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Chemoprevention of Colon Cancer by a Glutathione Conjugate of 1,4-Phenylene*bis*(methylene)selenocyanate, a Novel Organoselenium Compound with Low Toxicity[1]

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ABSTRACT

We have consistently shown that several synthetic Organoselenium compounds are superior cancer chemopreventive agents and less toxic than selenite or certain naturally occurring selenoamino acids. 1,4-Phenylene*bis*(methylene)selenocyanate (*p*-XSC) is the lead Organoselenium compound in that it has been shown to be the most effective and the least toxic agent in several experimental cancer models. It is not known whether *p*-XSC or one of its metabolites is responsible for its chemopreventive efficacy. As an initial step, we synthesized one of its putative metabolites, *i.e.*, the glutathione conjugate of *p*-XSC (*p*-XSe-SG), and determined its stability in the pH range from 2 to 8 and in the diet under normal feeding conditions. We also assessed its maximum tolerated dose and examined its chemopreventive efficacy against azoxymethane (AOM)-induced colon carcinogenesis in male F344 rats. *p*-XSe-SG proved to be very stable over the pH range tested. The maximum tolerated dose of *p*-XSe-SG determined in a 6-week subchronic toxicity study was found to be >210 ppm (>40 ppm selenium) when the compound was added to AIN-76A high-fat diet. To assess the efficacy of this agent in the postinitiation period of colon carcinogenesis, male F344 rats 6 weeks of age were fed the high-fat diet, and at beginning of weeks 7 and 8, all of the rats intended for carcinogen treatment were given AOM at a dose of 15 mg/kg body weight by s.c. injection. Two days after the carcinogen treatment, the groups of rats consuming the high-fat control diet began their respective high-fat experimental diet regimens with 0, 56, or 84 ppm *p*-XSe-SG (0.1, 10, and 15 ppm of selenium) supplementation. All animals continued on their respective diets for 38 weeks after the AOM-treatment and were then killed. Colon tumors were evaluated histologically using routine procedures and were also analyzed for cyclooxygenase (COX)-1 and COX-2 expression and enzymatic activities. The results indicate that *p*-XSeSG administered during the post-initiation stage significantly inhibited both the incidence ($P < 0.05–0.01$) and the multiplicity ($P < 0.05–0.005$) of AOM-induced colon adenocarcinomas. This agent also greatly suppressed the multiplicity ($P < 0.01–0.001$) of AOM-induced exophytic adenocarcinomas in a dose-dependent manner. Feeding of 56 or 84 ppm *p*-XSe-SG in the diet significantly suppressed total COX activity ($P < 0.02$ to -0.01) and COX-2 specific activity ($P < 0.005–0.0005$) but had minimal effect on the protein expression levels of COX-1 and COX-2. These results suggest that the newly developed synthetic Organoselenium compound, *p*-XSe-SG, is stable in the diet and at wide pH ranges, inhibits colon carcinogenesis when administered during the postinitiation stage, and inhibits COX activity. Compared with previous efficacy studies and considering the toxicity associated with selenium, *p*-XSe-SG seems to be the least toxic Organoselenium chemopreventive agent thus far tested in the experimental colon carcinogenesis. Studies are in progress to delineate whether *p*-XSe-SG is also effective when administered during the progression stage of colon carcinogenesis.

INTRODUCTION

Colon cancer is one of the leading causes of cancer death in Western countries, including North America. In the United States alone, nearly 56,000 deaths are attributed to this cancer annually (1). Risk reduction by nutritional intervention alone may not always be effective in individuals at high-risk for colon cancer; however, an alternate approach in secondary prevention has been to identify agents with chemopreventive efficacy (2, 3). Progress in chemoprevention research has brought about innovative approaches to the prevention and control of colon cancer (4, 5). Epidemiological studies have pointed to an inverse association between dietary intake of selenium and colorectal cancer risk in humans (6, 7). A clinical trial by Clark *et al.* (8) demonstrated that administration of selenium-enriched yeast significantly inhibited colon cancer incidence in humans. A series of experiments in laboratory animals also showed that supplementation of sodium selenite inhibits carcinogenesis in the colon, mammary gland, pancreas, and liver (7, 9). Generally, humans ingest organic forms of selenium, such as SM and SC; however, cancer prevention studies in preclinical models have not revealed any significant differences between the inorganic forms of selenium and those naturally occurring forms of selenium (10–12). A recent study from our laboratory indicates that dietary SM, one of the major forms of selenium in the selenium-enriched yeast, lacks chemopreventive efficacy against AOM[3]-induced colon carcinogenesis in F344 rats (13). There are also studies to indicate that chronic feeding of inorganic and certain organic forms of selenium at >5 ppm induces toxic effects (14, 15). Therefore, substantial efforts have been made to develop Organoselenium compounds such as *p*-XSC with maximal chemopreventive efficacy but low toxicity. Studies in our laboratory have shown that certain synthetic Organoselenium compounds hold great promise as chemopreventive agents (10, 12) against cancers of the colon, mammary gland, lung, and oral cavity in preclinical models (16–21). These Organoselenium agents were found to be superior to historically used selenium compounds, such as sodium selenite, and the naturally occurring SM.

