Biochemical and Biophysical Research Communications 383 (2009) 151–155

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Modulations of benzo[*a*]pyrene-induced DNA adduct, cyclin D1 and PCNA in oral tissue by 1,4-phenylenebis(methylene)selenocyanate

Kun-Ming Chen [a], Peter G. Sacks [d], Thomas E. Spratt [a], Jyh-Ming Lin [a], Telih Boyiri [a], Joel Schwartz [b], John P. Richie [c], Ana Calcagnotto [c], Arunangshu Das [a], James Bortner [a], Zonglin Zhao [d,e], Shantu Amin [f], Joseph Guttenplan [d,e], Karam El-Bayoumy [a,*]

[a] *Department of Biochemistry and Molecular Biology, Penn State College of Medicine, 500 University Drive, Hershey, PA 17033, USA*
[b] *University of Illinois, College of Dentistry, Chicago, IL 60612, USA*
[c] *Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA 17033, USA*
[d] *Department of Basic Sciences, College of Dentistry, New York University, New York, NY 10010, USA*
[e] *Department of Environmental Medicine, School of Medicine, New York University, New York, NY 10010, USA*
[f] *Department of Pharmacology, Penn State College of Medicine, Hershey, PA 17033, USA*

ARTICLE INFO

Article history:
Received 26 March 2009
Available online 1 April 2009

Keywords:
Chemoprevention
Selenium
p-XSC
B[*a*]P

ABSTRACT

Tobacco smoking is an important cause of human oral squamous cell carcinoma (SCC). Tobacco smoke contains multiple carcinogens include polycyclic aromatic hydrocarbons typified by benzo[*a*]pyrene (B[*a*]P). Surgery is the conventional treatment approach for SCC, but it remains imperfect. However, chemoprevention is a plausible strategy and we had previously demonstrated that 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) significantly inhibited tongue tumors-induced by the synthetic 4-nitroquinoline-*N*-oxide (not present in tobacco smoke). In this study, we demonstrated that *p*-XSC is capable of inhibiting B[*a*]P-DNA adduct formation, cell proliferation, cyclin D1 expression in human oral cells *in vitro*. In addition, we showed that dietary *p*-XSC inhibits B[*a*]P-DNA adduct formation, cell proliferation and cyclin D1 protein expression in the mouse tongue *in vivo*. The results of this study are encouraging to further evaluate the chemopreventive efficacy of *p*-XSC initially against B[*a*]P-induced tongue tumors in mice and ultimately in the clinic.

Introduction

Tobacco smoking and alcohol consumption are major etiological factors in the development of oral cancer; these agents are responsible for about 75% of oral cancer in the USA [1]. In the USA, over 30,000 cases and about 8000 deaths from oral cancer occur annually [2]. Oral cancer constitutes up to 25% of all types of cancer in developing countries, especially in southern Asia (e.g., India) as well as in China and certain regions of Brazil where the highest rates of oral cancer are observed [3,4]. The oral cancer incidence and mortality rates have been increasing in the USA, Japan, Germany, and Scotland, especially among young males [3,4]. Histologically, the most frequent cancers of the oral cavity are squamous cell carcinomas. Oral cancer is generally treated with surgery; however, surgery usually involves high degree of morbidity and high recurrence rates and does not address the development of multifocal neoplastic lesions in the entire epithelial field at risk [5]. Prevention is obviously preferential to treatment, and preventive strategies can be tailored to the status of the individuals. An obvious means of preventing this disease would be to remove the etiological factors; however, this strategy is very difficult to implement due to the addictive power of etiological agents (tobacco products and alcohol).

The use of naturally occurring or synthetic agents that can inhibit the appearance of clinically detectable tumors is an attractive and plausible approach to the prevention of cancer [6]. Indeed, some success has been obtained with retinoids in the treatment of premalignant lesions of the oral cavity [7]. An important goal in the development of cancer prevention strategies is the identification of molecular pathways by which normal cells progress to the first definable stage of cancer. Preclinical animal models of oral cancer can provide important mechanistic insights into the role of cancer chemopreventive agents [8,9]. The induction of oral cancer in laboratory animals by the synthetic carcinogens 4-nitroquinoline-*N*-oxide (4-NQO) in rats and mice, and 7,12-dimethylbenz[*a*]anthracene (DMBA) in hamster have served as *in vivo* models to examine the etiology of the disease and to identify agents that suppress oral carcinogenesis [6]. However, neither 4-NQO nor DMBA is found in the human environment. While it is

* Corresponding author. Fax: +1 717 531 0002.
E-mail address: kee2@psu.edu (K. El-Bayoumy).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.03.145

not clear which compounds in tobacco and tobacco smoke are involved in the oral cancer development in smokers, polycyclic aromatic hydrocarbone (PAH) and tobacco-specific nitrosamine (TSNA) are recognized as potential etiological agents [10].

