (*21*). Although there have been no reports of toxicity in human volunteers enrolled in intervention studies, careful monitoring of liver and kidney function is required until the risk of toxic events associated with tea catechins is established in humans.

Dietary Flavonoids, Effects on DNA, and Fetal Leukemia Risk

In 1994, Ross and Colleagues hypothesized that maternal intake of dietary topoisomerase inhibitors II, including flavonoids, could increase the risk of leukemia in offspring (*22*). A subsequent epidemiological study suggested that a link did indeed exist between maternal consumption of these compounds and acute myeloid leukemia (AML) (*23*). There was a statistically significant positive association (P trend = 0.04) between AML and increasing the consumption of DNA topoisomerase II inhibitor-containing foods (odds ratio [OR] = 9.8, 95% confidence interval (CI) = 1.1−84.8; OR = 10.2 and CI = 1.1−96.4 for medium and high consumption, respectively). No such association was observed between maternal flavonoid intake and acute lymphocytic leukemia. Cell line studies with purified flavonoids (including quercetin, luteolin, and kaempferol) have suggested that the mechanistic basis for this increased risk is inhibition of topoisomerase II activity in the fetus, which results in chromosomal translocation at chromosome 11q23 involving the mixed-lineage leukemia (*MLL*) gene (*24*). The most potent inhibitors were quercetin (25 μM) and fisetin (25 μM).

Although studies have shown that administration of higher doses of purified flavonoids could result in serum levels equivalent to or higher than those necessary to cause chromosomal translocation in cell line studies, it is not clear whether such concentrations are achieved in fetal tissues at normal, dietary flavonoid consumption (*24*). For example, a recent study has reported the pharmacokinetics of orally administered green tea catechins in the plasma and fetuses of pregnant rats (*25*). Following treatment with 55 mg/kg ig green tea extract, epicatechin (EC) had the highest and (−)-gallocatechin-3-gallate (GCG) had the lowest C_{max} (maximal concentration) in the maternal plasma at 9.8 and 0.04 μM, respectively. These levels

were 10 and 50−100 times higher than those in the placenta and the fetus, respectively. EGCG was the most abundant catechin in the placenta (3077.4 pmol/g) and the fetus (159.3 pmol/g). Similarly low fetal plasma concentrations of genistein were observed following repeated administration of 40 mg/kg ig genistein to pregnant Sprague−Dawley rats (*26*). The authors found that whereas the C_{max} for free genistein in the maternal plasma was 137.0 pmol/mL, the levels in the fetal plasma were 43.6 pmol/mL. Genistein was largely present as the glucuronide conjugate in both the maternal and the fetal plasma with C_{max} values of 10438 and 1525.0 pmol/mL. These levels were significantly lower than those required to induce chromosomal translocation in cell culture, and further studies in vivo are required to establish the increased risk, if any, of leukemia due to maternal flavonoid consumption.

Concluding Remarks

Although consumption of dietary phytochemicals such as flavonoids has been suggested to have beneficial biological effects including the prevention of cancer and heart disease, there is considerable evidence to suggest that such compounds are not without risk of adverse effects. The risk of adverse effects is likely increased by the use of pharmacological doses in prevention/treatment and supplement situations and genetic polymorphisms or drug−drug interactions that increase the bioavailability of test compounds. Such situations should be the subject of extensive future animal studies. A clear understanding of the potential adverse effects of dietary phytochemicals, including polyphenols, is necessary. Only when such data are compared to the evidence for beneficial health effects can a balanced judgment be made regarding the potential utility of these compounds for disease prevention and treatment.

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