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GREEN TEA AND TEA POLYPHENOLS IN CANCER PREVENTION

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1. ABSTRACT

The cancer-preventive effects of green tea and its main constituent (-)-epigallocatechin gallate [(-)-EGCG] are widely supported by results from epidemiological, cell culture, animal and clinical studies in the recent decade. *In vitro* cell culture studies show that tea polyphenols potently induce apoptotic cell death and cell cycle arrest in tumor cells but not in their normal cell counterparts. Green tea polyphenols affect several signal transduction pathways, including growth factor-mediated, the mitogen-activated protein kinase (MAPK)-dependent, and ubiquitin/proteasome degradation pathways. Epidemiological studies have suggested that the consumption of green tea lowers the risk of cancer. Various animal studies have revealed that treatment by green tea inhibits tumor incidence and multiplicity in different organ sites such as skin, lung, liver, stomach, mammary gland and colon. Phase I and II clinical trials were carried out recently to explore the anticancer effects of green tea in patients with cancer. At this time, more mechanistic research, animal studies, and clinical trials are necessary to further evaluate the role of green tea in cancer prevention.

2. INTRODUCTION

Annually, more than 5 million people are diagnosed with cancer and more than 3.5 million people die from cancer worldwide (1). In the United States, more than 1 million people are diagnosed with cancer and more than

½ million deaths are attributable to cancer per annum (2). When analysis of cancer incidence by racial group is performed for many types of cancers, Asian and islander populations have significantly reduced incidence and mortality due to cancer that seems to correlate with dietary intake of green tea and soy (3-9). The attraction of green tea as a cancer chemopreventative and/or a chemotherapeutic agent is self-evident. Tea consumption is not associated with toxic effects. Populations that practice extensive tea consumption have demonstrated reduced incidence and mortality due to cancer. The principle components of tea exhibit a wide array of cancer preventing activities (3-9).

The history of tea began in ancient China over 5,000 years ago. Teas of all kinds are the most widely consumed beverages in the world today, consumed by 1/3 of the world's population. Green tea, black tea, oolong tea are all derived from the *Camellia sinensis* plant. All of these teas contain a variety of compounds, the most significant of which are the polyphenols. The differences between green, black, and oolong tea lie in the fermentation process. Green tea undergoes no fermentation, while black tea is completely fermented, and oolong tea contains both a mixture of fermented and non-fermented leaves. The major polyphenols of green tea include (-)-epigallocatechin-3-gallate [(-)-EGCG], (-)-epigallocatechin [(-)-EGC], (-)-epicatechin-3-gallate [(-)-ECG], and (-)-epicatechin [(-)-EC] (see Figure 1). Of these, (-)-EGCG is the most abundant and has been extensively studied and implicated as a cancer preventative agent (3, 10). In addition, (-)-EGCG, in particular, is known to inhibit telomerase, urokinase, nitric-oxide synthase, tumor necrosis factor alpha, the proteasome (11-15), and others. The total amount of these polyphenols in green tea varies between 15 to 25% (dry weight basis) (16). Many chemical changes, among which are the oxidation of the polyphenols to the theaflavins and other oligomers, take place during the tea fermentation processes (16). As a result, the amount of (-)-EGCG in black tea is greatly reduced, which may account for the differences in the biological activities between green and black teas (16).

This review will examine studies using green tea, from *in vitro* cell models, animal studies, epidemiological evidence, to clinical trials. While the exact mechanisms of action and targets of (-)-EGCG and the other polyphenols remain to be elucidated, we will review numerous potential candidates. Possible molecular mechanisms include inhibition of tumor growth (17), angiogenesis (18, 19), metastasis (20), cell cycle arrest (15, 21), and apoptosis induction (15, 21, 22). The potential targets include: p53 (23), Bcl-X_L (24), vascular endothelial growth factor (25, 26), heterogenous nuclear ribonucleoprotein B1 (27), NF-κB (28), the Ras-MAP kinase pathway (29), and proteasome (15, 21, 22).

3. ANIMAL STUDIES

A growing amount of evidence from studies in laboratory animal models demonstrates that green tea and its components, green tea polyphenols (GTP), have an inhibitory effect on carcinogenesis in different animal models and different organ sites. These studies have been conducted in a wide variety of organs including the skin, liver, and lung. Other studies have been conducted using biomarkers or cancer-specific proteins, rather than just tumor response (30-36).

3.1. Green tea and skin cancer

The preventative potential of tea on skin cancer has been extensively studied in animals. Carcinogens mostly used to generate rodent skin carcinoma are ultraviolet A or B light (UVA or UVB) or 7,12-dimethylbenz[a]anthracene (DMBA) as an initiator and 12-*O*-tetradecanoylphorbol- 13-acetate (TPA) or mezerein (MEZ) as a promotor.

In one experiment, SKH-1 mice were exposed to UVB light (30 mJ/cm^2) twice weekly for 22 weeks, followed by oral administration of green tea (6 mg tea solids/ml) for an additional 23 weeks (37). It was found that green tea decreased the incidence of skin tumor formation from 89% (in water-fed control group) to 61% (in green tea-fed group) in the UVB-pretreated high-risk SKH-1 mice, decreased the number of tumors/mouse from 6.9/mouse (in control group) to 3.3/mouse (in green tea-fed group), and decreased tumor diameter by 28%. Decaffeinated green tea was less effective inhibitor of incidence of skin tumor formation (75% incidence, compared with 61% in green tea-fed group), but it decreased the tumor size. Adding caffeine back to the decaffeinated teas restored the inhibitory activity (37).

