Comparison of selenium and sulfur analogs in cancer prevention

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Several organoselenium compounds have been shown to have powerful anticarcinogenic activity. In view of certain similarities between selenium and sulfur biochemistry, we have evaluated the chemopreventive efficacy of three pairs of analogs using the 7,12-dimethylbenz[a]anthracene (DMBA)-induced mammary tumor model in rats. The compounds tested were selenocystamine/cysteamine, Se-methylselenocysteine/S-methylcysteine, selenobetaine/sulfobetaine. In the first study, each agent was added to the basal AIN-76A diet and was given before and continued after DMBA treatment until the end. All three selenium compounds were active; a 50% inhibition was achieved at ~25 × 10^{-6} mol/kg with Se-methylselenocysteine and selenobetaine and at ~40 × 10^{-6} mol/kg with selenocystamine. In the sulfur series, only cysteamine and S-methylcysteine produced anticancer activity, and the levels required for comparable responses were 500- to 750-fold higher compared to the corresponding selenium analogs. Sulfobetaine was inactive even when present at near maximally tolerated levels. In the second study, Se-methylselenocysteine and S-methylcysteine were chosen for further examination during the initiation and post-initiation phases of mammary carcinogenesis. Se-Methylselenocysteine was effective when it was given either before or after DMBA administration. In contrast, S-methylcysteine was effective only after DMBA treatment. Thus, compared to the sulfur structural analogs, selenium compounds are much more active in cancer protection and may have a multimodal mechanism in preventing cellular transformation as well as in delaying or inhibiting the expression of malignancy after carcinogen exposure.

Introduction

Several organoselenium compounds have been shown to have powerful anticarcinogenic activity in animal tumor models (1–5). Increasing information is also available in the literature on the cancer protective effect of naturally occurring sulfur compounds, which are present in greater abundance than selenium in foods. These sulfur compounds include allyl group containing sulfides, disulfides and trisulfides from garlic extract (6,7) as well as benzyl and phenethyl isothiocyanates from cruciferous vegetables (8–10). There are certain common features betewen sulfur and selenium biochemistry. Plants synthesize the sulfur amino acids and their derivatives from sulfite and sulfate. Likewise, they also synthesize selenoamino acids, such as selenomethionine, selenocysteine, selenocystathionine and Se-methylselenocysteine, from selenite and selenate. Humans consume a substantial portion of their dietary selenium in these organic forms, and little or none

as inorganic selenium. Sulfur is directly above selenium in the periodic table, and both elements commonly occur in forms corresponding to oxidation states of +6, +4 or −2. Both elements have similar covalent radii and possess the ability to form multiple bonding. In animals, ingested selenium is metabolized through a series of methylation reactions (11). Monomethylated selenide might form mixed selenenylsulfide derivatives of proteins (PS-SeCH$_3$), analogous to inactivation of proteins through mixed disulfide formation with methylmercaptan (CH$_3$SH), a toxic product of methionine metabolism. In view of these similarities between selenium and sulfur, it is important to establish the specificity of selenium in cancer prevention and the differences in dose response between selenium and sulfur compounds.

In the present study, we have evaluated the cancer chemopreventive efficacy of three pairs of selenium and sulfur analogs using the 7,12-dimethylbenz[a]anthracene (DMBA*) induced mammary tumor model in rats. The compounds tested were selenocystamine/cysteamine, Se-methylselenocysteine/S-methylcysteine and selenobetaine/sulfobetaine. Se-Methylselenocysteine has been identified in Astragalus, which is known to accumulate selenium (12). We have previously reported that Se-methylselenocysteine is active in cancer chemoprevention (3); it would therefore provide a positive control for comparison with S-methylcysteine, a non-volatile sulfur compound found in onion and beans (13,14). Selenobetaine and sulfobetaine are synthetic '-onium' compounds with two methyl groups attached to a positively charged selenium or sulfur moiety. Radiolabeled substrate studies have suggested that selenobetaine tends to lose a methyl group first before conversion to methylselenol (CH$_3$SeH) and ultimately to trimethylselenonium through the selenium detoxification pathway (15). Sulfobetaine forms trimethylsulfonium in a similar way (15). Selenobetaine has also been described by us to be effective as an anticarcinogenic agent (2) but no such information is available on sulfobetaine. Thus the objectives of this paper are (i) to determine if the corresponding sulfur analog of the selenium compound is active in cancer prevention; (ii) if so, to find out the dose needed to produce a comparable inhibitory response; and (iii) to elucidate whether both selenium and sulfur have similar modes of action in the initiation and post-initiation phases of chemical carcinogenesis.

