ABSTRACT

Tannic acid inhibits the mutagenicity of several polycyclic aromatic hydrocarbons (PAHs) and their bay-region diol-epoxides. Our prior studies have shown that when applied topically to Sencar mice, tannic acid caused substantial inhibition of epidermal PAH metabolism, subsequent PAH-DNA adduct formation, and PAH-induced skin tumorigenesis (H. Mukhtar et al., Cancer Res., 48: 2361–2365, 1988, and references therein). In this study the effects of tannic acid supplementation in the diet (1%, w/w, inAIN-76 diet) of Sencar mice on benzo(a)pyrene (BP) metabolism and its subsequent DNA binding and tumorigenesis in lung and forestomach were evaluated. Animals receiving a tannic acid-containing diet showed diminished aryl hydrocarbon hydroxylase and 7-ethoxyresorufin O-deethylase activities in the forestomach and lung. Elevated glutathione S-transferase and NAD(P)H: quinone reductase activities were observed in these tissues. Maximum effects occurred after 45 days of feeding. Administration of [3H]BP p.o. to animals resulted in lower covalent binding to DNA in forestomach and lung of animals receiving tannic acid-containing diet as compared to animals receiving AIN-76 control diet. Tumor induction studies in forestomach and lung revealed significant protection against BP-induced tumorigenesis in animals fed tannic acid-supplemented diet as compared to animals fed control diet. The mice fed tannic acid-supplemented diet developed 3.3 forestomach tumors/mouse compared to 5.2 tumors/mouse in animals receiving control diet. The numbers of pulmonary tumors per mouse in animals fed tannic acid-supplemented diet and control diet were 1.6 and 3.1, respectively. Topical application of 7,12-dimethylbenz(a)anthracene to animals fed tannic acid-supplemented diet did not result in significant protection against skin tumorigenesis. However, a slight delay in the onset of skin tumor formation occurred in tannic acid-fed animals when compared to animals receiving control diet. Our data suggest that dietary supplementation with tannic acid affords protection against BP-induced forestomach and lung tumorigenesis in rodents.

INTRODUCTION

In recent years, there has been a growing interest in identifying naturally occurring minor dietary constituents capable of protecting against the development of some forms of cancer (1–6). In this regard several plant phenols have been shown to inhibit the mutagenicity and/or tumorigenicity of several PAHs and their bay-region diol-epoxides (7–11). Our previous studies have shown that topical application of several plant phenols to murine skin inhibits PAH metabolism, PAH-DNA adduct formation, and skin tumorigenicity (11–14). In these studies, all of the plant phenols tested, tannic acid was shown to possess the most protective effects. Since tannic acid is usually consumed in the diet, it was considered important to study its protective effect when part of the diet. In this study we assessed the effect of dietary supplementation of tannic acid on PAH-metabolizing enzymes, subsequent binding of PAH metabolites to DNA, and tumorigenicity in the skin, forestomach, and lung of Sencar mice. Our data show that addition of tannic acid to the diet of Sencar mice affords protection against BP-induced forestomach and lung tumorigenesis.

MATERIALS AND METHODS

Chemicals. Gold label BP, resorufin, and tannic acid were obtained from Aldrich Chemical Co. (Milwaukee, WI). 7-Ethoxyresorufin was purchased from Pierce Chemicals. NADPH, NADH, protease (type XI), m-cresol, 8-hydroxyquinoline, calf thymus DNA (type I), RNase A (type II-A), 2,6-dichlorophenolindophenol, 1-chloro-2,4-dinitrobenzene, and bovine serum albumin were obtained from Sigma Chemical Co. (St. Louis, MO). [7-H]BP (specific activity, 25 Ci/mmol) was purchased from Amersham Searle (Chicago, IL). Prior to use, radiolabeled BP was purified first on a silica gel (Partisol 10 μm; Waters Associates) column with hexane as the eluting solvent and subsequently by reverse-phase high-performance liquid chromatography using a DuPont Zorbax octadecylsilane column (76.2 mm × 25 cm) eluted with methanol:water (19:1, v/v). The purity of BP was >99% as judged by high-performance liquid chromatography. All other chemicals were obtained in the purest form commercially available.

