1,4-Phenyleneis(methylene)selenocyanate Exerts Exceptional Chemopreventive Activity in Rat Tongue Carcinogenesis

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Abstract

Among the organoselenium compounds, 1,4-phenyleneis(methylene) selenocyanate (p-XSC) is reported to exert the most effective chemopreventive effect on chemically induced carcinogenesis in the mammary glands, colon, and lung of laboratory animals. This study was designed to test the inhibitory effects of dietary p-XSC (5 and 15 ppm as selenium) during the initiation phase (1 week before, during, and up to 1 week after the carcinogen exposure) and the postinitiation phase (1 week after carcinogen administration until termination) on the formation of neoplasms of the tongue in male F344 rats by 4-nitroquinoline-1-oxide (4-NQO). The doses of p-XSC were 20% (5 ppm selenium) and 60% (15 ppm selenium) of maximum tolerated dose levels. At 6 weeks of age, all rats except those given p-XSC alone and those in untreated groups were treated with 4-NQO (20 ppm in the drinking water for 8 weeks). Dietary p-XSC, administered at selenium levels of 5 and 15 ppm during either the initiation or postinitiation phases, significantly reduced the incidence of carcinoma of the tongue. p-XSC was especially effective when it was administered at 15 ppm selenium during the postinitiation phase, in which case it completely inhibited the development of tongue carcinoma (from 47% in the dietary control to 0%). Glutathione S-transferase activities in the liver and tongue of rats treated with 4-NQO and p-XSC were significantly elevated compared to those in rats treated with 4-NQO alone. Similarly, quinone reductase activity was significantly elevated in the liver but decreased in the tongue (posterior portion). Such modulation by p-XSC in the phase II enzyme activities of the liver and tongue might be related to inhibition of the initiation. In addition, the expression of cell proliferation biomarkers, such as polyamine level, ornithine decarboxylase activity, 5-bromo-2'-deoxyuridine-labeling index, and argyrophilic nucleolar organizer's protein number, in the epithelium of the tongue was significantly altered in cell proliferation through modulation of ornithine decarboxylase activity and polyamine biosynthesis in the tongue epithelium might be related to inhibition occurring in the postinitiation phase of carcinogenesis. The dose levels of p-XSC used induced no toxicity or alteration in body weight gain. Although the precise mechanisms of p-XSC-induced inhibition of tongue carcinogenesis remains to be elucidated, it is evident that p-XSC has powerful chemopreventive efficacy against tongue carcinogenesis.

Introduction

Oral cancer progresses from hyperplastic lesions through dysplasia and carcinoma in situ to invasive carcinoma (1–3). Tobacco and alcohol use are the major risk factors in the development of oral cancer. Also, the combination of exposure to both tobacco and alcohol results in increased cancer incidence (4–6). Overall survival of patients with head and neck cancer remains poor, despite improvements in diagnosis and treatment of oral cancer. Furthermore, a significant number of patients cured of primary tumors will develop a second cancer within a few years, usually in the head and neck region, lung, or esophagus. Many people consuming tobacco and alcohol increase their risk of developing simultaneous or subsequent second primary cancers of these regions (7–9). The concept that common carcinogen exposure affects the entire epithelial lining of the lungs and upper aerodigestive tract is now known as "field cancerization" (10, 11). An understanding of it is needed to embrace a bright, new idea that can change the prognosis of this malignancy. One such promising approach is chemoprevention (12). The induction of oral cancer in laboratory animals by the carcinogens 4-NQO and DMBA has served as an in vivo model to identify agents that suppress oral carcinogenesis. Compared to the DMBA-induced buccal pouch carcinogenesis model in the hamster, 4-NQO-induced oral lesions appear to have distinct advantages (13). 4-NQO, a water-soluble quinoline derivative, produces a spectrum of preneoplastic and neoplastic lesions in the oral cavity, especially at the dorsal end of the posterior tongue of rats, when given in the drinking water. Because it is easily accessible for examination and follow-up of neoplastic changes, the oral cavity is an excellent target organ for preclinical chemoprevention studies (3). Using an animal model of 4-NQO-induced oral carcinogenesis for experimental chemoprevention, we have already reported some effective chemopreventive agents against oral carcinogenesis (3). However, the overall effectiveness of these agents leaves much to be desired. One would hope to completely inhibit tumor formation, i.e., achieve zero incidence at low levels of toxicity in assays with potent carcinogens. Therefore, efforts are being continued to develop novel, more effective, and less toxic chemopreventive agents than those already on hand.

