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Polyphenols as Cancer Chemopreventive Agents

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Abstract This article summarizes available data on the chemopreventive efficacies of tea polyphenols, curcumin and ellagic acid in various model systems. Emphasis is placed upon the anticarcinogenic activity of these polyphenols and their proposed mechanism(s) of action.

Tea is grown in about 30 countries and, next to water, is the most widely consumed beverage in the world. Tea is manufactured as either green, black, or oolong; black tea represents approximately 80% of tea products. Epidemiological studies, though inconclusive, suggest a protective effect of tea consumption on human cancer. Experimental studies of the antimutagenic and anticarcinogenic effects of tea have been conducted principally with green tea polyphenols (GTPs). GTPs exhibit antimutagenic activity *in vitro*, and they inhibit carcinogen-induced skin, lung, forestomach, esophagus, duodenum and colon tumors in rodents. In addition, GTPs inhibit TPA-induced skin tumor promotion in mice. Although several GTPs possess anticarcinogenic activity, the most active is (–)-epigallocatechin-3-gallate (EGCG), the major constituent in the GTP fraction. Several mechanisms appear to be responsible for the tumor-inhibitory properties of GTPs, including enhancement of antioxidant (glutathione peroxidase, catalase and quinone reductase) and phase II (glutathione-*S*-transferase) enzyme activities; inhibition of chemically induced lipid peroxidation; inhibition of irradiation- and TPA-induced epidermal ornithine decarboxylase (ODC) and cyclooxygenase activities; inhibition of protein kinase C and cellular proliferation; antiinflammatory activity; and enhancement of gap junction intercellular communication.

Curcumin is the yellow coloring agent in the spice turmeric. It exhibits antimutagenic activity in the Ames *Salmonella* test and has anticarcinogenic activity, inhibiting chemically induced preneoplastic lesions in the breast and colon and neoplastic lesions in the skin, forestomach, duodenum and colon of rodents. In addition, curcumin inhibits TPA-induced skin tumor promotion in mice. The mechanisms for the anticarcinogenic effects of curcumin are similar to those of the GTPs. Curcumin enhances glutathione content and glutathione-*S*-transferase activity in liver; and it inhibits lipid peroxidation and arachidonic acid metabolism in mouse skin, protein kinase C activity in TPA-treated NIH 3T3 cells, chemically induced ODC and tyrosine protein kinase activities in rat colon, and 8-hydroxyguanosine formation in mouse fibroblasts.

Ellagic acid is a polyphenol found abundantly in various fruits, nuts and vegetables. Ellagic acid is active in antimutagenesis assays, and has been shown to inhibit chemically induced cancer in the lung, liver, skin and esophagus of rodents, and TPA-induced tumor promotion in mouse skin. Ellagic acid functions through a variety of mechanisms, including inhibition of microsomal P-450 enzymes, stimulation of glutathione-*S*-transferase, scavenging the reactive metabolites of carcinogens, and direct binding to DNA, thus potentially masking sites that would normally interact with ultimate carcinogens.

GTP, curcumin and ellagic acid exhibit potent antioxidant effects. This property, coupled with their other effects, make them effective chemopreventives against both the initiation and promotion/progression stages of carcinogenesis.

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Epidemiological, experimental and metabolic studies are providing convincing evidence that nutrition plays an important causative role in the initiation, promotion and progression of several types of human cancers [1]. It is also becoming clear that, in addition to substances that pose a cancer risk, the human diet also contains agents which are capable of affording protection against some forms of cancer [2]. Chemoprevention of cancer is a means of cancer control in which the occurrence of the disease, as a consequence of exposure to carcinogenic agents, can be slowed, completely blocked, or reversed by the administration of one or several naturally occurring or synthetic compounds. Such chemopreventive compounds are known as anticarcinogens, and ideally they should be non-toxic [1,2]. Among the more extensively studied chemopreventive agents are the polyphenols, including tea polyphenols, curcumin and ellagic acid. The present report summarizes the known inhibitory effects of these polyphenols and their likely mechanisms of action.

TEA POLYPHENOLS

Tea is grown in about 30 countries and, next to water, is the most widely consumed beverage in the world [3]. Of the approximately 2.5 million metric tons of dried tea manufactured annually, only 20% is green tea and 2% is oolong tea; the remainder is black tea [3]. In recent years, many investigations conducted in various organ-specific animal tumor bioassay systems have shown that polyphenolic compounds present in tea are capable of affording protection against cancer induced by both chemical and physical agents. Although the majority of these studies have focused on green tea, some studies have also shown that black tea may have similar effects.

Green tea is produced in relatively few countries, and is consumed primarily in China, Japan, India and a few countries in North Africa and the Middle East [3]. 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 compounds which include flavonols. Green tea contains 35–52% (measured in weight % of extract of solids) catechins and flavonols combined. Of the four major catechins in green tea (Fig. 1), *i.e.*, (–)-epicatechin (EC), (–)-epicatechin-3-gallate (ECG), (–)-epigallocatechin (EGC), and (–)-epigallocatechin-3-gallate (EGCG), EGCG is the major component, accounting for greater than 40% of the total polyphenolic mixture. About 78% of the world's tea consumption is the beverage prepared from black tea, generally consumed in the Western countries and some Asian countries. Basic steps in black tea production are plucking, withering, maceration (rolling) and drying. During this process, the polyphenols undergo oxidative polymerization by a process known as fermentation, resulting in the conversion of catechins to the aflavins and thearubigins (Fig. 1). A typical black tea beverage contains 3–10% catechins, 3–6% theaflavins, 12–18% thearubigins, and other components.

Tea and Chemoprevention

In recent years, a wide range of studies have demonstrated that a polyphenolic fraction isolated from green tea (GTP), a water extract of green tea (WEGT), and epicatechin derivatives present in green tea possess strong anticarcinogenic effects in skin and other tissues (Table I). Studies have also shown that a water extract of black tea (WEBT) has similar inhibitory effects, and it is speculated that these effects are associated with the polyphenols present therein.