Diet and Animals. Six-week-old female Sencar mice, obtained from the NCI-Frederick Cancer Research Facility, Bethesda, MD, were used in this study. Tannic acid-supplemented diet was custom prepared by I CN Biochemicals, Cleveland, OH, by mixing 1%, w/w, tannic acid in AIN-76 semipurified diet. AIN-76 semipurified diet was used as the control diet. Both diets were obtained in pellet form.

Treatment of Animals for Metabolic Studies. On arrival in our animal facility the animals were fed AIN-76 semipurified diet for 7 days after which they were divided into two groups. One group of animals continued receiving this diet whereas the animals of the other group were shifted to tannic acid-supplemented diet. A close estimate of the diet consumption was monitored by twice weekly weighing of the unconsumed feed. Both groups of animals consumed between 4.5 and 5.5 g of diet per day. Thus each animal on tannic acid-supplemented diet consumed approximately 50 mg tannic acid per day. The mice were weighed weekly during the course of the experiment. No significant differences in weight gain occurred in the two groups of animals. Furthermore, none of the animals receiving tannic acid-supplemented diet showed any signs of toxicity. Animals were withdrawn at 0, 30, 45, 60, and 90 days of feeding; shaved with electric clippers; and decapitated with surgical scissors. Lung, forestomach, and epidermis were removed, cleaned free of blood and extraneous material, and processed for the preparation of cytosol and microsomes.

Preparation of Microsomal and Cytosolic Fractions. Forestomach, lung, and epidermal microsomal and cytosolic fractions were prepared according to established procedures described earlier (13). The cytosolic fractions were stored at −80°C until use and the microsomal pellets were overlaid with buffer A [100 mM potassium phosphate, pH 7.4, containing 10 mM dithiothreitol, 10 mM EDTA, and 20% (v/v) glycerol] frozen at −170°C under liquid nitrogen. For the determination of microsomal enzyme activities the frozen pellets were thawed slowly (within 3–5 days of tissue preparation) in an ice bucket and used as the enzyme source. Enzyme activities were stable under these storage conditions for at least 3 weeks.

Enzyme Assays. AHCh activity was determined by a modification of the method of Neber and Gelboin (15), the details of which have been...
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Results

Effect of Dietary Tannic Acid on Enzyme Activities. It is believed that BP requires metabolism by sequential reactions catalyzed by cytochrome P-450 and epoxide hydrolase to BP diol-epoxide I which interacts with the target tissue DNA to initiate tumorigenesis (26). Therefore, inhibition of the activities of the specific enzymes responsible for the metabolism of BP might lead to diminished carcinogenic response in target organs. The effect of feeding tannic acid-supplemented diet to mice on microsomal AHH and ERD activities in forestomach and lung is shown in Figs. 1 and 2, respectively. Feeding of tannic acid to mice resulted in substantial lowering of AHH and ERD activities in lung and forestomach. Greater inhibitory effects were observed in the forestomach than in the lung. The maximum depletion of enzyme activities in forestomach was observed after 45 days of feeding and persisted up to 60 days. In lung, however, the enzyme activities showed a gradual normalizing trend.

The enzymes GST and QR play an important role in the detoxification and/or elimination of carcinogenic intermediates of PAHs (17, 27). The effect of tannic acid-supplemented diet on forestomach and pulmonary GST and QR activities in mice is shown in Figs. 1 and 2. Feeding of tannic acid significantly induced GST and QR activities in forestomach while no effect is shown in Figs. 1 and 2. Feeding of tannic acid-supplemented diet to mice on microsomal AHH and ERD activities in forestomach and pulmonary GST and QR activities in mice is shown in Figs. 1 and 2. Feeding of tannic acid significantly induced GST and QR activities in forestomach while no effect

![Fig. 1. Effect of feeding 1% tannic acid-supplemented diet to Sencar mice on enzyme activities in lung. Each value represents the mean of at least three determinations. Bars, SEM. For each determination 4 animals were pooled.](image-url)

![Fig. 2. Effect of feeding 1% tannic acid-supplemented diet to Sencar mice on enzyme activities in stomach. Each value represents the mean of at least three determinations. Bars, SEM. For each determination 4 animals were pooled.](image-url)
Effect of Feeding Tannic Acid in Diet on Skin Tumorigenesis. The effect of feeding tannic acid-supplemented diet on BP-induced pulmonary neoplasia is shown in Table 2. Feeding of tannic acid-supplemented diet to mice prior to tumor initiation resulted in a significant decrease in the number as well as in the incidence of forestomach tumors. In animals fed control diet and tannic acid-supplemented diet, the number of tumors per mouse were 5.2 ± 0.7 and 3.3 ± 0.6, respectively. Histological examination showed that the tumors in both the groups were benign papillomas. Tumors in animals receiving control diet was invariably larger than those in the animals fed tannic acid-supplemented diet.