One important class of chemopreventive compounds is that of the organic and inorganic forms of selenium (14). Increasing epidemiological evidence shows a protective role of selenium in human cancers (15, 16), including oral cancer (17–19). Supplementation of selenium in diet or drinking water is known to inhibit carcinogenesis in several animal models for mammary cancer, as well as cancer of the colon, lung, pancreas, liver, and skin (see Ref. 14 for review). Although inorganic selenium compounds can inhibit carcinogenesis, they are toxic. Naturally occurring selenium-containing amino acids, such as selenomethionine and selenocysteine, are not much more effective than inorganic selenium in cancer prevention and have comparable toxicity (14). Thus, the development of new selenium compounds with high anticarcinogenic efficacy but better tolerance continues to be a priority in chemopreventive research involving selenium. A series of novel organoselenium compounds has been synthesized in an effort to develop forms of selenium that are devoid of toxic side effects but retain effective chemopreventive activity (20–23). On the basis of a chemopreventive index (the ratio of dietary concentration of the test compound that inhibits carcinogenesis by 50% to the dose that is maximally tolerated without exerting an effect on body
weight gain), one compound, p-XSC (Fig. 1), was found to be far more effective than other selenium compounds evaluated in mammary tumorigenesis (24). p-XSC was also effective in inhibiting colon (25, 26), mammary gland (27), and lung (28) carcinogenesis (see Ref. 23 for review). Thus, p-XSC became a candidate for further evaluation in preclinical and, eventually, in clinical prevention trials.

To test the cancer protective effect of p-XSC in organs other than breast, colon, and lung, here we have evaluated its chemopreventive efficacy during the initiation and postinitiation phases in 4-NQO-induced oral carcinogenesis in rats. The effects of p-XSC on expression of proliferation biomarkers, such as ODC activity, polyamine level, BrdUrd-labeling index, and AgNORs number per nucleus were also assessed to clarify the underlying mechanism(s) of modification. In addition, GST and QR (also known as DT-diaphorase) were assayed in the liver and tongue to determine whether p-XSC could affect the tumor incidence via modification of these enzymes.

Materials and Methods

Animals, Diets, and Carcinogenesis. Four-week-old F344 rats were purchased from Japan SLC Inc. (Hamamatsu City, Japan), and 4-NQO was purchased from Tokyo Kasei Organic Chemicals Co., Ltd. (Tokyo, Japan). The basal diet, powdered CE-2, was bought from CLEA Japan Inc. (Tokyo, Japan). The rats were quarantined for 14 days before they were transferred to a holding room that was maintained under controlled environmental conditions, a 12-h light/dark cycle, 23 ± 2°C room temperature, and 50 ± 10% relative humidity.

p-XSC was synthesized by reacting KSeCN with α,α'-dibromo-p-xylene as described (29). Its purity was >99.9%, according to high-performance liquid chromatography analysis. The stability of p-XSC in the diet at room temperature was confirmed by high-performance liquid chromatography analysis, and the recovery of p-XSC was >97% under the bioassay feeding conditions. Previously, the MTD of p-XSC was reported to be 50 ppm (25 ppm as selenium; Ref. 25).