Our recent study also indicates that *p*-XSC significantly suppresses the genetically predisposed intestinal tumor formation in APC^{min} mice, thus the chemopreventive efficacy of this agent is not limited to chemically induced cancers (22). A recent study by Tanaka *et al.* (23) demonstrated that administration of *p*-XSC in the diet suppressed the lung metastasis of melanoma cells in mice. Our observations and those of others described in the literature indicate that the chemopreventive efficacy of selenium depends on the chemical form in which it is administered, and that metabolism of such compounds is a

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[3] The abbreviations used are: AOM, azoxymethane; APC, adenomatous polyposis coli; BSC, benzyl selenocyanate; *p*-XSC, 1,4-phenylene*bis*(methylene)selenocyanate; *p*-XSe-SG, glutathione conjugate of *p*-XSC or *N,N′*-[1,4-phenylene*bis*[methyleneselenothio[(*1R*)-1-[[(carboxymethyl)amino]carbonyl]-2,1-ethanediyl]]]*bis*-L-glutamine; SM, selenomethionine; SC, selenocysteine; MTD, maximum tolerated dose; PG, prostaglandin; Tx, thromboxane; COX, cyclooxygenase; HPLC, high-performance liquid chromatography; AA, arachidonic acid; NSAID, nonsteroidal anti-inflammatory drug.

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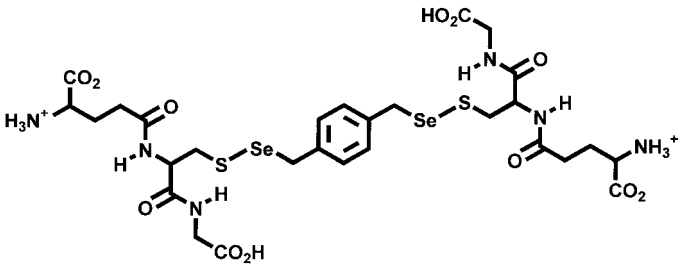
prerequisite for their cancer preventive potential (9, 12, 16, 24). There is evidence in support of the concept that the reductive metabolism of selenite by glutathione, which leads to the formation of the primary metabolite, selenodiglutathione, is a prerequisite for its antiproliferative effect (24). Hasegawa *et al.* (24) identified SC-glutathione selenyl sulfide as the selenium-containing metabolite in the small intestine of mice treated with SC. Therefore, we hypothesize that glutathione conjugates are putative metabolites that will be more effective than their parent Organoselenium compounds. In this connection, it is noteworthy that the glutathione conjugate of BSC was found to be a better inhibitor of colonic preneoplastic lesions, aberrant crypt foci, than BSC (25). On the basis of these studies, we hypothesize that *p*-XSe-SG is a primary metabolite of *p*-XSC mediating the chemopreventive activity and is a better inhibitor of colon carcinogenesis than other Organoselenium compounds thus far evaluated for their efficacy (Fig. 1).

The mechanisms by which Organoselenium compounds inhibit tumorigenesis are not fully known, but there is evidence that they act at several stages of the multistep carcinogenesis processes (5, 9, 10). COX isoforms, which are rate-limiting enzymes catalyzing the conversion of arachidonic acid into eicosanoids, play a significant role in tumorigenesis (26–28). COX-2 has been characterized as an early-response gene that is rapidly induced subsequent to stimulation of quiescent cells by mitogenic stimuli (29). Studies in our laboratory and elsewhere have demonstrated that colonic tumors of laboratory animals and humans have increased expression and/or activities of COX isoforms, particularly COX-2, when compared with levels in adjacent normal mucosa (30–32). Tsujii and DuBois (29) have reported that intestinal epithelial cells overexpressing the *COX-2* gene develop altered adhesion properties and resist apoptosis. Taken together, these observations imply that COX enzymes play a critical role in colon tumor growth and progression. In the present study, we tested the hypothesis that colon tumor modulation by *p*-XSe-SG may be mediated, in part, through the changes in the activities and expression of COX isoforms in colonic tumors.