Materials and methods

Animals and mammary tumor induction

Pathogen-free female Sprague–Dawley rats were purchased from Charles River Breeding Laboratories, Raleigh, NC. They were maintained on the AIN-76A diet (substituting dextrose for sucrose) as described previously (16) for the entire duration of the experiment. The AIN-76 mineral mix used in the diet provided 0.1 p.p.m. Se as sodium selenite. Mammary tumors were induced by i.g. administration of 10 mg of DMBA (Sigma) at ~55 days of age (17). Rats were palpated weekly to determine the appearance and location of tumors. At autopsy, the mammary gland was exposed for the detection of non-palpable tumors. Only confirmed adenocarcinomas were reported in the results. Tumor incidences were compared by chi-squared analysis and the total tumor yield compared by frequency distribution analysis as described previously (18). Multiple comparisons among groups were made in each of the five experiments described.

*Abbreviation: DMBA, 7,12-dimethylbenz[a]anthracene.

Experimental design

Two series of experiments were carried out. In the first series (experiments 1−3) each selenium or sulfur compound was added to the basal diet at different concentrations, with the given supplementation started at 2 weeks before DMBA administration and continued until the end of the experiment (23−25 weeks after DMBA). Each batch of diet was prepared fresh every week and food was changed every 2 days. A total of three pairs of selenium and sulfur analogs were evaluated for their chemopreventive activity. The source of these compounds is indicated below. In the second series (experiments 4 and 5), only Se-methylselenocysteine and S-methylcysteine were chosen for further examination during the initiation and post-initiation phases of mammary carcinogenesis. A single dose of each agent was given in the diet either from 2 weeks before DMBA to 1 week after DMBA (−2 to +1 weeks) or from 1 week after DMBA to the end of the experiment (+1 to +24 weeks).

Source of test chemicals

Cysteamine [$HS-CH_2-CH_2-NH_2$], selenocystamine (as a dimer) and S-methylcysteine [$CH_3-S-CH_2-CH(NH_2)-COOH$] were purchased from Sigma. These compounds were used without further purification. The method for the synthesis of selenobetaine [$(CH_3)_2-Se^+-CH_2-COO^-$, chloride form] and Se-methylselenocysteine has been described in detail in our previous publications (2,3). Sulfobetaine was prepared by the method of Maw (19). Monochloroacetic acid (258 g; 2.7 mol) was heated with equimolar dimethylsulfide (200 ml) plus 50 ml dimethyl ether at 35°C in a round-bottomed flask fitted with a reflux condensor. The solid product was recrystallized from 99% ethanol and analyzed by titration with standard sodium hydroxide. Further characterization was done by subjecting a 1 M solution to thin layer electrophoresis (formic acid/acetic acid/water, 150:100:750) followed by detection with Dragendorff spray reagent (20). A single '-onium' positive spot was observed. No spot was detectable in the position where trimethylsulfonium would migrate.

Results

The data comparing the chemopreventive efficacy of the various selenium and sulfur analogs (experiments 1−3) are summarized in Table I. In our previous reports on selenium compounds, we usually described the level of supplementation in the diet in p.p.m. Se. Since the atomic weight for selenium is 78.9 versus 32 for sulfur, it would be more appropriate in the present study to denote the concentration of each agent on a molar basis. As indicated in Table I, the levels of selenocystamine, Se-methylselenocysteine and selenobetaine added to the diet were in the range 25−40 × 10^{-6} mol/kg. This is equivalent to ~2−3 p.p.m. Se, a range used in most selenium chemoprevention studies. Results in Table I show that all three selenium compounds were active in mammary tumor suppression with these levels of supplementation. Preliminary studies indicated that much higher quantities of sulfur compounds were required to produce a similar magnitude of tumor inhibition as that observed with the selenium compounds. Consequently, with respect to the sulfur analogs, only the effective doses (in terms of cancer chemoprevention) or the highest tolerable doses are presented in Table I.