Experimental Procedure. Rats were divided into seven groups. At 7 weeks of age, rats in groups 1–5 were given 20 ppm 4-NQO in drinking water for 8 weeks. Starting at 6 weeks of age, rats in groups 2 and 3 were fed the diets containing 5 ppm selenium (20% MTD) and 15 ppm selenium (60% MTD) p-XSC, respectively, for 10 weeks. The regimen then switched to the basal diet without p-XSC, which was kept for 22 weeks. Rats in groups 4 and 5 received the diets containing p-XSC at dose levels of 5 and 15 ppm selenium, respectively, starting 1 week after the cessation of 4-NQO administration, and were kept on these diets for 22 weeks. Rats in group 6 were fed the diet mixed with 15 ppm selenium throughout the experiment; those in group 7 were given the basal diet alone. The latter group served as the untreated control group. The respective experimental and control diets and tap water of the rats were freely available during the study. All rats were carefully observed daily, and the consumption of the drinking water containing 4-NQO and of the diets mixed with or without p-XSC was recorded. The experiment was terminated after 32 weeks, and all animals were sacrificed. At necropsy, all organs, especially the oral cavity, were carefully inspected for preneoplastic and neoplastic lesions. Tongues were cut into halves: one portion was used for polyamine assay and measurement of ODC activity, and the other was used for histopathology and cell proliferation biomarker analysis. For histological confirmation, tissue and gross lesions were fixed in 10% buffered formalin and embedded in paraffin blocks. The sections were stained with H&E. Epithelial lesions (hyperplasia, dysplasia, and neoplasia) in the oral cavity were diagnosed according to the criteria described by Bánočzy and Csiba (30) and Kramer et al. (31).

Assays for Polyamine Levels and ODC Activity of Tongue Tissue. The polyamine (diamine, spermine, and spermidine) levels in the oral cavity tissues obtained at necropsy were determined by an enzymatic differential assay (32). ODC activity was measured by the method described by Calhoun et al. (33).

Determination of Proliferative Activity in the Tongue Epithelium by BrdUrd-labeling Index and AgNORS Number. To assess the proliferative activity, the BrdUrd-labeling index and the number of AgNORs molecules per nucleus of squamous epithelium of the tongue in all rats were quantified. For measuring the BrdUrd-incorporated nuclei, all rats were given an i.p. injection of 50 mg/kg body weight BrdUrd (Sigma Chemical Co., St. Louis, MO) 1 h prior to sacrifice. Three serial sections from one-half of the tongue were made after embedding in paraffin. On one section, the immunohistochemical staining for BrdUrd incorporation was done using an immunohistochemical analysis kit (Dako, Kyoto, Japan). The labeling indices of BrdUrd (%) were calculated by counting the labeled nuclei of 100 cells in normal or nonneoplastic tongue epithelium of each rat under ×400 magnification. On another section, a one-step silver colloid method for AgNORs staining was carried out, and computer-assisted image analysis quantification was based on the use of the image analysis system SPICCA II (Japan Abionics Co., Ltd., Tokyo, Japan), with an Olympus BH-2 microscope (Olympus Optical Ind. Co., Ltd., Tokyo, Japan) and a color charged-coupled device camera (Hamamatsu Photonics Co., Hamamatsu City, Japan). This was performed on 100 nuclei of interfuse cells from nonneoplastic areas. The remaining tongue section was used for histopathological diagnosis.

Measurement of GST and QR Activities of the Liver and Tongue. GST and QR activities were determined from five rats in each group at the end of the study. At sacrifice, the livers were perfused with saline to remove blood, excised immediately, and minced. The tongue epithelium was collected by scraping with a microscope knife. Aliquots of minced liver and of the mucosal scrapings of the tongue were processed for cytotoxic fraction as described (34, 35). The activities of GST with 1-chloro-2,4-dinitrobenzene as a substrate and of QR with NADH and menadione as substrates were determined as described (36–38). All spectrophotometric assays were based on absorption at 340 nm, and all samples were measured in triplicate. One unit of enzyme activity is the amount of enzyme catalyzing the conversion of 1 μmol of substrate to product per min at 25°C. Cytotoxic protein concentrations were determined by the Bradford method (39), using BSA as the standard.

Statistical Analysis. For statistical analyses on the incidence of lesions, we used Fisher’s exact probability test or χ² test. The measurements of body weight, liver weight, the polyamine assay, ODC assay, BrdUrd-labeling index, and AgNORs count were compared by Student’s t test. The results were considered statistically significant if P < 0.05.