In brief, topical application of GTP to mouse skin has been shown to result in protection against 3-methylcholanthrene (MCA)-induced skin tumorigenesis [4], 7,12-dimethylbenz(*a*)-anthracene (DMBA)-induced skin tumor initiation [3,5], 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion in DMBA-initiated skin [6,7], and benzoyl peroxide- and 4-nitroquinoline-*N*-oxide (4-NQO)-enhanced malignant progression of nonmalignant lesions [8]. WEGT has been shown to result in partial regression of established skin papillomas in mice [9]. Similarly, chronic oral feeding of GTP and WEGT has been shown to result in protection against both chemical carcinogen- and ultraviolet B (UVB) radiation-induced skin tumorigenicity [10,11]. Recent studies have shown that WEBT

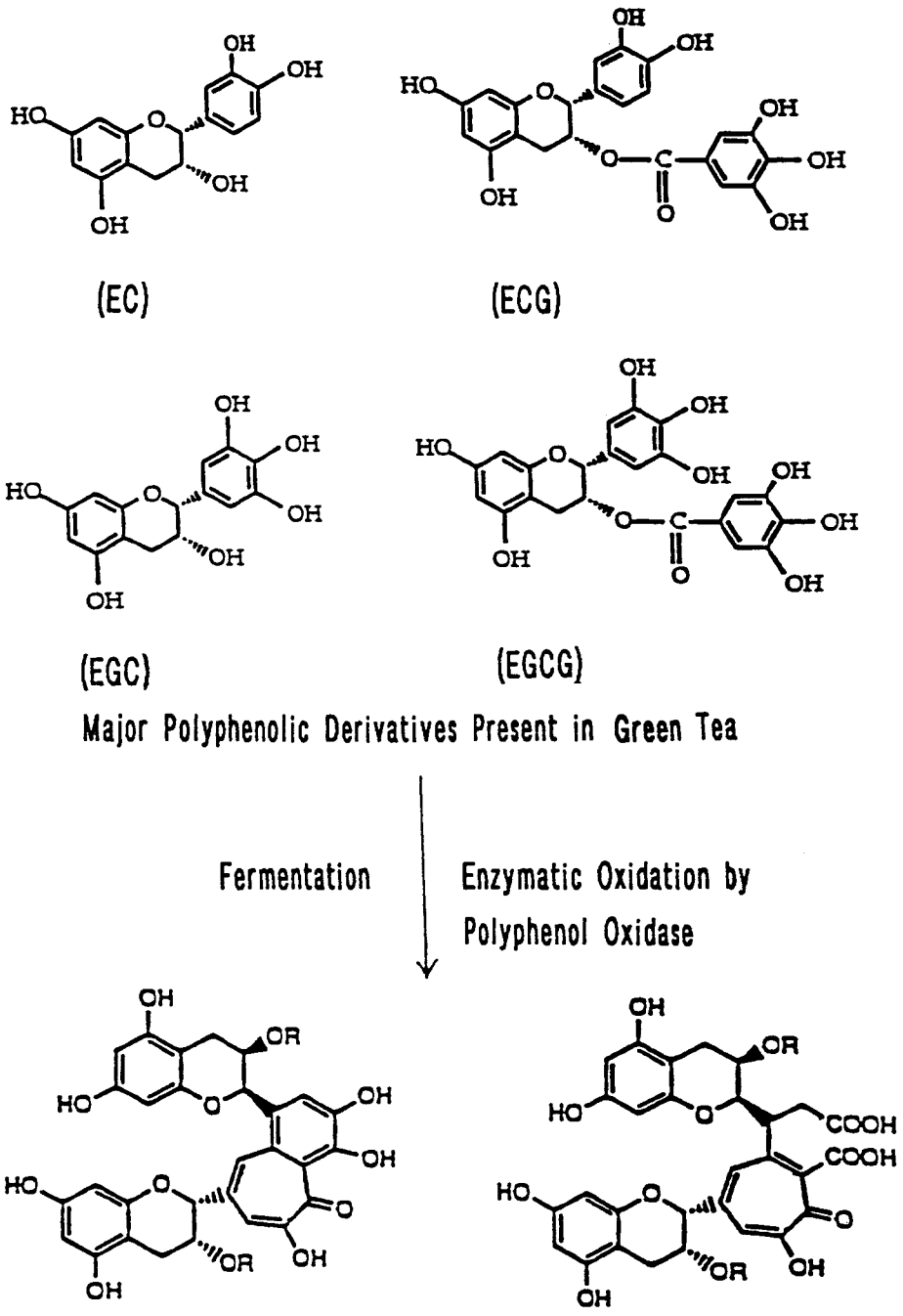


Fig. 1. Major polyphenolic derivatives present in green and black tea.

TABLE I. Tea Polyphenols: Summary of Anticarcinogenic Effects[a]

Organ	Treatment	References
Skin	Initiation: PAH, UVB Promotion: DMBA/TPA Benzoyl peroxide/TPA 4-NQO/TPA	3–8,10–12
Lung	B(*a*)P, NDEA, NNK	13–18
Forestomach	B(*a*)P, NDEA	13–15
Esophagus	NMBA, Nitrososarcosine	20,21
Duodenum	ENNG	19
Colon	Azoxymethane	22,23
Liver	Aflatoxin B_1, NDEA	24,25
Pancreas	*N*-nitroso-bis(2-oxypropyl)-amine	26
Mammary Gland	DMBA	27

[a] See text for discussion; [b] PAH, UVB, DMBA, TPA, 4-NQO, B(*a*)P, NDEA, NNK, NMBA, ENNG — see text for names of abbreviated compounds.

also possesses cancer chemopreventive effects in the mouse skin tumor model [12]. Collectively, these data suggest that green and black tea components possess significant chemoprotective effects against each stage of carcinogenesis, and that they may be useful against inflammatory responses associated with the exposure of skin to chemical tumor promoters as well as to solar radiation [3].

In addition to skin, several studies have been conducted to assess whether oral consumption of GTP or WEGT produces preventive effects against carcinogenesis induced in internal organs. Oral administration of WEGT (1.2–2.5% w/v) or GTP (0.2%, w/v) in the drinking water to A/J mice during the initiation, post-initiation and entire period of tumorigenesis protocols resulted in a significant reduction in the multiplicity of both lung and forestomach tumors induced by benzo(*a*)pyrene (B(*a*)P) [13–15]. Similarly, oral administration of WEGT (0.63–1.25%, w/v) in the drinking water to A/J mice reduced the incidence and multiplicity of lung and forestomach tumors caused by po administration of *N*-nitrosodiethylamine (NDEA) [14]. Wang *et al.* [16] showed that oral feeding of 0.6% WEGT or WEBT to A/J mice prior to or after challenge with NDEA or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) resulted in significant protection in terms of lung tumor incidence and multiplicity. Similar results were reported by Xu *et al.* [17], where inhibitory effects of WEGT and EGCG, the major epicatechin derivative present in green tea/GTP/WEGT, against NNK-induced lung tumorigenicity in A/J mice were demonstrated. In a recent study, the effects of oral administration of decaffeinated green tea or black tea on NNK-induced lung tumorigenesis were also investigated [18]. Significant protection against lung tumor formation occurred when tea was given either during or after NNK treatment.

