The Biochemistry of Green Tea Polyphenols and Their Potential Application in Human Skin Cancer

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ABSTRACT

Green tea contains many polyphenol substances with exceptional antioxidant activity, and is consumed widely throughout the world. Green tea aqueous extract and its polyphenols, of which epigallocatechin gallate (EGCG) predominates, have been studied extensively in animal models for their anti-neoplastic properties. EGCG and green tea polyphenols (GTP) have been shown to inhibit skin tumor initiation, promotion, and progression by a number of mechanisms, including, but not limited to: inhibition of DNA binding by carcinogens, radical scavenging, inhibition of cytochrome P-450, maintenance of cellular communication, and inhibition of arachidonic acid metabolism. It is evident that the consumption of green tea is beneficial in the prevention of cancer in these models; however, the question remains whether use of oral or topical green tea preparations will have a preventative effect on human skin cancers, and should be answered by well-designed human clinical and epidemiological studies.
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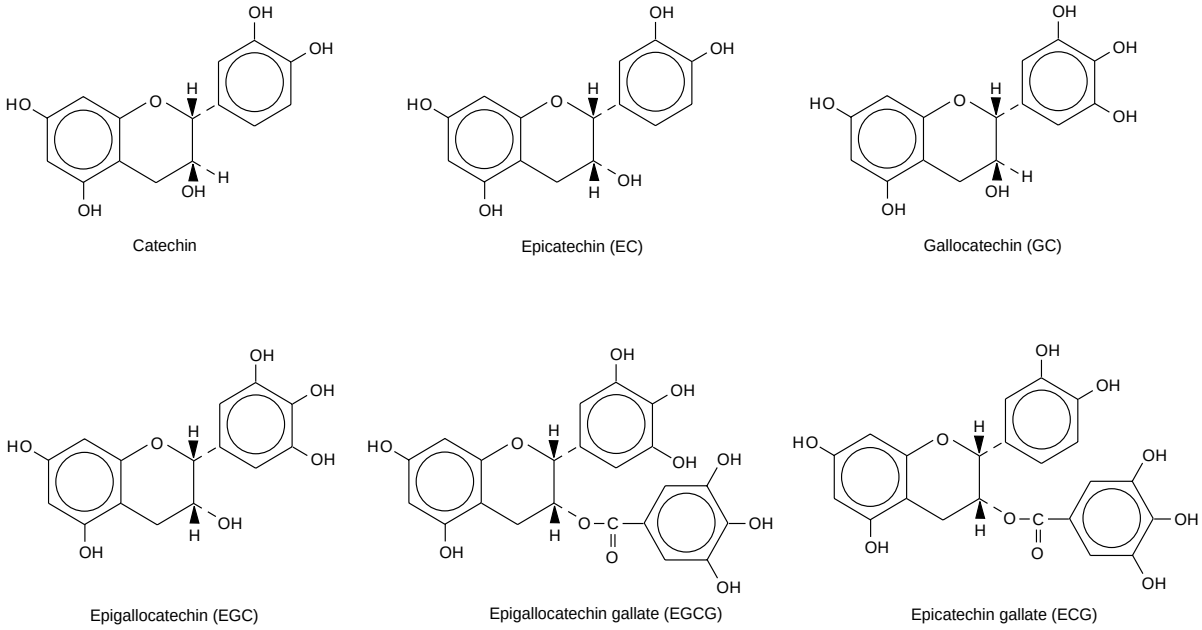
Introduction

Tea (*Camellia Sinensis*) is one of the most popular beverages consumed in the world today, second only to water. Green tea is prepared in such a way as to preclude the oxidation of polyphenols, unlike black tea in which oxidation is promoted, and oolong tea in which partial oxidation is promoted.[1] Green tea, therefore, retains its antioxidant ability, as well as other properties that make it a potent inhibitor of tumorigenesis.