The effects of topical application of GTP to the skin of DMBA-initiated SEN-CAR mice were also investigated (38). The results of this study showed that topical application of GTP (6 mg/animal) to the skin of DMBA-initiated mice 30 minutes prior to promotion by TPA or MEZ resulted in significant protection against skin tumor formation in terms of tumor incidence (32–60%), multiplicity (49–63%) and tumor volume/mouse (73–90%). Results from other studies have also demonstrated that green tea or tea polyphenols potently inhibit skin carcinogenesis in animal models (39-42).

3.2. Green tea and hepatocarcinogenesis

The inhibitory effect of green tea on hepatocarcinogenesis in animal models has also been investigated. It was reported that the incidence of hepatocellular tumors was 73.3% in mice given pentachlorophenol (PCP) as carcinogen at concentration of 600 ppm for 23 weeks following treatment with the initiator diethylnitrosamine (DEN) treatment at 20 ppm for 8 weeks (43). However, in the group of mice given the green tea infusion both before the start of the PCP treatment and until the end of the experiment, the incidence of hepatocellular tumors decreased to 33.3%. A study on implanted tumor animal models demonstrated that dietary powdered green tea significantly reduced the solid tumor volume and weight in a time-dependent manner (44). The absolute weight of solid tumors in the green tea group was lowered by 27% (44). The hepatoma-induced endogenous

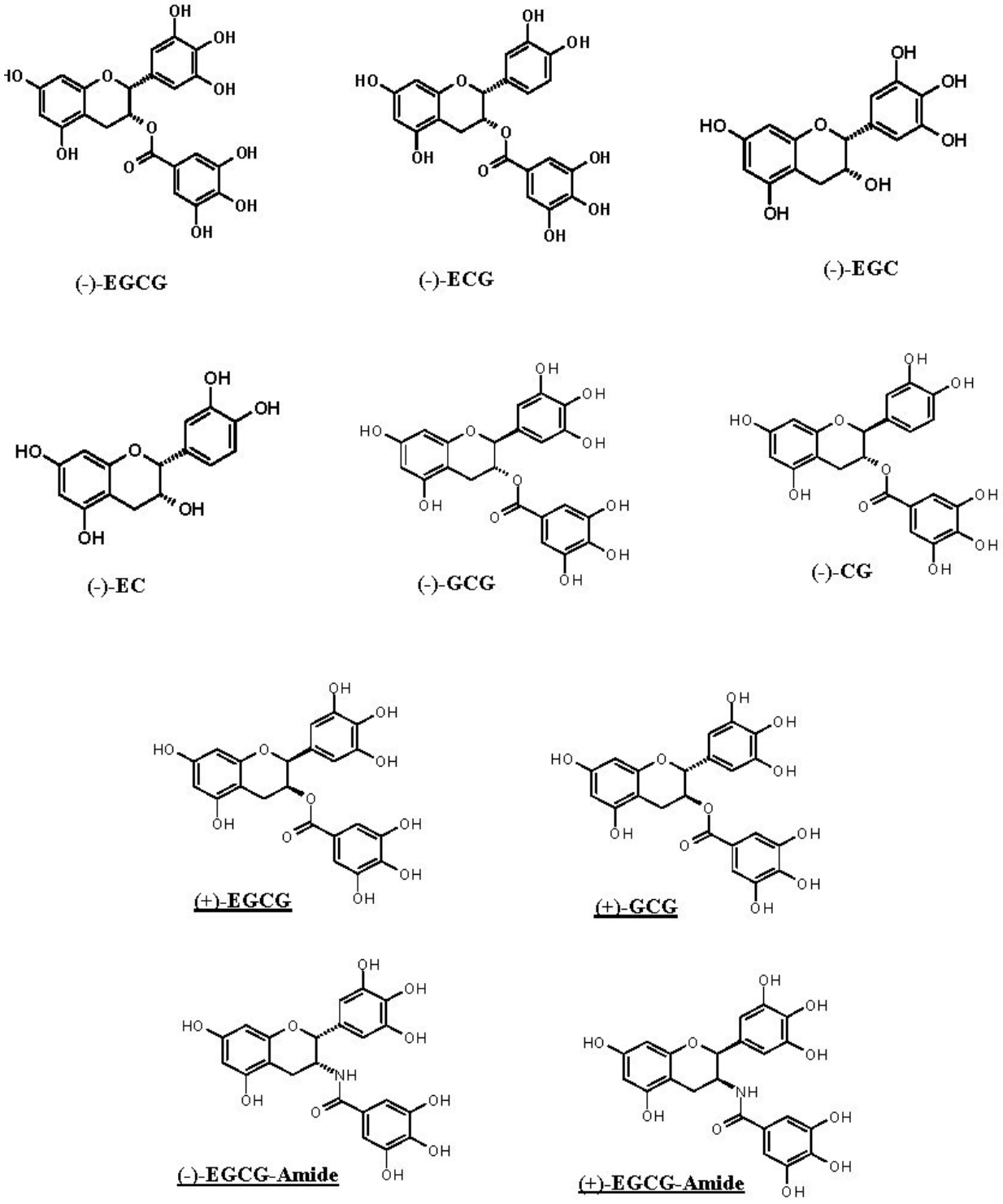

Figure 1. Structures of natural and synthetic green tea polyphenols. The names of the synthetic green tea polyphenols are underlined to distinguish them from the natural compounds.

