Mechanism of Benzylselenocyanate Inhibition of Azoxymethane-induced Colon Carcinogenesis in F344 Rats

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ABSTRACT

Benzylselenocyanate (BSC), a novel organoselenium compound, has been found to inhibit azoxymethane (AOM)-induced colon carcinogenesis in rats during initiation. To investigate its mechanism of action, we examined the effects of BSC feeding on the following parameters: (a) metabolism of [14C]AOM to 14CO2 in vivo; (b) metabolic activation of AOM to MAM and of MAM to formic acid and methanol by rat liver microsomes in vitro; and (c) AOM-induced DNA methylation in rat livers and colons. Five-week-old male F344 rats were fed modified (23% corn oil) AIN-76A diets containing BTC (control), 25, or 50 ppm of BSC or benzylthiocyanate (BTC), a sulfur analogue of BSC which does not inhibit the colon carcinogenicity of AOM. After 3 weeks, rats were either sacrificed for the isolation of liver microsomes or were given 15 mg/kg of [14C]AOM s.c. to determine the rate of carcinogen metabolism in vivo. No difference in [14C]AOM metabolism was found between rats fed the BTC diets and those fed the control diet. In contrast, the rate of [14C] AOM metabolism, as determined by exhaled radioactivity, was 2-3 times higher in rats fed the BSC diets. While liver microsomes from rats fed the BTC diets metabolized AOM and MAM at rates not significantly different from those obtained with control liver microsomes, the metabolic activation of AOM as well as of MAM was stimulated severalfold when assayed with liver microsomes from rats fed the BSC diets. An increase in total liver cytochrome P-450 was also observed in the BSC-fed rats. Following the administration of 15 mg/kg AOM, significantly less N6- methylguanine and 7-methylguanine was present in the colon DNA from rats consuming the BTC diets than in rats fed the BTC or control diets. The body weight gains of rats fed the 25- and 50-ppm BSC-containing diets for 3 weeks were less (27 and 43%, respectively) than those of rats fed either the control or BTC-containing diets. These results indicate that dietary BSC significantly inhibits the hydroxylation of AOM and the oxidation of MAM in rat liver. An increase in the rates of AOM and MAM metabolism in the liver due to enzyme induction by BSC will result in decreased delivery of MAM to the colon via the bloodstream. This may be reflected in decreased DNA alkylation, as observed, and is likely to be a major factor in the inhibition of AOM-induced colon carcinogenesis by BSC.

INTRODUCTION

BSC (Fig. 1) has been shown to be an effective inhibitor of AOM-induced colon carcinogenesis in F344 rats (1) when included in their diet at a level of 25 ppm. The organoselenium compound also significantly inhibited benzo(a)pyrene-induced forestomach tumors in CD-1 mice (2) and dimethylbenz(a)anthracene-induced mammary tumors in female Sprague-Dawley rats (3). In these studies, equivalent dietary levels of BTC (Fig. 1), an analogue of BSC in which the selenium atom is replaced by sulfur, had no tumor-inhibitory effects. Inorganic selenium, administered in the drinking water or in the diet as sodium selenite, has also been reported to inhibit colon carcinogenesis induced in rats by the metabolically related (4) carcinogens 1,2-dimethylhydrazine (5, 6), AOM (7), and MAM acetate (8) and the unrelated carcinogen bis(2-oxopropyl)nitrosamine (9). In the case of 1,2-dimethylhydrazine, it has been suggested that inorganic selenium may act in part by inhibiting the metabolism of the carcinogen in the liver and by decreasing the rate of DNA synthesis in the colon (10), whereas in the case of bis(2-oxopropyl)nitrosamine, the inhibition of colon carcinogenicity was ascribed in part to enhanced repair of colonic DNA damage (9). Although both BSC and inorganic selenium have the ability to inhibit chemical carcinogenesis in various animal organs and, clearly, the presence of selenium in BSC is essential to its effects, it is not certain to what extent, if any, the mechanisms of chemoprevention by inorganic selenium and BSC overlap. On a molar basis, the acute toxicity of BSC in male F344 rats is approximately 3.4 times less than that of sodium selenite, suggesting that extensive metabolic conversion of BSC to selenite or to some other common toxic intermediate does not occur in vivo. For this reason, and taking into account previously published studies by others (10-14), it is probable that the chemopreventive effects of BSC are intrinsic to the compound and are separable from those of inorganic selenium. Thus, it becomes important to delineate the mechanism of action of BSC, since this may facilitate attempts to design even more effective organoselenium chemopreventive agents.