The multistep nature of carcinogenesis provides many opportunities for intervention with chemopreventive agents targeted at specific mechanisms involved in the initiation, promotion, and progression of cancers. Determining the efficacy of these agents during the postinitiation stage, at which point the premalignant lesions are known to have developed, is very important with regard to the eventual clinical use of these agents in the secondary prevention of colon cancer among patients with polyps. Therefore the present study was designed to evaluate the potential chemopreventive efficacy of *p*-XSe-SG administered in a high-fat diet during the postinitiation phase of colon carcinogenesis. The rationale for using high-fat diet in this study was to simulate a Western diet regimen. The dose selection of *p*-XSe-SG was based on a subchronic toxicity study with several dose levels of *p*-XSe-SG.



***p*-XSe-SG**

Fig. 1. Structure of *p*-XSe-SG.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. AOM (CAS:25843-45-2) was purchased from Ash Stevens (Detroit, MI). Weanling male F344 rats were bought from the Charles River Breeding Laboratories (Kingston, NY), and all ingredients of the semipurified diet were obtained from Dyets, Inc. (Bethlehem, PA) and stored at 4°C before the preparation of diets. *p*-XSe-SG was synthesized as described by Sohn *et al.*,[4] and its structure was confirmed on the basis of ^{1}H NMR and high resolution mass spectra. The purity of this agent was found to be ≥99% as ascertained by HPLC analysis. Although the MTD of p-XSe-SG has been found to be >210 ppm, dose selection was based on our previous study in which 20 ppm *p*-XSC (10 ppm as selenium) achieved significant inhibition of colon carcinogenesis in this model assay (20). The composition of the high-fat semipurified diet was as follows: casein, 23.5%; DL-methionine, 0.35%; corn starch, 32.9%; dextrose, 8.32%; Alphacel, 5.9%; corn oil, 23.5%; mineral mix, 4.11%; vitamin mix, 1.18%; and Choline bitartrate, 0.24% (20). *p*-XSe-SG was incorporated into the control diet with a *V*-blender after premixing each level of test agent with a small quantity of diet in a food mixer to insure its uniform distribution. The stability of *p*-XSe-SG in the diet was determined periodically in multiple samples by HPLC,[4] and the compound was found to be stable in the diet under the experimental conditions used here. The recovery of *p*-XSe-SG from the diet was >96%. The control diet and the experimental diets containing *p*-XSe-SG were prepared weekly in our laboratory and stored in a cold room.

Stability of *p*-XSe-SG at Various pH Levels. The objective of this part of the study was (*a*) to determine the stability of the agent at physiological pH levels such as those found in the human stomach (pH 2), urine (pH 6), and blood (pH 7.4); (*b*) to examine whether, upon oral administration, the selenium from this agent can be effectively delivered to the colon; and (*c*) to determine the stability of this agent in the diet under feeding conditions. To determine the stability of *p*-XSe-SG at various pH concentrations, a suspension of *p*-XSe-SG (1.6 mg; 1.8 μmol) in DSMO (2 ml) was heated at 60°C until completely dissolved. The solution was filtered through glass wool, dispensed in glass vials (100 μl), and incubated with pH-2.0, -6.0, or -7.4 buffers (300 μl) at 37°C for 30 min under anaerobic and aerobic conditions. The solutions were cooled to room temperature and immediately analyzed by reverse phase HPLC. *p*-XSe-SG was quantified in each sample by chromatographic peak area (254 nm; 0.5 absorbance units range) and the percentage of *p*-XSe-SG decomposed was calculated using the following equation: [1-(Peak area at each pH/Peak area of control) 100]. To determine the stability of this agent in the diet under feeding conditions, aliquots of diets containing *p*-XSe-SG were extracted and analyzed by reverse phase HPLC as described above.

Determination of MTD of *p*-XSe-SG. To estimate the effective dose level of *p*-XSe-SG for a long-term efficacy study, the MTD level was determined in male F344 rats by feeding different concentrations of the agent in a 6-week subchronic toxicity study. The MTD is defined as the highest dose that causes no more than a 10% weight decrement as compared with the appropriate 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. At 7 weeks of age, groups of male F344 rats (6 rats/group) were fed the experimental diets containing 0, 56, 84, 105, 126, 147, or 210 ppm of *p*-XSe-SG. Body weights were recorded twice weekly for 6 weeks. All animals were examined daily for any symptoms (physical signs) of toxicity, such as ill-kept appearance, circling rashes, tremors, roughened coat, rhinitis, chromodacryorrhea, and prostration, to cite a few. At the end of 6 weeks, all animals were killed, and oral cavity, colon, small intestine, stomach, liver, and kidney were examined grossly under a dissection microscope for any abnormalities.