Epidemiologic, and preclinical studies, and some clinical intervention trials indicate that selenium-containing compounds is an important class of chemopreventive agents. Studies in our laboratories indicated that the organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate (*p*-XSC) is a highly effective chemopreventive agent in animal models including oral cancer [11]. We showed that *p*-XSC, administered during the initiation or post-initiation phases of carcinogenesis, significantly reduced the incidence of carcinoma in the tongue of rats treated with 4-NQO [12]. Recently, several potential mechanisms by which *p*-XSC inhibited 4-NQO-induced tongue tumors in the rats were proposed based on the effects of *p*-XSC in inhibiting DNA damage, mutagenesis, and p53 protein expression [13]. However, to date, there are no studies of the effectiveness of this compound against oral carcinogenesis induced by a tobacco smoke relevant carcinogen. In a previous report, Culp et al. [14] showed that the human carcinogen, B[*a*]P (100 ppm in the diet, for 2 years) produced a 48% incidence in tongue tumors in B6C3F1 mice. In the present investigation, using a short-term B[*a*]P-induced bioassay in mice, and human oral cancer cells, we examined the effects of *p*-XSC on B[*a*]P-DNA adduct formation, cell proliferation, induction of apoptosis, expression of PCNA and cyclin D1. The results of the present investigation suggest that *p*-XSC is a promising chemopreventive candidate against the development of tongue tumors-induced by B[*a*]P in mice.

Materials and methods

Chemicals. B[*a*]P was obtained commercially (Sigma Chemical Co.), 7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) and N^2-(7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-10-yl)-2′-deoxyguanosine (N^2-BPDE-dG) were obtained from National Cancer Institute Chemical Carcinogen Reference Standard Repository at the Midwest Research Institute, Kansas City, MO. *p*-XSC was synthesized as reported previously [15].

Cell survival measured by MTT assay. The human oral squamous cell carcinoma SCC 1483 cells were maintained in a 1:1 mixture of DMEM/F12 supplemented with 10% FBS and penicillin/streptomycin (50 μg/mL). The MSK-Leuk1 cell line was established from a dysplastic leukoplakia lesion adjacent to a squamous cell carcinoma of the tongue [16]. Squamous cell carcinoma SCC1483 or oral keratinocyte MSK-Leuk1 cells (10,000/well) were seeded and grown in a 96-well plate for 24 h, and then treated with different doses of *p*-XSC for 24 h. Methylthiazoletetrazolium (MTT) (50 μg/100 μl) was added to the cells in each well for 4 h at 37 °C. MTT was aspirated and 100 μl of DMSO was added to each well and absorbance at 570 nm was measured to quantify the number of surviving cells.

Apopototic cell death detection. A cell death detection enzyme-linked immunosorbent assay kit (Roche Diagnostics, Indianapolis, IN) was used to quantitatively determine cytoplasmic histone-associated DNA oligonucleosome fragments associated with apoptotic cell death according to the manufacturer's manual. Briefly, after cells were lysed, and incubated for 30 min at room temperature, 20 μl of supernatant was transferred into the streptavidin-coated microtiter plate, and 80 μl of the immunoreagent was added to each well. After incubation at room temperature for 2 h, the solution was decanted, and each well was rinsed three times with incubation buffer. Color development was carried out by adding 100 μl of ABTS solution, and absorbency was measured at 405 nm in a microtiter plate reader against ABTS solution as a blank.

Western blot analysis. SCC1483 cells treated with various doses of *p*-XSC (2.5, 5 and 10 μM) for 1 h, were harvested by scraping, and washed with phosphate-buffered saline. Cellular proteins were isolated with cell lysis buffer purchased from Cell Signaling (Beverly, MA). Equal amounts of protein (30 mg) was taken, boiled for 5 min, electrophoresed on a 10% SDS–PAGE at 100 V for 110 min, then electro-transferred to a nitrocellulose membranes. Antibodies used for Western blots were cyclin D1 and actin from Santa Cruz. Band expressions were developed using ECL reagents from Amersham, Piscataway, NJ, and analyzed by Quantity One Analysis Software v4.5.0 (Bio-Rad Laboratories, Richmond, CA).