hyperlipidemia, characterized by rises in serum cholesterol (hypercholesterolemia) and triglyceride (hypertriglyceridemia) levels, was significantly suppressed by powdered green tea treatment as well. The inhibitory effect of (-)-EGCG on spontaneous hepatoma in C3H/HeNCrj mice was also investigated (34). (-)-EGCG reduced the incidence of hepatoma-bearing mice from 83.3% (control) to 56.0% (0.05% EGCG) and 52.2% (0.1% EGCG), and also reduced the average number of hepatomas per mouse from

1.83 (control) to 0.72-0.91 (EGCG groups) at week 65 (34).

3.3. Green tea and lung cancer

The study of the effects of green tea infusion on the spontaneous formation of lung tumors and rhabdomyosarcomas in A/J mice demonstrated that the mice given 1% green tea exhibited a significantly lower lung tumor multiplicity from 0.72/mouse to 0.41/mouse (45). For the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumorigenesis in A/J mice, 2% green tea or 560 ppm of (-)-EGCG in drinking water for 13 weeks decreased lung tumor multiplicity from 22.5 lung adenomas per mouse to 12.2 ($P< 0.01$) and 16.1 ($P <0.05$), respectively (46).

3.4. Green tea and multi-organ cancers

In multi-organ carcinogenesis animal models, the cancer chemopreventive effects of water extract of green tea and GTP against N-nitrosodiethylamine (DEN)- and benzo[a]pyrene (BP)-induced forestomach and lung tumorigenesis in A/J mice (35) have been tested. The evidence in this study showed that oral feeding of 0.2% GTP in drinking water to mice decreased 39-66% and 68-82% of the percentage of mice with tumors in forestomach induced by DEN- and BP, respectively. In case of pulmonary tumor multiplicity caused by DEN and BP, the protective effects of GTP were between 38-43 and 25-46%, respectively (35).

In another study of multi-organ carcinogenesis animal models (47), male C3H mice were given decaffeinated green or decaffeinated black tea in their drinking water prior to, during, and after carcinogen treatment with diethylnitrosamine (DENA, 50 mg/kg i.p., once per week for 8 weeks). After 40 weeks of tea treatment, mice treated with both DENA and tea displayed a significant decrease in the mean number of lung and liver tumors compared to DENA-only treated animals. Mice receiving DENA and either 0.63 or 1.25% green tea or 1.25% black tea showed a decrease in the mean number of lung tumors of 40, 46, and 34%, respectively, compared with DENA-only treated mice. Mice that received 0.63 or 1.25% green tea or 1.25% black tea exhibited a reduction in liver tumor numbers of 54, 50, and 63%, respectively. This study showed a dose-dependent chemoprevention of both lung and liver tumors by both green and black tea in DENA-treated C3H mice (47).

3.5. Biomarkers in animal studies using green tea

Studies on tumors serve well to examine the anti-tumor effects of a compound. However, cancer preventative agents may not possess the potency necessary to eliminate tumors so much as preventing tumors from forming initially. From a preventative point of view changes in cancer-specific proteins and/or enzymes indicative of cancer formation or metastasis are preferable to studies focused directly on tumors. Use of biomarkers provides information on the pre-tumor state effects of a given agent that is a better indicator of the agent's potential as a cancer preventative. This has been especially true with green tea polyphenols.

3.5.1. Connexin32 (Cx32) and gap junctional intercellular communication (GJIC)

Gap junctions are channels in the plasma membrane composed of connexons, which are hexamers of connexins (Cxs). These junctions allow for the exchanging of ions and small molecules, including sugars, nucleotides, amino acids, and the second messenger cAMP between adjacent cells (48). It has been suggested that a loss of intercellular communication *via* gap junctions may contribute to multistage carcinogenesis (49). Data shows that one-year-old male and female mice deficient for Cx32, a major Cxs molecule expressed in the liver, had 25-fold more and 8-fold more spontaneous liver tumors, respectively, than wild-type mice. The results suggest that blocking down-regulation of GJIC could be critical for preventing tumor promotion (50). Sai *et al*. explored the potential preventive effects of green tea against the promoting action of PCP in mouse hepatocarcinogenesis and examined whether drinking green tea prevents down-regulation of GJIC inhibition in the liver caused by tumorigenic doses of PCP (51). The results showed that GJIC was inhibited by 45 and 60% in livers of the mice treated by 300 ppm [PCP (L)] and 600 p.p.m [PCP (H)], respectively, compared with the control group. However, in the one week pretreatment group plus 2 weeks of co-treatment of green tea with PCP, the inhibition of GJIC by PCP was only reduced ~10% of the control with both PCP (L) and PCP (H), compared with control group. The authors further investigated the effect of green tea on Cx32 levels in livers of mice and found that PCP treatment reduced the density of Cx32 plaques compared with the high density of plaques observed in the control, and co-treatment with green tea significantly prevented the reduction in Cx32 plaques in the liver compared with the PCP treatment alone (51). These findings suggest that Cx32 could be one of the biomarkers for evaluating effects of chemo-prevention and treatment in hepatoma animal models.

