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

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ABSTRACT

Progression of benign tumors to malignant cancer is critical since cancerous lesions are capable of metastatic spread and eventually causing death. Inhibitors of the conversion process, therefore, would likely be useful as cancer chemopreventive agents. In this study, we assessed the protective effect of topical application of a polyphenolic fraction isolated from green tea (GTP) against spontaneous as well as benzo(a)pyrene (BPO)- and 4-nitroquino line-N-oxide (4-NQO)-enhanced malignant conversion of chemically induced skin papillomas in SENCAR mice. Papillomas were induced in SENCAR mice by topical application of 7,12-dimethylbenz(a)anthracene as a tumor-initiating agent followed by twice a week application of 12-O-tetradecanoylphorbol-13-acetate as a tumor-promoting agent. Beginning at the 20th week, when papilloma yield was stabilized, enhanced malignant conversion was achieved by twice weekly topical application of either BPO or 4-NQO, whereas spontaneous malignant conversion was associated with topical application of acetone. In these protocols, preapplication of GTP (6 mg/animal) 30 min prior to skin application of acetone, BPO, or 4-NQO resulted in 14, 31, and 29% protection, respectively, in terms of percentage of mice with carcinomas, and 20, 35, and 43% protection in terms of number of carcinomas/mouse. In these experiments, a BPO- and 4-NQO-enhanced rate of malignant conversion was also found to be decreased significantly by the skin application of GTP; however, such effects of GTP were less profound in the cases of spontaneous malignant conversion. The results of this study suggest that, in addition to its chemopreventive effects against tumor initiation and promotion stages of multistage carcinogenesis, green tea also possesses significant protective effects against tumor progression, specifically tumor progression induced by BPO and 4-NQO.

INTRODUCTION

Carcinogenesis in murine skin, and possibly in human skin and other tissues, is a multistage process composed of initiation, promotion, and progression (1). Among these stages, the tumor progression stage in which nonmalignant lesions convert into malignancy is of greatest concern since malignant lesions are capable of metastatic spread and eventually causing death. The naturally occurring dietary inhibitors of this process, therefore, could offer an attractive approach for cancer chemoprevention. While at least 25 classes of dietary inhibitors that offer promise against initiation and promotion stages of carcinogenesis are known (2), far less is known about the inhibitors of the tumor progression stage of carcinogenesis.

In recent years, we and others have reported the cancer-chemopreventive effects of green tea, specifically GTP (3), WEGT, and EGCG, in various animal tumor bioassay systems using a diversified class of chemical carcinogens as well as UV. Studies from our laboratory have shown that p.o. feeding in drinking water or topical application of GTP or EGCG affords protection against benzo(a)pyrene-, DMBA-, and 3-methylcholanthrene-induced tumor initiation and complete carcinogenesis and UVB radiation-induced photocarcinogenesis in murine skin (3–5). Subsequently, similar observations were also reported by others (6) using WEGT. Huang et al. (7) and Katiyar et al. (8) have also shown that topical application of GTP protects against TPA-caused tumor promotion in DMBA-initiated murine skin; the mechanism of this effect involves the inhibition of tumor promoter-caused induction of epidermal cyclooxygenase, lipoxygenase, and ornithine decarboxylase activities, edema, and hyperplasia (7–10). In another recent study, it has also been shown that green tea inhibits the growth of established skin papillomas in mice (11). By using different animal model systems, several other studies have also shown that p.o. feeding of GTP, WEGT, or EGCG protects against chemical carcinogen-induced tumorigenesis in lung, forestomach, colon, duodenum, and esophagus (Ref. 12 and references therein). Recently, we have shown that oral feeding of WEGT or GTP to A/J mice during initiation, postinitiation, and the entire period of tumorigenesis results in significant protection against benzo(a)pyrene- and N-nitrosodimethylamine-induced forestomach and lung tumorigenesis (13).