Measurement of BPDE-DNA adduct in vitro. Levels of BPDE-DNA adduct in SCC1483 cells that had been treated with B[*a*]P were measured by competitive ELISA using monoclonal antibody 5D11 according to a published method [17]. Anti-BPDE-DNA adduct antibody 5D11 was obtained from Dr. Santella, Columbia University. Briefly, the genomic DNA isolated from cultured cells using the Qiagen genomic DNA isolation kit (Valencia, CA) was analyzed. Polyvinyl flexible flat bottom 96-well plates (BD Falcon, Franklin Lakes, NJ) were coated with 50 ng per well of the BPDE-DNA in 100 μL of 50 mM Tris–buffer (pH 7.5) at 4 °C overnight. The plate was then washed with 200 μL of PBS containing 0.05% Tween-20 and 0.02% NaN_3 as washing buffer. Non-specific binding to the plate was minimized by incubating the plates with 200 μL of

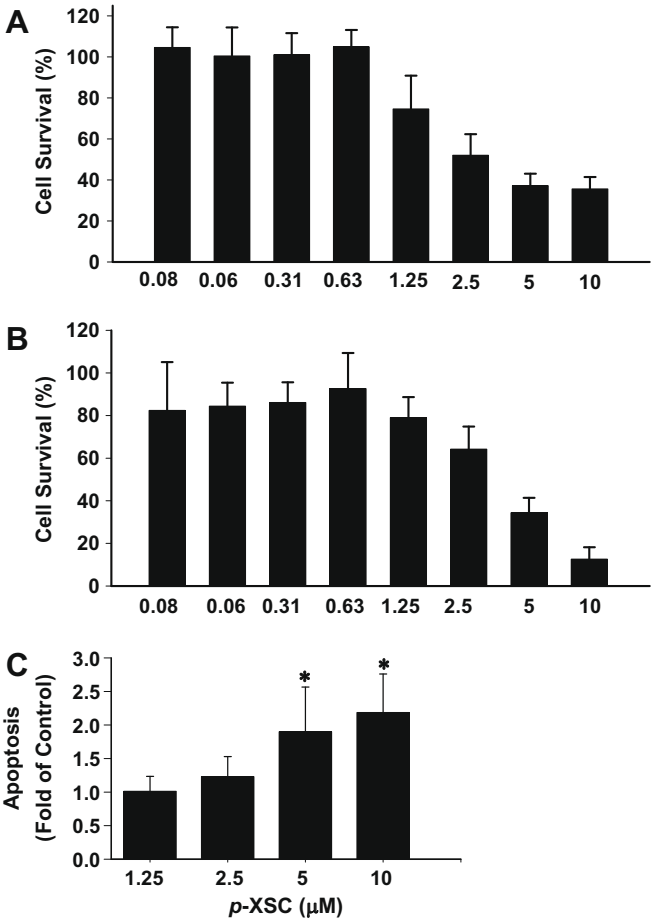

Fig. 1. The effect of *p*-XSC on cell survival and induction of apoptosis in oral cell lines. SCC 1483 (A) or MSK-Leuk1 cells (B) were incubated with *p*-XSC for 24 h and cell survival was determined by the MTT assay. (C) SCC 1483 Cells were incubated with *p*-XSC for 24 h and apoptotic cell cell-death was determined by ELISA. Results were quantitated from three independent experiments. Data are presented as means ± SD. * statistically significant ($P < 0.01$) as compared with untreated controls.

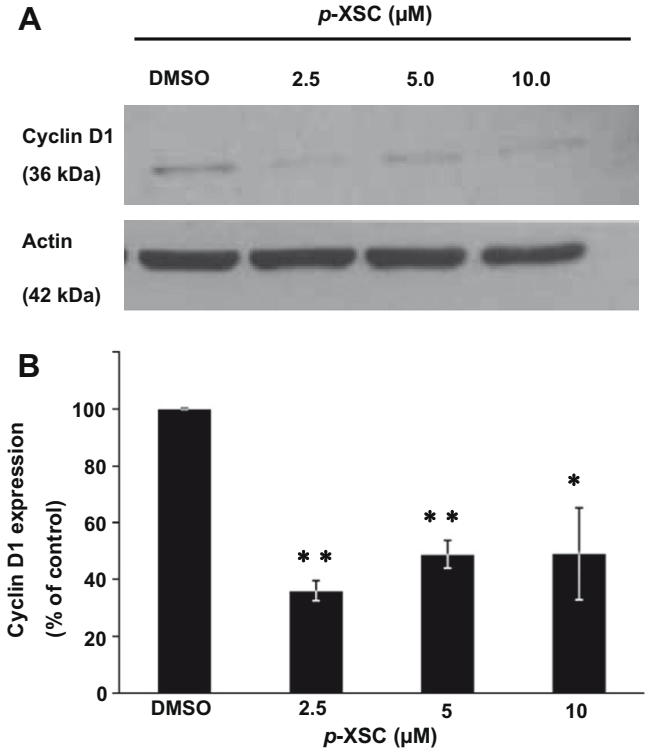

Fig. 2. The effect of *p*-XSC on cyclin D1 expression in squamous cell carcinoma SCC1483 cells. A representative illustration of (A) the Western blot analysis, and a comparison between various doses of *p*-XSC of (B) is provided. Levels of protein expression were quantified from three independent experiments. Results are presented as means ± SD. ∗ statistically significant ($P < 0.05$) and ∗∗ statistically significant ($P < 0.01$) as compared with untreated controls.