In the case of cysteamine (experiment 1), three levels of this compound were evaluated at 15 × 10^{-3}, 30 × 10^{-3} and 60 × 10^{-3} mol/kg in the diet. It can be seen that supplementation of cysteamine at a concentration of 30 × 10^{-3} mol/kg resulted in a tumor inhibitory response close to that produced by selenocystamine at a concentration of 40 × 10^{-6} mol/kg. Increasing the level of cysteamine to 60 × 10^{-3} mol/kg did not seem to lead to further suppression. The activities of Se-methylseleno-cysteine and S-methylcysteine were compared in experiment 2. Similar to the results in experiment 1, a much higher concentration of S-methylcysteine was needed in order to reduce the total tumor yield to a number comparable to that produced by Se-methylselenocysteine. For example, a level of 15 × 10^{-3} mol/kg of S-methylcysteine was approximately equivalent in potency to only 25 × 10^{-6} mol/kg of Se-methylseleno-cysteine. Experiment 3 was designed to examine dimethylated selenonium and sulfonium analogs in the form of selenobetaine and sulfobetaine respectively. Selenobetaine at 25 ×

10^{-6} mol/kg in the diet produced about a 50% reduction in the total number of tumors. On the other hand, sulfobetaine was totally inactive in mammary cancer prevention even at a concentration of 20 × 10^{-3} mol/kg in the diet. We did not test concentrations >20 × 10^{-3} mol/kg because this amount was found to be maximally tolerated by the animals with no depression of growth.

It should be pointed out here that in experiments 1−3, none of the treatment groups with selenium or sulfur compounds suffered any reduction in weight gain compared to the controls (data not shown). The doses of these compounds were chosen to stay within the acceptable range so that the tumor data would not be confounded by changes in growth of the animals. With all three selenium compounds, a concentration of 40 × 10^{-6} mol/kg is near the limit of the acceptable range without the manifestation of any adverse effects. Sulfur compounds, on the other hand, are much better tolerated than the corresponding selenium analogs, as evidenced by the higher doses used in this study. However, the chemical structure of the sulfur compound is also clearly a determining factor in this regard. In experiment 1, we had used up to 60 × 10^{-3} moles of cysteamine per kg

Table I. Effect of selenium or sulfur analog supplementation on inhibition of DMBA-induced mammary carcinogenesis

Exp.	Treatment group[a]	Supplementation in diet (mol/kg)	Tumor incidence	Total tumor yield
1	control	−	18/25 (72%)	58
	selenocystamine	25 × 10^{-6}	14/25 (56%)	43
	selenocystamine	40 × 10^{-6}	11/25 (44%)[b]	31[b]
	cysteamine	15 × 10^{-3}	17/25 (68%)	44
	cysteamine	30 × 10^{-3}	12/25 (48%)	34[b]
	cysteamine	60 × 10^{-3}	13/25 (52%)	39[b]
2	control	−	22/25 (88%)	66
	Se-methylselenocysteine	25 × 10^{-6}	10/25 (40%)[b]	27[b]
	S-methylcysteine	5 × 10^{-3}	19/25 (76%)	54
	S-methylcysteine	15 × 10^{-3}	13/25 (52%)[b]	32[b]
3	control	−	20/25 (80%)	51
	selenobetaine	25 × 10^{-6}	11/25 (44%)[b]	20[b]
	sulfobetaine	5 × 10^{-3}	19/25 (76%)	55
	sulfobetaine	10 × 10^{-3}	21/25 (84%)	49
	sulfobetaine	20 × 10^{-3}	18/25 (72%)	45

[a]Supplementation was started at 2 weeks before DMBA administration and continued until the animals were killed.
[b]$P < 0.05$, compared to the corresponding control value in each experiment.

Table II. Inhibitory effect of Se-methylselenocysteine or S-methylcysteine on the initiation and post-initiation phases of DMBA-induced mammary carcinogenesis

Exp.	Treatment group[a]	Duration of supplementation	Tumor incidence	Total tumor yield
4	control	−	25/30 (83.3%)	76
	Se-methylselenocysteine	−2 to +1 weeks	17/30 (56.7%)[b]	48[b]
	Se-methylselenocysteine	+1 to +24 weeks	15/30 (50.0%)[b]	41[b]
5	control	−	23/30 (76.6%)	81
	S-methylcysteine	−2 to +1 weeks	21/30 (70.0%)	74
	S-methylcysteine	+1 to +24 weeks	14/30 (46.6%)[b]	47[b]

[a]Se-Methylselenocysteine (exp. 4) or S-methylcysteine (exp. 5) was added to the diet at 25 × 10^{-6} mol/kg or 15 × 10^{-3} mol/kg respectively.
[b]$P < 0.05$, compared to the corresponding control value in each experiment.

of diet with no ill effects. As a matter of fact, an initial toxicological study suggested that even a dose of 100 × 10^{-3} mol/kg was still within the safe and tolerable range (data not shown). The same was true for S-methylcysteine. Unlike the two above, sulfobetaine was less well tolerated since as noted in the previous paragraph, a concentration of 20 × 10^{-3} mol/kg was near the maximum amount that could be given to the animals without producing growth depression.