Other organ systems in which tea and its components have demonstrated inhibitory effects include the duodenum, esophagus, colon, liver, pancreas and mammary gland. Fujita *et al.* [19] reported the chemopreventive effects of EGCG against *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine (ENNG)-induced duodenal tumorigenicity in C57BL/6 mice. Green tea has been shown to afford protection against *N*-nitrosomethylbenzyl-

amine (NMBA)- [20] and nitrososarcosine-induced [21] esophageal tumorigenesis in rats and mice, respectively. Yamane *et al.* [22] showed that oral feeding of 0.01 or 0.1% GTP in drinking water resulted in significant protection against azoxymethane (AOM)-induced colon cancer in Fischer rats. Narasiwa and Fukara [23] showed that a very low dose of GTP administered in drinking water prevented *N*-nitrosomethylurea (NMU)-induced colon carcinogenesis in F-344 rats. Chen *et al.* [24] and Li [25] provided evidence for cancer protective effects of green tea against aflatoxin B_1 and NDEA-induced hepatocarcinogenesis. Harada *et al.* [26] showed antipromotion effects of green tea extracts against *N*-nitroso-bis(2 -oxopropyl)amine-induced pancreatic cancer in Syrian golden hamsters. Hirose *et al.* [27] demonstrated inhibition of DMBA-induced mammary gland carcinogenesis in rats by diet containing green tea. These studies amply demonstrate that tea components afford protection in various animal tumor bioassay systems.

Mechanistic Studies of Anticarcinogenic Effects of Tea

Tea and its components have been shown to inhibit carcinogenesis by several mechanisms. With respect to inhibition of biochemical markers of tumor initiation, GTP and its constituent polyphenols (Fig. 1) have been shown to interact with cytochrome P-450 and inhibit associated monooxygenase activities [28]. Administration of GTP, either topically or orally, to SENCAR mice inhibited carcinogen-DNA adduct formation in epidermis after topical application of [^{3}H]B(*a*)P or [^{3}H]DMBA [4]. Chronic oral administration of GTP to mice for four 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 [29]. GTP interacts with B(*a*)P-7,8-diol-9,10-epoxide-2 (BPDE), the ultimate carcinogenic metabolite of B(*a*)P, suggesting that it could have a "scavenging effect" [30]. Sohn *et al.* [31] determined the effect of administering 2% (w/v) solutions of green or black tea to F-344 rats on hepatic xenobiotic metabolizing enzymes. These treatments resulted in significant increases in hepatic P-450 1A1, 1A2 and 2B1 activities, but no changes in P-450E1 and 3A4 activities. Of the phase II enzymes, UDP-glucuronyltransferase was found to be increased, but not glutathione-*S*-transferase. In a recent study Shi *et al.* [18] showed that addition of green tea and black tea extracts and their fractions to lung microsomes *in vitro* inhibited NNK oxidation and NNK-induced DNA methylation. In this same study, EGCG was also found to inhibit the catalytic activities of several P-450 enzymes related to P-450 1A and 2B1. Inhibition of phase I enzymes involved in cancer initiation and enhancement of enzymes that play a role in carcinogen detoxification may be expected to protect against carcinogenesis.

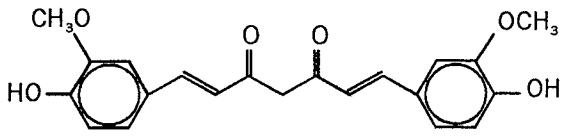
Tea components have also been shown to influence biochemical markers of tumor promotion/progression. Topical application of GTP to mouse skin inhibits TPA-mediated induction of epidermal ODC activity in a dose-dependent manner [32]. GTP application to SENCAR mice also inhibits the induction of epidermal ODC activity caused by several structurally different mouse skin tumor promoters [32]. Prior application of GTP to mouse skin resulted in significant inhibition of TPA-induced epidermal edema and hyperplasia [33], as well as both cyclooxygenase- and lipoxygenase-catalyzed metabolism of arachidonic acid [3]. Ruch *et al.* [34] showed that GTP prevented TPA-induced cytotoxicity and inhibition of intercellular communication in normal human epidermal keratinocytes. Inhibition of these pathways, either alone or in combination, may contribute to the overall antitumor promoting effects of tea.

CURCUMIN

Turmeric, the powdered rhizome from the root of the plant *Curcuma longa* Linn. has long been used as a spice in foods and as a naturally occurring medicine for the treatment of inflammatory diseases. Turmeric has a somewhat bitter taste and gives Indian curry dishes a characteristic yellow color. Curcumin (diferuloylmethane), the yellow pigment in turmeric, has also been used as a coloring agent and/or spice in foods, as well as in cosmetics and drugs [35]. It is the major phenolic antioxidant and antiinflammatory agent in turmeric, and can be extracted from turmeric with ethanol or other organic solvents. The chemical, biological and pharmacological properties of curcumin have been reviewed [35–38] and its structure is given in Figure 2.

Curcumin and Chemoprevention

Recent investigations have demonstrated that curcumin possesses strong anticarcinogenic effects in several tissues (Table II). The initial report of its antitumor effects was published by Huang *et al.* [39], in which 1, 3 or 10 μmol curcumin applied topically to the skin of CD-1 mice inhibited TPA-induced tumor promotion in DMBA-initiated skin. This observation was consistent with the previously reported antioxidant and antiinflammatory effects of curcumin [35]. In an extensive study by Nagabhushan and Bhide [40], 200 nmol curcumin applied topically to the skin of each Swiss mouse was found to inhibit DMBA-induced—as well as TPA-promoted–skin tumors. In addition, when administered by gavage at a level of 1 mg per mouse, curcumin reduced the occurrence of B(*a*)P-induced forestomach tumors. Subsequent investigations demonstrated the ability of curcumin to inhibit the development of precancerous lesions, *i.e.*, DMBA-induced hyperplastic nodules in cultured rat mammary gland tissues [41] and azoxymethane-induced crypts in rat colon [42]. In a recent study employing several mouse strains, curcumin added to the diet at concentrations ranging from 0.5% to 4% inhibited B(*a*)P-induced forestomach tumors in A/J mice, ENNG-induced duodenal tumors in C57BL/6 mice, and azoxymethane-induced colon tumors in CF-1 mice [43]. Collectively, these studies clearly demonstrate that curcumin affords protection in various animal model bioassay systems.