blocking buffer containing 1% FCS in washing buffer. BPDE-DNA standards and DNA samples (4 μg in 50 μL/well) were mixed with 50 μL of 5D11 (1:100 dilution), and added into each duplicate well. Wells were incubated and washed before adding goat anti-mouse IgG alkaline phosphatase conjugate (100 μL, 1:100 dilution), then the plate was incubated for 90 min at 37 °C. The plate was washed and 200 μL *p*-nitrophenyl phosphate (1 mg/mL in 1 M diethanolamine, pH 8.6) was then added. After incubation for 30 min, the color development at 405 nm was measured by microtiter plate reader.

Diet preparation and animal treatment. Female 8-week old B6C3F1 mice were obtained from Harlan Sprague–Dawley Inc. (Indianapolis, IN). Mice were housed in an animal facility maintained on a 12-h light/dark cycle, at a constant temperature (22 ± 2 °C) and relative humidity (55 ± 15%) controlled room. *p*-XSC was given to the animals in the diet (AIN-76A) at 5 and 20 ppm over the entire course of the experiment beginning 2 weeks prior to B[*a*]P exposure and during carcinogen exposure until termination. B[*a*]P in corn oil was administered in five doses over a 2 weeks period. Twenty-four hours after the last administration of B[*a*]P, the animals were sacrificed and their tongues were isolated. The tongue was sliced lengthwise; one half was used for DNA adduct analysis and the other half was used for PCNA and cyclin D1 examinations. DNA was isolated from the epithelial layer of the tongue by a modified Marmur method as described [18].

Immunohistochemical detection. The epithelial cells from the tongue of mice were carefully removed and placed into phosphate-buffered saline, washed and fixed with 1.0% paraformaldehyde. The primary immunohistochemical staining of cells was analyzed for levels of PCNA and cyclin D1 (AbCam, Cambridge, MA, USA). A rabbit anti-mouse secondary antibody (Zymed; San Francisco, CA) with a fluoresceinated isothiocyanate conjugate (FITC) was used to detect the primary antibody. The primary antibody was added for 1 h and washed at least twice with 1× PBS and a secondary detection antibody was added and washed at least twice with 1× PBS. Cells were analyzed using a laser scanning cytometer purchased from CompuCyte (Cambridge, MA, USA). The fluorescence was detected at 480 nm using an argon laser. The laser scans the total slide area which is gated in the instrument.

Measurement of BPDE-DNA adduct in mouse tongue. Levels of B[*a*]P-DNA adduct in the tongue of mouse that had been treated with B[*a*]P was analyzed by the ^{32}P-postlabeling assay [19]. DNA isolated from tissue was hydrolyzed to nucleoside 3′-monophosphates with micrococcal endonuclease and spleen phosphodiesterase, followed by nuclease P1 treatment to convert only the unmodified nucleotides to nucleosides. The B[*a*]P-modified nucleotides were radiolabeled with [γ-^{32}P] ATP and polynucleotide kinase. The 3′,5′-bisphosphates were separated by Macherey–Nagel polyethyleneimine cellulose TLC Plate (Alltech Associates, Deerfield, IL) using N^2-BPDE-dG as standard, and exposed to Cronex X-ray film (Dupont, Wilmington, DE) for visualization of adducts.

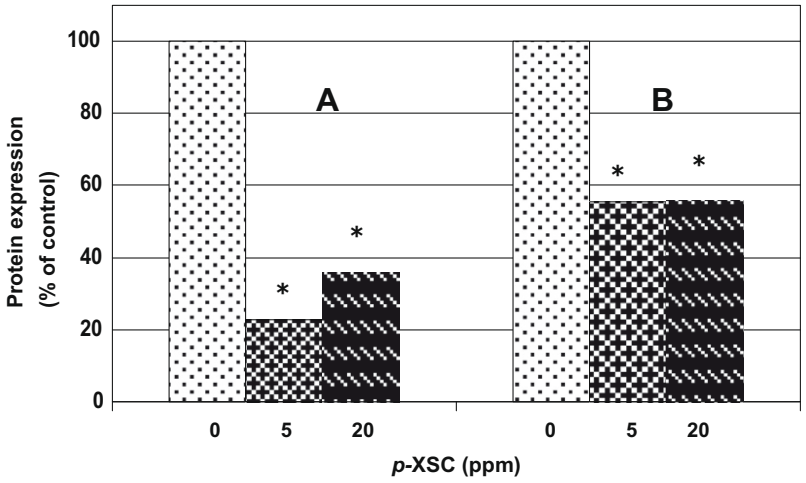

Fig. 3. Inhibition of B[*a*]P-induced cell proliferation and cyclin D1 by *p*-XSC in the mouse tongue. Cell proliferation (PCNA) (A) and cyclin D1 (B) were measured by immunohistochemistry. Data are presented as percentage relative to the effect of B[*a*]P treatment. Data are presented as means ± SD. ∗ statistically significant ($P < 0.01$) as compared with B[*a*]P-treated controls.

