Preclinical Efficacy Studies of Green and Black Tea Extracts (44367)

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Tea (*Camellia sinensis*) is one of the most popular beverages consumed worldwide. Tea leaves are primarily manufactured as green, black, or oolong, with black tea representing approximately 80% of tea products consumed. Green tea is the nonoxidized/nonfermented product and contains several polyphenolic components such as epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate (EGCG). EGCG is the major green tea polyphenol (GTP) (>40% dry weight). The major components of black tea (the fermented product) are theaflavins (1%–2% dry weight) and thearubigins (10%–20% dry weight). Theaflavins, which determine the quality and flavor of the tea, are formed by oxidation of quinones derived from the epicatechins. Thearubigins are highly colored flavonol oxidation products that are often bound to peptides or proteins. Oolong tea is the partially oxidized/fermented product that retains a considerable amount of the original polyphenolic material (1).

In the National Cancer Institute (NCI) testing effort, tea extracts, tea polyphenol–enriched fractions, isolated catechin fractions, and a theaflavin-rich fraction were used (Table I). The black and green tea extracts were tested in both caffeinated and decaffeinated forms. The percentages of polyphenols in both green and black teas are approximately the same; however, levels of EGCG and caffeine in green tea are 4- to 5-fold that of black tea. Theaflavins are unique to black tea due to the oxidation process.

In an effort to determine the cancer chemopreventive activity of tea compounds, the NCI Chemoprevention Branch initially conducted *in vitro* mechanistic assays and *in vitro* cell transformation assays to screen for efficacy of these compounds. The mechanistic assays measure: a) inhibition of DNA adduct formation; b) inhibition of free radical formation; and c) enhancement of glutathione (GSH), glutathione-*S*-transferase (GST), ornithine decarboxylase (ODC), and NAD(P)H:quinone reductase (QR) activity. The *in vitro* assays measure: a) inhibition of morphological transformation in rat tracheal epithelial (RTE) cells; b) inhibition of anchorage independence in human lung tumor (A427) cells; and c) inhibition of hyperplastic alveolar nodule formation in mouse mammary organ cultures (MMOC). Following the observation of significant *in vitro* activity, animal efficacy studies in the rat azoxymethane (AOM)-induced colon and N-nitrosomethylbenzylamine (NMBA)-induced esophageal carcinogenesis models were begun (2–5) with these compounds.

Chemopreventive Activity of the Tea Compounds in the *In Vitro* Mechanistic Studies

Significant inhibitory activity was also observed in the *in vitro* mechanistic assays measuring inhibition of formation of DNA adducts, free radicals, and ODC and enhancement of GSH, GST, and QR.

In the DNA adduct inhibition assay, significant inhibitory activity was observed with BTE, BTP, the theaflavins, EGCG, and other catechins; GTP was less active than these compounds. In the free radical formation inhibition assay, BTE, the theaflavins, GTE, GTP, epicatechin gallate, and epigallocatechin were effective. GTE, GTP, and the catechins, except for EGCG, demonstrated significant activity in the ODC assay.

Several tea extracts were positive in the GST assay including BTE, BTP, GTP, epicatechin gallate, epigallocatechin, and EGCG; only two of the extracts (BTP, GTE) were effective in increasing the GSH levels. Most of the tea compounds, except for BTE, theaflavins, and EGCG, were positive in the QR assay.

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Table I. Constituents of Tea Tested by the NCI, Chemoprevention Branch

Compound[a]	Polyphenol fraction (%)	EGCG (%)	Theaflavins (%)	Caffeine (%)	Unknown (%)
BTE, caffeinated	44.94	2.95	2.15	5	44.96
BTE, decaffeinated	41.9	2.67	3.39	<0.1	52.04
GTE, caffeinated	44.06	11.16	—	0.56	44.22
GTE, decaffeinated	44.72	10.33	—	<0.1	44.95
BTP	32 (unknown polyphenols) 2 (nonepicatechins) 5 (flavonol glycosides) 16 (other gallates) 9 (catechins)	17	8	1.06	—
GTP	2 (nonepicatechins) 13 (other polyphenols) 1 (flavonol glycosides) 36 (catechins)	43	—	0.82	—
Theaflavin Mixture	—	—	30.59 (theaflavin gallate A) 19.45 (theaflavin gallate B) 32.17 (theaflavin digallate) 7.65 (other theaflavins)	—	6.14 4 (water)
Epicatechin (EC) MW: 290.28, purity: 98% Epigallocatechin (EGC) MW: 306.28, purity: 95% Epicatechin gallate (ECG) MW: 442.28, purity: 91.67% Epigallocatechin gallate (EGCG) MW: 458.28, purity: 92.3%					

[a] Freeze dried, hot water extract.

