Green Tea and Skin—Anticarcinogenic Effects

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Because of its special aroma, green tea is a popular beverage consumed by some human populations worldwide. In recent years, many laboratory studies have shown that in a variety of animal tumor bioassay systems the administration of green tea, specifically the polyphenolic fraction isolated from green tea leaves (green tea polyphenols), affords protection against cancer induction. In mouse skin tumor bioassay systems, topical application of green tea polyphenols to skin has been shown to result in protection against a) 3-methylcholanthrene-induced skin tumorigenesis, b) 7,12-dimethylbenz(a)anthracene (DMBA)-induced skin tumor initiation, c) 12-O-tetradecanoylphorbol-13-acetate and other tumor promoters caused tumor promotion in DMBA-initiated skin, and d) benzoyle peroxide- and 4-nitroquinoline N-oxide caused enhanced malignant progression of nonmalignant lesions. Green tea extract has also been shown to cause partial regression of established skin papillomas in mouse. Similarly, chronic oral feeding of green tea polyphenols or water extract of green tea has also been shown to result in protection against both chemical carcinogen— and ultraviolet B radiation—induced skin tumorigenicity. Collectively these data suggest that green tea possesses significant chemopreventive effect against each stage of carcinogenesis, and that it may be useful against inflammatory responses associated with the exposure of skin to chemical tumor promoters as well as to solar radiation. Available data regarding the mechanism by which green tea affords these diversified effects is discussed. J Invest Dermatol 102:3–7, 1994

Tea is a popular beverage consumed worldwide and contains several polyphenolic antioxidants. Antioxidants are agents that have the ability to prevent oxidant-induced damage to cells. Because oxidants play an important role at least in initiation and promotion stages of multistage carcinogenesis [1], in recent years the role of antioxidants present in fruits, vegetables, and beverages humans consume has received considerable attention as cancer chemopreventive agents ([2–4] and references therein). Many laboratory studies have demonstrated the inhibitory effects of green tea polyphenols against tumor formation and growth in skin, lung, stomach, esophagus, duodenum and small intestine, colon, liver, pancreas, and mammary gland ([5–8] and references therein). In this review, we have summarized the laboratory studies on the effect of green tea consumption on the prevention of skin carcinogenesis and inflammation.

GENERAL CONCEPTS

It is becoming increasingly clear that dietary factors play a major role in a variety of human cancers [9]. It is also becoming clear that, in addition to substances that pose cancer risk, human diet also contains agents that are capable of affording protection against cancer ([2–4,10] and references therein). Such substances are known as anticarcinogens or chemopreventive agents. It is generally accepted that carcinogenesis is a multistep process that requires both initiating and promoting substances for the development of cancer ([11,12] and references therein). The initiation stage is essentially an irreversible step in which genetic changes occur in gene(s) controlling differentiation [11,12]. The promotion stage leads to the development of visible non-malignant lesions through epigenetic mechanisms [11,12]. Because tea (Camellia sinensis) is one of the most popular beverages consumed worldwide, and because it was shown that water extract of green tea (WEGT) is antimutagenic in bacterial test systems [13,14], we undertook studies to evaluate potential anticarcinogenic effects of green tea. Because inflammation is an early event in tumor promotion, we extended the studies to assess whether green tea also possesses anti-inflammatory effects.

GREEN TEA

The term “green tea” refers to the product manufactured from fresh tea leaves by steaming or drying at elevated temperatures with the precaution to avoid oxidation of the polyphenolic components, which include flavanols. Mostly, commercial tea is manufactured from the leaf and bud of the plant; it is estimated that about 2.5 million metric tons of dried tea are manufactured annually. Of this production, about 20% is green tea, mainly consumed in Asian countries, Japan, China, Korea, and India; about 78% is black tea, mainly consumed in the Western countries and some Asian countries; and about 2% is oolong tea, mainly produced and consumed in southeastern China [15]. Basic steps in black tea production are plucking, withering, maceration (rolling), and drying. During this process, polyphenol oxidase—dependent oxidative polymerization by a process known as fermentation occurs, which results in the conversion of catechins to theaflavins and thearubigins. A typical black tea beverage contains 3–10% catechins, 3–6% theaflavins, 12–18% thearubigins, and other components.