Chemistry of Green Tea

Flavonoids, flavonols and phenolic acids make up approximately 30% of dried *Camellia Sinensis* leaves by weight.[1,2] Most of the polyphenols present are flavonols commonly known as catechins, with epicatechin and its derivatives being the most predominant forms. The gallic acid ester epigallocatechin gallate (EGCG) is present in the highest concentration, making up over 61% of the epicatechin derivatives included in green tea leaves.[3-4] Figure 1 shows the structure of these flavonols. Other green tea polyphenols include flavonoids and their glycosides, depsides such as chlorogenic acid and coumarylquinic acid, and a phenolic acid unique to tea, theogallin. Caffeine makes up an additional 3%, and there are trace amounts of the methylxanthines theophylline and theobromine, and an amino acid unique to tea, theanine.[1,2]

FIGURE 1
Epicatechin Derivatives of *Camellia Sinensis*



Multistage Carcinogenesis in Mouse Skin

Carcinogenesis in mouse skin is essentially a three-stage process of initiation, promotion, and progression.[5-7] Initiation is defined as permanent alteration of the cell genotype with no neoplastic phenotype.[7] Initiation alone is insufficient for skin tumorigenesis unless amplification of the mutated oncogene is triggered by tumor promoters.[7] Initiating chemicals bind covalently to DNA,[8] causing point mutations[9] or translocations.[10] Subsequently, initiation may result from the inability of the DNA to repair itself or from errors in the repair process.[11] Initiated cells can lay dormant for decades or a lifetime without ever expressing their neoplastic potential.[7]

Polycyclic aromatic hydrocarbons (PAH), including 7,12-dimethylbenz [a] -anthracene (DMBA) and benzoyl peroxide (BP), are one class of carcinogens that possess initiating and complete carcinogenesis activity.[6] There are conflicting reports as to the effects of BP. It has been described as a strict promoter[7,12,13] and an initiator with complete carcinogenesis activity.[5,6] BP has been listed as an initiator because it is used exclusively as an initiator in studies of green tea and skin cancer in mice.

Skin tumor initiation is inhibited by green tea aqueous extract (GTA),[14] green tea polyphenols (GTP)[15-20] and by EGCG.[4] Possible mechanisms of initiation inhibition by green tea include direct scavenging of initiators, inhibition of the cytochrome P-450 enzymes, scavenging of radical oxygen species (ROS), and inhibition of DNA binding by initiators. These will be explained herein.

Promotion is split into 2 "stages" called Stage I (conversion) and Stage II (propagation).[6] Promoting chemicals confer a growth advantage on initiated cells.[6] Cellular changes seen dur-

ing Stage I include induction of dark basal keratinocytes (DBK), increased prostaglandins, and increased growth factors.[5,6] During Stage II, ornithine decarboxylase (ODC) activity increases, polyamine levels increase and the cell proliferates.[5,6] During promotion, scientists have observed increased phospholipid synthesis,[5,21] sequential stimulation of RNA, protein and DNA synthesis,[21,22] increased phosphorylation of histones,[21,23] induced protein kinase C (PKC),[24] increased permeability between dermis and epidermis,[25] decreased intercellular communication,[26,27] and increased protease activity.[28] Promotion of initiated mouse skin causes inflammation, edema, leukocytic infiltration,[29,30] and a 5- to10-fold increase in the percentage of DBK's in the epidermis.[12,13,31] Effective chemical promotion results in visible papillomas (10^5-10^6 cells).[7]

12 -*O*-tetradecanoylphorbol- 13-acetate (TPA), a phorbol ester isolated from croton oil is the most potent and commonly used tumor promoter.[3,31,32] H_2O_2 is a free radical and a generator of other ROS, which also induces promotion.[13] Skin tumor promotion is inhibited by GTA,[14,33] GTP,[14-20,29,33,34] and EGCG.[4,33] Possible mechanisms of promotion inhibition by green tea include inhibition of ODC activity, inhibition of the arachidonic acid cascade, inhibition of DNA synthesis and cellular proliferation, and protection against decreased cellular communication. These will be explained herein.

Progression is associated with malignancy and includes invasion, metastasis, and further genetic changes.[5,6] Progression can be chemically induced or may occur spontaneously.[5,6] Skin tumor progression is inhibited by GTP[35] and EGCG.[36] Mechanisms of progression inhibition are unknown.