Curcumin

Fig. 2. Curcumin

Mechanistic Studies of the Anticarcinogenic Effects of Curcumin

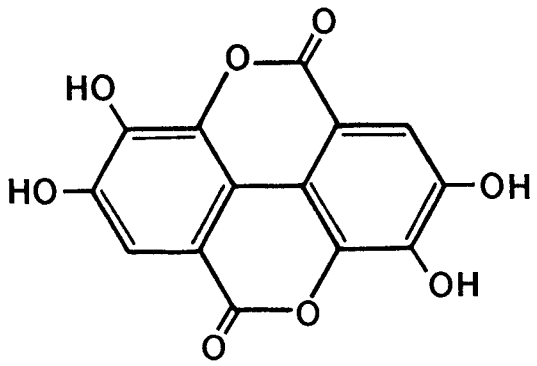
Experimental studies have shown that curcumin inhibits carcinogenesis by several mechanisms. With respect to biomarkers of tumor initiation, curcumin has been reported to inhibit the metabolic activation and promote the detoxification of carcinogens. For example, curcumin inhibited the metabolic activation of B(*a*)P to mutagens *in vitro*, the metabolic activation of B(*a*)P to B(*a*)P-DNA adducts in mouse skin *in vivo* [44–46], and the formation of B(*a*)P-DNA adducts

TABLE II. Curcumin: Summary of Anticarcinogenic Effects[a]

Organ	Treatment	References
Skin	Initiation: DMBA Promotion: DMBA/TPA B(*a*)P/TPA	39,40
Forestomach	B(*a*)P	40,43
Mammary Gland (*in vitro*)	DMBA	41
Colon		
(*in vitro*)	AOM	42
(*in vivo*)	AOM	43
Duodenum	ENNG	43

[a] See text for discussion.

or single strand breaks in DNA in the forestomach or liver of mice [47]. In additional studies, dietary administration of curcumin to rats or mice was reported to increase the levels of hepatic phase I and phase II enzymes [45]. Curcumin has also been shown to increase the rate of DNA repair in yeast [48]. With respect to the post-initiation phase of carcinogenesis, curcumin has been found to inhibit TPA-induced ODC activity, cell proliferation, and tumor promotion in mouse epidermis [37,39]. Curcumin has strong antioxidant and free radical-scavenging activity [49,50], inhibits epidermal arachidonic acid metabolism *via* lipoxygenase and cyclooxygenase pathways [51], inhibits the inflammatory action of arachidonic acid [51], and inhibits TPA-induced inflammation in mouse skin [39]. Recent studies have revealed an inhibitory effect of dietary curcumin on AOM-induced increases in ODC activity, tyrosine protein kinase activity and arachidonic acid metabolism in rat colon, consistent with its ability to inhibit the formation of aberrant crypt foci in this tissue [42].



Ellagic Acid

Fig. 3. Ellagic Acid

ELLAGIC ACID

Ellagic acid (Fig. 3) is a naturally occurring phenolic constituent of many species from a diversity of flowering plant families. It is present in plants in the form of hydrolyzable tannins called ellagitannins. Ellagitannins are esters of glucose with hexahydroxydiphenic acid; when hydrolyzed, they yield ellagic acid, the dilactone of hexahydroxydiphenic acid [52]. The content of ellagic acid in a series of fruits and nuts has been determined, and the highest amounts were found in blackberries, raspberries, strawberries, cranberries, walnuts and pecans [53]. Ellagic acid, a very stable compound, is moderately soluble in dimethylsulfoxide, slightly soluble in other organic solvents, and relatively insoluble in water. It is pharmacologically active and has been found to control hemorrhage in animals and in humans, presumably as a result of its ability to activate Hageman factor [54].

Ellagic Acid and Chemoprevention

Initial studies of the antitumor activity of ellagic acid were conducted in mice using lung and skin tumors as endpoints (Table III). Lesca [55]

TABLE III. Ellagic Acid: Summary of Anticarcinogenic Effects

Organ	Treatment	Reference
Lung	B(*a*)P, BPDE, NNK	55-57
Skin	Initiation:	58
	MCA	56
	Promotion:	78,79
	B(*a*)P/TPA	
	DMBA/TPA	
Esophagus	NMBA	61–63
Liver	FAA	64

[a] See text for discussion.

investigated the effect of ellagic acid on B(*a*)P-induced lung tumors in A/J mice. When administered ip or as a dietary admixture, ellagic acid decreased the multiplicity of B(*a*)P-induced tumors; however, significant toxicity was observed following ip administration of ellagic acid. Chang *et al.* [56] showed that treatment of preweanling mice with a total dose of 300 nmol of ellagic acid shortly before ip injection of B(*a*)P diol-epoxide caused a 44–75% inhibition in the number of diol-epoxide-induced lung tumors. Similar treatment with ellagic acid had little or no effect on the occurrence of B(*a*)P-induced lung tumors. Boukharta *et al.* [57] reported that, at doses ranging from 0.06 to 4.0 g/kg diet, ellagic acid inhibited the multiplicity of lung tumors induced by NNK administered to A/J mice in the drinking water by 54%. In contrast, two related compounds, esculin and esculetin, had no effect on lung tumorigenesis. Mukhtar *et al.* [58] showed that topical application of ellagic acid to the skin of BALB/c mice exerted strong protective effects against MCA-induced skin carcinogenesis. In a separate experiment using a similar dosing regimen, Smart *et al.* [59] did not observe any inhibitory effect of ellagic acid on MCA-induced skin carcinogenesis in CD-1 or in BALB/c mice. Chang *et al.* [56] observed that topical application of 2,500 nmol of ellagic acid shortly before a tumor-initiating dose of B(*a*)P diol-epoxide caused a 59-66% inhibition in the number of skin tumors per mouse after promotion with TPA. Similar treatment of mice with ellagic acid before the application of B(*a*)P inhibited the mean number of skin tumors by 28–33% after promotion with TPA, but this decrease was not significant. The data of Chang *et al.* [56] in both mouse skin and lung are consistent with the report of Sayer *et al.* [60], who showed that ellagic acid can bind to the diol-epoxide of B(*a*)P *in vitro*. If similar binding occurs *in vivo*, then one would expect ellagic acid to be more effective against B(*a*)P diol-epoxide tumorigenesis than against B(*a*)P tumorigenesis.

