Chemoprevention of 4-Nitroquinoline 1-Oxide-induced Oral Carcinogenesis by Dietary Protocatechuic Acid during Initiation and Postinitiation Phases

Takuji Tanaka, Toshihiko Kawamori, Masami Ohnishi, Kiyohisa Okamoto, Hideki Mori, and Akira Hara

First Department of Pathology, Gifu University School of Medicine, 40 Tsukasa-machi, Gifu City 500 [T. T. K., M. O., K. O., H. M.], and Department of Biochemistry, Gifu Pharmaceutical University, Gifu City 502 [A. H.], Japan

ABSTRACT

The modifying effects of three doses of dietary protocatechuic acid (PCA) given the initiation and postinitiation phases of oral carcinogenesis initiated with 4-nitroquinoline 1-oxide (4-NQO) were investigated in male F344 rats. At 6 weeks of age, rats were divided into experimental and control groups and fed the diet containing PCA at various doses of 0 g/kg diet (basal diet alone), 0.5 g/kg diet (500 ppm), 1 g/kg diet (1000 ppm), and 2 g/kg diet (2000 ppm). At 7 weeks of age, all animals except PCA alone and control groups were given 4-NQO (20 ppm) in the drinking water for 8 weeks to induce oral cancer. Seven days after the 4-NQO exposure, groups of animals fed the PCA diets were switched to the basal diet and continued on this diet until the end of the study. Starting 1 week after the end of 4-NQO exposure, the groups given 4-NQO and a basal diet were switched to the diets containing PCA and maintained on these diets for 22 weeks. The other groups consisted of rats given 2000 ppm PCA alone or untreated rats. All animals were necropsied at the termination of the experiment (week 33). The incidences of tongue neoplasms and preneoplastic lesions, polyamine levels in the tongue tissue, and cell proliferation activity estimated by bromodeoxyuridine-labeling index and by morphometric analysis of silver-stained nucleolar organizer regions' protein were compared among the groups. Feeding of PCA at all doses during initiation or postinitiation phase significantly decreased the development of tongue neoplasms (squamous cell papilloma and carcinoma) and preneoplasia (hyperplasia and dysplasia) (P < 0.05). There were no such lesions in rats treated with 2000 ppm PCA alone or those in an untreated control group. Dietary administration of PCA also caused significant decreases in the labeling index of bromodeoxyuridine and the number and area of silver-stained nucleolar organizer regions per cell nucleus, known as cell proliferation indices, of the tongue squamous epithelium (P < 0.05). In addition, PCA exposure during either initiation or postinitiation phase decreased polyamine levels in the oral mucosa (P < 0.05). These results clearly indicated that PCA inhibited rat oral carcinogenesis in both initiation and postinitiation phases, when administered in these respective phases together with or following treatment with 4-NQO, and such inhibition might be related to suppression of cell proliferation by PCA.

INTRODUCTION

Oral cancer is a common neoplasm in some regions in the world. The highest rates occur in developing countries, particularly in the Southern Asia (India, Sri Lanka, South Vietnam, Papua New Guinea, the Philippines, Hong Kong, or Taiwan), China, and parts of Brazil (1–4). In the Southern Asian countries, up to 25% of all cancers are present in the oral cavity, and tobacco and betel nut chewing are responsible for this malignancy (5). In Europe, France has the highest incidence of oral and pharyngeal cancer. This variation in incidence is related to exposure to known etiological agents (6). Epidemiological data provide strong support for exogenous factors such as tobacco and alcohol use as being major causative agents (7, 8). Viral infection (9, 10) and contaminants such as nitrosamines (11), polycyclic hydrocarbons (12), or urethans (13) are also suggested to be causative factors. Although Japan has one of the lowest incidences of oral and pharyngeal cancer in the world, the number of patients with these malignancies has been increasing, accounting for 4,900 new cases and 1,825 deaths in 1980 and 11,000 new cases and 2,607 deaths in 1990 (14). It has been reported that patients with oral cancer have an increased incidence of second primary tumors of the oral cavity (15, 16). In fact, patients with early lesions have a high cure rate of their primary tumor but go on to succumb to the second malignancy. Approximately 10–40% of such patients will develop second primary tumors, a rate that is related to continued exposure to carcinogens or promoters (17). This is considered to be the result of a diffuse mucosal abnormality, often referred as “field cancerization” resulting from carcinogen exposure (18). Oral cancer is a multifocal disease and experimental studies indicated that such lesions develop through a multistage process (19, 20). Because of easy accessibility to examine and follow-up of the lesions in the oral cavity, the oral cavity is one of the excellent target organs for experimental chemoprevention studies.