Green Tea and Cancer Studies

The studies listed in Table 1 show the protective effects of green tea and its epicatechins on tumorigenesis and their inhibitory effects on processes which are part of the mechanism of carcinogenesis.

In opposition to the studies in Table 1 are K.S. Kirby,[37] who in 1960 showed that tannin extracts can induce tumors; H.E. Kaiser,[38] who in 1967 showed that phenols in tea are cancer promoting; P. Bogovski,[39] who in 1977 showed that mouse skin treated topically with BP and then painted with green tea developed tumors earlier than those that were not; and G. Kapadia,[40] who in 1976 was able to induce tumors in 66% of mice by injecting them subcutaneously with 8 mg of the tannin fraction of *Camellia Sinensis* for 58-68 weeks.

It is worth noting, however, that none of the mice (of various strains) used in the studies listed in Table 1, whether they received tea as an aqueous extract or specific epicatechin derivative, and whether the substance was applied topically or orally, displayed any signs of enhanced tumor growth or liver toxicity. Attempts to induce tumors with large doses of GTP were unsuccessful,[20] even after initiation with DMBA.[18] Application of polyphenols alone do not induce ODC activity.[3] The evidence from these newer studies, especially those using specific epicatechin derivatives, seems to override the negative conclusions from the older studies.

Cytochrome P-450

Cytochrome P-450 is a set of microsomal enzymes (monooxygenases), including aryl hydrogen hydrolase (AHH), 7-ethoxyresorufin-*O*-deethylase (ERD), and 7-ethoxycoumarin-*O*-deethylase (ECD), among others.[41] It is normally important for the detoxification of xenobiotic compounds[42] and is induced by

TABLE 1

Carcinogen	Promoter	Green Tea Form	Dose/Delivery	Results	Reference
DMBA UVB UVB	TPA TPA UVB	aqueous extract GTP EGCG	Various doses in drinking water	All forms of green tea at all tested doses inhibited the growth of, and caused partial regression of established papillomas.	(33)
	TPA H_2O_2 Mezerein (-)Indolactam V Anthralin BP0 n-Dodecane	GTP, EGC, EC, ECG, EGCG	2mg. GTP topical 4 µmol EGC, EC, ECG, or EGCG topical	All forms of green tea inhibited ODC induction by TPA. GTP inhibited ODC induction by all other promoters.	(3)
DMBA 4-NQOa BPOa	TPA	GTP	6 mg. topical	GTP inhibited malignant conversion of papillomas to squamous cell carcinomas after treatment with 4-NQO or BPO.	(35)
DMBA	TPA	GTP	3.6 mg. topical	GTP inhibited tumor growth by 94%.	(14)
UVB	UVB	aqueous extract	1.25 or 2.5% drinking water	Green tea delayed the time of appearance of tumors and inhibited tumor growth.	(14)
DMBA	UVB	aqueous extract	.63 to l.25% drinking water	Green tea inhibited tumor growth and protected against sunburn.	(14)
BPDE-2	TPA	GTP	24 g. per mouse topical	GTP delayed the time of appearance of tumors and inhibited tumor growth.	(15)
BP DMBA		EGCG	5 µmol topical	EGCG inhibited DNA binding induced by BP and DMBA by 48% and 40%, respectively.	(4)
TPA		EGCG	5 µmol topical	EGCG inhibited ODC activity induced by TPA by 90%.	(4)
DMBA	TPA	EGCG	5 µmol topical	EGCG delayed the time of appearance of tumors and inhibited tumor growth.	(4)
UVB	UVB	GTP	10 mg. topical .1 %/ drinking water	GTP delayed the time of appearance of tumors and inhibited tumor growth.	(16)
3-MC		GTP	1 .2 mg. topical	GTP delayed the time of appearance of tumors and inhibited tumor growth.	(17)
DMBA	TPA	GTP	10 mg. topical .5g. per liter drinking water	GTP delayed the time of appearance of tumors and inhibited tumor growth.	(17)
BP	DMBA	GTP	10 mg. topical 5g. per liter drinking water	GTP inhibited binding to epidermal DNA by BP and DMBA by 42-62%.	(17)
TPA		GTP	.3mg topical	GTP inhibited edema and hyperplasia induced by TPA by 75% and 90%, respectively.	(29)
BP DMBA	TPA	GTP	1.2 or 3.6mg. topical	GTP inhibited tumor initiation by BP . and DMBA	(18)
TPA		GTP	1.2 - 5mg. topical	GTP inhibited TPA-induced edema, ODC activity, hyperplasia, and H_20_2 production in epidermis.	(18)
DMBA	TPA	GTP	l-24 mg. topical	GTP delayed the time of appearance and inhibited tumor growth in a dose-dependent manner. GTP inhibited cyclooxygenase and lipoxygenase activity, edema, and hyperplasia.	(19)
DMBA	TPA mezerein	GTP	6mg. topical	GTP inhibited tumor multiplicity and growth at both Stage I and Stage 11 of tumor promotion.	(34)
UVB DMBA	TPA UVB	GTP	1.25% or 2.5% in drinking water 25, 50, 75, and 100% of GTP orally	GTP inhibited UVB-induced skin lesions, DMBA/UVB-induced tumors, DMBA/TPA-induced tumors, and UVB/TPA-induced tumors.	(20)