Other tissues in which ellagic acid has been shown to exhibit anticarcinogenic effects include the esophagus and liver. Mandal and Stoner [61] reported inhibitory effects of ellagic acid (0.4 and 4.0 mg/kg diet) on NMBA-tumorigenesis in the esophagus of F-344 rats. The ellagic acid inhibited the development of both preneoplastic and neoplastic lesions by 25–50%. These results were confirmed in a subsequent experiment by Daniel and Stoner [62]. In a recent experiment in our laboratory, ellagic acid was found to be an effective inhibitor of NMBA-tumorigenesis in the rat esophagus only when administered before, during and after the carcinogen; there was no significant inhibition of esophageal tumorigenesis when the inhibitor was administered in the post-initiation phase only [63]. Tanaka *et al.* [64] investigated the effect of ellagic acid on hepatocarcinogenesis induced by *N*-2-fluorenylacetamide (FAA) in male ACI/N rats. Rats were fed a diet containing 400 ppm of ellagic acid before, during and after administration of FAA in the diet. The ellagic acid reduced the number of altered foci and the incidence of hepatocellular neoplasms in carcinogen-treated rats.

Mechanistic Studies of the Anticarcinogenic Effects of Ellagic Acid

The inhibition of carcinogenesis by ellagic acid appears to occur through a number of mechanisms. With respect to biomarkers of tumor initiation, ellagic acid has been shown to inhibit the metabolic activation of polycyclic hydrocarbons (PAH) [*e.g.*, B(*a*)P, DMBA, and MCA], nitroso compounds (*e.g.*, NMBA and NNK) and aflatoxin B_1 into forms that induce DNA damage [65–71]. It could also promote carcinogen detoxification by stimulating the activity of various isoforms of the enzyme, glutathione-*S*-transferase [72–73]. A third mechanism by which ellagic acid could inhibit tumor initiation is through its potential role as a scavenger of the reactive metabolites of carcinogens. Ellagic acid reacts *in vitro* with B(*a*)P diol-epoxide by taking a sterically favorable position to form a covalently linked product in which the reactive epoxide ring of the pyrene is opened, rendering the carcinogen harmless [60]. It is not known if this reaction occurs *in vivo* however, or whether the reactive metabolites of carcinogens other than B(*a*)P react with ellagic acid. A fourth mechanism by which ellagic acid could inhibit tumor initiation is through occupation of sites in DNA that might otherwise react with carcinogens or their metabolites [74]. In one study, ellagic acid inhibited the binding of NMU to salmon sperm DNA by reacting with the O^6 position in guanine and preventing methylation at that site [75]. A subsequent report however,

failed to confirm this observation [76]. Barch and Fox [77] found that dietary ellagic acid selectively blocked methylation of the O^6 position of guanine in rats treated with NMBA. They suggested that this was due to the binding of ellagic acid to DNA since, in their experiments, the inhibitor had no effect on the metabolic activation of NMBA or the ability of NMBA to methyl-ate DNA.

With respect to the effects of ellagic acid on biomarkers of tumor promotion/progression, recent studies in the laboratory of Perchellet [78–79] have confirmed earlier reports of the ability of ellagic acid to inhibit TPA-induced tumor promotion in mouse skin. Moreover, these studies demonstrated that ellagic acid, applied topically to mouse skin, inhibited TPA-induced ODC activity, hydroperoxide production and DNA synthesis, all markers of skin tumor promotion [78–81]. Ellagic acid is also a potent inhibitor of free radical-induced lipid peroxidation which could contribute to its anti-promotion effects [82].

SUMMARY

The data presented in this report suggest that the tea polyphenols, curcumin and ellagic acid all have significant promise as chemopreventive agents, and that they have similar modes of action. Their ultimate use in chemoprevention however, may depend upon the extent of their bioavailability. To our knowledge, there are no reports on the pharmacokinetics of uptake and distribution of the tea polyphenols; studies to evaluate their biodistribution should be undertaken. There are conflicting reports on the uptake and distribution of curcumin however, it would appear that more than 50% of an oral dose is absorbed and small amounts appear in the urine [83–85]. Pharmacokinetic studies with ellagic acid indicate that its bioavailability is minimal; approximately 20% of an ip or oral dose excreted within the urine in 24 hours, and tissue concentrations in ppm of the administered dose [57,86, 87]. These observations in animal models suggest that the use of ellagic acid in humans may be severely compromised by its rather poor bioavailability.

REFERENCES

1. Morse MA, Stoner GD: Cancer chemoprevention: Principles and prospects. Carcinogenesis 14:1737–1746, 1993.
2. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC: Chemopreventive drug development: Prospectives and progress. Cancer Epidemiol Biomarkers Prev 3:85–98, 1994.
3. Mukhtar H, Katiyar SK, Agarwal R: Green tea and skin—anticarcinogenic effects. J Invest Dermatol 102: 3–7, 1994.
4. Wang ZY, Khan WA, Bickers DR, Mukhtar H: Protection against polycyclic aromatic hydrocarbon-induced skin tumor initiation in mice by green tea polyphenols. Carcinogenesis 10:411–415, 1989.
5. Yang CS, Wang ZY: Tea and cancer. J Natl Cancer Inst 85:1038–1049, 1993.
6. Katiyar SK, Agarwal R, Wood GS, Mukhtar H: Inhibition of 12-*O*-tetradecanoyl-phorbol-13-acetate-caused tumor promotion in 7,12-dimethylbenz(*a*)anthracene-initiated SENCAR mouse skin by a polyphenolic fraction isolated from green tea. Cancer Res 52:6890–6897, 1992.
7. Huang M-T, Ho C-T, Wang ZY, Ferraro T, Finnegan-Olive T, Lou Y-R, Mitchell JM, Laskin JD, Newmark H, Yang CS, Conney AH: Inhibitory effect of topical application of a green tea polyphenol fraction on tumor initiation and promotion in mouse skin. Carcinogenesis 13:947–954, 1992.
8. Katiyar SK, Agarwal R, Mukhtar H: Protection against malignant conversion of chemically-induced benign skin papillomas to squamous cell carcinomas in SENCAR mice by a polyphenolic fraction isolated from green tea. Cancer Res 53:5409–5412, 1993.
9. Wang ZY, Huang M-T, Ho C-T, Chang R, Ma W, Ferraro T, Reuhl KR, Yang CS, Conney AH: Inhibitory effect of green tea on the growth of established skin papillomas in mice. Cancer Res 52:6657–6665, 1992.
10. Wang ZY, Agarwal R, Bickers DR, Mukhtar H: Protection against ultraviolet B radiation-induced photocarcinogenesis in hairless mice by green tea polyphenols. Carcinogenesis 12:1527–1530, 1991.
11. Wang ZY, Huang M-T, Ferraro T, Wong C-Q, Lou Y-R, Iatropoulos M, Yang CS, Conney AH: Inhibitory effect of green tea in the drinking water on tumorigenesis by ultraviolet light and 12-*O*-tetradecanoyl-phorbol-13-acetate in the skin of SKH-1 mice. Cancer Res 52:1162–1170, 1992.
12. Wang ZY, Huang M-T, Lou Y-R, Xie J-G, Reuhl KR, Newmark HL, Ho C-T, Yang CS, Conney AH: Inhibitory effects of black tea, green tea, decaffeinated black tea, and decaffeinated green tea on ultraviolet B light-induced skin carcinogenesis in 7,12-dimethylbenz(*a*)anthracene-initiated SKH-1 mice. Cancer Res 54:3428–3435, 1994.
13. Wang ZY, Agarwal R, Khan WA, Mukhtar H: Protection against benzo(*a*)pyrene and *N*-nitrosodiethylamine-induced lung and forestomach tumorigenesis in A/J mice by water extracts of green tea and licorice. Carcinogenesis 13:1491–1494, 1992.
14. Katiyar SK, Agarwal R, Zaim MT, Mukhtar H: Pro-