The term “cancer chemoprevention” refers to the prevention of cancer by intervention using nontoxic synthetic chemicals or chemicals from natural substances before malignancy (21). A number of micronutrients (22), macronutrients (23), and nonnutrients (24) have been reported as inhibiting or chemopreventive agents in chemical carcinogenesis in rodents. In our search for chemopreventive agents for cancer development using several experimental animal models, some natural products with antioxidative property from edible plants, herbs, or fruits have appeared to exert tumor-inhibiting effects in digestive organs (25, 26). Experimental studies on cancer chemoprevention in the oral cavity have mainly been conducted using hamster buccal pouch carcinogenesis model with a carcinogen, 7,12-dimethylbenz[a]anthracene, and chemopreventives have been limited to some vitamins (27–29). Our previous works revealed inhibitory and chemopreventive effects of several natural and synthetic compounds on 4-NQO-induced oral carcinogenesis (30–33). These include indole-3-carbinol, sinigrin, plant phenolics (caffeic, ellagic, chlorogenic, and ferulic acids), synthetic antioxidants (butylated hydroxytoluene and butylated hydroxyanisole), nonsteroidal antiinflammatory drugs (indomethacin and piroxicam), and disulfiram. An ODC inhibitor, DL-α-difluoromethylornithine, also exerted remarkable suppressing effects on 4-NQO-induced rat carcinogenesis (34). Some of these agents have been shown to inhibit cell proliferation (32, 33), which plays an important role in the multistage carcinogenesis (35–37), in the target organs including oral cavity (38), and such property is suggested to be one of the mechanisms of their tumor-inhibitory potential (39). More recently, we have proposed a new chemopreventive agent, PCA, in liver and colon carcinogenesis (40, 41). Epidemiological observations suggest a statistically significant inverse association between oral cancer and the consumption of fruits and/or vegetables (42, 43). PCA is one of the constituents in edible plants,
fears, and vegetables. Recently, PCA from the rind of Citrus reticulata BLANCO has been reported to have a strong antioxidative property (44). Therefore, a modifying (possibly inhibiting) effect of PCA on oral carcinogenesis might be suspected.

In the present study, a possible inhibitory effect of dietary exposure of PCA during initiation and postinitiation stage on 4-NQO-induced oral carcinogenesis was investigated in male F344 rats to obtain further evidence that PCA possesses cancer chemopreventive property. Dose-related efficacy of PCA, the effects of PCA on polyamine levels in the oral cavity, and the alteration of proliferative potential of the oral mucosa by measuring BrdUrd labeling index and AgNORs number were also assessed to clarify underlying mechanism(s), if PCA possessed an inhibitory effect on oral tumorigenesis.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. Male F344 rats, 4 weeks old, were purchased from Japan SLC, Inc. (Hamamatsu City, Japan). After quarantine for 2 weeks, a total of 193 rats (6 weeks of age) for determining MTD and the modifying effect of PCA were transferred to the holding room under controlled condition at 23 ± 2°C (SD) temperature, 50 ± 10% humidity, and a 12-h light/dark cycle and randomized into experimental and control groups. They were housed three or four to a wire cage. Powdered CE-2 (CLEA Japan, Inc., Tokyo, Japan) was used as basal diet during the experiment. It contained 50.4% crude carbohydrate, 24.8% crude protein, 4.6% crude fat, 2.7% ash, 4.2% crude cellulose, and 8.8% water but did not plant phenolics present from plant foods. 4-NQO (CAS: 56-57-5, 98% pure) was obtained from Wako Pure Chemical Ind. Co., Ltd., Osaka, Japan. PCA (CAS: 99-50-3, 97% pure) was purchased from Aldrich Chemical Co., Milwaukee, WI. Experimental diets mixed with PCA at various concentrations and 4-NQO solution (20 ppm) were prepared on a weekly basis and stored in a cold room (4°C) until used. The stability test of PCA in the diet at room temperature was not done since this chemical is quite stable as was confirmed in the previous studies (the recovery of PCA was more than 98% under the bioassay feeding conditions) (40, 41).