Results

We observed that *p*-XSC can inhibit cell survival in both squamous cell carcinoma SCC1483 cells and oral leukoplakia keratinocyte MSK-Leuk1 cells in a dose-dependent fashion (Fig. 1A and B). The IC_{50} for *p*-XSC is approximately 2.5 μM for both cell lines. The induction of apoptosis by *p*-XSC is shown (Fig. 1C) in SCC 1483 cells. Compared to control with no *p*-XSC added, increasing the concentration to 10 μM resulted in more than double the rates of apoptosis. The inhibitory effect of *p*-XSC on the expression of cyclin D1 was also observed in SCC 1483 cells. The level of cyclin D1 was found to be decreased after 1 h treatment of cells with 2.5, 5 and 10 μM of *p*-XSC (Fig. 2); however, the effect was not dose-dependent.

To determine whether *p*-XSC could affect cell proliferation of the oral tissue *in vivo*, the expression of PCNA was analyzed by immunohistochemistry. *p*-XSC at both doses (5 and 20 ppm in diet) significantly inhibited the expression of PCNA ($P < 0.01$, 2-tailed *T*-test) in B[*a*]P treated female B6C3F1 mice (Fig. 3A). The expression of cyclin D1 in the tongue of mice treated with B[*a*]P and *p*-XSC was also determined; *p*-XSC at both doses significantly inhibited the expression of cyclin D1 ($P < 0.01$, 2-tailed *T*-test) (Fig. 3B), but the effect was not dose-dependent.

In SCC 1483 cells, B[*a*]P treatment resulted in the formation of BPDE-DNA adducts in a dose-dependent fashion. *p*-XSC treatment was found to decrease the level of BPDE-DNA adducts induced by B[*a*]P treatment (Fig. 4A); however, the effects was not significant. Fig. 4B–F show autoradiogram of a thin layer chromatogram of ^{32}P-labeled nucleotides from DNA of tongues of mice treated with B[*a*]P. Consistent with our observations in SCC1483 cells, dietary *p*-XSC at 20 ppm (10 ppm as selenium) significantly inhibited the formation of N^2-BPDE-dG adduct induced by B[*a*]P treatment in mouse tongue ($P < 0.05$). No significant inhibition of adduct formation was observed at the lower dose of *p*-XSC (5 ppm). Fig. 4F shows a representative quantitative comparison of the autoradiogram with and without 20 ppm *p*-XSC.