Efficacy Study. Two dose levels, 56 and 84 ppm, equivalent to 10 and 15 ppm as selenium, were evaluated for chemopreventive efficacy. Previously, we had established that, in a number of investigations, *p*-XSC at 20 ppm, equivalent to 10 ppm selenium, inhibited chemically induced colon carcinogenesis. Studies were designed to determine the efficacy of 56 and 84 ppm *p*-XSe-SG administered during the postinitiation stage of AOM-induced colon carcinogenesis. Male F344 rats received at weaning were quarantined for 10 days and had unrestricted access to modified AIN-76A control diet. After quarantine, all

[4] Sohn, O. S., Rosa, J.G., Fiala, E. S., and El-Bayoumy, K. Synthesis, excretion profiles and tissue distribution of selenium derived from a glutathione conjugate of 1,4-phenylenebis(methylene)selenocyanate, submitted for publication.

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rats were randomly distributed by weight into various groups (Fig. 2; Table 1) and transferred to an animal holding room. They were housed in plastic cages with filter tops (3 rats/cage) under controlled conditions of a 12-h light and dark cycle at 50% relative humidity and 21°C. At 5 weeks of age, all rats were randomly assigned to various experimental groups (36 animals/group) and fed the high-fat control diet (Fig. 2). Two weeks later, animals intended for carcinogen treatment received s.c. injections of 15 mg AOM/kg body weight once weekly for 2 successive weeks. Vehicle-treated groups (6 rats/group) received no AOM but an equal volume of normal saline. Two days after the second injection of AOM or normal saline, the groups of rats receiving the control diet were switched to diets containing 56 or 84 ppm *p*-XSe-SG for the remainder of the experiment. One group continued on control diet. Body weights were recorded every 2 weeks until the 16th week and then every 4 weeks until the termination of the experiment 38 weeks after the last AOM treatment. Animals who were moribund were killed and necropsied. Surviving animals were killed as scheduled. All organs, including the intestines, were examined grossly under the dissection microscope. Colon tumors with a diameter >0.4 cm were cut into halves; one portion of the tumor was used to determine the expression and activities of COX isoforms and the other half was used for histopathological evaluation. Portions of colon tumors intended for biochemical determinations were frozen quickly in liquid nitrogen and stored at −80°C until analysis. Intestinal tumors were fixed in 10% neutral buffered formalin and were processed by routine histological methods as described previously (33). The histological criteria for colon tumor classification were also described previously (34). According to the criteria, most of the colon tumors in this study were adenocarcinomas, either invasive or noninvasive. The invasive adenocarcinomas were mostly the signet-ring mucinous type, invading the muscularis mucosa deep into the intestinal wall and beyond. The noninvasive adenocarcinomas were those growing outward toward the intestinal lumen and not invading the muscularis mucosa. They were usually well-differentiated adenocarcinomas.

Biochemical Analysis. To minimize the sample-to-sample variation, colonic tumors from each treatment group were selected on the basis of the histopathology of the individual tumors. Because most of the AOM-induced colonic tumors are tubular adenocarcinomas, we used these tumors for the analysis of COX-isoform expression and activities. Sample preparation for analyses of COX activities and/or expression were performed as described previously (22, 32). Samples of colonic tumors were homogenized in 1:3 (w/v) volumes of homogenizing buffer containing 30 mM Tris-HCl (pH 7.4), 140 mM NaCl, 5 mM KCl, 20 μm EDTA, 10 μg/ml leupeptin, 50 μg/ml trypsin inhibitor, and 1 mM phenylmethylsulfonyl fluoride. The homogenates were centrifuged at 100,000 × *g* at 4°C for 1 h. The resulting pellet fraction was used to measure COX isoform activity and expression.

Western Blot Analyses of COX-1 and COX-2. COX-1 and COX-2 purified proteins, which were purchased from Cayman Chemicals (Ann Arbor, MI), were used as electrophoresis standards. The proteins were separated on 8% PAGE-gel and then electroplated on polyvinylidene difluoride membranes as described (32). After blocking membranes in 5% nonfat dry milk, they were incubated with antibodies of COX-1 and COX-2 for 1 h. The membranes were washed three times and incubated once more with secondary horseradish peroxidase-linked antigoat or antirabbit *g*G antibody at a final concentration of 1:2000. The membranes were developed in an enhanced chemiluminescence system and exposed to Kodak XAR5 film. Intensities of each band were scanned by a computing densitometer.

Table 1 *Chemopreventive effect of p-XSe-SG on AOM-induced colon adenocarcinoma incidence and multiplicity in male F344 rats*

Experimental group	Adenocarcinoma incidence[a]	Adenocarcinoma multiplicity[b]
AOM-treated		
Control diet	83%	2.0 ± 0.30
56 ppm p-XSe-SG	52%[c] (37.4)[d]	1.30 ± 0.23[e] (35)
84 ppm p-XSe-SG	55%[c] (33.7)	0.96 ± 0.16[f] (52)
Vehicle-treated		
Control diet	0	0
84 ppm p-XSe-SG	0	0

[a] Percentage of rats with adenocarcinomas.
[b] No. of adenocarcinomas/rat.
[c] Significantly different from the control group by χ^2 test; $P < 0.02–0.05$.
[d] Values in parentheses are percentage inhibition from control.
[e] Significantly different from the control group by Student's *t*-test; $P < 0.03$.
[f] $P < 0.002$.