drugs, steroids, chemicals, pesticides, herbicides, food preservatives, and certain dyes used as coloring agents.[43] However, it also converts carcinogens into a chemically reactive form.[42-44]

Many initiators, including PAH's, require metabolic activation by cytochrome P-450 monooxygenase enzymes.[43,44] They are converted to a variety of primary metabolites (oxidation products), such as epoxides, dihydrodiols, quinones, phenols and diol-epoxides.[45] BP, for example, is activated by cytochrome P-450 and epoxide hydrolase to active forms which include BP-diols, BP-phenols and BP-diol-epoxides, each with varying carcinogenic and mutagenic effects.[6,43,45,46] It is these ultimate carcinogens that bind covalently to macromolecules such as proteins and DNA, leading to carcinogenesis.[6,43,45,46] Inhibitors of the cytochrome P-450 enzymes have been shown to diminish tumorigenesis in mouse skin.[47,48]

Green tea epicatechins and (+)-catechin interact with and inhibit cytochrome P-450 enzymes and epoxide hydrolase in skin and liver, with EGCG being the most effective.[35,41,49] Theories of flavonoids' ability to decrease cytochrome P-450 activity include (1) increasing the V_{max} and decreasing the K_m for microsomal monooxygenases,[49] (2) enhancing the interaction of NADPH-cytochrome P-450 reductase with cytochrome P-450,[48] and (3) binding the catalytic sites of cytochrome P-450.[41] Tests suggest that the phenolic hydroxyl groups on the phenyl substituent of (-)-epicatechins and other plant phenols are essential for such activities.[41,48]

Ornithine Decarboxylase

ODC catalyzes the first and controlling step in polyamine biosynthesis (converts ornithine to putrescine).[21,51] Increased ODC leads to increased putrescine, spermidine and spermine.[6,51,52] These molecules play an essential role in cell proliferation and differentiation.[6,32,51] Elevated ODC is one of the important and characteristic biochemical parameters of TPA-induced tumor promotion.[6,21,32,52]

Benign tumors have been shown to have 20-30 times normal ODC levels.[52] In malignant tumors, ODC levels of 100 times normal have been reported,[52] and the larger the tumor, the higher the level of ODC, putrescine, spermine and spermidine.[53] The magnitude of ODC activation is directly proportional to the promoting ability of phorbol esters and other promoters.[52]

Inhibitors of ODC, including n-propyl gallate, flavonoids, retinoids, and antioxidants, are capable of inhibiting tumor promotion in mouse skin.[5,7,54] Topical application of GTP or EGCG inhibits ODC activity[3,4,18,19] in a dose-dependent manner, with EGCG displaying maximum inhibition (to 90%).[3,4] Six other polyphenols inhibit ODC activity[55] but none are as potent as EGCG.[4]