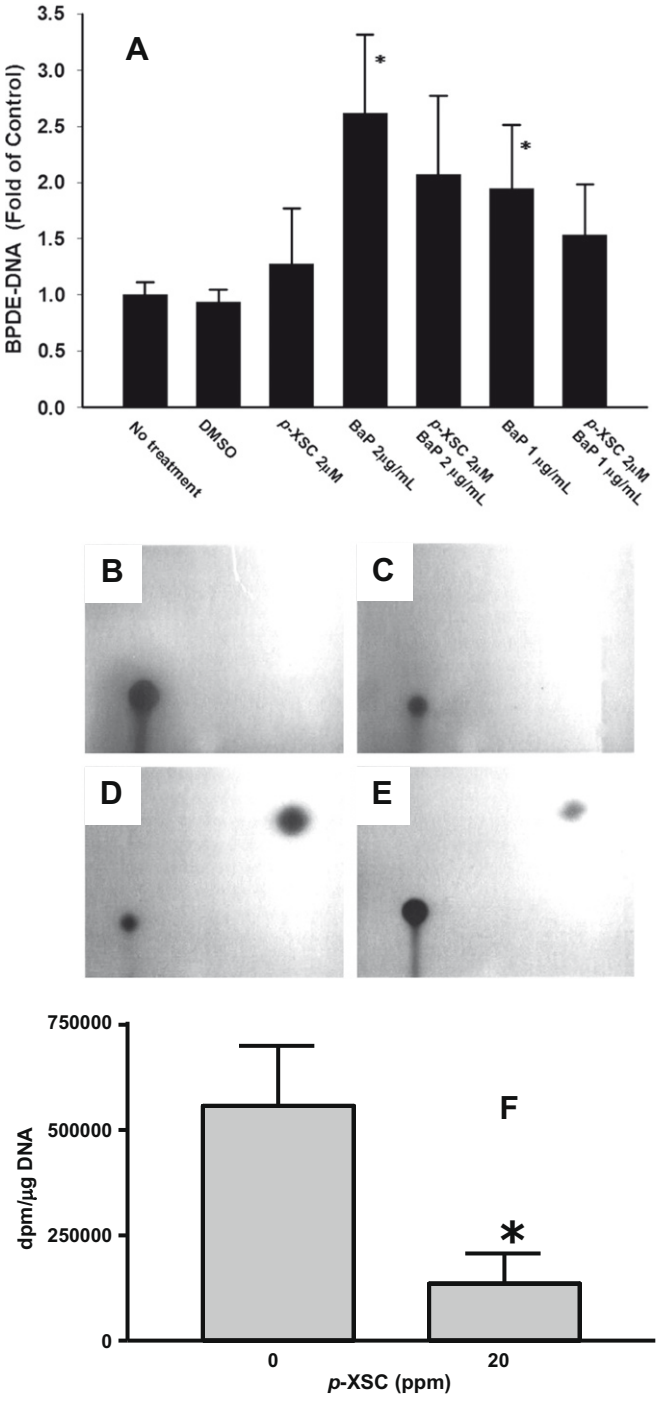

Fig. 4. The effects of p-XSC on the BPDE-DNA adduct formation. (A) BPDE-DNA isolated from Squamous cell carcinomas SCC1483 cells. (B) Representative Autoradiographic profiles are presented for tongues from untreated mice, and mice treated with *p*-XSC (C), B[*a*]P (D), and B[*a*]P and *p*-XSC (E). The spot in the upper right of panels D and E coelute with standard N^2-BPDE-dG. A comparison of N^2-BPDE-dG adduct formation between groups of mice fed control and *p*-XSC supplemented diets ($n = 3$/group) is provided in panel E. Levels of BPDE-DNA adducts were quantitated from three independent experiments. Results are the means ± SD. of three separate experiments. ∗statistically significant ($P < 0.05$) as compared with untreated controls.

Discussion

Detailed studies aimed at identification of critical biochemical and molecular markers during the progression from normal, hyperplasic lesions, through dysplasia and carcinoma [8] could lead to improved diagnostic techniques for cancer prevention. In this study we have demonstrated that *p*-XSC can inhibit many of these critical steps in cancer development in a relevant *in vivo* animal model using a carcinogen found in tobacco smoke. We showed that following a short-term treatment of mice with B[*a*]P resulted in the formation of B[*a*]P-induced DNA adduct in mouse tongue. Our results appear to be consistent with those reported previously that B[*a*]P is capable of inducing DNA adducts in human oral epithelial SCC-9 cells [20].

Consistent with other model systems, we found that *p*-XSC decreased B[*a*]P-induced DNA adduct formation using competitive ELISA assay *in vitro* and ^{32}P-postlabeling assay *in vivo*. In our previous studies, we found that total GST enzyme activity, as well as GST-π and GST-μ enzyme activities were significantly induced by *p*-XSC in both mouse lung and liver [21]; *p*-XSC also induced glutathione peroxidase activity in the mouse lung but not in the liver [21]. Although similar studies in the mouse oral tissues have not been performed, we have shown that dietary *p*-XSC induced GST activity and inhibited cell proliferation in the tongue of F344 rats [12]. Together, these results obtained in the liver, lung and tongue reinforce the suggestion that the induction of GST by *p*-XSC may, in part, account for the reduction of B[*a*]P-DNA adduct formation in both SCC 1483 cells and mouse tongue observed in the present investigation.

A major characteristic of cancer cells is uncontrolled cell growth which is due to the loss of cell cycle control. Additionally, cell proliferation (DNA replication) is necessary for the conversion of DNA

damage to mutations. Overexpression of cyclin D1 has been reported in a variety of human tumors, including breast carcinomas, mantle cell lymphomas, and squamous cell carcinomas derived from the oral cavity, larynx, and esophagus as well as from other sites [22]. Cyclin D1 was amplified in approximately 30% of HNSCC [23]. Immunohistochemical analysis of cyclin D1 performed by Nakahara et al. [24] on 78 oral SCC, 46 leukoplakia, and 20 normal mucosa samples found that the overexpression of cyclin D1 was not observed in normal mucosa and was observed in 36% of SCC. These results clearly indicate that alteration in gene expression varies according to the stage of disease. However, for cancer prevention of HNSCC, it is critically important to elucidate molecular changes in addition to cyclin D1 in well-defined and well-controlled animal models.

The effect of *p*-XSC on cell proliferation in the B[*a*]P-treated tongue of mice was assayed by PCNA as a proliferative marker, and cyclin D1 as a key factor in cell cycle control. Consistent with our previous study using 4-NQO in the rats [12]; we demonstrated that *p*-XSC can inhibit cell proliferation in cells in culture, and dietary *p*-XSC is also capable of inhibiting cell proliferation induced by B[*a*]P in the mouse tongue. Our results also demonstrate that *p*-XSC inhibits cyclin D1 protein expression, which may be, in part, responsible for its inhibitory effects on cell proliferation.