Total COX and COX-2 Synthetic Activity. COX activities in colon tumor samples were assayed by using a slight modification of a method published previously (32, 35). The microsomal pellet was resuspended in 50 mM potassium phosphate buffer (pH 7.4) for the assay of total COX and COX-2 activities. For determining total COX activity, 150 μl of reaction mixture containing 12 μM [^{14}C]AA (420,000 dpm), 1 mM epinephrine, 1 mM glutathione in 50 mM phosphate buffer, and 25–35 μg of tumor microsomal protein were incubated at 37°C for 15 min. For determining COX-2 activity, the reaction mixture was preincubated with 150 μM of aspirin to block COX-1 activity and to modify COX-2 activity. After incubation, the reaction was terminated by adding 40 μl of 0.2 M HCl. The COX metabolites of AA were extracted three times with 0.5 ml of ethyl acetate. The combined extracts were evaporated to dryness under N_2, redissolved in chloroform, and subjected to TLC on Silica G plates. The TLC plates were developed in a solvent system containing a mixture of chloroform:methanol:acetic acid:water (100:15:1.25:1, v/v/v/v) and were exposed in an iodide chamber for 5 min to visualize the standards. The metabolites of [^{14}C]AA corresponding to PGE_2, $PGF_{2\alpha}$, PGD_2, 6-keto-$PGF_{1\alpha}$, and TxB_2 were detected by their comigration with authentic standards for total COX activity and [^{14}C]-15 (*R*)-hydroxyeicosatetraenoic acid for COX-2 activity.

Statistical Analysis. Differences in body weights among the groups were analyzed by ANOVA. Tumor incidence (number of rats with tumors) among the dietary groups was compared by Armitage's χ^2 method. Tumor multiplicity (total number of tumors/rat) and COX isoforms expression and activities were analyzed by Student's *t* test. Differences were considered statistically significant at $P < 0.05$. Also, the dose-response effect of *p*-XSe-SG was analyzed by logistic regression analysis.

RESULTS

Stability of *p*-XSe-SG at Different pH Levels and in Diet. Our results indicate a quantitative recovery of this conjugate at different pH levels, suggesting that there is no evidence of decomposition of this agent at these physiological pH concentrations. We found that >96% of *p*-XSe-SG could be accounted for in feed samples stored in a cold room for 7 days and also in food cups under feeding conditions.

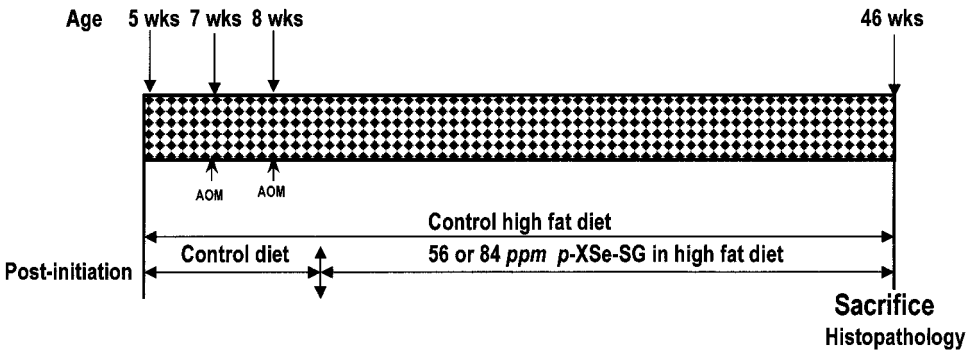

Fig. 2. Experimental design. Animals at 5 weeks of age were fed a high-fat control diet, and beginning at weeks 7 and 8, groups of rats were given AOM (15 mg/kg of body weight, by s.c. injection once weekly for 2 weeks). Two days after the last AOM-injection, designated groups of rats were transferred to experimental diets containing 0, 56, or 84 ppm of *p*-XSe-SG.

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MTD. *p*-XSe-SG, even at 210 ppm (~40 ppm of selenium) gave no indication of toxicity, based on gross observations, and no significant body weight loss (data not shown). On the basis of this study, the MTD of *p*-XSe-SG in male F344 rats is >210 ppm when it is administered in the AIN-76A high-fat diet.

General Observations. Body weights of AOM- and saline-treated animals fed the high-fat diet with or without *p*-XSe-SG were comparable (data not shown). In vehicle-treated rats, administration of *p*-XSe-SG did not produce any gross changes attributable to toxicity either in the liver, kidney, or lungs, suggesting that long-term administration of *p*-XSe-SG at those levels was tolerable.