Determination of MTD of PCA. Before the start of the chemopreventive experiment, the MTD of PCA was determined. At 5 weeks of age, groups of male F344 rats (10 rats/group) were fed the basal diet (CE-2) containing various levels of PCA (0, 0.3, 0.6, 1.2, 2.5, 5.0, and 10 g/kg diet). Body weights were recorded weekly for 6 weeks. All animals were inspected daily for any symptoms of toxicity. At the end of 6 weeks, all animals were sacrificed, and several organs (stomach, intestine, liver, kidney, and lung) were examined grossly and histopathologically for any abnormalities. Also, clinical chemistry was performed for aspartate transaminase, alanine transaminase, alkaline phosphatase, lactic dehydrogenase, and glucose-6-phosphatase. The MTD is defined as the highest dose that causes no more than a 10% weight decrement as compared to the control diet group and does not produce mortality or any external signs of toxicity that would be predicted to shorten the natural life span of the animal. The MTD value of PCA was over 10 g/kg PCA diet (10,000 ppm), since no clinical signs and histopathological changes for toxicity, weight gain retardation, and abnormalities of chemical profiles were noted in rats fed at various levels (data not shown).

Experimental Procedure for Chemopreventive Efficacy of PCA. The experiment was designed to examine the modifying effect of three doses of PCA during the initiation and postinitiation phases of 4-NQO-induced oral carcinogenesis in male F344 rats (Fig. 1). Based on the results of MTD study and those in previous studies (40, 41), the highest dose of PCA used in the present study was 2 g PCA/kg diet (<20% MTD).

A total of 173 rats were divided into nine groups as shown in the tables. At 7 weeks of age, rats in groups 1 through 7 were given 4-NQO (20 ppm) in the drinking water for 8 weeks. Groups 2, 3, and 4 were, respectively, given the diets containing 0.5 g PCA/kg diet (500 ppm), 1 g PCA/kg diet (1000 ppm), and 2 g PCA/kg diet (2000 ppm), since no clinical signs and histopathological changes for toxicity were observed. At 6 weeks of age until 1 week after the stop of the carcinogen exposure, they were then switched to the basal diet and maintained on this diet for 22 weeks. Groups 5, 6, and 7 were, respectively, fed the diets mixed with PCA at concentrations of 0.5 g/kg diet, 1 g/kg diet, and 2 g/kg diet, starting 1 week after the cessation of 4-NQO treatment and continued on these diets for 22 weeks. Group 8 was fed the diet containing 2000 ppm PCA alone during the experiment. Group 9 was given the basal diet and tap water throughout the experiment and served as an untreated control.

All rats were carefully observed daily and consumption of the drinking water containing 4-NQO or the diets mixed with PCA was recorded to estimate intake of chemicals. The experiment was terminated at 32 weeks and all animals were sacrificed by decapitation between 9 a.m. and noon, avoiding artificial changes from polyamine assay, to evaluate the incidences of preneoplastic and neoplastic lesions in the oral cavity. At necropsy, all organs, especially the oral cavity, were examined grossly and all organs except for tongue were fixed in 10% buffered formalin. Tongues were cut approximately into two halves; one portion was used for polyamine assays and the other was used for histopathology and cell proliferation counts. For histopathological confirmation, tissues and gross lesions were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by the conventional histological methods using hematoxylin and eosin stain. Epithelial lesions (hyperplasia, dysplasia, and neoplasm) in the oral cavity were diagnosed according to the criteria described by Bánóczy and Cala (45) and WHO (46).

Polyamine Levels of Tongue Tissue. The polyamines in the oral cavity tissues were measured by means of a new enzymatic method developed by Koide et al. (47). At sacrifice, one-half of the tongues of all rats were collected and the amounts of diamine, spermine, and spermidine were determined by an enzymatic differential assay. The results obtained by this method correlated well with those obtained by high performance liquid chromatography.