Results

General Observations. The rats in groups 1–7 tolerated the oral administration of 4-NQO and/or p-XSC quite well. Food intake for all groups was between 15.3 and 15.8 g/day/animal. There were no significant differences on the total intake of 4-NQO/rat among the five groups (data not shown). The mean body weights and mean and relative liver weights at the end of the study are indicated in Table 1. The differences in mean body weights among the groups were insignificant. The mean liver weights in groups 3 and 5 were significantly larger than those of rats in group 1 (P < 0.005 and P < 0.001, respectively). Similarly, the average relative liver weights (g/100 g body weight of rats in groups 3 and 5 were significantly higher than those in group 1 (P < 0.001). Here, dietary administration of p-XSC did not cause any clinical signs of low survival rates, poor conditions, or histological changes that would point to toxicity in the liver, kidney, and lung.

Incidence of Tumors and Preneoplastic Lesions. In this study, endophytic and exophytic tumors developed mainly in the dorsal region of the posterior tongue of rats in groups 1–7. Histologically, the former were well differentiated squamous cell carcinoma, and the latter were squamous cell papilloma. No preneoplastic or neoplastic lesions were observed in rats in groups 6 and 7. The incidence of tumors (squamous cell papilloma and carcinoma) in each group are shown in Table 2. In group 1 (4-NQO alone), the incidences of squamous cell carcinoma and squamous cell papilloma of the tongue were 47 and 21%, respectively.
On the other hand, only a few rats (11–21%) that were given p-XSC during 4-NQO administration (groups 2 and 3) or fed p-XSC after 4-NQO exposure (groups 4 and 5) had neoplasms of the tongue. No carcinomas developed in the tongues of rats in group 5. Statistical analysis revealed a significant decrease in the incidence of tongue carcinoma in rats that were fed the p-XSC-containing diet during the initiation phase (groups 2 and 3) or postinitiation phase (groups 4 and 5) when it was compared with that in group 1 (P < 0.01 or P < 0.001). A certain degree of hyperplasia or dysplasia with or without neoplasms was also observed in the tongue of rats in groups 1–5 (Table 2). The incidences of total hyperplasia (simple hyperplasia and papillary hyperplasia) in groups 3 (62%) and 4 (50%) were lower than in group 1 (89%; P < 0.05 and P < 0.005, respectively). Also, the frequencies of simple hyperplasia (43%) and papillary hyperplasia (21%) of rats in group 4 were significantly smaller than those of rats in group 1 (79 and 58%; P < 0.05). The incidences of total dysplasia in groups 3 (62%), 4 (57%), and 5 (32%) were significantly lower than that of group 1 (89%; P < 0.05, P < 0.005, and P < 0.001, respectively). Among various types of dysplasia, the frequencies of moderate dysplasia in groups 4 and 5 and the incidence of severe dysplasia in group 5 were significantly lower than those of group 1 (P < 0.05, P < 0.05, and P < 0.001, respectively).

Expression of Cell Proliferation Biomarkers. The data on expression of cell proliferation biomarkers of the tongue epithelium are shown in Table 3. Total polyamine (diamine, spermidine, and spermine) levels in rats given 4-NQO alone (group 1) were significantly higher than in nontreated rats (group 7; P < 0.005). Total polyamine levels of rats in groups 2–5 were significantly lower than those of rats in group 1 (P < 0.01, P < 0.05, P < 0.05, and P < 0.005, respectively). The mean BrdUrd-labeling index and the mean number of AgNORs in the tongue epithelium of rats exposed to 4-NQO alone (group 1) were highest among all groups (P < 0.005), including those in the untreated control group (group 7; P < 0.001). Dietary administration of p-XSC (groups 2–5) significantly decreased those indices compared with group 1 (P < 0.005, P < 0.05, P < 0.01, or P < 0.001). In groups 6 and 7, the measurements were comparable. The ODC activity of the tongue epithelium of rats treated with 4-NQO alone (group 1) was about 2-fold higher than that in the untreated controls (group 7; P < 0.01). Dietary administration of p-XSC either during or after 4-NQO exposure significantly reduced this elevated ODC level (P < 0.02 or P < 0.01).