Tumor Data. Table 1 summarizes the AOM-induced colon tumor incidence and multiplicity. In this study, >96% of colon tumors in rats receiving AOM and the high-fat control diet were adenocarcinomas; the remainder of the tumors were adenomas (data not shown). Administration of AOM induced colon adenocarcinomas in ~83% of the rats fed the high-fat control diet. Seventy percent of the rats had noninvasive adenocarcinomas. Administration of 56 or 84 ppm of *p*-XSe-SG in the high-fat diet during the postinitiation stage significantly inhibited the incidence of adenocarcinomas in the colon when compared with the incidence in animals fed the high-fat control diet ($P < 0.05$). As shown in Table 1, there was no significant difference in the incidence of colon adenocarcinomas between the rats fed the diets containing 56 and 84 ppm *p*-XSe-SG. Administration of 56 or 84 ppm *p*-XSe-SG in the high-fat diet significantly suppressed the multiplicity of colonic adenocarcinomas ($P < 0.03$–0.002). The dose-dependent effect of p-XSe-SG analyzed by the regression method indicates that administration of 56 and 84 ppm of this agent significantly inhibited the multiplicity of adenocarcinomas in a dose-dependent manner ($P < 0.01$). However, *p*-XSe-SG at higher dose levels suppressed the colon tumor multiplicity more than at lower doses, but no significant ($P \leq 0.115$) differences were observed in tumor multiplicity between these dose levels. The incidence and multiplicity of noninvasive adenocarcinomas of the colon were likewise greatly suppressed in rats receiving 56 or 84 ppm *p*-XSe-SG, but it had no effect on the invasive adenocarcinomas.

Expression and Activities of COX Isoforms. We investigated whether the inhibition of colon carcinogenesis by *p*-XSe-SG is associated with the modulation of COX isoforms expression and activities in the colon tumors. We found that the administration of 56 or 84 ppm *p*-XSe-SG had no significant effect on COX-1 and COX-2 protein expression levels in the colon tumor (Fig. 3), based on integrated absorbance from the laser densitometric scan data (6 samples/group). The effects of 56 and 84 ppm *p*-XSe-SG on total COX and COX-2 activities in the colon tumor are summarized in Table 2 and Fig. 4. Results summarized in Table 2 indicate that dietary 56 or 84 ppm *p*-XSe-SG significantly reduced the total COX activity leading to formation of low levels of AA metabolites ($P < 0.02$ to -0.012) in the tumor tissue. Importantly, in animals fed the 84 ppm *p*-XSe-SG supplemented diet, the formation of PGE_2 levels in the colon tumors

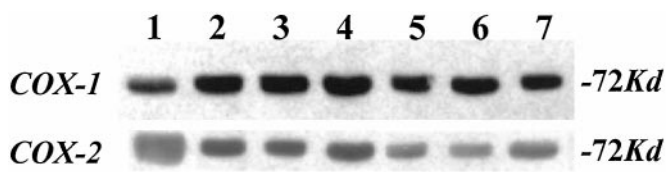

Fig. 3. Effect of *p*-XSe-SG on the AOM-induced colon adenocarcinoma COX-1 and COX-2 protein expression. *Lane 1*, standards of COX-1 and COX-2 proteins, each 10 ng; *Lanes 2*, *3*, and *4*, loaded with 50 μg protein from colonic tumors; and *Lanes 5*, *6*, and *7*, loaded with 30 μg protein from colonic tumors. *Lanes 2* and *5*, tumor samples of rats fed control diet; *Lanes 3* and *6*, tumor samples of rats fed 56 ppm *p*-XSe-SG diet; and *Lanes 4* and *7*, tumor samples of rats fed 84 ppm *p*-XSe-SG diet.

Table 2 *Effect of p-XSe-SG on AOM-induced colon tumor COX activity in male F344 rats*

Major COX metabolites	pmol of PGs and TxB_2 formed from [^{14}C]AA/mg protein/15 min		
	Control diet	56 ppm p-XSe-SG	84 ppm p-XSe-SG diet
PGE_2	878 ± 64[a]	642 ± 54[b] (−26.9)	518 ± 41[c] (−41)[d]
$PGF_{2\alpha}$	564 ± 36	513 ± 38 (−9.04)	422 ± 32[b] (−25.2)
PGD_2	375 ± 29	356 ± 30 (−5.1)	290 ± 26[e] (−22.6)
6-Keto $PGF_{1\alpha}$	807 ± 63	754 ± 51 (−15.1)	723 ± 57 (−10.4)
TxB_2	642 ± 47	545 ± 37 (−15.1)	543 ± 43 (−15.4)
Total AA metabolites[f]	3584 ± 228	2915 ± 217[e] (−18.6)	2770 ± 206[g] (−22.7)

[a] Mean ± SE ($n = 6$).
[b] Values are significantly different from the control diet group by Student's *t* test; $P < 0.007$.
[c] Values are significantly different from the control diet group by Student's *t* test; $P < 0.0004$.
[d] Values in the parentheses are percentage inhibition from the control diet group.
[e] Values are significantly different from the control diet group by Student's *t* test; $P < 0.03$.
[f] Total AA metabolites include major (as shown in table) and minor (not shown in table) metabolites.
[g] Values are significantly different from the control group by Student's *t* test; $P < 0.012$.