Effect of Dietary Tannic Acid on Lung Tumorigenesis. The effect of feeding tannic acid-supplemented diet on BP-induced pulmonary neoplasia is shown in Table 3. Feeding of tannic acid-supplemented diet to mice prior to tumor initiation resulted in a significant decrease in the number (1.6 ± 0.3 tumors/mouse in the tannic acid-supplemented diet group compared to 3.1 ± 0.5 tumors/mouse in the control diet group) as well as in the incidence (30% decrease) of lung tumors. All the tumors were identified histologically as adenomas and no evidence of malignancy was observed in any animal.

Effect of Feeding Tannic Acid in Diet on Skin Tumorigenesis. The effect of feeding tannic acid-supplemented diet on DMB-initiated and 12-O-tetradecanoylphorbol-13-acetate-promoted skin tumorigenesis is shown in Fig. 3. Tumor data are presented as the percentage of mice with tumors (Fig. 3A) and as the number of tumors per mouse (Fig. 3B) as a function of the number of weeks on test. Feeding of tannic acid-supplemented diet to mice prior to tumor initiation resulted in an increase in the latency period of tumor initiation. The latency period was 3 weeks in the control group as compared to 6 weeks in the tannic acid-fed group of animals. However, at time periods beyond 6 weeks on test no significant difference was found in tumor numbers or percentage of mice with tumors in the mice of the two groups. All skin tumors developed in both groups of animals were benign papillomas.

### DISCUSSION

A great deal of attention has recently focused on the role of diet in cancer etiology with a view to develop strategies for cancer prevention (28). Several epidemiological studies have suggested that diet influences human cancer risk (29). A large number of anticarcinogenic compounds have been identified in food. These are predominantly the plant products with highly diversified chemical structures which comprise a part of virtually every human diet (28–30). Polyhydroxy plant phenols are one important group of compounds in this category. One of the widely distributed members of this class is tannic acid, found in a variety of plants, some of which are also consumed in human diet (31). Tannic acid has been shown to have beneficial pharmaceutical effects (32, 33). The feeding of tannic acid in diet in the present study resulted in protection against BP-induced neoplasia of forestomach and lung while it showed only a slight delay in the onset of skin tumorigenesis. It appears that administration of tannic acid p.o. has its strongest effects in forestomach followed by lung. Previous reports from our laboratory and others have demonstrated that several plant phenols like quercetin (34), myricetin (14), anthraflavinic acid (10, 14), ellagic acid (35), and tannic acid (14) show inhibitory effects against PAH-induced tumorigenesis. Unlike the present study, in most of the previous studies the application of the plant phenol was topical although their actual uptake is through the diet (31). Therefore, the results of the present study simulate, to some extent, the actual influence of these compounds in diet.

The mechanisms by which these compounds exert their potential effects to inhibit chemical-induced tumorigenesis are not well understood (1, 2). However, some of these compounds inhibit certain P-450-dependent monooxygenase activities while others induce phase II drug-metabolizing enzymes like GST (9, 10, 12, 18). Since carcinogenic PAHs require metabolic activation by the P-450-dependent monooxygenase enzyme system to manifest their carcinogenic potential, the inhibition of P-450 and associated monooxygenase activities might lead to the inhibition of PAH metabolism and their subsequent binding to DNA, thus inhibiting their carcinogenic response. Feeding of tannic acid in the diet in the present study was found to inhibit P-450-dependent AH and ERD activities in forestomach and lung. This tumor-inhibitory response in the forestomach and lung reported in the present study may, therefore, be due in part to the inhibition of P-450 monooxygenase activities. The reduction in [3H]BP binding to DNA in target organs in the present study may also be due to the metabolic inhibition of P-450-dependent metabolism of PAH in these tissues. It has been suggested that the levels and persistence of specific carcinogen-DNA adducts, such as benzo(a)pyrene diol-epoxide 1-deoxyguanosine adduct in the target tissue, correlate with the susceptibility to BP-induced neoplasia (13, 26). In prior studies we have shown that topical applications of tannic acid and other...
Table 2 Effect of feeding 1% tannic acid-supplemented diet to Sencar mice on BP-induced forestomach neoplasia