3.5.2. Ornithine decarboxylase (ODC)

ODC is the rate-limiting enzyme of the polyamine pathway and is overexpressed in prostate cancer (52). Prostatic fluid in humans could serve as the target for prevention and therapy of human prostate cancer (52) because the prostate is known to secrete ODC (53). It has been found that androgens are important for the regulation of ODC activity in the prostate and that androgenic stimulation regulates the development and growth of both normal and tumorigenic prostate cells (54, 55). Gupta *et al*. reported that the administration of testosterone (10 mg/kg body weight, i.p.) to sham-operated and castrated Cpb:WU rats resulted in up to 38-fold increases in ODC activity, respectively, in the ventral prostate (4). Oral feeding of 0.2% GTP in drinking water for 7 days before testosterone administration resulted in 20 and 54% decreases in testosterone-caused induction of ODC activity in sham-operated and castrated rats, respectively. Similar results were obtained with C57BL/6 mice, where testosterone treatment at a similar dosage resulted in an increase in ODC activity in the ventral prostate and prior oral feeding with

0.2% GTPs resulted in 40% inhibition of ODC activity induction (4).

3.5.3. Proliferating cell nuclear antigen (PCNA)

Proliferating cell nuclear antigen (PCNA) plays an essential role in nucleic acid metabolism as a component of the replication and repair machinery, a requisite auxiliary protein for DNA polymerase δ-driven DNA synthesis, and a cell cycle regulatory protein (56). The effect of GTP on expressed levels of PCNA in transgenic adenocarcinoma of the mouse prostate (TRAMP) model was investigated (57). The results of this study revealed that all 10 mice (100%) in the water-fed control group developed severe prostate cancer with marked local invasiveness in the abdominal region, which was assessed by abdominal pelvic palpation and MRI. In contrast, only 3 of the 10 (30%) GTP-infused TRAMP mice developed palpable tumors. Further, the authors studied the effect of GTP infusion on the metastases to different site organs. The cumulative data at the termination of the experiment (32 weeks of age) from 20 animals in the water-fed group showed 100% invasive tumors, which metastasize to lymph (95% animals), lungs (65% animals), liver (40% animals), and bone (25% animals). In sharp contrast, the 20 mouse in the GTP-infused group exhibited no metastases to any of the organs studied. Western-blot analysis showed that 0.1% GTP (w/v) provided as the sole source of drinking fluid to TRAMP mice resulted in marked reduction in the protein expression of PCNA in the prostate compared with water-fed TRAMP mice (57).

3.5.4. Insulin-like growth factor (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3)

The insulin-like growth factors (IGFs) are mitogens that play a pivotal role in regulating cell proliferation, differentiation, and apoptosis (58). High circulating levels of insulin-like growth factor-I (IGF-I) is associated with increased risk for several common cancers, including breast, prostate, lung, and colorectum (59-62). The level of IGF-binding protein-3 (IGFBP-3), a major IGF-I-binding protein in serum that suppresses the mitogenic action of IGF-I, is inversely associated with the risk of these cancers (58). The effect of oral infusion of GTP on the level of IGF-I and IGFBP-3 in serum of TRAMP mice was investigated (57). The results demonstrated that levels of IGF-I in serum were significantly lowered in GTP-infused TRAMP mice compared with water-fed TRAMP mice. Serum IGFBP-3 levels that were lower in water-fed TRAMP mice were significantly restored in GTP-infused TRAMP mice (57, 58). These findings suggest that IGF-I/IGFBP-3 could serve as the target for prevention and therapy of human prostate cancer.

4. EPIDEMIOLOGICAL STUDIES

Epidemiological evidence regarding the association of green tea consumption to reduced cancer incidence is promising. Most of the studies have consistently shown that green tea polyphenols may inhibit the induction of a variety of cancers.

4.1. Digestive tract cancers

A case-control study design in Shanghai, China comprised of 190 cases of gastric and 42 cases of esophageal cancers, that occurred in 18244 men aged 45-64 years at recruitment with 12 years of follow-up, and 772 cohort controls (63). This study design was used to investigate the association between prediagnostic urinary tea polyphenol markers and subsequent risk of gastric and esophageal cancers. Urinary tea polyphenols, including EGC, EC and their respective metabolites including tri- (M4) and di-hydroxyphenol valerolactone (M6), were measured in all study subjects. Data were analyzed by standard set methods. Overall there was no association between the risk of gastric or esophageal cancer and positivity for any of the four urinary tea polyphenols. However, EGC displaced an evident inverse association with risk of gastric cancer alone or gastric and esophageal cancer combined as duration of follow-up lengthened. This study suggests that tea polyphenols may act as chemopreventive agents against gastric and esophageal cancer development (63).