tection against *N*-nitrosodiethylamine and benzo(*a*)-pyrene-induced forestomach and lung tumorigenesis in A/J mice by green tea. Carcinogenesis 14:849–855, 1993.

15. Katiyar SK, Agarwal R, Mukhtar H: Protective effects of green tea polyphenols administered by oral intubation against chemical carcinogen-induced forestomach and pulmonary neoplasia in A/J mice. Cancer Lett 73:167–172, 1993.
16. Wang ZY, Hong JY, Huang MT, Reuhl KR, Conney AH, Yang CS: Inhibition of N-nitrosodiethylamine- and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced tumorigenesis in A/J mice by green tea and black tea. Cancer Res 52:1943–1947, 1992.
17. Xu Y, Ho C-T, Amin SG, Han C, Chung FL: Inhibition of tobacco-specific nitrosamine-induced lung tumorigenesis in A/J mice by green tea and its major polyphenol as antioxidants. Cancer Res 52:3875–3879, 1992.
18. Shi ST, Wang Z, Theresa JS, Hong J, Chen W, Ho C, Yang CS: Effects of green tea and black tea on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone bioactivation, DNA methylation and lung tumorigenesis in A/J mice. Cancer Res 54:4641–4647, 1994.
19. Fujita Y, Yamane T, Tanaka M, Kuwata K, Okuzumi J, Takahashi T, Fujiki H, Okuda T: Inhibitory effect of (–)-epigallocatechin gallate on carcinogenesis with *N*-ethyl-*N*-nitro-*N*-nitrosoguanidine in mouse duodenum. Jpn J Cancer Res (Gann) 80:503–505, 1989.
20. Xu Y, Han C: The effect of Chinese tea on the occurrence of esophageal tumors induced by *N*-nitrosomethylbenzylamine formed *in vivo*. Biomed Environ Sci 3:406–412, 1990.
21. Gao GD, Zhou LF, Qi G: Initial study of antitumorigenesis of green tea: Animal test and flow cytometry. Tumor 10:42–44, 1990.
22. Yamane T, Hagiwara N, Tateishi M, Akachi S, Kim M, Okuzumi J, Kitao Y, Inagake M, Kuwata K, Takahashi T: Inhibition of azoxymethane-induced colon carcinogenesis in rat by green tea polyphenol fraction. Jpn J Cancer Res (Gann) 82:1336–1340, 1991.
23. Narasiwa T, Fukaura Y: A very low dose of green tea polyphenols in drinking water prevents *N*-methyl-*N*-nitrosourea-induced colon carcinogenesis in F344 rats. Jpn J Cancer Res (Gann) 84:1007–1009, 1993.
24. Chen ZY, Yan RQ, Qin GZ: Effect of six edible plants on the development of aflatoxin B_1-induced γ-glutamyltranspepidase positive hepatocyte foci in rats. Chung Hua Chung Liu Tsa Chih (Chinese J Cancer) 9:109–111, 1987.
25. Li Y: Comparative study on the inhibitory effect of green tea, coffee and levamisole on the hepatocarcinogenic action of diethylnitrosamine. Chung Hua Chung Liu Tsa Chih (Chinese J Cancer) 13:193–195, 1991.
26. Harada N, Takabayashi F, Oguini I: Anti-promotion effect of green tea extracts on pancreatic cancer in golden hamster induced by *N*-nitroso-bis(2-oxopropyl)amine. Intl Symp Tea Science (Japan) 1991.
27. Hirose M, Hoshiya T, Akagi K, Futakuchi M, Ito N: Inhibition of mammary gland carcinogenesis by green tea catechins and other naturally occurring antioxidants in female Sprague-Dawley rats pretreated with 7,12-dimethylbenz(*a*)anthracene. Cancer Lett 83:149–156, 1994.
28. Wang ZY, Das M, Bickers DR, Mukhtar H: Interaction of epicatechins derived from green tea with rat hepatic cytochrome P-450. Drug Metab Disp 16: 98–103, 1988.
29. Khan SG, Katiyar SK, Agarwal R, Mukhtar H: Enhancement of antioxidant and phase II enzymes by oral feeding of green tea polyphenols in drinking water to SKH-1 hairless mice: Possible role in cancer chemoprevention. Cancer Res 52:4050–4052, 1992.
30. Khan WA, Wang ZY, Athar M, Bickers DR, Mukhtar H: Inhibition of the skin tumorigenicity of (+)-7, β8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)-pyrene by tannic acid, green tea polyphenols and quercetin in SENCAR mice. Cancer Lett 42:7–12, 1988.
31. Sohn OS, Surace A, Fiala ES, Richie Jr. JP, Colosimo S, Zand E, Weisburger JH: Effects of green and black tea on hepatic xenobiotic metabolizing systems in the male F344 rat. Xenobiotica 24:119–127, 1994.
32. Agarwal R, Katiyar SK, Zaidi SIA, Mukhtar H: Inhibition of skin tumor promoter-caused induction of epidermal ornithine decarboxylase in SENCAR mice by polyphenolic fraction isolated from green tea and its individual epicatechin derivatives. Cancer Res 52: 3582–3588, 1992.
33. Katiyar SK, Agarwal R, Ekker S, Wood GS, Mukhtar H: Protection against 12-*O*-tetradecanoylphorbol-13-acetate-caused inflammation in SENCAR mouse ear skin by polyphenolic fraction isolated from green tea. Carcinogenesis 14:361–365, 1993.
34. Ruch RJ, Cheng SJ, Klaunig JE: Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 10:1003–1008, 1989.
35. Govindarajan VS: Turmeric chemistry, technology and quality. CRC Rev Food Sci Nutr 12:199–301, 1980.
36. Ammon HPT, Wahl MA: Pharmacology of *Curcuma longa*. Planta Med 57:1–7, 1990.
37. Huang M-T, Robertson FM, Lysz T, Ferraro T, Wang ZY, Georgiadis CA, Laskin JD, Conney AH: Inhibitory effects of curcumin on carcinogenesis in mouse epidermis. In Huang M-T, Ho C-T, Lee CY (eds): "Phenolic Compounds in Food and Their Effects on Health II: Antioxidants and Cancer Prevention." Washington, DC: American Chemical Society, 1992, pp 339–349.
38. Tonnesen HH: Chemistry, stability and analysis of curcumin, a naturally occurring drug molecule. Ph.D. Dissertation, The Institute of Pharmacy, University of Oslo, 1988.
39. Huang M-T, Smart RC, Wong C-Q, Conney AH: Inhibitory effect of curcumin, chlorogenic acid, caffeic acid and ferulic acid on tumor promotion in mouse