Protein Kinase C

PKC is an enzyme crucial in transmembrane signaling. It is normally stimulated by the hydrolysis of membrane phospholipids, producing diacylglycerol (DG) and inositol- ,4,5-triphosphate, and is involved in the regulation of cellular proliferation.[18] Activation of PKC leads to the induction of epidermal ODC,[56] and mimics the action of polypeptide growth factors.[57]

PKC is a receptor for TPA,[58-60] which can substitute for phospholipids, activating PKC directly in vitro.[61] By binding PKC directly, TPA can bypass normal cellular mechanisms for regulating cell proliferation, or can affect the interaction of growth factors with their receptors on keratinocytes.[62] TPA has also been suggested to be able to activate phospholipase

C, causing increased DG, and subsequent PKC activity.[63] X-irradiation and H_2O_2 also stimulate PKC via ROS formation.[64]

Arachidonic Acid Cascade

The cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism are involved in the mechanism of ODC induction by TPA.[3,6,7,32,65] The products of these two enzymes are suspected inducers of ODC activity.[65,66] Phorbol esters and other stimuli increase arachidonic acid release by skin.[67] This may be due to activation of phospholipase A_2, since inhibitors of that enzyme, such as mepacrine and tetracaine, inhibit ODC induction by TPA.[68] Epithelial cells treated with phospholipase C or diacylglycerol also results in ODC induction.[69,70] GTP has been shown to inhibit the TPA-induced increase in products of the arachidonic acid cascade *in vivo*.[9]

O_2 Free Radicals

Radical oxygen species (ROS) are produced during initiation and promotion. Activated PAHs are electrophiles; activation of PAHs may proceed through quinone derivatives and free radical intermediates, producing ROS.[72] Leukocytes, one of the main sources of ROS in the body, are increased and stimulated to release ROS, especially H_2O_2, due to inflammation.[5-7] Ionizing radiation damages DNA via free radical production.[73]

Oxidant stress may cause DNA damage either directly or by initiating lipid peroxidation.[7,57] When genetic material is the target of the oxidant attack,[74] mutations, DNA adducts, strand breaks or clastogenic effects can result.[57] TPA has been shown to increase the level of peroxides in initiated epidermal cells,[75,76] and induce xanthine oxidase (XO),[77] an 0_2- generating system, but has not been shown to be directly mutagenic and does not interact with DNA directly.[7] The activities of detoxifying enzymes SOD and CAT seem to be significantly depressed by tumor promoters.[78,79] TPA also inhibits glutathione peroxidase,[7] a potent enzyme catalyst for glutathione scavenging of ROS.[80]

Nothing compares to the antioxidant action of flavonoids.[81] GTP inhibits superoxide anion production,[82] scavenges 0_2^- and H_20_2 in a dose-dependent manner[2,27,83] and reduces 0_2^- production by X0.[27] The antioxidant ability of GTP is greater than vitamin E in scavenging 0_2^- produced by irradiation and greater than vitamins C and E in stimulated PMN systems.[83] GTP and EGCG scavenge ROS produced by PAH in situ more potently than BHT (a known antioxidant).[50,84] (+)-Catechin displayed the strongest antioxidant activity of 25 flavonoids tested.[80] Catechin gallates and gallocatechins have been shown to be even stronger antioxidants than (+)-catechin.[85] GTA, GTP, and EGCG stimulate the dose-dependent disappearance of BPDE from cell-free solutions[49] and in vivo,[15] with EGCG displaying the strongest scavenging ability.[49]

Possible mechanisms for the antioxidant activity of flavonoids include: (1) chelation of metal ions (Fe ions generate ROS by Fenton and Haber-Weiss reactions),[2] (2) scavenging R0S via reactions with their hydroxyl groups,[80,86] and (3) increasing the activity of endogenous antioxidant enzymes.[55]

Intercellular Communication

Decreased intercellular communication disrupts cell-to-cell growth control mechanisms and permits cell replication,[87] via induction of PKC.[26] GTP inhibits the decrease in intercellular communication induced by TPA.[27]

Mutagenesis

Inhibitors of mutagenic activity are:

(1) promoters of error-free DNA repair,
(2) inhibitors of error-prone DNA repair, and
(3) inhibitors of cell proliferation, increasing time for repair.[88]

Disease states which include defects in DNA repair (xeroderma pigmentosum and ataxia telangectasia) predispose to neoplasm.[89,90] Spontaneous mutations, normally due to error-prone DNA replication, involve an altered DNA-polymerase III.[91]

EGCG has been shown to interact with DNA-polymerase III, which may improve the fidelity of DNA replication.[91] EGCG has been shown to inhibit the mutagenicity of BP, aflatoxin B_1 (AFB_1), methanol extracts of coal tar pitch, and nitrosation products of methylurea in *Salmonella typhimurium* TA98 and TA100 in a dose-dependent manner, up to 95%.[49] EGCG inhibits the direct-acting mutagenicity of BP-diol-epoxide (activated form of BP) in vitro, inhibits mutations in Drosophila fed promutagens, and inhibits single strand breaks, in vitro, in cells exposed to ROS.[92] EGCG inhibits mutagenicity induced during lipid peroxidation in RBC membranes.[93] EGCG inhibits sister chromatid exchanges, AFB_1-induced chromosomal aberrations, and BP-induced mutations in Chinese hamster V79 cells in a dose-dependent manner.[49] Finally, of several hundred plant specimens, the homogenate of *Camellia Sinensis* exhibited the highest bio-antimutagenic activity in the screening plate using Bacillus subtilis. [11,25,91]

DNA Adduction

Carcinogen-DNA adducts correlate to susceptibility to skin tumor induction in mice,[89,90] and decreased adducts correlate with a chemoprotective effect on mutagenesis/carcinogenesis.[94] EGCG inhibits BP-DNA binding in calf thymus DNA,[49] and GTP inhibits BP and DMBA-induced epidermal DNA-adduct formation.[17]

The suggested methods of protection against DNA adduction by green tea polyphenols are: (1) protection of the nucleophilic site from binding of electrophiles by stearic inhibition[88] or (2) scavenging of electrophiles by antioxidant nucleophiles[80] before they bind DNA.

Epidemiological Studies

Epidemiological studies of tea and cancer of various organs have proven to be inconclusive and oftentimes contradictory,[2,71] and an exhaustive literature search failed to uncover any epidemiological studies of green tea consumption and skin cancer. The author of one review[71] does conclude that "tea consumption is likely to have beneficial effects in decreasing cancer risk," and the author of another[2] concludes "tea consumption is likely to have beneficial effects in reducing cancer risk."

Epidemiological studies, although inconclusive, suggest a protective effect of tea consumption on human cancer.

Relevance of Mouse Skin Studies to Human Cancer

Like skin cancer in mice, human skin cancer is also a multistep process.[95] Although exact sequences of events may differ between tissues and species, some mice and human skin cancers have been found to have similar characteristics. For instance, squamous cell carcinomas (SCC) in mouse skin and human skin are similar in histology and invasiveness,[6] and activation of the *ras* gene family has been seen in a significant proportion of mouse and human SCC, basal cell carcinomas, and melanoma.[6]

Dosage

The dosage of GTP given to mice is not easily translated to equivalent human dosage from the studies that administered tea ad libitum in drinking water. However, human epidemiological studies on other types of cancer suggest that a total daily intake of at least 10 cups of green tea (600-1250 mg GTP) might decrease the risk for certain cancers. Doses for topical application are even more difficult to extrapolate from mice studies.

Conclusion

GTP and EGCG have been shown to inhibit initiation, promotion and progression of skin cancer. They scavenge initiators, scavenge ROS, inhibit cytochrome P-450, inhibit DNA binding by carcinogens, maintain intercellular communication, and inhibit promoter-induced activity of ODC and the arachidonic acid cascade. It therefore seems reasonable to recommend that green tea polyphenols, especially EGCG, be subjected to human clinical trials and retrospective epidemiological studies. If proven to be effective, green tea could be included in any dietary regimen prescribed to prevent skin cancer, and EGCG could be included in sunscreens and other topicals intended to prevent skin cancer.

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