Although more relevant case-control and cohort studies show mixed results, several investigations point to the possibility of lowered risks of digestive tract cancers among tea drinkers, especially those consuming green tea (64). With a large series of stomach cancer cases, a population-based case-control study in Shanghai, China, found that green-tea consumption was associated with a lower risk of stomach cancer (65). A population-based case-control study in Yangzhong, China, with 133 stomach cancer cases, 166 chronic gastritis cases and 433 healthy controls, observed an inverse association between green tea drinking and chronic gastritis and stomach cancer risks (66). A comparative case-referent study at Aichi Cancer Center in Japan, comprised of 185 esophagus, 893 stomach, 362 colon, and 266 rectum cancer cases matched with 21,128 non-cancer controls, showed that the risk of stomach cancer decreased among green tea drinkers (7 cups or more per day), suggesting the potential for protective effects against site-specific digestive tract cancer by consumption of green tea (67). Some epidemiological studies on tea drinking and stomach cancer do not justify the claims that green tea exhibits a cancer-protective effects (68). However a protective effect on the development of colon cancer is suggested (68). A number of epidemiological studies conducted in diverse populations have investigated the effect of tea consumption on gastric/esophageal cancer development in China and Japan, where green tea is preferred. The study suggested a protected effect of green tea consumption on gastric/esophageal cancer risk and a decrease in this risk associated with increased green tea consumption (65, 66, 69-74).

4.2. Pancreatic cancer

A large population-based case-control study in Shanghai, China, included 931 colon, 884 rectum and 451 pancreas cancer cases matched with 1552 controls, found an inverse association with these types of cancer and increasing amounts of green tea consumption, with rectal and pancreatic cancers showing the strongest trends (75). The inverse association appeared to be stronger among women than men, but dose-response trends were evident for both sexes (75). A case-control study in Hokkaido,

Japan, comprised of 72 patients with pancreatic cancer who were matched to 142 controls by both sex and age, showed a significant decrease in risk, associated with consumption of green tea and raw vegetables (76). However, another multi-institute, hospital-based, case-control study on pancreatic cancer in Tokyo, Japan demonstrated that drinking green tea (5 cups or more per day) was positively associated with the risk of developing pancreatic cancer (77). Thus, further studies are required to determine the exact effects of green tea in regards to pancreatic cancer.

4.3. Breast cancer

A study in Los Angeles County of green tea drinkers showed a significantly reduced risk of breast cancer compared to non-green tea drinkers (7). It was observed that the risk of breast cancer was lowest among those who drank green tea only, intermediate among those who drank both green and black tea, and unchanged among those who drank black tea only (7). Two hospital-based studies from Japan suggested that green tea may favorably influence the risk of breast cancer recurrence (78, 79). A prospective cohort study in Saitama, Japan, involving a total of 472 breast cancer patients who were classified into stages I, II and III and who consumed 2-8 cups per day of green tea, found that among stages I and II patients, the group consuming 8 cups per day showed a lower recurrence rate, and a longer disease-free period than those consuming 2 cups of green tea per day. However, the stage III cancer patients did not show any significant associations (80).

4.4. Prostrate cancer

Geographical observations suggest that the incidence of prostate cancer is lower in Japanese and Chinese populations that consume green tea on a regular basis (55). It has been reported that drinking 6 cups of green tea per day significantly inhibits prostrate cancer development and metastasis (55). A case-control study was conducted in Southeast China during 2001-2002 that included 130 cases of prostatic adenocarcinoma and 274 controls without prostate cancer or any other malignancies (81). Among the cases, 55.4% were green tea drinkers compared to 79.9% for the controls. The results indicate that prostate cancer risk declined with increasing frequency, duration and quantity of green tea consumption (81).

4.5. Ovarian cancer

A case-control study in China during 1999-2000 that involved 254 cases of epithelial ovarian cancer and 652 controls found significant dose-response relationships and an inverse association between ovarian cancer and green tea consumption (82). These results indicated that increasing frequency and duration of tea drinking, especially green tea, can reduce the risk of ovarian cancer (82). It is also interesting that serous cell ovarian cancer appeared to have a stronger inverse association with green tea consumption than the other types of ovarian cancer (82).

4.6. Bladder cancer

A follow-up study of 258 urinary bladder cancer patients in Nagoya, Japan, did not find any significant associations between drinking green tea and the risk of developing bladder cancer (83). Two other population-based studies also observed no statistically significant association between the consumption of green tea and the risk of bladder cancer (84). However, a separate case-control study in Taiwan, comprised of 40 cases of bladder cancer and 160 matched controls, suggest that tea (black and/or green tea) consumption may be associated with increased bladder cancer risk (85).

4.7. Lung cancer

A population-based case-control study identified 649 cases of primary lung cancer among women matched with a randomly selected control group of 675 women from the Shanghai Residential Registry (86). It was observed that among nonsmoking women, consumption of green tea was associated with a reduced risk of lung cancer, and the risks decreased with increasing consumption of green tea (86). However, a retrospective study of 200 female lung cancer patients and 200 matched controls from the Chinese population in Hong Kong demonstrated a statistically significant increase in lung cancer risk among those who drank green tea possibly due to presence of mutagens in tea and other ingestants and inhalants in human cancer etiology (87).