Determination of Proliferative Activity in the Tongue Epithelium by AgNORs Enumeration and BrdUrd-labeling Index. To assess the proliferative activity of squamous epithelium of the tongue, the number and area of AgNORs per nucleus, and BrdUrd-labeling index of randomly selected five nonlesional areas of the tongue were quantified according to the methods described previously (19). For measurement of BrdUrd-incorporated nuclei, the animals were given an i.p. injection of 50 mg/kg body weight BrdUrd (Sigma Chemical Co., Ltd., St. Louis, MO) 1 h prior to killing. The tongue was removed and cut into two. One half was used for polyamine assay and the other was fixed in 10% buffered formalin for histopathology, AgNORs counting, and BrdUrd-labeling index. Three serial sections (3 mm thick) were made after embedding in paraffin. On the one section, a one-step silver colloid method for AgNORs staining (19) was carried out and computer-assisted image analysis quantification using an image analysis system (SPICCA II (Japan Abionics Co., Tokyo, Japan) with a Olympus BH-2 microscope (Olympus Optical Ind. Co., Ltd., Tokyo, Japan) and a color-charged coupled device camera (Hamamatsu Photonics Co., Hamamatsu City, Japan) was performed on 100 nuclei of interphase cells from nonlesional areas (25). The other section was used for
Inhibition of oral cancer by Protocatechuic Acid

### RESULTS

#### General Observations.
Animals in groups 1–8 tolerated well the p.o. administration of 4-NQO and/or PCA. There were no significant differences on total intake of 4-NQO/rat among seven groups (groups 1–7; data not shown). Total intakes of PCA/rat in groups 2–4 or groups 5–7 were increased in proportion as the dose increased (data not shown). The mean body and liver weights at the end of the study are indicated in Table 1. The mean body weights of rats in all groups given 4-NQO and/or PCA except groups 6 and 7 were comparable with that of a control group (group 9). The mean body weights of rats in groups 6 and 7 were significantly lower than that of group 1 (P < 0.001 and P < 0.02). The mean liver weight of animals in group 7 was significantly lower than that of group 1 (P < 0.02 and P < 0.001) and the mean liver weight of animals in group 5 was significantly lower than that of group 1 (P < 0.05). Also, significant differences in the mean relative liver weight were obtained between group 1 and groups 2 through 7. Dysplasia present in the present study could be classified into three degrees (mild, moderate, and severe dysplasia) and the frequencies of various grades of dysplasia are shown in Table 4. The incidences of moderate and severe dysplasia in groups 2–7 were lower than those of group 1 and in rats of group 4 there were no moderate and severe dysplasia (group 1 versus group 4: P < 0.05). Also no moderate dysplasia were found in rats of groups 6 and 7 (group 1 versus group 6 or 7: P < 0.05). On the other hand, the incidences of mild dysplasia in groups 5 and 6 were higher than that of group 1 with no statistical differences.

#### Polyamine Levels.
The results of polyamine assay of tongue epithelium are summarized in Table 5. Total polyamine and spermine levels in rats of each group were located at the dorsal site of the tongue and mostly were well-differentiated squamous cell carcinoma, microscopically. The incidences of tongue tumors (squamous cell papilloma and carcinoma) in all groups are shown in Table 2. In group 1 (4-NQO alone), the incidences of tongue squamous cell carcinoma and squamous cell papilloma were 58% and 16%, respectively. Combined incidence of these tumors in this group was 58%. On the other hand, only a few rats given PCA during 4-NQO administration (groups 2–4) or those fed PCA diets after 4-NQO exposure (groups 5–7) possessed tongue neoplasms. No neoplasms developed in rats of groups 8 and 9. Statistical analysis revealed significant differences on the incidence of tumors between group 1 and groups 2–7 (P < 0.05–P < 0.001). Besides these neoplasms, a number of preneoplastic lesions (hyperplasia and dysplasia) were present in the tongue of rats in groups 1–7. The incidences of such lesions are indicated in Table 3. The incidences of rats with hyperplasia and dysplasia in group 1 were 89 and 58%, respectively. In rats fed PCA together with or after 4-NQO exposure (groups 2–7), the incidences of these lesions were smaller compared with those of group 1 (4-NQO alone). Significant differences in the incidence of preneoplasia were obtained between group 1 and groups 2 through 7 (P < 0.05–P < 0.001). Dysplasia present in the present study could be classified into three degrees (mild, moderate, and severe dysplasia) and the frequencies of various grades of dysplasia are shown in Table 4. The incidences of moderate and severe dysplasia in groups 2–7 were lower than those of group 1 and in rats of group 4 there were no moderate and severe dysplasia (group 1 versus group 4: P < 0.05). Also no moderate dysplasia were found in rats of groups 6 and 7 (group 1 versus group 6 or 7: P < 0.05). On the other hand, the incidences of mild dysplasia in groups 5 and 6 were higher than that of group 1 with no statistical differences.