Chemistry of Green Tea The chemical composition of green tea is approximately similar to that of fresh leaves, and contains flavanols, flavonoids, and phenolic acids. These compounds may
Table I. Summary of Laboratory Studies Demonstrating the Preventive Effects of Green Tea Against Murine Skin Tumorigenesis

<table>
<thead>
<tr>
<th>Tumorigenesis Protocol</th>
<th>Mouse Strain</th>
<th>Carcinogen</th>
<th>Promotor</th>
<th>Dose of GTP/EGCG Treatment</th>
<th>Mode of Application</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete</td>
<td>Balb/C</td>
<td>3-MC</td>
<td>TPA</td>
<td>1.2 mg GTP</td>
<td>Topical</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>SKH-1</td>
<td>UVB</td>
<td>TPA</td>
<td>10.0 mg GTP</td>
<td>Topical</td>
<td>[26]</td>
</tr>
<tr>
<td></td>
<td>SKH-1</td>
<td>UVB</td>
<td>TPA</td>
<td>0.1% GTP</td>
<td>Drinking water</td>
<td>[26]</td>
</tr>
<tr>
<td>Multistage, given during initiation</td>
<td>SENCAR</td>
<td>DMBA</td>
<td>TPA</td>
<td>10 mg GTP</td>
<td>Topical</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>SENCAR</td>
<td>DMBA</td>
<td>TPA</td>
<td>0.05% GTP</td>
<td>Drinking water</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>SKH-1</td>
<td>UVB</td>
<td>TPA</td>
<td>5 μEGCG</td>
<td>Topical</td>
<td>[17]</td>
</tr>
<tr>
<td>Multistage, given during promotion</td>
<td>SENCAR</td>
<td>DMBA</td>
<td>TPA</td>
<td>1–24 mg GTP</td>
<td>Drinking water</td>
<td>[27]</td>
</tr>
<tr>
<td></td>
<td>CD-1</td>
<td>DMBA</td>
<td>TPA</td>
<td>3.6 mg GTP</td>
<td>Topical</td>
<td>[18]</td>
</tr>
<tr>
<td>Stage I</td>
<td>SENCAR</td>
<td>DMBA</td>
<td>Teledodin</td>
<td>EGCG</td>
<td>Topical</td>
<td>[19]</td>
</tr>
<tr>
<td>Stage II</td>
<td>SENCAR</td>
<td>DMBA</td>
<td>Okadaic acid</td>
<td>EGCG</td>
<td>Topical</td>
<td>[20]</td>
</tr>
<tr>
<td>Stage II, given during progression</td>
<td>SENCAR</td>
<td>4-NQO*</td>
<td>TPA</td>
<td>EGCG</td>
<td>Topical</td>
<td>[21]</td>
</tr>
<tr>
<td>Chemotherapeutic effect</td>
<td>CD-1</td>
<td>DMBA/UVB</td>
<td>BPO*</td>
<td>6.0 mg GTP</td>
<td>Topical</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td>CD-1</td>
<td>DMBA/UVB</td>
<td>TPA</td>
<td>Varying doses of WEGT/EGCG</td>
<td>Drinking water/ip</td>
<td>[28]</td>
</tr>
</tbody>
</table>

*Used to enhance the rate of malignant conversion.

ANTICARCINOGENIC EFFECT OF GREEN TEA

Protection Against Chemical Carcinogen-Induced Tumor Initiation/Tumorigenesis Utilizing several tumor bioassay protocols, studies from this laboratory, subsequently verified by other investigators, have shown that topical application or oral feeding of a polyphenolic fraction isolated from green tea, hereafter referred to as GTP, to SENCAR, CD-1, and Balb/C mice results in significant protection against skin tumorigenesis [5–8]. In a complete carcinogenesis protocol, topical application of GTP onto the back of Balb/C mice for 7 d prior to that of 3-methylcholanthrene, was found to result in significant protection against the development of skin tumors [16]. We also assessed whether GTP possesses anti-tumor initiating effects. For these studies a two-stage skin carcinogenesis protocol in SENCAR mice was used. Topical application of GTP for 7 d prior to the single application of 7,12-dimethylbenz(a)anthracene (DMBA) as the initiating agent followed by twice weekly applications of the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) was found to result in significant protection against tumorigenesis [16]. In this study, in GTP-treated animals considerable delay in the appearance of the first tumor and subsequent tumor growth was also observed. Oral feeding of 0.05% (w/v) GTP in drinking water (this dose approximately corresponds to four cups of tea drinking per day by an adult human) for 50 d prior to the DMBA-TPA treatment or its continuous feeding during the entire period of two-stage carcinogenesis protocol was also found to result in significant protection (40%) in terms of the number of tumors per mouse when compared with non-GTP-fed animals (Table I). Among the five major epicatechin derivatives present in green tea (Fig 1), only EGCG has been assessed for its anti-skin tumor initiating activity [17]. Topical application of EGCG to SENCAR mice before the challenge with DMBA as tumor initiator was found to result in significant reduction in tumor incidence and tumor multiplicity following TPA application [17].