4.8. Others

Finally, a prospective cohort study of a Japanese population comprising 8,552 individuals over 40 years of age with daily consumption of green tea ($\leq$ 3 cups, 4-9 cups, $\geq$ 10 cups), identified 384 cancer cases (male, 220; female, 164) during the 9 years of follow-up study (71,248.5 person-years) (88). There was a slowdown in increase of cancer incidence, especially among females, drinking $\geq$ 10 cups a day, indicating that the greater the green tea consumption, the later the onset of cancer (88).

5. GREEN TEA AND CLINICAL TRIALS

All research findings from tumor cell cultures, animal models, and epidemiological studies have shown the potent effects of green tea and tea polyphenols in cancer prevention. However, this should be confirmed in clinical trials in order to gain more knowledge of the relationship between green tea consumption and cancer chemoprevention and treatment.

The beneficial effects on humans were also evaluated in clinical trials. In 1997, the US Food and Drug Administration (FDA) granted permission for a Phase I clinical trial with green tea capsules. The purpose of this trial was to determine the maximum-tolerated dose, toxicity, and pharmacology of oral green tea extract once or three times daily. A total of 49 cancer patients were studied (89). There were two treatment regimens in this clinical trial: 0.5 to 5.05 g/m^2 once daily dose and 1.0 to 2.2 g/m^2 three-times-a-day for six months. Mild to moderate toxicities were seen at most dose levels, and dose-limiting toxicities were caffeine-related, including neurologic and gastrointestinal effects (89). The maximum-tolerated dose was 4.2 g/m^2 once daily or 1.0 g/m^2 three times daily. This clinical trial concluded that a dose of 1.0 g/m^2 three times

daily is recommended for future studies and that oral green tea extract at the doses studied can be taken safely for at least 6 months (89).

Another phase I clinical trial with green tea was initiated to assess the potential for green tea to be used as a colorectal cancer chemopreventive agent (90). After the volunteers drank 0.6, 1.2, or 1.8 g of green tea powder dissolved in warm water, rectal biopsies were obtained at 4, 8, and 24 h. As a colorectal carcinogenesis biomarker, prostaglandin E2 (PGE2) levels were analyzed by ELISA assay in rectal mucosa. The results showed that 10 of 14 subjects (71%) demonstrated a response to green tea by at least 50% inhibition of PGE2 levels at 4 h (90). The data demonstrated that green tea constituents have biological activity in inhibiting PGE2 synthesis, suggesting a study of green tea as a colorectal chemopreventive agent in more long-term Phase II trials.

The result of a phase II trial to explore anti-cancer effects of green tea in patients with androgen-independent prostate carcinoma was reported in 2003 (91). In this trial, patients were instructed to take 6 grams of green tea per day orally in 6 divided doses and were monitored monthly for response and toxicity. The result of this study showed that tumor response, defined as a decline ≥50% in the baseline prostate-specific antigen (PSA) value, occurred in a single patient, or 2% of the cohort. This trial concluded that green tea carries limited antineoplastic activity, as defined by a decline in PSA levels, among patients with androgen-independent prostate carcinoma. However, the patients selected in this clinical trial were androgen-independent metastatic prostate carcinoma. Data in this study demonstrated that drinking green tea could not inhibit the progression of this type of prostate carcinoma at this late clinical stage (91). It is therefore important to assess whether green tea has an inhibitory or chemopreventive effect on other cancers at various clinical stages.

A double-blind intervention trial was reported in 1999 (92). In this trial, 59 patients of both sexes, mostly smokers with oral leukoplakia and a preneoplastic lesion, were randomly divided into treated (3 g mixed tea oral administration and topical treatment) and control (placebo and glycerin treatment) groups. Results showed that oral lesions were significantly reduced in 38% of the 29 patients treated with the tea mixture, as compared with 10% of the 30 patients in the control group. The incidence of micronucleated, exfoliated oral mucosa cells in the tea-treated group (5.4 per 1000 cells) was lower than that in the control group (11.3 per 1000 cells) ($P < 0.01$). This study also reported that the micronuclei and chromosome aberration rate in the peripheral blood lymphocytes showed the same results (92). In pathological examinations, there were significant differences ($P < 0.05$) in the number and total volume of the silver-stained Nucleolar Organizer Regions and the proliferating index of PCNA in oral mucosa cell nuclei between the tea-treated group and the control group. This indicates that cell proliferation decreased in the treated patients (92). The findings of this study provided valuable direct evidence on the protective effects of tea in oral cancer.

Another phase II trial was reported in 2003 (93). This randomized controlled tea intervention trial was designed to study the effect of high consumption (4 cups/day) of decaffeinated green or black tea on oxidative DNA damage as measured by urinary 8-hydroxydeoxyguanosine (8-OHdG) among smokers over a 4 month-period. A total of 143 heavy smokers, aged 18-79 years old, were randomized to drink green tea, black tea, or water. Levels of plasma and urinary catechins and urinary 8-OHdG were measured monthly. A total of 133 of 143 smokers completed the 4-month intervention. Assessment of urinary 8-OHdG after adjustment for baseline measurements and other potential confounders revealed a highly significant decrease in urinary 8-OHdG (31%) after 4 month of drinking decaffeinated green tea ($P = 0.002$) (93). These data suggest that regular green tea drinking might protect smokers from oxidative damage and could reduce cancer risk or other diseases caused by free radicals associated with smoking.