### Table 1 Body, liver, and relative liver weights in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Body wt (g)</th>
<th>Liver wt (g)</th>
<th>Relative liver wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-NQO</td>
<td>19</td>
<td>360 ± 23</td>
<td>15.7 ± 2.5</td>
<td>4.37 ± 0.57</td>
</tr>
<tr>
<td>2</td>
<td>4-NQO + 500 ppm PCA</td>
<td>20</td>
<td>356 ± 39</td>
<td>13.9 ± 2.9d</td>
<td>3.87 ± 0.58</td>
</tr>
<tr>
<td>3</td>
<td>4-NQO + 1000 ppm PCA</td>
<td>19</td>
<td>369 ± 46</td>
<td>15.2 ± 3.2</td>
<td>4.08 ± 0.61</td>
</tr>
<tr>
<td>4</td>
<td>4-NQO + 2000 ppm PCA</td>
<td>21</td>
<td>359 ± 28</td>
<td>15.8 ± 2.2e</td>
<td>4.40 ± 0.45</td>
</tr>
<tr>
<td>5</td>
<td>4-NQO → 500 ppm PCA</td>
<td>21</td>
<td>355 ± 26</td>
<td>15.1 ± 1.7</td>
<td>4.24 ± 0.24</td>
</tr>
<tr>
<td>6</td>
<td>4-NQO → 1000 ppm PCA</td>
<td>19</td>
<td>327 ± 30f</td>
<td>13.1 ± 1.7f</td>
<td>4.01 ± 0.43f</td>
</tr>
<tr>
<td>7</td>
<td>4-NQO → 2000 ppm PCA</td>
<td>20</td>
<td>335 ± 38g</td>
<td>13.4 ± 2.3g</td>
<td>3.99 ± 0.50g</td>
</tr>
<tr>
<td>8</td>
<td>2000 ppm PCA</td>
<td>18</td>
<td>344 ± 27</td>
<td>13.3 ± 1.8</td>
<td>3.87 ± 0.35</td>
</tr>
<tr>
<td>9</td>
<td>No treatment</td>
<td>18</td>
<td>348 ± 21</td>
<td>13.6 ± 1.5</td>
<td>3.91 ± 0.32</td>
</tr>
</tbody>
</table>

* Mean ± SD.

a Significantly different from group 9 by Student's t test.

b Significantly different from group 9 by Fisher's exact probability lest.

c Significantly different from group 1 by Student's t test.

d Significantly different from group 1 by Fisher's exact probability lest.

### Table 2 Incidence of tongue neoplasms in rats of each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Total</th>
<th>Papilloma</th>
<th>Carcinoma</th>
<th>No. of rats with tongue neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-NQO</td>
<td>19</td>
<td>11 (58)*</td>
<td>3 (16)</td>
<td>1 (5)*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>2</td>
<td>4-NQO + 500 ppm PCA</td>
<td>20</td>
<td>3 (15)*</td>
<td>3 (15)</td>
<td>1 (5)*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>3</td>
<td>4-NQO + 1000 ppm PCA</td>
<td>19</td>
<td>2 (11)*</td>
<td>2 (11)</td>
<td>0*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>4</td>
<td>4-NQO + 2000 ppm PCA</td>
<td>21</td>
<td>2 (10)*</td>
<td>2 (10)</td>
<td>0*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>5</td>
<td>4-NQO → 500 ppm PCA</td>
<td>21</td>
<td>2 (10)*</td>
<td>2 (10)</td>
<td>0*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>6</td>
<td>4-NQO → 1000 ppm PCA</td>
<td>19</td>
<td>5 (26)*</td>
<td>4 (21)</td>
<td>2 (11)*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>7</td>
<td>4-NQO → 2000 ppm PCA</td>
<td>20</td>
<td>2 (10)*</td>
<td>2 (10)</td>
<td>0*</td>
<td>4 (10)</td>
</tr>
<tr>
<td>8</td>
<td>2000 ppm PCA</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>No treatment</td>
<td>18</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, percentage.