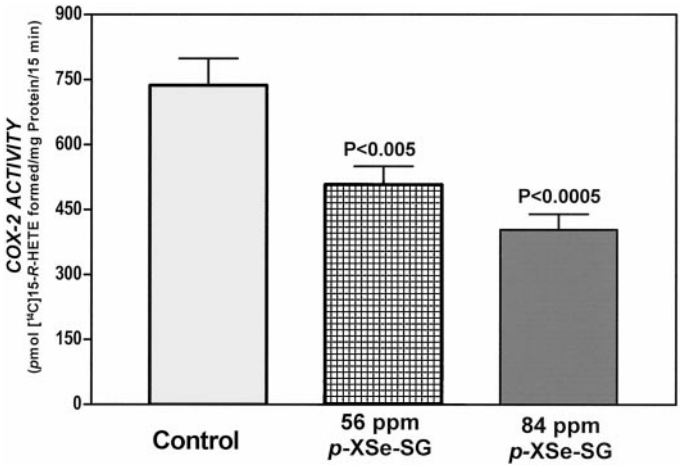

Fig. 4. Effect of *p*-XSe-SG on the AOM-induced colon tumor *COX-2* activity in male F344 rats (Six samples/group).

was inhibited >41% ($P < 0.0004$) compared with the effects of the control diet. Colonic tumors of rats treated with 56 ppm *p*-XSe-SG contained reduced levels of PGE_2 (−26.9%, $P < 0.008$); however it had no significant effect on other PGs and TxB_2 levels (Table 2). Also, 84 ppm *p*-XSe-SG diet significantly inhibited $PGF_{2\alpha}$ and PGD_2 levels ($P < 0.03$–0.01) but had no significant effect on the 6-keto-$PGF_{1\alpha}$ and TxB_2 levels. Interestingly, administration of *p*-XSe-SG suppressed COX-2 activity in the colon tumor tissues in a dose-dependent manner, as shown in Fig. 4.

DISCUSSION

The results of the present investigation are of considerable interest, because this is the first demonstration of chemopreventive efficacy of *p*-XSe-SG against colon carcinogenesis during the postinitiation phase. In addition to *p*-XSe-SG chemopreventive efficacy, this newly developed agent possesses significantly less toxicity when compared with other selenium compounds developed in our laboratory. Results generated from our previous studies that used the same experimental design enabled a comparison of the MTD levels of sodium selenite, SM, BSC, *p*-XSC, and *p*-XSe-SG. The results showed that the subchronic MTD dose of *p*-XSe-SG in rats is >20-fold higher than that of Na_2SeO_3, ~14-fold higher than that of SM, >8-fold higher than BSC and 4-fold greater than that of *p*-XSC, its parent compound.

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Importantly, the efficacy of both *p*-XSC and *p*-XSe-SG are comparable; however, the latter agent appears to be a better candidate, considering its remarkably low toxicity. The chemopreventive index is defined as the ratio of the MTD of an agent under study to the dose that inhibits 50% tumor multiplicity. The chemopreventive indices of sodium selenite, SM, BSC, *p*-XSC, and *p*-XSe-SG were 1.3, 1.0, 2.5, 3.3, and >3.5, respectively. The high index value for *p*-XSe-SG signifies that this compound is well tolerated at doses required for chemoprevention and is superior to sodium selenite, SM, BSC, and *p*-XSC.

Previously, we have shown that several Organoselenium compounds, such as BSC and/or *p*-XSC, inhibit colon carcinogenesis in F344 rats and also in APC^{min} mice (12, 16, 22). Recently, we have shown that *p*-XSC administered with a high-fat diet significantly suppresses chemically induced colonic adenocarcinoma in male F344 rats, and the effect was more pronounced when the agent was administered with a low-fat diet (20). The literature testifies to the existence of several successful studies of the chemopreventive efficacy of *p*-XSC against mammary (18), lung (19), and oral cavity (21) carcinogenesis in preclinical models. The results of this study are of particular interest because *p*-XSe-SG, with its very low toxicity, is very effective during the postinitiation stage of colon carcinogenesis when compared with other known selenium-containing chemopreventive agents (12, 13, 17, 20). Also, results of the present study are potentially important because higher levels of *p*-XSe-SG can be administered with no apparent toxicity. The high chemopreventive efficacy of *p*-XSe-SG during the post-initiation stage of colon carcinogenesis and its very low toxicity by comparison with all previously known selenium compounds provides a rational basis for designing studies with this agent in the clinical setting.