GST and QR Activities in the Liver and Tongue. The results of GST and QR assays in the liver and tongue are summarized in Table 4. The GST activities in the liver and (posterior and anterior) tongue of group 1 were slightly decreased in rats in group 7. The liver and tongue GST activities in groups 2–5 were significantly increased by comparison to group 1 (P < 0.01 or P < 0.001). On the other hand, QR activity in the posterior tongue of rats in group 1 was significantly elevated by comparison to those in group 7 (P < 0.05). The QR activities in the posterior tongue of rats in groups 2–5 were significantly decreased when compared to those in group 1 (P < 0.05). However, the QR activities in the anterior tongue of rats that were treated with 4-NQO and/or p-XSC did not significantly differ from those of untreated rats.

Discussion

The present study clearly demonstrates that dietary administration of p-XSC to rats during the initiation or postinitiation phase effectively blocks or suppresses 4-NQO-induced oral carcinogenesis without any toxicity and pathological alteration of other organs. It is remarkable that no tongue carcinomas developed in rats that were given the higher dose (15 ppm selenium) of p-XSC after 4-NQO exposure. Similar chemopreventive ability of dietary p-XSC during the initiation and postinitiation phases of carcinogenesis was observed in the mammary glands (24) and colon of rats (25, 26). Recently, Reddy et al. (40, 41) reported that p-XSC and analogues suppressed the incidence of precursor lesions of colon carcinoma, such as aberrant crypt foci. Here, feeding of p-XSC also suppressed the development of preneoplastic lesions in oral carcinogenesis. Feeding of p-XSC during either the initiation or postinitiation phase significantly inhibited the expression of cell proliferation biomarkers (BrdUrd-labeling index, AgNORs number, polyamine levels, and ODC activity). Also, p-XSC feeding affected the activities of GST and QR in the liver and tongue. This is the first report on the ability of p-XSC to modulate the phase II enzymes, GST, and QR in the tongue.

### Table 1 Body and liver weights

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>No. of rats examined</th>
<th>Body weight (g)</th>
<th>Liver weight (g)</th>
<th>Relative liver weight (g/100 body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-NQO alone</td>
<td>19</td>
<td>375 ± 17</td>
<td>15.1 ± 1.5</td>
<td>4.02 ± 0.43</td>
</tr>
<tr>
<td>2</td>
<td>4-NQO + 10 ppm p-XSC</td>
<td>28</td>
<td>370 ± 27</td>
<td>14.5 ± 1.6</td>
<td>3.93 ± 0.42</td>
</tr>
<tr>
<td>3</td>
<td>4-NQO + 30 ppm p-XSC</td>
<td>26</td>
<td>375 ± 20</td>
<td>17.0 ± 2.0</td>
<td>4.55 ± 0.50</td>
</tr>
<tr>
<td>4</td>
<td>4-NQO → 10 ppm p-XSC</td>
<td>28</td>
<td>365 ± 24</td>
<td>15.6 ± 1.8</td>
<td>4.25 ± 0.45</td>
</tr>
<tr>
<td>5</td>
<td>4-NQO → 30 ppm p-XSC</td>
<td>28</td>
<td>368 ± 28</td>
<td>17.5 ± 2.3</td>
<td>4.74 ± 0.50</td>
</tr>
<tr>
<td>6</td>
<td>30 ppm p-XSC</td>
<td>12</td>
<td>372 ± 18</td>
<td>16.8 ± 1.2</td>
<td>4.50 ± 0.33</td>
</tr>
<tr>
<td>7</td>
<td>No treatment</td>
<td>8</td>
<td>382 ± 12</td>
<td>16.3 ± 1.9</td>
<td>4.25 ± 0.44</td>
</tr>
</tbody>
</table>

a Mean ± SD.
b Significantly different from group 1 by Student's t test or Welch's t test (P < 0.005).
c Significantly different from group 1 by Student's t test or Welch's t test (P < 0.001).