** Significantly different from group 1 by Fisher's exact probability lest.

*. Significantly different from group 1 by Fisher's exact probability lest.

Downloaded from cancerres.aacrjournals.org on June 9, 2017. © 1994 American Association for Cancer Research.
AgNORs Enumeration and BrdUrd-labeling Index. The results of morphometric analysis of AgNORs and BrdUrd-labeling indices in the squamous epithelium are listed in Table 4. The mean number of AgNORs in group 8 (2000 ppm PCA alone) were almost similar to those of group 9 (untreated control).

DISCUSSION

The results in the present study indicated that dietary PCA administration during the initiation or postinitiation phase effectively suppressed oral carcinogenesis initiated with 4-NQO as revealed by reduced the incidences of neoplasms and preneoplasia in the tongue. Such inhibition was more remarkable when rats were given PCA together with 4-NQO. In particular, no malignant tongue neoplasms developed in rats given 4-NQO together with 1000 or 2000 ppm PCA diet. Also, no malignant tumors occurred in the tongue of rats fed the diet mixed with 2000 ppm PCA after 4-NQO exposure. The lowest dose of PCA used in the present study is almost 4 times larger than that consumed by humans, if humans daily consume 10 g lettuce and/or strawberry that contains 10–40 mg PCA/100 g.

The results in the current study are in agreement with those in our earlier works showing a suppressing effect of PCA on chemically induced liver and colon carcinogenesis in rats (40, 41). In liver carcinogenesis, feeding of PCA at 500 and 1000 ppm during initiation and postinitiation phases significantly reduced the occurrence of preneoplasia (liver cell foci) and hepatocellular neoplasms induced by diethylnitrosamine in rats (40). In addition, livers of rats fed PCA had lowered ODC activity compared with those treated with diethylnitrosamine alone (40). In azoxymethane-initiated rat colon carcinogenesis, PCA feeding at three dose levels (250, 500, and 1000 ppm) significantly inhibited the development of intestinal tumors when fed at a dose of 1000 ppm during initiation and fed at levels of 500 and 1000 ppm after azoxymethane exposure (41). The inhibitory effect of PCA in the present study was more remarkable than that found in liver
and colon carcinogenesis (40, 41), since the highest dose of PCA (2000 ppm) completely inhibited tongue cancer occurrence when fed during either the initiation or the postinitiation phase. Such tumor-reducing effect by PCA exposure during either initiation or postinitiation stage was observed parallel with decreased ODC activity of colonic mucosa and AgNORs counts in crypt cell nuclei (41). Thus, the results in the current study provide the additional evidences that a natural product, PCA, could effectively inhibit tumor development without any toxicity and PCA might be a possible cancer chemopreventive agent in the oral cavity in addition to other organs (colon and liver).

Various types of inhibitors of mutagenesis or carcinogenesis and their mechanisms have been postulated in available reviews (24, 39, 48–52). Initiation might be inhibited by preventing the formation of carcinogens from precursors, blocking the metabolic activation of carcinogens, increasing the detoxification of carcinogens by increasing the level of the enzymes involved (e.g., glutathione S-transferase), interception of carcinogens before their reaction with DNA, stimulation of error-free DNA repair, or suppression of cell proliferation. Chemopreventive agents that inhibit such processes have been categorized as “blocking agents” by Wattenberg (21, 49). Other types of chemopreventives are “suppressing agents” that prevent the development of neoplasms after carcinogen exposure (21, 49). The results in the current and previous studies may suggest that PCA possesses both blocking and suppressing properties in carcinogenesis (21, 49), although biochemical studies on modifying effects of dietary PCA on the phase I and phase II enzymes that affect the metabolism, detoxification, and elimination of carcinogens should be done. Such chemopreventives acting as blocking and suppressing agents from environment have been reported. These include an omega-3 fatty acid docosahexaenoic acid (53), benzyl isothiocyanate (54), and 1,4-phenylenedioxy(methylene)selenocyanate (55) in colon carcinogenesis, d-limonene in mammary carcinogenesis (21), and garlic or garlic compounds (56). Our series of chemopreventive study in oral carcinogenesis revealed similar properties of indole-3-carbinol and sinigrin (32). Indole-3-carbinol could also suppress the occurrence of spontaneous endometrial cancer in female Donryu rats by altering estradiol 2-hydroxylation (57). Although the mechanisms by which PCA exert its inhibitory effect on 4-NQO-induced rat oral carcinogenesis remain to be elucidated, several mechanisms could be considered. The results that PCA feeding during the initiation phase inhibited tongue tumor development may support the hypothesis that some plant phenolics with an antioxidative effect act as blocking agents (52, 58). As for 4-NQO-induced carcinogenesis, metabolic activation by DT-diaphorase [NAD(P)H dehydrogenase] in several organs including liver, lung, and stomach to 4-HAQO which is considered to be a proximate carcinogen (59); adduct formation of 4-HAQO with DNA (N-2-guanine, C-8-guanine, and N-6-adenine adducts) (60); and accessions of 4-NQO and/or 4-HAQO to the target tissues are necessary. Therefore, the mechanism(s) by which PCA administration during initiation stage suppressed tongue neoplasms might be its blocking capability of one or more of such processes.