Protection Against Tumor Promotion Because without promoting substances usually cancer does not result, the inhibitors of the tumor-promotion stage of carcinogenesis are most likely to be of value as chemopreventive agents for the human population. Phor-
regimen, compared to non-GTP-treated positive controls, GTP application was found to inhibit significantly tumor growth/development. These data indicate that GTP inhibits both stage I and stage II of skin-tumor promotion, and that the inhibition of tumor promotion depends on the duration of GTP treatment [22].

**Protection Against Malignant Conversion of Benign Skin Papillomas to Carcinomas** Progression of benign tumors to malignant cancer is the most critical step in carcinogenesis because malignant lesions are capable of metastatic spread and eventual death [23 and references therein]. Inhibitors of the conversion process, therefore, would likely be useful as cancer chemopreventive agents. To assess the possible role of GTP in protecting against conversion of skin papillomas to squamous cell carcinomas, SENCAR mice were initiated by topical application of DMBA followed by twice weekly applications of TPA as tumor-promoting agent. Beginning at the twentieth week, when papilloma yield was stabilized, enhancement of rate of malignant conversion was achieved by twice weekly topical applications of either free-radical generating compound, benzoyl peroxide, or carcinogenic agent, 4-nitroquinoline-N-oxide, whereas spontaneous malignant conversion was associated with topical application of acetone. In these protocols, application of GTP 30 min prior to skin application of acetone, benzoyl peroxide or 4-nitroquinoline-N-oxide was found to result in significant protection when assessed for the conversion of papilloma to carcinomas. These results of this study suggest that green tea possesses significant protective effects against tumor progression induced by free-radical generating compounds and genotoxic agents [24].

**Protection Against Photocarcinogenesis** Ultraviolet B (UVB) radiation present in the solar spectrum is one of the major risk factors for skin cancer in humans [25]. We assessed the effect of oral feeding of GTP in drinking water as well as its topical application to SKH-1 hairless mice on UVB radiation--induced photocarcinogenesis [26]. Chronic oral feeding of 0.1% GTP in drinking water (w/v) to mice during the entire period of UVB exposure was found to result in significantly lower tumor burden compared to non-GTP-fed animals [26]. Topical application of GTP before UVB radiation exposure was also found to afford some protection against photocarcinogenesis; observed protection was, however, lower than that observed after oral feeding of GTP in drinking water. This occurred when tumor data was assessed in terms of percentage of mice with tumors or as number of tumors per mouse [26]. In another study [27], it was shown that infusion of green tea extracts as a sole source of drinking water (1.25%, w/v) to mice afforded protection against UVB radiation-induced intensity of red color and area of skin lesions, as well as UVB radiation-induced tumor initiation and tumor promotion. Based on the laboratory studies in murine skin, we speculate that consumption of green tea may reduce the risk of some forms of human cancers induced by solar radiation.

**Effect on Growth of Skin Tumors** Experiments have been conducted to demonstrate that WEGT or GTP in addition to resulting in decreased tumor formation and their multiplicity, also markedly reduced the tumor size [28]. In this study it was also shown that feeding WEGT, or GTP or EGCG given intraperitoneal, inhibited tumor growth and caused partial regression of established skin papillomas in female CD-1 mice [28]. These data suggest that green tea may possess chemotherapeutic effects. In Table I, a summary of major laboratory studies demonstrating the preventive effects of green tea or its components on initiation, promotion, progression, and regression of skin tumors is provided.