Recently, *p*-XSC was found to reduce the expression of NF-κB and several target genes regulated by NF-κB including cyclin D1 in non-small cell lung cancer cells (NSCLC) [25]. Furthermore, we have shown that *p*-XSC can inhibit the NF-κB-DNA binding in both oral squamous cell carcinoma (SCC 1483) and non-small cell lung cancer NCI-H460 [26]; and by using MALDI-TOF/TOF and computer modeling studies, we have demonstrated that the Cys62 residue in the active site of NF-κB (p50) protein was modified by the organoselenocyanates, (e.g., *p*-XSC, benzyl selenocyanate) through the formation of a selenium–sulfur bond, which would subsequently hinder binding of the DNA substrate [26]. Control of cell growth and differentiation by transcriptional regulation of cyclin D1 via NF-κB has been reported by Guttridge et al. [27]. Based on these reports, *p*-XSC may inhibit the activation of NF-κB, and lead to down-regulation of cyclin D1 expression, thereby inhibiting cell proliferation.

In conclusion, the results of this study together with those reported in the literature by us and by others, suggest several potential mechanisms by which *p*-XSC would inhibit B[*a*]P-induced oral carcinogenesis. The mechanisms are based on the ability of the synthetic organoselenium compound of a thin layer chromatogram of ^{32}P-labeled nucleotides from DNA of tongues of mice treated with B[*a*]P to inhibit one or more of the following processes: inhibition of B[*a*]P-DNA adduct formation, inhibition of cell proliferation, and induction of apoptosis. Our results encourage future studies examine the chemopreventive activity of *p*-XSC against B[*a*]P-induced tongue tumors in mice.

Acknowledgments

Grant Support: NCI Grant R01-CA100924.