Because chemical carcinogenesis in mouse skin by initiation and promotion provides a useful model for the study of the progression of benign papillomas to malignant squamous cell carcinomas (14), we used a multistage model of experimental carcinogenesis to evaluate the chemopreventive effect of GTP against the tumor progression stage of carcinogenesis. Studies were designed to assess such effects under a spontaneous malignant conversion protocol, as well as those enhanced by the free radical-generating compound BPO and the carcinogenic agent 4-NQO.

MATERIALS AND METHODS

DMBA and BPO were from Aldrich Chemical Co., Milwaukee, WI, and 4-NQO and TPA were from Sigma Chemical Co., St. Louis, MO. GTP was prepared from green tea leaves as described earlier (9).

Animals. Six-week-old female SENCAR mice, obtained from Harlan-Sprague Dawley, Indianapolis, IN, were acclimatized for 1 week before use and subjected to a 12-h light/12-h dark cycle, housed at 24 ± 2°C and 50 ± 10% relative humidity in a room with 12–15 cycles of air exchanges/h, and fed Purina chow diet and water ad libitum.

Papilloma-Carcinoma Induction in SENCAR Mouse Skin. The dorsal skin of SENCAR mice was shaved using electric clippers and only those animals which were in the resting phase of their hair cycle were used in this study. As described earlier (15), benign skin papillomas were induced by a single topical application of DMBA (25 nmol in 0.2 ml of acetone/animal), and 1 week following initiation, all the mice were promoted with twice weekly topical applications of TPA (4.0 nmol in 0.2 ml of acetone/animal) for 18 weeks. A few of the tumors, which were verified histologically, were benign papillomas.

As summarized in Table 1, beginning at the 20th week, the tumor-bearing SENCAR mice were divided into 6 groups; 20 animals bearing benign skin papillomas were randomly assigned to each group in a way that the total...
PROTECTION AGAINST MALIGNANT CONVERSION BY GREEN TEA

Table 1 Protection against the conversion of chemically induced benign skin papillomas to carcinomas by GTP

<table>
<thead>
<tr>
<th>Treatments^a</th>
<th>% of mice with carcinomas^b</th>
<th>% Protection</th>
<th>No. of carcinomas/mouse^b</th>
<th>% of protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone</td>
<td>35</td>
<td>0.45</td>
<td>2.25</td>
<td>1.0</td>
</tr>
<tr>
<td>GTP + acetone</td>
<td>30</td>
<td>1.4</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>BPO</td>
<td>15</td>
<td>3.1</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>GTP + BPO</td>
<td>45</td>
<td>3.1</td>
<td>2.0</td>
<td>3.5</td>
</tr>
<tr>
<td>4-NQO</td>
<td>70</td>
<td>3.5</td>
<td>2.0</td>
<td>13.5</td>
</tr>
<tr>
<td>GTP + 4-NQO</td>
<td>50</td>
<td>29</td>
<td>2.0</td>
<td>43.0</td>
</tr>
</tbody>
</table>

^a Beginning at the 20th week, SENCAR mice bearing benign skin papillomas were divided into 6 subgroups of 20 animals. The mice in different subgroups were treated twice weekly either with 0.2 ml of acetone, 20 mg of BPO, or 250 μg of 4-NQO in 0.2 ml of acetone/mouse. To study the protective effect of GTP, 6 mg of GTP in 0.2 ml of acetone were applied topically 30 min prior to the application of acetone, BPO, or 4-NQO, respectively. The selection of doses of BPO and 4-NQO was based on our prior studies in which the topical application of GTP (6 mg) showed substantial protective effects against skin tumorigenesis (8).

^b Data represent the protective effect of GTP in terms of the percentage of mice with carcinomas and the number of carcinomas per mouse at the termination of the experiment, i.e., 52nd week.

RESULTS

The two-stage initiation-promotion protocol detailed in “Materials and Methods” was used. By 20 weeks, the papilloma yield had stabilized since no new papillomas were induced and the size of the papillomas did not increase with further treatment with TPA. During the tumor progression experiment, no increase in the number of papillomas per mouse and no increase in the cumulative number of papillomas per group was evident, suggesting that no new tumors appeared as a result of the use of BPO or 4-NQO during the progression stage (data not shown).