**MECHANISTIC STUDIES REGARDING ANTICARCINOGENIC EFFECTS**

**Inhibition of Biochemical Markers of Tumor Initiation**

Cytochrome P-450 (P-450) is the major enzyme system responsible for the metabolism of procarcinogens to their DNA binding metabolites [29,30] and references therein]. This binding to DNA is considered essential for tumor initiation [29,30]. We studied the interaction of GTP and its constituent polyphenols (−)-epicatechin, (−)-epicatechin-3-gallate, (−)-epigallocatechin, and EGCG with P-450 and associated monoxygenase activities [31]. The addition of all these catechin derivatives and GTP to micromoles prepared from rat liver resulted in a dose-dependent inhibition of P-450--dependent aryl hydrocarbon hydroxylase, 7-ethoxycoumarin-O-deethylase, and 7-ethoxyresorufin-O-deethylase activities [31]. It was observed that the administration of GTP, either topically or orally, to SENCAR mice inhibited carcinogen-DNA adduct formation in epidermis after topical application of [3H]benz(a)pyrene (BP) or [3H]DMBA [16]. In another study, we showed that epidermal aryl hydrocarbon hydroxylase activity and epidermal enzyme-mediated binding of BP and DMBA to DNA was inhibited by these polyphenols [32]. We also showed that chronic oral administration of GTP to mice for 4 weeks resulted in moderate to significant enhancement in glutathione peroxidase, catalase, NADPH-quinone oxidoreductase, and glutathione S-transferase activities in small bowel, lung, and liver [33]. Enhancement of these enzymatic pathways that play a role in detoxification of carcinogenic metabolites formation by P-450 and other enzymes by green tea and/or its ability to inhibit enzymatic pathways that are key determinant for cancer initiation may be expected to have protective functions against carcinogenesis. These pathways alone or in combination may contribute to overall protective effects of green tea against cancer.

**Scavenging of Activated Metabolites of Carcinogens**

Flavonoids are group of chemicals that possess strong nucleophilic centers at two positions. This property provides an opportunity for the flavanols to react with electrophilic carcinogenic species to form flavonol-carcinogen adducts that may result in prevention of tumor genesis. In general, the initial step in carcinogenesis is the metabolic activation of chemical carcinogens by the P-450--dependent biotransformation reaction. For example, the ubiquitous environmental pollutant BP is known to cause cancer of the skin and other body sites in experimental animals only after its metabolic activation to highly reactive molecules [1,29,30]. The ultimate carcinogenic metabolite of BP is BP-7,8-diol, 9,10-epoxide-2, the formation of which is catalyzed by successive enzymatic steps catalyzed by P-450 and epoxide hydrolase [29,30]. We have shown that tea polyphenols interact with BP-7,8-diol, 9,10-epoxide-2, and that topical application of GTP prior to BP-7,8-diol 9,10-epoxide-2 treatment results in inhibition of skin tumor initiation [34].

**Inhibition of Biochemical Markers of Tumor Promotion**

Topical application of phorbol esters like TPA on mouse skin results in epidermal hyperplasia, inflammation, increase in the number of dark basal keratinocytes, and induction of epidermal ornithine decarboxylase (ODC) activity followed by an increase in the levels of polyamines [11,12]. It has not been possible to establish which of these parameters or many others are obligatory or sufficient for the process of tumor promotion. It is, however, accepted that the induction of ODC activity is closely associated, though not sufficient, with the tumor-promoting activity of a variety of tumor promoters [11,12]. ODC also plays an essential role in cell proliferation and differentiation [11,12]. The induction of inflammation in skin mediated by TPA is believed to be governed by cyclooxygenase and lipoxygenase catalyzed metabolites of arachidonic acid, specifically prostaglandins and hydroxyeicosatetraenoic acids, respectively [1,11,12]. The importance of induction of epidermal ODC, cyclooxygenase, and lipoxygenase activities in skin-tumor promotion is evident from the fact that several inhibitors of these enzymes inhibit the tumor promotion in murine skin [1,3,5,36] and references therein.

Topical application of GTP to mouse skin was found to inhibit TPA-mediated induction of epidermal ODC activity in a dose-dependent manner [36]. The inhibitory effect of GTP was also dependent on the time of its application relative to TPA treatment. GTP application to SENCAR mice was also found to inhibit the induc-
tion of epidermal ODC activity caused by several structurally dif-
ferent mouse skin tumor promoters [36]. Prior application of GTP
to mouse skin was found to result in significant inhibition of TPA-
induced epidermal edema and hyperplasia [18]. As quantitated by
the formation of prostaglandin and hydroxyeicosatetraenoic acid
metabolites from respectively cyclooxygenase- and lipoxygenase-
catalyzed metabolism of arachidonic acid, skin application of GTP
to SENCAR mice was also found to result in significant inhibition
of TPA-caused effects on these two enzymes [18]. Ruch et al [37]
showed that GTP prevented TPA-induced oxygen radical-induced
cytotoxicity and inhibition of intercellular communication in nor-
mal human epidermal keratinocytes. GTP was also shown to inhibit
TPA-induced protein kinase C activity [20]. Inhibition of all of
these pathways alone or in combination may contribute to overall
anti-tumor-promoting effects of green tea.