<table>
<thead>
<tr>
<th>Addition to AIN-76 diet</th>
<th>No. of mice at risk</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>30</td>
<td>90</td>
<td>5.2 ± 0.7</td>
</tr>
<tr>
<td>1% tannic acid</td>
<td>30</td>
<td>63</td>
<td>3.3 ± 0.6*</td>
</tr>
</tbody>
</table>

* Female Sencar mice (6–8 weeks old) were fed AIN-76 semipurified diet (control) or AIN-76 semipurified diet supplemented with 1% tannic acid for 45 days after which they received BP (100 mg/kg body weight) in 0.2 ml of corn oil by intubation p.o. twice weekly for 4 weeks. At the end of the fourth week the animals were switched to control diet on which they were maintained until the termination of the experiment at 36 weeks. Data represent the mean ± SEM of 30 animals.

Table 3 Effect of feeding 1% tannic acid-supplemented diet to Sencar mice on BP-induced pulmonary adenoma formation

<table>
<thead>
<tr>
<th>Addition to AIN-76 diet</th>
<th>No. of mice at risk</th>
<th>% of mice with tumors</th>
<th>No. of tumors/mouse</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>30</td>
<td>90</td>
<td>3.1 ± 0.5</td>
</tr>
<tr>
<td>1% tannic acid</td>
<td>30</td>
<td>60</td>
<td>1.6 ± 0.3*</td>
</tr>
</tbody>
</table>

* Female Sencar mice (6–8 weeks old) were fed AIN-76 semipurified diet (control) or AIN-76 semipurified diet supplemented with 1% tannic acid for 45 days after which they received single injection of BP (100 mg/kg body weight) in 0.2 ml of corn oil and were then maintained on control diet until the termination of the experiment at 36 weeks. Data represent the mean ± SEM of 30 animals.

![Fig. 3. Effect of feeding 1% tannic acid (TA)-supplemented diet to Sencar mice on DMBA-initiated and 12-O-tetradecanoylphorbol-13-acetate-promoted skin tumorigenesis. Female mice (6–8 weeks old) were fed AIN-76 (control) or 1% tannic acid-supplemented AIN-76 diet for 45 days after which they received a single topical application of an initiating dose of DMBA (10 μg/mouse). Ten days later, the animals received twice weekly topical applications of 12-O-tetradecanoylphorbol-13-acetate (3.24 nmol). On the first day of 12-O-tetradecanoylphorbol-13-acetate application, all animals were shifted to AIN-76 control diet. The percentage of mice with tumors (A) and the number of tumors per mouse (B) were plotted as a function of the number of weeks on test. None of the animals in the DMBA alone, 12-O-tetradecanoylphorbol-13-acetate alone, or acetone alone groups developed neoplasms.

Experimental Design

- Plant phenols to murine skin resulted in the reduction of PAH-DNA binding, particularly in the inhibition of benzo(a)pyrene diol-epoxide I-deoxyguanosine adduct formation (13). Thus, the inhibition in forestomach and pulmonary tumorigenesis by dietary tannic acid in the present study may be due to the inhibition of [3H]BP-DNA binding in forestomach and lung. Similarly, induction of GST in forestomach and lung might also lead to increased conjugation leading to faster excretion of the reactive intermediary metabolite(s). The induction of QR activity may be helpful in reducing levels of toxic quinone derivatives of the carcinogen. Thus, the induction in the activities of both enzymes might contribute to the protection afforded by tannic acid against BP-induced neoplasia. However, other possible mechanisms such as the antioxidant action of tannic acid and its direct interaction with carcinogenic reactive metabolite(s) cannot be ruled out.

In conclusion our data show that dietary supplementation with low levels of tannic acid affords protection against PAH-induced forestomach and lung neoplasia in rodents. The mechanism(s) of inhibition of tumorigenesis may be due to its inhibitory effect on microsomal monooxygenase activity, subsequent PAH-DNA binding, and its ability to induce detoxification enzymes.

ACKNOWLEDGMENTS

Thanks are due to Daniel P. Bik and James D. Steele for technical assistance and to Sandra Evans for preparing the manuscript.

REFERENCES

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