skin by 12-*O*-tetradecanoylphorbol-13-acetate. Cancer Res 48:5941–5946, 1988.
40. Nagabhushan M, Bhide SV: Curcumin as an inhibitor of cancer. J Am Coll Nutr 11:192–198, 1992.
41. Mehta RG, Moon RC: Characterization of effective chemopreventive agents in mammary gland *in vitro* using an initiation-promotion protocol. Anticancer Res 11:593–596, 1991.
42. Rao CV, Simi B, Reddy BS: Inhibitory effect by dietary curcumin of azoxymethane-induced ornithine decarboxylase, tyrosine protein kinase, arachidonic acid metabolism and aberrant crypt foci formation in the rat colon. Carcinogenesis 14:2219–2225, 1993.
43. Huang M-T, Lou Y-R, Ma W, Newmark HL, Reuhl KR, Conney AH: Inhibitory effects of dietary curcumin on forestomach, duodenal, and colon carcinogenesis in mice. Cancer Res 54:5841–5847, 1994.
44. Huang M-T, Wang ZY, Georgiadis CA, Laskin JD, Conney AH: Inhibitory effects of curcumin on tumor initiation by benzo[*a*]pyrene and 7,12-dimethylbenz-[*a*]anthracene. Carcinogenesis 13:2183–2186, 1992.
45. Azuine MA, Bhide SV: Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer 17:77–83, 1992.
46. Mukundan MA, Chacko MC, Annapurna VV, Krishnaswamy K: Effect of turmeric and curcumin on BP-DNA adducts. Carcinogenesis 14:493–496, 1993.
47. Lahiri M, Maru GB, Amonkar AJ, Bhide SV: Modulation of benzo[*a*]pyrene induced DNA damage by some chemopreventive agents. In Bhide SV, Maru GB (eds): "Chemoprevention of Cancer." New Delhi: Omega Scientific Publishers, 1992, pp 152–161.
48. Polasa K, Averback D, Krishnaswamy K: Studies on the influence of curcumin on the induction and repair of DNA single strand breaks (SSb) produced by genotoxic agents. Mutat Res, 1994 (in press).
49. Rao TS, Basu N, Siddiqui HH: Anti-inflammatory activity of curcumin analogues. Indian J Med Res 75:574–578, 1982.
50. Kunchandy E, Rao MNA: Oxygen radical scavenging activity of curcumin. Int J Pharmaceut 58:237–240, 1990.
51. Huang M-T, Lysz T, Ferraro T, Abidi TF, Laskin JD, Conney AH: Inhibitory effects of curcumin on *in vitro* lipoxygenase and cyclooxygenase activities in mouse epidermis. Cancer Res 51:813–819, 1991.
52. Bate-Smith EC: Detection and determination of ellagitannins. Phytochemistry 11:1153–1156, 1972.
53. Daniel EM, Krupnick AS, Heur Y-H, Blinzler JA, Nims RW, Stoner GD: Extraction, stability, and quantitation of ellagic acid in various fruits and nuts. J Food Comp Anal 2:338–349, 1989.
54. Botti RE, Ratnoff OD: Studies on the pathogenesis of thrombosis: An experimental "hypercoagulable" state induced by the intravenous injection of ellagic acid. J Lab Clin Med 64:385–398, 1964.
55. Lesca P: Protective effects of ellagic acid and other plant phenols on benzo(*a*)pyrene-induced neoplasia in mice. Carcinogenesis 4:1651–1653, 1983.
56. Chang RL, Huang MT, Wood AW, Wong CQ, Newmark HL, Yagi H, Sayer JM, Jerina DM, Conney AH: Effect of ellagic acid and hydroxylated flavonoids on the tumorigenicity of benzo(*a*)pyrene and (±)-7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(*a*)-pyrene on mouse skin and in the newborn mouse. Carcinogenesis 6:1127–1133, 1985.
57. Boukharta M, Jalbert G, Castonguay A: Biodistribution of ellagic acid and dose-related inhibition of lung tumorigenesis in A/J mice. Nutr Cancer 18:181–189, 1992.
58. Mukhtar H, Das M, Del Tito Jr. BJ, Bickers DR: Protection against 3-methylcholanthrene-induced skin tumorigenesis in BALB/c mice by ellagic acid. Biochem Biophys Res Commun 119:751–757, 1984.
59. Smart RC, Huang MT, Chang RL, Sayer JM, Jerina DM, Wood AW, Conney AH: Effect of ellagic acid and 3-*O*-decylellagic acid on the formation of benzo-(a)pyrene-derived DNA adducts *in vivo* and on the tumorigenicity of 3-methylcholanthrene in mice. Carcinogenesis 7:1669–1675, 1986.
60. Sayer JM, Yagi H, Wood AW, Conney AH, Jerina DM: Extremely facile reaction between the ultimate carcinogen benzo(*a*)pyrene-7,8-diol 9,10-epoxide and ellagic acid. J Am Chem Soc 104:5562–5564, 1982.
61. Mandal S, Stoner GD: Inhibition of *N*-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats by ellagic acid. Carcinogenesis 11:55–61, 1990.
62. Daniel EM, Stoner GD: The effects of ellagic acid and 13-*cis*-retinoic acid on *N*-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats. Cancer Lett 56:117–124, 1991.
63. Siglin JC, Barch DH, Stoner GD: Effects of phenethyl isothiocyanate, ellagic acid, sulindac and supplemental dietary calcium on the induction and progression of esophageal carcinogenesis in rats induced by short-term administration of *N*-nitrosomethylbenzylamine. Carcinogenesis, 1995 (in press).
64. Tanaka T, Iwata H, Niwa K, Mori Y, Mori H: Inhibitory effect of ellagic acid on *N*-2-fluorenylacetamide-induced liver carcinogenesis in male AC1/N rats. Jpn J Cancer Res (Gann) 79:1297–1303, 1988.
65. Dixit R, Teel RW, Daniel FB, Stoner GD: Inhibition of benzo[*a*]pyrene and benzo(*a*)pyrene-trans-7,8-diol metabolism and DNA binding in mouse lung explants by ellagic acid. Cancer Res 45:2951–2956, 1985.
66. Teel RW, Dixit R, Stoner GD: The effect of ellagic acid on the uptake, persistence, metabolism and DNA-binding of benzo[*a*]pyrene in cultured explants of strain A/J mouse lung. Carcinogenesis 6:391–395, 1985.
67. Singletary K, Liao C: Ellagic acid effects on the carcinogenicity, DNA-binding and metabolism of 7,12-dimethylbenz(*a*)anthracene (DMBA). In Vivo 3:173–176, 1989.
68. Mukhtar H, Del Tito Jr. BJ, Marcelo CL, Das M, Bickers DR: Ellagic acid: A potent naturally occurring inhibitor of benzo[*a*]pyrene metabolism and its subsequent glucuronidation, sulfation and covalent binding to DNA in cultured BALB/c mouse keratin-