### Table 2 Incidence of tongue lesions in rats treated with 4-NQO and p-XSC

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hyperplasia</th>
<th>Dysplasia</th>
<th>Neoplasms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Simple</td>
<td>Papillary</td>
</tr>
<tr>
<td>4-NQO</td>
<td>17/19 (89%)</td>
<td>15/19 (79%)</td>
<td>2/19 (11%)</td>
</tr>
<tr>
<td>4-NQO + 10 ppm p-XSC</td>
<td>22/28 (89%)</td>
<td>24/28 (86%)</td>
<td>2/28 (7%)</td>
</tr>
<tr>
<td>4-NQO → 10 ppm p-XSC</td>
<td>16/26 (62%)</td>
<td>14/26 (54%)</td>
<td>2/26 (8%)</td>
</tr>
<tr>
<td>4-NQO → 30 ppm p-XSC</td>
<td>14/28 (50%)</td>
<td>12/28 (43%)</td>
<td>2/28 (7%)</td>
</tr>
<tr>
<td>4-NQO + 30 ppm p-XSC</td>
<td>18/28 (64%)</td>
<td>22/28 (79%)</td>
<td>10/28 (36%)</td>
</tr>
</tbody>
</table>

* SCC, squamous cell carcinoma.

\(^{a}\) Significantly different from group 1 by Fisher's probability test (P < 0.005).
\(^{b}\) Significantly different from group 1 by Fisher's probability test (P < 0.01).
\(^{c}\) Significantly different from group 1 by Fisher's probability test (P < 0.001).
\(^{d}\) Significantly different from group 1 by Student's t test (P < 0.05).
\(^{e}\) Significantly different from group 1 by Fisher's probability test (P < 0.001).
Here, feeding of p-XSC-containing diets (15 ppm selenium) did not cause retardation of body weight gain. No significant pathological alterations in liver, including centrilobular hypertrophy with mild fatty change (42), were found in rats fed the diet containing 15 ppm selenium formulated as p-XSC during the study. In addition, no pathological abnormalities were seen in other organs, including kidney, lung, heart, testis, prostate, brain, and others. These results confirm the low toxicity of p-XSC. This is important because the ultimate application of cancer chemopreventive compounds is that in clinical trials for humans for which long-term parenteral treatment is much less practical than oral administration.

In vivo (43) and in vitro (44) studies suggest that inhibition of cell proliferation of preneoplastic lesions is a fundamental mechanism by which selenium inhibits or delays tumorigenesis. Most of the possible chemopreventive agents against 4-NQO-induced oral carcinogenesis could suppress cell proliferation activity (3). ODC is the rate-limiting enzyme in the polyamine biosynthesis pathway, which plays an essential role in cell proliferation and differentiation. Here, we estimated cell proliferation using polyamine levels and the BrdUrd-labeling index in the tongue epithelium. The results for both of these proliferation biomarkers indicate that p-XSC has suppressing effects on cell proliferation in the target organ. Moreover, p-XSC feeding resulted in a significant decrease of ODC activity of the tongue mucosa. Therefore, one of the mechanisms in the chemopreventive activities of p-XSC may be related to suppression of cell proliferation in the target tissue, especially when the compounds are given during the postinitiation phase.

Certain selenium compounds can modulate events in the initiation phase of neoplastic development. In fact, selenium compounds, including p-XSC, have been reported to suppress the formation of DNA adducts in the target tissues (27, 45, 46). The liver is the major organ for the activation and detoxification of xenobiotics. The tongue is known to be able to metabolize 4-NQO (47). In general, the various oxidation steps resulting in the formation of DNA-damaging electrophiles are carried out by members of the cytochrome P450 system, which are commonly designated as phase I enzymes. However, many of the metabolic reactions catalyzed by cytochrome P450 do not lead to activation but to the formation of more water-soluble, nonelectrophilic detoxification products. Defense against carcinogenic events is also provided by phase II enzymes (48) that are involved in the removal of electrophilic, as well as by nonelectrophilic metabolites through conjugation with glutathione or glucuronic acid. p-XSC has no effect on liver cytochrome P450, but it induces UDP-glucuronyltransferase in the liver (23). Unlike other chemical carcinogens, 4-NQO requires metabolic activation, not by cytochrome P450 enzymes, but by DT-diaphorase (a phase II enzyme) to exert its carcinogenic activity. Here, QR (DT-diaphorase) activity in the posterior tongue was significantly increased in rats treated with 4-NQO alone. However, QR activity in the anterior tongue of rats given 4-NQO was comparable to that in untreated rats. This may explain why 4-NQO induces tongue tumors specifically in the posterior tongue. Dietary p-XSC, administered during either the initiation or postinitiation stage in the current study, decreased QR activity in the posterior tongue, whereas it did not affect the level of QR in the anterior tongue. Feeding of p-XSC significantly enhanced the activity of GST activity in the liver and tongue and increased QR activity in the liver. Thus, alterations in QR and GST activities in the liver and tongue may