In this work, we examined the mechanism of BSC action by determining the effects of two dietary levels of the organoselenium compound and its sulfur analogue on the in vivo and in vitro metabolic activation of AOM. We find that BSC, but not BTC, is a powerful inducer of rat liver enzymes that catalyze the hydroxylation of AOM to MAM and the further oxidation of MAM. The feeding of BSC, but not BTC, also results in decreased colon DNA methylation by AOM. These observations lead us to propose that the inhibition of AOM colon carcinogenicity by BSC depends on the increased rate of liver metabolism of the carcinogen, resulting in decreased availability of MAM, a more proximate carcinogenic metabolite of AOM, for further metabolic activation in the colon mucosa.

MATERIALS AND METHODS

Chemicals. AOM and MAM acetate were purchased from Ash Stevens, Inc. (Detroit, MI), and from Starks Associates (Buffalo, NY), respectively. [1,2-14C]AOM and [1,2-14C]MAM acetate were obtained from NEN Research Products (Boston, MA). For in vitro metabolism assays, [1,2-14C]MAM was prepared from [1,2-14C]MAM acetate by hydrolysis with porcine liver esterase (Sigma Chemical Co., St. Louis, MO); otherwise the conditions were the same as those described by Feinberg and Zedeck (15). The material was purified by HPLC (16) prior to use. BTC was obtained from Aldrich Chemicals (Milwaukee, WI). BSC was synthesized as previously described (2).

4 As determined by C. C. Conaway (unpublished) the 50% lethal dose p.o. in male F344 rats of sodium selenite is 0.21 mmol/kg; the 50% lethal dose of BSC under the same conditions is 0.72 mmol/kg.

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3 The abbreviations used were: AOM, azoxymethane; BSC, benzylselenocyanate; BTC, benzylthiocyanate; MAM, methylazoxymethanol; HPLC, high-performance liquid chromatography.
BENZYLSELENOCYANATE (BSC)

CH₂-Se-C≡N

BENZYLTHIOCYANATE (BTC)

CH₂-S-C≡N

Fig. 1. Chemical structure of organoselenium compound BSC and its sulfur analogue, BTC.

RESULTS

Body Weight Gains and in Vivo Metabolism of AOM. During the 3-week feeding period, the body weight gains of the animals increased as follows: control diet, from 92 ± 5 (SEM) to 188 ± 9 g; 25-ppm BTC diet, from 91 ± 3 to 188 ± 7 g; 25-ppm BSC diet, from 93 ± 2 to 161 ± 10 g; 50-ppm BTC diet, from 97 ± 6 to 195 ± 7 g; 50-ppm BSC diet, from 98 ± 5 to 154 ± 5 g. Thus, the body weight gain of the rats fed the control or BTC-containing diets was approximately the same, i.e., 101 to 107%, whereas the body weight gains of the rats fed the 25- and 50-ppm BSC diets were only 73 and 57%, respectively.