References

[1] M.T. Canto, S.S. Devesa, Oral cavity and pharynx cancer incidence rates in the United States, 1975–1998, Oral Oncol. 38 (2002) 610–617.
[2] A. Jemal, R. Siegel, E. Ward, Y. Hao, J. Xu, T. Murray, M.J. Thun, Cancer statistics, 2008, CA Cancer J. Clin. 58 (2008) 71–96.
[3] I. Magrath, J. Litvak, Cancer in developing countries: opportunity and challenge, J. Natl. Cancer Inst. 85 (1993) 862–874.
[4] D.M. Parkin, P. Pisani, J. Ferlay, Global cancer statistics, CA Cancer J. Clin. 49 (1999) 33–64. 1.
[5] V.A. Papadimitrakopoulou, J.J. Lee, W.N. William Jr., J.W. Martin, M. Thomas, E.S. Kim, F.R. Khuri, D.M. Shin, L. Feng, W.K. Hong, S.M. Lippman, Randomized trial of 13-cis retinoic acid compared with retinyl palmitate with or without beta-carotene in oral premalignancy, J. Clin. Oncol. 27 (2009) 599–604.
[6] K. El-Bayoumy, R. Sinha, Molecular chemoprevention by selenium: a genomic approach, Mutat. Res. 591 (2005) 224–236.
[7] S.M. Lippman, J. Sudbo, W.K. Hong, Oral cancer prevention and the evolution of molecular-targeted drug development, J. Clin. Oncol. 23 (2005) 346–356.
[8] R. Czerninski, P. Amornphimoltham, V. Patel, A.A. Molinolo, J.S. Gutkind, Targeting mammalian target of rapamycin by rapamycin prevents tumor progression in an oral-specific chemical carcinogenesis model, Cancer Prev. Res. (Phila Pa) 2 (2009) 27–36.
[9] K.K. Wong, Oral-specific chemical carcinogenesis in mice: an exciting model for cancer prevention and therapy, Cancer Prev. Res. (Phila Pa) 2 (2009) 10–13.
[10] K. El-Bayoumy, F.L. Chung, J. Richie Jr., B.S. Reddy, L. Cohen, J. Weisburger, E.L. Wynder, Dietary control of cancer, Proc. Soc. Exp. Biol. Med. 216 (1997) 211–223.
[11] K. El-Bayoumy, C.V. Rao, B.S. Reddy, Multiorgan sensitivity to anticarcinogenesis by the organoselenium 1,4-phenylenebis(methylene)selenocyanate, Nutr. Cancer 40 (2001) 18–27.
[12] T. Tanaka, H. Makita, K. Kawabata, H. Mori, K. El-Bayoumy, 1,4-phenylenebis(methylene)selenocyanate exerts exceptional chemopreventive activity in rat tongue carcinogenesis, Cancer Res. 57 (1997) 3644–3648.
[13] J. Guttenplan, K.M. Chen, M. Khmelnitsky, W. Kosinska, J. Hennessy, R. Bruggeman, D. Desai, S. Amin, Y.W. Sun, T.E. Spratt, K. El-Bayoumy, Effects of 1,4-phenylenebis(methylene)selenocyanate on mutagenesis and p53 protein expression in the tongue of lacI rats treated with 4-nitroquinoline-*N*-oxide, Mutat. Res. 634 (2007) 146–155.
[14] S.J. Culp, D.W. Gaylor, W.G. Sheldon, L.S. Goldstein, F.A. Beland, A comparison of the tumors induced by coal tar and benzo[*a*]pyrene in a 2-year bioassay, Carcinogenesis 19 (1998) 117–124.
[15] K. El-Bayoumy, Y.H. Chae, P. Upadhyaya, C. Meschter, L.A. Cohen, B.S. Reddy, Inhibition of 7,12-dimethylbenz(a)anthracene-induced tumors and DNA adduct formation in the mammary glands of female Sprague–Dawley rats by the synthetic organoselenium compound, 1,4-phenylenebis(methylene)selenocyanate, Cancer Res. 52 (1992) 2402–2407.
[16] P.G. Sacks, Cell, tissue and organ culture as in vitro models to study the biology of squamous cell carcinomas of the head and neck, Cancer Metastasis Rev. 15 (1996) 27–51.
[17] R.M. Santella, A. Weston, F.P. Perera, G.T. Trivers, C.C. Harris, T.L. Young, D. Nguyen, B.M. Lee, M.C. Poirier, Interlaboratory comparison of antisera and immunoassays for benzo[*a*]pyrene-diol-epoxide-I-modified DNA, Carcinogenesis 9 (1988) 1265–1269.
[18] J. Marmur, P. Doty, Thermal renaturation of deoxyribonucleic acids, J. Mol. Biol. 3 (1961) 585–594.
[19] A.K. Roy, K. El-Bayoumy, S.S. Hecht, 32P-postlabeling analysis of 1-nitropyrene-DNA adducts in female Sprague–Dawley rats, Carcinogenesis 10 (1989) 195–198.
[20] X. Wen, T. Walle, Preferential induction of CYP1B1 by benzo[*a*]pyrene in human oral epithelial cells: impact on DNA adduct formation and prevention by polyphenols, Carcinogenesis 26 (2005) 1774–1781.
[21] B. Prokopczyk, J.G. Rosa, D. Desai, S. Amin, O.S. Sohn, E.S. Fiala, K. El-Bayoumy, Chemoprevention of lung tumorigenesis induced by a mixture of benzo(a)pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by the organoselenium compound 1,4-phenylenebis(methylene)selenocyanate, Cancer Lett. 161 (2000) 35–46.
[22] P. Sicinski, J.L. Donaher, S.B. Parker, T. Li, A. Fazeli, H. Gardner, S.Z. Haslam, R.T. Bronson, S.J. Elledge, R.A. Weinberg, Cyclin D1 provides a link between development and oncogenesis in the retina and breast, Cell 82 (1995) 621–630.
[23] D. Sidransky, Cancer of the head and neck* molecular biology of head and neck tumors, in: V.T. DeVita Jr., S. Hellman, S.A. Rosenberg (Eds.), Cancer: Principles & Practice of Oncology, Lippincott Williams & Wilkins, Philadelphia, PA, 2001, pp. 789–796.
[24] Y. Nakahara, S. Shintani, M. Mihara, A. Kiyota, Y. Ueyama, T. Matsumura, Alterations of Rb, p16(INK4A) and cyclin D1 in the tumorigenesis of oral squamous cell carcinomas, Cancer Lett. 160 (2000) 3–8.
[25] K. El-Bayoumy, A. Das, B. Narayanan, N. Narayanan, E.S. Fiala, D. Desai, C.V. Rao, S. Amin, R. Sinha, Molecular targets of the chemopreventive agent 1,4-phenylenebis (methylene)-selenocyanate in human non-small cell lung cancer, Carcinogenesis 27 (2006) 1369–1376.
[26] K.M. Chen, T.E. Spratt, B.A. Stanley, D.A. De Cotiis, M.C. Bewley, J.M. Flanagan, D. Desai, A. Das, E.S. Fiala, S. Amin, K. El-Bayoumy, Inhibition of nuclear factor-kappaB DNA binding by organoselenocyanates through covalent modification of the p50 subunit, Cancer Res. 67 (2007) 10475–10483.
[27] D.C. Guttridge, C. Albanese, J.Y. Reuther, R.G. Pestell, A.S. Baldwin Jr., NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1, Mol. Cell. Biol. 19 (1999) 5785–5799.