Table 3 Expression of cell proliferation indices

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment</th>
<th>AgNORs/nucleus(a)</th>
<th>BrdUrd-labeling index(a) (%)</th>
<th>Polyamine levels(d) (mmol/mg tissue)</th>
<th>ODC activity(a) (pnmol/mg protein/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4-NQO alone</td>
<td>3.35 ± 1.09(a)</td>
<td>7.31 ± 1.54(a)</td>
<td>3.74 ± 0.28(b)</td>
<td>134.08 ± 38.04(d)</td>
</tr>
<tr>
<td>2</td>
<td>4-NQO + 10 ppm p-XSC</td>
<td>2.44 ± 0.84(a)</td>
<td>6.26 ± 1.36()</td>
<td>3.16 ± 0.15(b)</td>
<td>72.80 ± 12.8(b)</td>
</tr>
<tr>
<td>3</td>
<td>4-NQO+30 ppm p-XSC</td>
<td>2.42 ± 1.05(a)</td>
<td>5.95 ± 0.87()</td>
<td>3.15 ± 0.28(b)</td>
<td>59.90 ± 12.7(b)</td>
</tr>
<tr>
<td>4</td>
<td>4-NQO→10 ppm p-XSC</td>
<td>2.16 ± 0.48(a)</td>
<td>5.33 ± 0.92()</td>
<td>3.28 ± 0.28()</td>
<td>59.24 ± 20.9(b)</td>
</tr>
<tr>
<td>5</td>
<td>4-NQO→30 ppm p-XSC</td>
<td>2.17 ± 0.73(a)</td>
<td>5.19 ± 1.04()</td>
<td>3.18 ± 0.16()</td>
<td>58.60 ± 15.2(b)</td>
</tr>
<tr>
<td>6</td>
<td>30 ppm p-XSC</td>
<td>2.10 ± 0.72</td>
<td>5.10 ± 0.99</td>
<td>3.15 ± 0.27</td>
<td>60.88 ± 13.8</td>
</tr>
<tr>
<td>7</td>
<td>No treatment</td>
<td>2.09 ± 0.38</td>
<td>5.08 ± 1.15</td>
<td>2.96 ± 0.22</td>
<td>56.70 ± 13.38</td>
</tr>
</tbody>
</table>

\(a\) Mean ± SD.
\(b\) Significantly different from 4-NQO alone group by Student’s t test or Welch’s t test (P < 0.005).
\(c\) Significantly different from 4-NQO + 10 ppm p-XSC group by Student’s t test or Welch’s t test (P < 0.001).
\(d\) Significantly different from 4-NQO + 30 ppm p-XSC group by Student’s t test or Welch’s t test (P < 0.001).
\(e\) Significantly different from 4-NQO→10 ppm p-XSC group by Student’s t test or Welch’s t test (P < 0.001).
\(f\) Significantly different from 4-NQO→30 ppm p-XSC group by Student’s t test or Welch’s t test (P < 0.001).