6. MOLECULAR TARGETS OF GREEN TEA

6.1. Tumor suppressor p53

The tumor suppressor protein p53 is a critical protein in cancer study. This protein assists in the regulation of cellular division and coordinates the decision for cells to enter apoptosis, or programmed cell death (94). Mutation of p53 is found in 50% or greater of all human cancers (95). In 2000, Lu *et al.*. demonstrated *in vivo* upregulation of p53 by green tea (96). In this experiment, SKH-1 mice were administered with 0.6% green tea for two weeks then exposed to UV light (96). Treatment resulted in increased numbers of p53-positive, apoptotic cells induced by UV exposure (96). Other studies have also found that green tea, and in particular the polyphenol (-)-EGCG, can induce and stabilize p53 (23, 97). In the liver cancer cell line HepG2 and prostate cancer cell line LNCaP, (-)-EGCG was capable of inducing cell cycle arrest at the G_1 phase and subsequent apoptosis (23, 97). Similar results were found in bovine aortic smooth muscle cells after treatment with tea polyphenols or (-)-EGCG in the 40 – 50 μM range (98).

6.2. Bcl-X_L

Bcl-X_L is an antiapoptotic member of the Bcl-2 family of proteins. Down regulation of the hyperphosphylated form of Bcl-X_L by GTP or (-)-EGCG has been implicated in the induction of apoptosis in prostate cancer cell lines PC-3 and LNCaP (24). Treatment with 50 μg/mL GTP or 50 μM (-)-EGCG resulted in cytochrome C release, a hallmark of apoptosis, within 3 hours (24). Coincident with apoptosis induction was loss of hyper-phosphorylated Bcl-X_L (24). However the specific mechanism involved is still unclear.

6.3. Vascular Endothelial Growth Factor (VEGF)

Angiogenesis, a neo-vascularization process, is required for the growth of tumors beyond a few millimeters in diameter and for invasion and metastasis (99, 100). GTP

and (-)-EGCG were found to reduce VEGF secretion, its transcription and promoter activity in human breast cancer and umbilical vein endothelial cells (25). Chung *et al.* found that 30.7b Ras 12 cells treated with 20 μM (-)-EGCG showed a significant decrease in levels of phospho-Erk 1/2 and –Mek 1/2 (29). Furthermore, (-)-EGCG treatment resulted in reduced association of Raf-1 and Mek1 and prevented phosphorylation of Elk-1 (29). In another study, mice were injected with HT29 colon cancer cells and then intraperitoneally injected 1.5 mg (-)-EGCG or (-)-EC after 22 days, followed by analysis of tumor masses and characteristics (26). The results showed that mice treated with (-)-EGCG had a 61% reduction in tumor volume and a 55% reduction in tumor weight as well as increases in apoptosis and reduction in tumor vessel counts (26). *In vitro* studies by Jung *et al.* also demonstrated a loss of phosphorylated Erk-1 and –2 in serum-deprived HT29 cells and overall reduction of VEGF expression (26).

6.4. Heterogenous nuclear ribonucleoprotein B1 (hnRNP B1)

hnRNP A2/B1 is an RNA-binding protein that is required for maturation of mRNA precursors (101). It was reported that hnRNP A2/B1 was overexpressed from the early clinical stage of lung cancer (102). Immunohistochemical staining with the hnRNP B1 antibody revealed that hnRNP B1 protein was specifically stained in the nuclei of human cancer cells but not in those of normal adjacent lung epithelial cells (101). This protein may therefore serve as a biomarker for early detection of a variety of lung cancers. When combined with sulindac (an antioxidant and a cancer-preventive drug), green tea extract was found to have a synergistic effect resulting in a greater than 50% reduction in the number of tumors and a significant decrease in the size of tumors in male C57BL/6J-Min/+ mice (27). Furthermore, treatment with green tea extract resulted in reduced expression of hnRNP B1, suggesting a loss of tumor development or progression (27).

6.5. Telomerase

Telomeres, the ends of the DNA strand, are maintained by the enzyme telomerase. The activity of this enzyme is typically absent in somatic cells but is found in tumors, and it aids in allowing immortalization of the cancer cells (103, 104). There is additional evidence suggesting that telomerase has DNA repair capabilities and may be an anti-apoptotic enzyme (103, 104). (-)-EGCG was found to have a significant inhibitory effect on telomerase at concentrations around 5 μM (13). If (-)-EGCG was allowed to incubate in media, it underwent rapid degradation, accompanied by greatly increase in its telomerase-inhibitory activity (13). Reduction of telomerase activity would result in loss a proliferation capability of tumor cells.

6.6. Oxidative stress and apoptosis

It has been shown that many of the antiproliferative effects of (-)-EGCG are attributable to its antioxidant properties (105). (-)-EGCG is also known to directly initiate apoptosis as demonstrated by loss of mitochondrial membrane potential, cytochrome C release, DNA laddering, and dramatic increase in caspase-3 activity (106). However, it has been demonstrated that (-)-EGCG can generate oxidative stress. HT-29 colon carcinoma cells, pre-treated with antioxidants (reduced glutathione and *N*-acetyl-L-cysteine) and subsequently treated with (-)-EGCG, demonstrated reduced mitogen activated protein kinase activation and reduced cytochrome C release and apoptosis although this could not be blocked by catalase (106).