The malignant conversion of chemically induced papillomas to squamous cell carcinomas at different time intervals after the treatment with acetone, BPO, or 4-NQO, with or without prior application of GTP, is shown in Figs. 1 and 2. In terms of both the percentage of mice with carcinomas (Fig. 1) and the number of carcinomas per mouse (Fig. 2) as a function of the number of weeks on test, the application of GTP prior to the application of BPO and 4-NQO resulted in significant protection (P < 0.03 to 0.05, Wilcoxon rank sum test) against the conversion of benign skin papillomas to carcinomas in SENCAR mice. GTP application also afforded protection against spontaneous malignant conversion of benign papillomas to carcinomas (Figs. 1 and 2); however, in this case the protective effect of GTP was not statistically significant, specifically when compared with that observed in the cases of BPO- and 4-NQO-enhanced malignant conversion. As shown in Table 1, at the termination of the experiment at 52 weeks, the application of GTP prior to the application of BPO or 4-NQO resulted in 31 and 29% protection, respectively, in terms of the percentage of mice with carcinomas, and 35 and 43% protection, respectively, in terms of the number of carcinomas per mouse.

The protective effects of GTP against the spontaneous, BPO-enhanced, and 4-NQO-enhanced rate of malignant conversion are shown in Fig. 3. During the progression stage, it was observed that the rate of malignant conversion is stabilized at 40 weeks in the case of BPO and at 44 weeks in the case of 4-NQO. At each time point, however, the BPO- and 4-NQO-enhanced rate of malignant conversion was significantly higher when compared with the spontaneous malignant conversion rate (Fig. 3). The application of GTP prior to the application of BPO and 4-NQO showed a significant protection against the malignant conversion rate throughout the experiment (P < 0.001 to 0.005, Fisher-Irwin exact test). However, no significant difference was observed in the magnitude of protection afforded by GTP against the spontaneous conversion rate (Fig. 3). It is also evident from the data shown in Fig. 3 that GTP also delayed the latent period...
Earlier studies suggest that the transformation of benign skin papillomas to carcinomas requires further genetic changes in papilloma cells, and it can be achieved by repeated treatment with tumor-initiating agents (14, 21). These findings suggest that the changes necessary to influence the rate of malignant transformation seem to be identical with the changes involved in initiation (14, 21). Our study also confirms that 4-NQO, a tumor initiator as well as a complete carcinogen, significantly increases the rate of malignant conversion substantially inhibited by pretreatment with GTP, suggesting that GTP may also protect against the malignant conversion of skin papillomas to carcinomas caused by a variety of carcinogens.

The ineffectiveness of GTP against spontaneous malignant conversion supports the hypothesis that at least two different populations of cells occur during the initiation stage of carcinogenesis, giving rise to different populations of papillomas (14, 21). It has been suggested that one group of papillomas are programmed genetically to progress into carcinomas, whereas the other group progresses into carcinoma only after additional insult to the genome of tumor cells (14, 21). Such additional mutations may be induced by either free radical-generating compounds or genotoxic agents (Refs. 14, 16–18, and 21 and references therein). In this study, treatment with GTP seems to be ineffective in preventing the conversion of papillomas already programmed genetically to progress into carcinomas. However, GTP appears to be effective in suppressing the effect of BPO and 4-NQO toward enhanced genetic instability, subsequently leading to additional insult to the genome. This process results in protection against the increased transformation of benign skin papillomas into carcinomas.

In summary, these results suggest that topical application of GTP afforded protection against BPO- and 4-NQO-enhanced malignant conversion of benign skin papillomas to squamous cell carcinomas in SENCAR mice. The protective effects were evident in terms of the percentage of mice with carcinomas and the number of carcinomas per mouse as well as the rate of malignant conversion.

REFERENCES
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