Table 4 GST and QR activities of liver and tongue of rats treated with 4-NQO and/or p-XSC

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Tissues</th>
<th>4-NQO alone</th>
<th>4-NQO + 10 ppm p-XSC</th>
<th>4-NQO + 30 ppm p-XSC</th>
<th>4-NQO → 10 ppm p-XSC</th>
<th>4-NQO → 30 ppm p-XSC</th>
<th>30 ppm p-XSC</th>
<th>Untreated control</th>
</tr>
</thead>
<tbody>
<tr>
<td>GST-CDNS</td>
<td>Liver</td>
<td>470.0 ± 73.0</td>
<td>613.3 ± 8.6(c)</td>
<td>576.2 ± 20.7()</td>
<td>628.2 ± 79.9(d)</td>
<td>678.0 ± 14.3(d)</td>
<td>531.6 ± 30.5</td>
<td>543.7 ± 13.9</td>
</tr>
<tr>
<td>Tongue (anterior)</td>
<td>41.08 ± 6.11</td>
<td>51.69 ± 3.69(c)</td>
<td>54.40 ± 3.92(d)</td>
<td>55.97 ± 4.06()</td>
<td>57.45 ± 4.53(d)</td>
<td>57.84 ± 4.28(d)</td>
<td>46.37 ± 5.95</td>
<td></td>
</tr>
<tr>
<td>QR-NADH</td>
<td>Liver</td>
<td>29.75 ± 2.52</td>
<td>41.46 ± 4.93(d)</td>
<td>44.82 ± 1.09()</td>
<td>40.11 ± 3.18()</td>
<td>45.39 ± 4.91(d)</td>
<td>33.76 ± 0.73</td>
<td>32.06 ± 5.23</td>
</tr>
<tr>
<td>Tongue (anterior)</td>
<td>45.87 ± 4.91(d)</td>
<td>47.39 ± 0.54</td>
<td>57.78 ± 6.03()</td>
<td>78.62 ± 9.41(d)</td>
<td>95.26 ± 8.72(d)</td>
<td>189.15 ± 17.8(d)</td>
<td>115.2 ± 15.4</td>
<td></td>
</tr>
<tr>
<td>QR-NADH</td>
<td>Tongue (anterior)</td>
<td>415.0 ± 25.2(d)</td>
<td>257.6 ± 12.1(d)</td>
<td>230.7 ± 27.0(d)</td>
<td>230.9 ± 44.0()</td>
<td>215.7 ± 13.3(d)</td>
<td>291.2 ± 34.4</td>
<td>212.8 ± 25.6</td>
</tr>
<tr>
<td>QR-NADH</td>
<td>Tongue (anterior)</td>
<td>460.6 ± 54.0</td>
<td>461.0 ± 21.3</td>
<td>426.5 ± 21.2</td>
<td>449.1 ± 21.2</td>
<td>428.2 ± 51.5</td>
<td>428.8 ± 34.0</td>
<td>413.4 ± 43.7</td>
</tr>
</tbody>
</table>

\(a\) Mean ± SD.
\(b\) Significantly different from 4-NQO only group by Student’s t test or Welch’s t test (P < 0.02).
\(c\) Significantly different from 4-NQO alone group by Student’s t test or Welch’s t test (P < 0.005).
\(d\) Significantly different from 4-NQO alone group by Student’s t test or Welch’s t test (P < 0.001).
\(e\) Significantly different from 4-NQO alone group by Student’s t test or Welch’s t test (P < 0.0001).
\(f\) Significantly different from untreated control by Student’s t test or Welch’s t test (P < 0.001).
\(g\) Significantly different from untreated control by Student’s t test or Welch’s t test (P < 0.05).

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contribute to the chemopreventive activity of p-XSC that was observed in the current study, although there are also other mechanisms by which p-XSC may inhibit tumor formation. These include its capability to increase selenium-dependent glutathione peroxidase (25), to inhibit tyrosine kinase activity (49), to inhibit activities of protein kinases A and C (50), to inhibit cell growth and induce apoptosis (44, 51), and to modulate activation of Jun-N-kinases (52).

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References


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1,4-Phenylenebis(methylene)selenocyanate Exerts Exceptional Chemopreventive Activity in Rat Tongue Carcinogenesis

Takuji Tanaka, Hiroki Makita, Kunihiro Kawabata, et al.


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