The effects of feeding the modified AIN-76A diet only (control diet) or the modified AIN-76A diet containing 25 or 50 ppm of BTC or BSC for 3 weeks on the in vivo metabolism of [1,2-14C]AOM are shown in Fig. 2. During the 4-h period following the administration of the labeled carcinogen, the rate of carcinogen metabolism was approximately the same in rats fed the control diet as in rats fed diets containing 25 or 50 ppm BTC. In contrast, a significant enhancement of [14C]AOM metabolism was observed in rats fed the 25- or 50-ppm BSC diets, compared to rats fed either the corresponding levels of BTC or the control diet. For example, 2 h following [14C]AOM administration, 15.1 ± 2.9% (SEM) and 11.1 ± 0.7% of the carcinogen was metabolized to 14CO2 by rats fed the 25- and 50-ppm BTC diets, respectively, whereas, at the same time point, 35.1 ± 1.9% and 30.6 ± 0.61% of the carcinogen was metabolized by rats fed the 25- and 50-ppm BSC diets, respectively. In these instances, the corresponding differences were significant at P < 0.01. It is apparent that feeding of rats for 3 weeks with BSC at 25- or 50-ppm dietary levels increases the rate of in vivo AOM metabolism approximately 2-3-fold with respect to rats fed the control diet or the control diet containing the same amounts of BTC.

An examination of Fig. 2 reveals an apparent paradox in that the cumulative amount of radioactivity exhaled reaches a peak about 5 h after labeled carcinogen administration and decreases slightly at 6 and 7 h. A possible explanation of this effect may be that [14C]AOM is partially metabolized in vivo to a minor volatile species other than 14CO2, which is then transiently trapped by aqueous NaOH solution. HPLC analysis of the contents of the gas washers failed to indicate the presence of unmetabolized [14C]AOM. At this time, the identity of the putative volatile AOM metabolite is not known. One likely possibility is ethane, since evidence for the presence of ethane in the exhaled air of rats treated with 1,2-dimethylhydrazine, a metabolic precursor of AOM (4), has been presented by others (25, 26). The identification of this species and its quantitation are subjects under investigation in this laboratory.

In Vitro Metabolism of AOM and MAM. The increased rate of AOM metabolism in vivo by rats fed diets containing BSC was reflected in enhanced conversion of AOM to MAM and of MAM to methylethylguanine, two minor metabolites of AOM, isolated from the livers of these animals. As shown in Fig. 3, liver microsomes from rats fed the control diet or the control diet containing BTC at levels of 25 or 50 ppm metabolized AOM to MAM at a rate of approximately 0.8–1 nmol AOM/mg microsomal protein/min. In contrast, liver microsomes from rats fed the BSC diet at levels of 25 or 50 ppm metabolized AOM to MAM at rates of about 5.3 or 7.5 nmol/mg protein/min (P < 0.01), respectively. Similarly, as shown in Fig. 4, the rate of metabolism of MAM by liver microsomes from rats fed the BTC or control diets was approximately 0.4–0.7 nmol/mg protein/min, whereas the rate of this reaction increased to about 2.5–5.0 nmol/mg protein/min following feeding of rats the BSC diet.
Fig. 2. In vivo metabolism of [14C]AOM. Rats fed control, BTC-containing, or BSC-containing diets for 3 weeks received s.c. injections of 15 mg/kg [14C]AOM and were enclosed in metabolism cages, and exhaled radioactive volatile metabolites (for the most part, 14CO2) trapped in 1 N NaOH were determined. Points, means of 3-4 experiments, each using one animal; bars, SEM.

Fig. 3. In vitro metabolism of [14C]AOM to [14C]MAM by liver microsomes from rats fed control, BTC-containing, or BSC-containing diets for 3 weeks. The [14C]MAM product was determined by HPLC as described in “Materials and Methods.” Columns, means of 3-4 determinations, each using liver microsomes from one animal; bars, SD.

Fig. 4. In vitro metabolism of [14C]MAM to labeled methanol and formic acid by liver microsomes from rats fed control, BTC-containing, or BSC-containing diets for 3 weeks. Products of MAM metabolism were determined by HPLC as described in “Materials and Methods.” Columns, means of 3-4 separate determinations, each using liver microsomes from one animal; bars, SD.