Chemopreventive Activity of the Tea Compounds in the *In Vitro* Studies

Several tea compounds including the catechins were tested in the RTE, A427, and MMOC assays. These assays measure the ability of the potential chemopreventive agents to inhibit benzo(*a*)pyrene [B(*a*)P]-induced malignant cell transformation, reduce phenotypic characteristics of transformation (anchorage independence), and prevent dysplasia in 7,12-dimethylbenz(*a*)anthracene (DMBA)–treated mouse organ cultures, respectively.

In the RTE assay, BTE, GTE and their decaffeinated extracts, BTP, GTP, EGCG, and epicatechin gallate were positive. BTE (decaffeinated) was the most effective agent tested. In the A427 assay, all the tea compounds except for GTE and its decaffeinated extract were positive. The most effective components were the theaflavins. BTE, BTE, and GTE decaffeinated extracts, GTP, and EGCG were positive in the MMOC assay; the most effective were BTE and its decaffeinated extract.

Chemopreventive Activity of Tea Compounds in the *In Vivo* Studies

In the rat NMBA esophageal carcinogenesis model, the tea compounds were administered in the drinking water at concentrations of 360 and 1200 ppm for 2 weeks before administration of the carcinogen (subcutaneously, three times weekly for 5 weeks) for 25 weeks (4). At the end of the study (Week 25), tumor multiplicity was significantly ($p < 0.05$) reduced in the group receiving 1200 ppm theaflavins. At 20 weeks, similar results were obtained in the high-dose (1200 ppm) EGCG group. High-dose (1200 ppm) BTP, GTP, and EGCG also reduced tumor multiplicity significantly ($p < 0.05$) at 15 weeks. Tumor incidence was not affected by any of the tea compounds at any time during the study.

In the rat AOM-induced colon carcinogenesis model, none of the tea compounds was effective (5). In this model, F344 rats received daily administration of 360 or 3600 ppm black or green tea extracts (BTE, GTE); 360 or 1800 ppm EGCG; 360 or 1800 ppm black tea polyphenol (BTP); or GTP in drinking water followed by two subcutaneous doses of 15 mg/kg AOM. The proposed explanation of these results is lack of activity of the tea compounds on cytochrome P450 2 E1, which is involved in the metabolism of AOM.

However, the chemopreventive activity of tea compounds was also evaluated in an AOM-induced colon model that measures the occurrence of premalignant lesions (ACF) (Table II). In this model, low-dose BTP (360 ppm in drinking water) and high-dose EGCG (1200 ppm in drink-

Table II. Effects of Tea Extracts on Azoxymethane-Induced Colon Crypts

Group	Dose	Route	Result
BTE	4.5 g/kg diet	Diet	NE
	9.0 g/kg diet	Diet	NE
BTP	360 ppm	Water	+(26% reduction)[a]
	1200 ppm	Water	±(14% reduction)
GTE	4.5 g/kg diet	Diet	NE
	9.0 g/kg diet	Diet	NE
GTP	360 ppm	Water	NE
	1200 ppm	Water	±(16% reduction)
EGCG	360 ppm	Water	±(17% reduction)
	1200 ppm	Water	+(35% reduction)[a]

[a] $p < 0.05$ (Wargovich *et al.* Unpublished data.)

ing water) inhibited the formation of ACF by 26% and 35%, respectively (2).

Further Development of the Tea Compounds

Results from these and other studies indicate the promising potential of the tea compounds for further development as cancer chemopreventive drugs in human trials. Currently, these compounds are under evaluation in the 28- and 90-day rat and dog toxicology studies. Phase I, II, and III clinical studies are being planned with the tea compounds. These include Phase I studies with BTP, GTP (*e.g.*, Polyphenon E), and EGCG, and Phase II and III trials in cohorts at high risk for colon, lung (smokers), esophageal, and skin (actinic keratosis) cancer.

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