In the present study, the proliferation activity of the tongue mucosal epithelium was estimated and PCA feeding lowered the cell proliferation activity. These results were comparable to our earlier experiments testing the chemopreventive efficacy of several natural phenolic antioxidants (25, 33) and a synthetic compound L-α-difluoromethylornithine (34). In rodent and human oral carcinogenesis, increased polyamine levels and/or ODC activity that are essential for cellular proliferation (61) were reported (34, 62). Cell proliferation is suggested to play an important role in multistage carcinogenesis including oral tumorigenesis (35–38). Presently, dietary PCA administration in either the initiation or the postinitiation stage reduced cell proliferation in the tongue epithelium and polyamine levels in the tongue tissue with or without preneoplastic and neoplastic lesions. Indole-3-carbinol and sinigrin, which possess some antioxidative property and antitumor activity in oral carcinogenesis, also exert their antitumor effects through suppression of cell proliferation in the oral squamous epithelium (32). Such an effect of PCA might also partly contribute to the inhibition of tongue tumorigenesis, especially when PCA diet was fed during the postinitiation phase. An isomer of PCA, 2,3-dihydroxybenzoic acid diacetate (dipropycol), is a known analgesic or antipyretic agent, presumably by inhibiting arachidonic metabolism to prostaglandins, leukotrienes, thromboxanes, etc., Thus, PCA may act as a tumor antipromoter by inhibition of arachidonic acid metabolism in oral epithelial cells, like other nonsteroidal antiinflammatory agents (31). Ongoing study using two-stage mouse skin carcinogenesis model will clarify such an antipromoter effect of PCA.

Since an important element in the evaluation of the possible role of chemopreventive compounds is the assessment of preclinical toxicity (48), the safety and toxicity of the possible chemopreventives deserve more attention. The results in this study and those in previous experiments in our laboratory (40, 41) indicated that a dose as low as 500 ppm (<5% MTD) of PCA in diet was found to be effective. Moreover, no toxicity was observed in rats fed the diet mixed with PCA at a dose of 10,000 ppm. PCA is a widely distributed phenolic acid. Several fruits and plants like citrus fruit, Illicium anisatum, and Illicium verum contain a small amount of PCA. However, very few epidemiological
studies on the relationship between the intake of foods rich in PCA and cancer incidence are available.

The significant reduction in the incidence of tongue preneoplastic lesions by PCA feeding may also indicate that PCA could be applied for the prevention of second primary neoplasms in the oral cavity, since such lesions are believed to precede tumor development in the oral cavity and to account for the development of local recurrence and second primary malignancies (18). Additional study on cancer chemoprevention of PCA is now under way in our laboratory using a rat bladder carcinogenesis model that is also considered to be a "field carcinogenesis" model.

In summary, dietary administration of PCA during the initiation and postinitiation phases significantly inhibited 4-NQO-induced oral carcinogenesis in rats, although the exact mechanisms by which PCA exerts its chemopreventive property are not known. The available evidences in this study warranted further research on the possible role of PCA in the prevention of cancer development.

REFERENCES

INHIBITION OF ORAL CANCER BY PROTOCATECHUIC ACID


Chemoprevention of 4-Nitroquinoline 1-Oxide-induced Oral Carcinogenesis by Dietary Protocatechuic Acid during Initiation and Postinitiation Phases

Takuji Tanaka, Toshihiko Kawamori, Masami Ohnishi, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/54/9/2359

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.