2.3 and 3.3 nmol/mg/min, respectively, when liver microsomes from rats fed the 25- or 50-ppm BSC diets were used. In these in vitro experiments, radioactivity recoveries of close to 100% were routinely obtained, indicating that the conversion of AOM or MAM to the putative volatile metabolite suspected of being produced in vivo does not occur in vitro under the incubation conditions used.

Effect on Total Liver Cytochrome P-450 Level. In addition to the significant increase of enzyme activities in rat liver microsomes which catalyze the hydroxylation of AOM and the oxidation of MAM (28), feeding of BSC (but not BTC) resulted in an increase in total liver cytochrome P-450 content as shown in Fig. 5. This effect appeared to be dose dependent; in rats fed the 25-ppm BSC diet, total cytochrome P-450 increased by 35% (P < 0.01) and 73% (P < 0.01) in rats fed the 50-ppm BSC diet compared to control diet-fed rats. There were no significant effects on liver cytochrome P-450 in rats fed either the 25- or 50-ppm BTC diet.

AOM-induced Methylation of DNA Guanine in Rat Liver and Colon. As shown in Fig. 6, compared to feeding with the control diet, feeding rats with BSC or BTC for 3 weeks at levels of 25 or 50 ppm in the diet did not significantly alter the levels of O6-methylguanine or 7-methylguanine in liver DNA assayed 6 h after the administration of 15 mg/kg of AOM. Under these conditions, approximately 6.2 ng 7-methylguanine and 0.88 ng O6-methylguanine/µg guanine were present in liver DNA from rats fed the control diet. The failure of BSC to affect the level of AOM-induced DNA methylation in the liver is in contrast to the inhibition it produced on AOM-induced DNA guanine methylation in the colon (Fig. 7). As in the case of the liver, feeding of BTC at 25 or 50 ppm produced no change in the degree of guanine methylation in colon DNA. However, the same dietary levels of BSC caused a greater than 50% inhibition (P < 0.01) of AOM-induced DNA guanine methylation in the colon at both levels of the compound in the diet. The differences in the methylated guanine content of colon DNA between the two dietary levels were not statistically significant.
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Fig. 6. Liver DNA guanine (G) methylation induced by AOM in rats fed control, BTC-containing, or BSC-containing diets for 3 weeks. AOM was administered s.c. at a dose of 15 mg/kg, and the rats were killed 6 h later. Columns, means of 3–4 experiments, each using one animal, except in the case of the 25 ppm BSC diet, where livers from only 2 animals became available for the study; bars, SD. MeG, methylguanine.

Fig. 7. Colon DNA guanine (G) methylation induced by AOM in rats fed control, BTC-containing, or BSC-containing diets for 3 weeks. Columns were obtained from the same animals used for the results presented in Fig. 6. Columns, means of 3–4 separate determinations, each using one animal; bars, SD. MeG, methylguanine.

DISCUSSION

The inhibition of chemical carcinogenesis by chemopreventive agents can proceed through diverse mechanisms (29); during the stage of tumor initiation, two of the most common are inhibition of carcinogen activation and increased metabolic detoxication. One example of the former process is the complete inhibition of 1,2-dimethylhydrazine-induced colon carcinogenesis in mice by thiono-sulfur compounds such as disulfiram (30) and carbon disulfide (31). Metabolic studies, generally similar to those described in the present work, determined that this effect was primarily due to a block in the metabolic activation of the carcinogen, specifically, inhibition of enzymes catalyzing the N-oxidation of azomethane to AOM (32). In the case of the chemopreventive agent BSC, it is apparent that a completely different mechanism is operating. Examination of the data in Fig. 2 shows that the rate of in vivo metabolism of AOM by rats fed diets containing BSC at levels of 25 or 50 ppm is almost tripled compared to that of control rats or rats fed equivalent amounts of the sulfur-containing analogue BTC. These results suggest that BSC induces enzymes of AOM metabolism. This is confirmed by in vitro studies (Figs. 3 and 4) which indicate that liver microsomes from rats fed BSC are severalfold more active than liver microsomes from control or BTC-fed rats in metabolizing AOM and MAM. The consequence of increased rate of metabolism of AOM and MAM by the liver is decreased availability of MAM for further metabolic activation in the colon, as reflected in decreased DNA methylation (Fig. 7) and inhibition of AOM-induced colon carcinogenesis (1). These relationships are illustrated schematically in Fig. 8. In rat liver, AOM and MAM are activated, at least in part, by the same enzyme (20), recently identified as cytochrome P-450IIE1 (28). The identity of enzymes which further activate MAM in the rat colon is not certain, but it is probable that dehydrogenases, such as alcohol dehydrogenase, as originally suggested by Schoenthal (33) and Zedeck et al. (34), rather than cytochrome P-450s, may play a major role.