ANTI-INFLAMMATORY EFFECTS OF GREEN TEA

Protection Against TPA-Caused Inflammatory Responses

Because edema and hyperplasia are often used as an early marker of
skin-tumor promotion, we assessed the effect of preapplication of
GTP on these parameters. As described above, skin application of
GTP to SENCAR mice was found to result in significant protection
against TPA-caused effects on cyclooxygenase and lipoxygenase
activities [18]. Prior application of GTP onto the dorsal skin of mice
resulted in significant inhibition of TPA-mediated epidermal
edema and hyperplasia [18]. In further studies, we found that single
or multiple applications of GTP to SENCAR mouse ear skin prior
to or after the application of TPA afforded significant protection
against TPA-mediated edema [38]. Preapplication of GTP also affor-
ded significant protection against TPA-induced hyperplasia in
the ear skin [38]. The percentage protection by GTP both in terms
of epidermal thickness and vertical cell layers was 75 and 90%,
respectively [18,38]. Preapplication of GTP also afforded pro-
tection against TPA-caused infiltration of polymorphonuclear
leukocytes [38].

Protection Against UVB Radiation- Caused Inflammatory Responses

In a recently completed study, we assessed whether GTP possesses
protective effects against UVB radiation- caused changes in murine skin. Chronic oral feeding of 0.26% GTP (w/v) as
the sole source of drinking water for 30 d to SKH-1 hairless mice
followed by irradiation with UVB (900 mJ/cm²) was found to result
in significant protection against UVB radiation- caused cuta-
neous edema, and depletion of antioxidant-defense system in epider-
minus [39]. The oral feeding of GTP also afforded protection against
UVB radiation- caused induction of epidermal ODC and
cyclooxygenase activities in a time-dependent manner [39].

Implications of Anti-Inflammatory Effects

Collectively, the studies outlined above suggest that green tea may be useful against inflammatory responses associated with the exposure of skin to UV solar radiation ([39,40] and references therein). The validity of these studies to humans exposed to low level of UV radiations chronically
through solar radiation remains to be established.

EPIDEMIOLOGICAL STUDIES ON TEA AND CANCER

The epidemiologic studies reviewed by the Working Group of the
International Agency for Research on Cancer on consumption of
tea and cancer, yielded inconsistent and inconclusive results. On
a positive note, this study concluded that there is inadequate evidence
for the carcinogenicity in humans of tea drinking and also inade-
quate evidence for the carcinogenicity of tea in experimental ani-
imals [41]. Also, available epidemiologic information provides no
indication that tea consumption has a statistically significant causa-
tive effect on human cancers. However, possible harmful effects
of the consumption of excessive amount of tea, at very high tem-
perature, or salted tea, cannot be ruled out. This in our opinion is
an important issue relevant to human health. A case-control study in
Kyushu, Japan showed that individuals consuming green tea more
frequently or in larger quantities tended to have a lower risk for
gastric cancer [42]. Epidemiologic studies conducted in Shizuoka
Prefecture, Japan indicated that the cancer death rate in this tea-pro-
ducing area, especially from stomach cancer, was lower than the
national average [43]. On the basis of many epidemiologic observa-
tions and laboratory studies on animals, we believe that tea con-
sumption is likely to have beneficial effects in reducing cancer risk.
To our knowledge, no prospective or retrospective epidemiologic
study has assessed a relationship between tea consumption and
human skin.

CONCLUSIONS AND FUTURE RESEARCH PROSPECTS

Changing life style, as reflected in dietary habits, has been recog-
nized as a major factor for human cancer risk ([2-10] and references
therein). For these reasons, changes in dietary habits with the intake
of more cancer chemopreventive agents appear to be a practical
approach for cancer prevention. Based on numerous epidemiologic
observations, and studies on laboratory animals, it can be concluded
that green tea consumption may have beneficial effects in reducing
cancer risk. Although considerable body of information is accumu-
lated on the preventive effects of green tea on cancer, a clear un-
derstanding of the mechanisms by which green tea components may
affect the induction, growth, and subsequent progression of specific
cancers is essential. For this reason more well-designed case-control,
and cohort studies are needed to address under what conditions
green tea consumption may inhibit the development of specific
cancers in specific populations. In other words, a clear understand-
ing of applicability of animal data to human situation is essential to
making recommendations to human populations. Because consid-
erable experimental data on the protection of mouse skin carcinogi-
ness and inflammation by GTP and its constituents exist, an
intervention study on human skin carcinogenesis and inflammatory
responses would be of great importance. Because GTP contains four
major catechines, it is important to establish which of them posses-
ses biologic activity in what biologic function. After this is
established, decisions regarding their use in cosmetics, toiletries and
ointments can be made.

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