6.7. Proteasome

The proteasome is a 700 kDa multicatalytic complex that constitutes the principle degradation machinery of the cell (107). Many cell cycle and apoptosis regulators are known targets for the proteasome, including p53, pRB, p21, $p27^{Kip1}$, IkB-α, and Bax (107). The mammalian 20S proteasome has three activities: chymotrypsin-like, trypsin-like, and peptidyl-glutamyl peptide hydrolyzing (PGPH or caspase-like) (107). Inhibition of the proteasome chymotrypsin-like activity is associated with induction of tumor cell apoptosis (107). Proteasome inhibitors have greatly reduced effects on normal cells, making the proteasome an attractive target for chemotherapy.

Recent studies have shown that green tea polyphenols, and specifically those with ester bonds, (-)-EGCG, (-)-ECG, (-)-GCG, (-)-CG, have potent proteasome-inhibitory properties selective for the chymotrypsin-like activity (15 and Figure 1). This inhibition was accompanied by apoptosis induction and was limited to cancer cells (15, 21, 22). Though in normal cells, proteasome inhibition did lead to an accumulation of cells in the G_1 phase of cell cycle (15).

We have studied the mechanism of proteasome inhibition by tea polyphenols. Given the structure of (-)-EGCG (Figure 1), the ring system was reminiscent of a tyrosine mimic. As such it was speculated that (-)-EGCG may bind directly into the binding cleft of the chymotrypsin-like active site on the β5 subunit (21). It has been hypothesized that the ester-carbon on the polyphenol could be subject to nucleophilic attack by the hydroxyl group of the N-terminal threonine of the β5 subunit (which is responsible for the chymotrypsin-like catalytic activity) (21). To determine the possibility of (-)-EGCG binding directly to the active site, computational modeling studies were employed (21). These studies compared the binding energies and IC_{50} values of a variety of (-)-EGCG analogs including those where an amide bond substituted the ester bond (20, 21 and Figure 1). The results indicated that the ester carbon of (-)-EGCG would line up with the hydroxyl of the N-terminal threonine placing the carbonyl carbon within 3 – 4 Å at an optimal distance for nucleophilic attack (21). Furthermore the actual IC_{50}s of the EGCG analogs were predicted by the binding energies of the compounds as determined by the computational model (21). This provides strong support for (-)-EGCG (and other ester-bond containing polyphenols of green tea) as direct inhibitors of the proteasome.

Given the evidence surrounding the anticancer and preventative effects of the natural tea polyphenols, it

became desirable to produce synthetic analogs. Such analogs would allow for determination of the mechanistic activities of the polyphenols and generation of more potent and stable pharmacological agents. We have synthesized (+)-EGCG, (+)-GCG, and a benzyl-protected form of (+)-EGCG; [Bn-(+)-EGCG], and examined their proteasome inhibitory properties (22 and Figure 1). We found that (+)-EGCG and (+)-GCG were indistinguishable from the natural counterparts in their proteasome inhibition activities. However, Bn-(+)-EGCG possessed no inhibitory properties (22). As with the natural compounds, the synthetic epimers [except Bn-(+)-EGCG] could not inhibit trypsin or calpain activities and did inhibit colony formation in soft agar assays (22). These results suggest that it is possible to synthesize analogs of the tea polyphenols that may lead to compounds with greater anticancer effects and/or stability in serum.

Green tea has been shown to possess an array of anticancer activities mostly from the actions of its polyphenols. The targets of green tea include many enzymes directly associated with cancer (eg, proteasome, telomerase, growth factors, MAPKs, etc.). Given the wide variety of targets and non-toxic and highly consumed nature, green tea holds substantial promise as a leading means of cancer prevention and/or chemotherapy.

7. FUTURE DIRECTIONS

Epidemiological and animal studies have provided evidence that green tea and tea polyphenols reduce the risk of certain types of cancer. Many laboratory and clinical studies are now underway with the goal of assessing the properties and mechanisms of green tea and tea polyphenols to serve as a chemopreventive and/or therapy agent for human cancers. Studies focusing on the purified tea polyphenol compound (-)-EGCG should continue to provide researchers an improved understanding of tea polyphenol absorption, distribution, role in anti-cancer reactions, metabolism and anti-cancer mechanisms. On the other hand, work should continue on synthesizing and evaluating more analogs of green tea polyphenols to find more potent, stable and specific polyphenol proteasome inhibitors as novel anti-cancer agents (21, 22). In contrast to this “single agent approach”, scientists could direct their attention upon the diverse array of different natural and synthetic products as a complex mixture, a “cocktail approach”, which together may have synergistic anti-cancer benefits (108). Different combinations of diverse agents could be tested including (-)-EGCG (15), genistein (109, 110), selenium (111), curcumin (112), resveratrol (113), quercetin (114), extracts of apple and grape (115), and vitamin or mineral supplements (116). This approach should continue to be explored in clinical, laboratory, animal, and epidemiological studies in the future. A major challenge of cancer prevention is to integrate new molecular findings into clinical practice. Identification of molecular targets or biomarkers, whose changes are associated with inhibition of malignantly transformed cell properties, is paramount to cancer prevention and treatment by green tea and will greatly assist in a better understanding of anti-cancer mechanisms by green tea.

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