Based on the outcome of experiment 2, we then selected Se-methylselenocysteine and S-methylcysteine for further examination of their protective activity during the initiation and post-initiation phases of mammary carcinogenesis. The data of this study are summarized in Table II. It can be seen from experiment 4 that Se-methylselenocysteine was effective in tumor suppression when it was given either around the time of DMBA (−2 to +1 weeks) or after DMBA administration (+1 to +24 weeks). In contrast, experiment 5 showed that S-methylcysteine was effective only during the post-initiation period (+1 to +24 weeks).

Discussion

Of the three selenium compounds we have tested in this study, Se-methylselenocysteine and selenobetaine appeared to be slightly more active than selenocystamine (Table I). It is possible that the former two compounds might be better precursors in generating methylated selenium intermediates (15,20); this explanation would be consistent with our current hypothesis that metabolism of selenium through the methylation reactions is critical in producing certain active species in cancer protection (2,3). In the sulfur series, only cysteamine and S-methylcysteine resulted in anticancer activity. However, the levels of these sulfur compounds required to produce a comparable magnitude of tumor suppression were 500- to 750-fold higher than that of the selenium analogs. Thus on an equimolar basis, selenium compounds are far more active than structurally similar sulfur compounds in cancer protection, suggesting that the effect of selenium is specific. In general, selenium undergoes reductive metabolism and forms methylated excretory products (11), whereas sulfur tends to form more oxidized excretory products (21). It remains to be determined whether this difference in selenium and sulfur metabolism could account for the chemopreventive activity of these two classes of compounds.

As far as we are aware, this is the first report which shows that S-methylcysteine is an effective anticarcinogenic agent. High concentrations of S-methylcysteine occur in seeds of lima bean and kidney bean; it is ineffective as a source of labile methyl groups but produces toxicity effects comparable to methionine when high levels are fed to rats, which may be related to release of methanethiol following metabolism to 2-methylthioacetate (21). Members of the *Allium* family also contain S-methylcysteine and related nonvolatile sulfur compounds (13). S-Allylcysteine has recently been reported to inhibit 1,2-dimethylhydrazine-induced colon cancer when given orally to mice before carcinogen administration (7).

Sulfobetaine was not as well tolerated as S-methylcysteine and cysteamine and was totally inactive in tumor suppression, even when administered in an amount close to the maximally tolerated level. Sulfobetaine does not occur in nature, but long ago was shown to be an effective methyl donor; it produces toxic effects comparable to methionine when fed to rats, in contrast to betaine and other 'onium'-type methyl donors (22). Whether the lack of anticancer activity and the relatively low tolerance are characteristic features of other sulfonium compounds needs to be studied further.

The present study showed that Se-methylselenocysteine was effective in cancer chemoprevention when given either before or after DMBA administration. In contrast, S-methylcysteine was effective only after DMBA treatment. Recent data from Milner's laboratory have suggested that selenite feeding at 1 p.p.m. Se in the diet is effective in inhibiting DMBA−DNA adduct formation in mammary epithelial cells (23). Further investigation is necessary to determine whether Se-methylselenocysteine is also capable of interfering with DMBA metabolism and binding to DNA. At the present time, it is unclear how Se-methylseleno-cysteine or S-methylcysteine might act to retard the proliferation of transformed cells during the promotion phase of carcinogenesis. A variety of sulfur compounds, both naturally occurring as well as synthetic, have been found to have anticarcinogenic activity. These include substituted dithiolethiones and arylalkyl isothiocyanates, as well as allyl sulfides and sulfur amino acid derivatives (24). Most of these sulfur compounds suppress tumor induction in the initiation phase by either reducing carcinogen activation or facilitating its detoxification. In comparing the efficacy of selenium and sulfur in chemoprevention, our study convincingly demonstrated that molecule for molecule, selenium is much more active than sulfur and that selenium may have a multi-modal mechanism in preventing cellular transformation as well as in delaying or inhibiting the expression of malignancy after carcinogen exposure.

Acknowledgements

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