The present study also demonstrated that administration of *p*-XSe-SG significantly suppresses COX-2 activities and, to some extent, total COX in colonic tumors. COX metabolites play a very important role in colon tumor growth and progression (26–29). It is noteworthy that several human epidemiological studies have demonstrated that intake of NSAIDs, and, among them, especially aspirin, reduces the risk of colon cancer (36, 37). Laboratory animal assays have supported this concept in studies with several NSAIDs (28, 33, 38, 39). One of the mechanisms by which NSAIDs inhibit colon carcinogenesis is *via* inhibition of COX enzymes, which, in a rate-limiting step, catalyze the conversion of arachidonic acid into PGs (26, 27, 40). The latter are potent biological mediators of diverse normal physiological effects and are implicated in various pathological conditions, including inflammation and neoplastic transformation (26, 28, 41). In addition, the byproducts of PG biosynthesis might be relevant mutagens (26, 29). In the present study, administration of *p*-XSe-SG had no significant effect on the protein expression of COX-1 and COX-2 but modulates the COX-activities, particularly COX-2 activity, at the posttranslational level rather than at the protein-expression level. Preferential modulation of COX-2 activity by *p*-XSe-SG has not been described in the literature. In support of the above, our recent study with *p*-XSC suggests that administration of this agent to the APC^{min} mouse significantly suppresses the COX-2 activity rather than the protein expression in intestinal tumors (22).

Other mechanisms by which *p*-XSe-SG inhibits the development of colonic adenocarcinoma formation are not known. It is likely that this agent intervenes at more than one level in the cellular and molecular events that lead to the inhibition of colon tumorigenesis. The general mechanisms of tumor inhibition that have been proposed for selenium compounds include the inhibition of lipid peroxidation and facilitation of peroxide decomposition, free radical scavenging, the repair of molecular damage, the inhibition of protein kinase C, DNA synthesis, and incorporation into enzymes with protective functions for the cell, *e.g.,* glutathione peroxidase (42–46), an enzyme responsible for preventing oxidative damage attributable to peroxidation. Recently, Ganther (47) reviewed selenium metabolism and possible mechanisms leading the cancer prevention. In addition, studies have demonstrated that dietary *p*-XSC inhibits colonic cell proliferation, COX-2 activity, and PGE_2 and induction of apoptosis in colonic tumor tissues (17, 48, 49). Also, when added to mammary tumor cell lines, *p*-XSC was capable of inhibiting thymidine kinase and cell proliferation, whereas equal concentrations of selenium in the form of sodium selenite had no effect (50). This suggests that cellular responses to these agents may contribute to chemopreventive effects (42–50). In addition, *p*-XSC has been shown to suppress β-catenin expression and COX-2 activity in intestinal polyps of APC^{min} mice (22). It is possible that colon tumor inhibition by *p*-XSe-SG may be mediated through one or more of the above mechanisms. A recent study of incubation of *p*-XSe-SG with cecal contents containing microflora under anaerobic conditions suggests that the reductive metabolism of the conjugate leads to the formation of tetracycloselenophane, presumably *via* the formation of an aromatic selenol intermediate (4). In fact, when *p*-XSC was incubated with the cecal microflora under identical anaerobic conditions, it was recovered intact. These results indicate that *p*-XSe-SG can liberate aromatic selenol more effectively than the parent compound, *p*-XSC. Selenols have been proposed to be responsible for cancer prevention by selenium compounds (47). Additional studies are warranted to pinpoint the mechanisms involved in the chemoprevention of colon carcinogenesis by *p*-XSe-SG.

In conclusion, the study described here demonstrates that dietary administration of a putative metabolite of *p*-XSC namely, *p*-XSe-SG, during the post-initiation stage of AOM-induced carcinogenesis in rats significantly suppresses colon adenocarcinoma formation in the colon in a dose-dependent manner. Furthermore, this new agent possesses very low-toxicity, COX-2-inhibitory activity and relatively higher chemopreventive index compared with previously known Organoselenium compounds tested in the experimental colon cancer using similar experimental design and protocols. Although an understanding of the mechanisms of the chemopreventive action of *p*-XSe-SG is very important, the development of preventive strategies using agents with low toxicity and preferential COX-2-inhibitory activity during the postinitiation stage of tumorigenesis will serve as a practical approach toward the design of chemoprevention trials in humans. Our findings support the application of this strategy to studies of the prevention of colorectal tumors in populations at high risk, including patients with preclinical familial adenomatous polyps and sporadic polyps.

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