ocytes. Carcinogenesis 5:1565–1571, 1984.
69. Mandal S, Shivapurkar NM, Galati AJ, Stoner GD: Inhibition of *N*-nitrosobenzylmethylamine metabolism and DNA binding in cultured rat esophagus by ellagic acid. Carcinogenesis 9:1313–1316, 1988.
70. Pepin P, Rossignol G, Castonguay A: Inhibition of NNK-induced lung tumorigenesis in A/J mice by ellagic acid and butylated hydroxyanisole. Cancer J 3:266–273, 1990.
71. Mandal S, Ahuja A, Shivapurkar NM, Cheng S-J, Groopman JD, Stoner GD: Inhibition of aflatoxin B_1 mutagenesis in *Salmonella typhimurium* and DNA damage in cultured rat and human tracheobronchial tissues by ellagic acid. Carcinogenesis 8:1651–1656, 1987.
72. Das M, Bickers DR, Mukhtar H: Effect of ellagic acid on hepatic and pulmonary xenobiotic metabolism in *mice: Studies* on the mechanism of its anticarcinogenic action. Carcinogenesis 6:1409–1413, 1985.
73. Barch DH, Rundhaugen LM: Ellagic acid induces glutathione *S*-transferase Ya through activation of the antioxidant responsive element of the 5' regulatory region of the glutathione *S*-transferase Ya gene. Hepatology 16:561, 1992.
74. Teel RW: Ellagic acid binding to DNA as a possible mechanism for its antimutagenic and anticarcinogenic action. Cancer Lett 30:329–336, 1986.
75. Dixit R, Gold B: Inhibition of *N*-methyl-*N*-nitrosourea-induced mutagenicity and DNA methylation by ellagic acid. Proc Natl Acad Sci (USA) 83:8039–8043, 1986.
76. Lord HL, Snieckus VA, Josephy PD: Re-evaluation of the effect of ellagic acid on dimethylnitrosamine mutagenicity. Mutagenesis 4:453–455, 1989.
77. Barch DH, Fox CC: Selective inhibition of methylbenzylnitrosamine-induced formation of esophageal O^6-methylguanine by dietary ellagic acid in rats. Cancer Res 48:7088–7092, 1988.
78. Perchellet JP, Gali HU, Perchellet EM, Laks PE, Bottari V, Hemingway RW, Scalbert A: Antitumor-promoting effects of gallotannins, ellagitannins, and flavonoids in mouse skin *in vivo*. In Huang M-T, Osawa T, Ho C-T, Rosen RT (eds): "Food Phytochemicals for Cancer Prevention. I—Fruits and Vegetables." Washington, DC: American Cancer Society, 1994, pp 303–327.
79. Perchellet J-P, Gali HU, Perchellet EM, Klish DS, Armbrust AD: Antitumor-promoting activities of tannic acid, ellagic acid, and several gallic acid derivatives in mouse skin. In Hemingway RW, Laks PE (eds): "Plant Polyphenols." New York: Plenum Press, 1992, pp 783–801.
80. Gali HU, Perchellet EM, Perchellet J-P: Inhibition of tumor promoter-induced ornithine decarboxylase activity by tannic acid and other polyphenols in mouse epidermis *in vivo*. Cancer Res 51:2820–2825, 1991.
81. Gali HU, Perchellet EM, Klish DS, Johnson JM, Perchellet J-P: Hydrolyzable tannins: potent inhibitors of hydroperoxide production and tumor promotion in mouse skin treated with 12-*O*-tetradecanoylphorbol-13-acetate *in vivo*. Int J Cancer 51:425–432, 1992.
82. Majid S, Khanduja KL, Gandhi RK, Kapur S, Sharma RR: Influence of ellagic acid on antioxidant defense system and lipid peroxidation in mice. Biochem Pharmacol 42:1441–1445, 1991.
83. Wahlstrom B, Blennow G: A study on the fate of curcumin in the rat. Acta Pharmacol Toxicol 43:86–92, 1978.
84. Ravindranath V, Chandrasekhara N: Absorption and tissue distribution of curcumin in rats. Toxicology 16: 259–265, 1980.
85. Ravindranath V, Chandrasekhara N: Metabolism of curcumin—studies with [^{3}H]curcumin. Toxicology 22:337–344, 1982.
86. Teel RW: Distribution and metabolism of ellagic acid in the mouse following intraperitoneal administration. Cancer Lett 34:165–171, 1987.
87. Teel RW, Martin RM: Disposition of the plant phenol ellagic acid in the mouse following oral administration by gavage. Xenobiotica 4:397–405, 1988.