It is interesting to note that, in producing these effects, BSC appears to differ considerably from inorganic selenium. For instance, Banner et al. (12) concluded that 4 ppm sodium selenite in drinking water had no effect on the activation of MAM, and Harbach and Swenberg (10) found no effect on the metabolism or colon DNA-methylating ability of 1,2-dimethylhydrazine, the metabolic precursor of AOM and MAM. In addition, a single dose of inorganic selenium caused a decrease in total rat liver cytochrome P-450 by increasing heme oxygenase (11), and continuous administration (4 ppm in drinking water for 30 days) was found to cause no change in the level of total liver cytochrome P-450 in male rats (14).

As noted previously during the induction of metabolism of N-nitrosodimethylamine (35) and MAM (36) by ethanol, increased metabolism of AOM in the livers of BSC-fed animals does not lead to increased DNA methylation in that organ (Fig. 6). It is apparent that, in the liver, the extent of DNA methylation following an acute dose of AOM (or MAM or N-nitrosodimethylamine) does not depend on the rate of metabolism but rather on the total amount of carcinogen metabolized (i.e., the dose).

The administration of BSC to rats at 25 or 50 ppm is not without its side effects. Due to decreased food consumption during the 3 weeks of feeding, the weight gain of the animals fed the BSC diet was significantly less than that of the animals fed the control diet or the diet containing BTC. Significantly decreased body weight gain has also been reported in rats consuming diets high in inorganic selenium (13). BSC has a pronounced odor somewhat similar to that of burnt rubber; presumably, diets containing the compound are not palatable to the animals. Since the induction of cytochrome P-450IIE1 in rat liver can be produced by overnight starvation (37), it was of interest to determine whether the increased rate of AOM metabolism in the BSC-fed animals was due to what was, in effect, dietary restriction. In a separate study (results not

Fig. 8. Schematic representation of relationship between AOM activation in rat liver and colon. Induction of enzymes metabolizing AOM and MAM in the liver occurring after feeding of BSC will limit the amount of MAM reaching the colon. This results in decreased MAM activation in that organ, decreased DNA methylation, and inhibition of AOM-induced carcinogenesis.
examined the effects of a 30% dietary restriction on the induc- shown), using a diet identical to the control diet used here, we tion of enzymes of AOM and MAM metabolism in male F344 rats. Although the weight gain of rats submitted to this degree of dietary restriction was essentially the same as that of rats on the 50-ppm BSC dietary regimen, no significant effects on total liver cytochrome P-450 levels or on AOM metabolism by liver microsomes could be detected. In addition to metabolic effects, dietary (or caloric) restriction has been reported to result in a decreased rate of cell turnover in various rat organs, including the colon (38); this factor may also contribute to the inhibition of colon carcinogenesis by dietary BSC. The relationships among these factors and their contributions to the inhibitory effect may turn out to be complex. Nevertheless, the present work indicates clearly that, under conditions very similar to those used previously to demonstrate inhibition of AOM-in-duced colon carcinogenesis by BSC (39), a major effect of BSC feeding is inhibition of AOM-induced colon DNA alkylation, and therefore tumor initiation, through increased carcinogen metabolism in the liver.

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