Chemical Form of Selenium, Critical Metabolites, and Cancer Prevention

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ABSTRACT

Methylated selenides are prominent metabolites at the dietary levels used for obtaining anticarcinogenic effects with selenium. The present study reports the chemopreventive activities of 2 novel selenium compounds, Se-methylselenocysteine and dimethyl selenoxide, in the rat dimethylbenzaanthracene-induced mammary tumor model. Other treatment groups were supplemented with either selenite or selenocystine for comparative purposes. Each selenium compound was tested at different levels and was given to the animal starting 1 week before dimethylbenz(a)anthracene administration and continued until sacrifice. Results of the carcinogenesis experiments showed that the relative efficacy with the four compounds was Se-methylselenocysteine > selenite > selenocystine > dimethyl selenoxide. In correlating the chemical form and metabolism of these selenium compounds with their anticarcinogenic activity, it is concluded that: (a) selenium compounds that are able to generate a steady stream of methylated metabolites, particularly the monomethylated species, are likely to have good chemopreventive potential; (b) anticarcinogenic activity is lower for selenoamino acids, such as selenocysteine following conversion from selenocysteine, which have an escape mechanism via random, nonstoichiometric incorporation into proteins; and (c) forms of selenium, as exemplified by dimethyl selenoxide, which are metabolized rapidly and quantitatively to dimethyl selenide and trimethylselenonium and excreted, are likely to be poor choices. We undertook a separate bioavailability study using Se-methylselenocysteine, dimethyl selenoxide, and trimethylselenonium as the starting compounds for delivering selenium with one, two, or three methyl groups, and measured the ability of these compounds to restore glutathione peroxidase activity in selenium-depleted animals. All three compounds were able to fully replete this enzyme, although with a wide range of efficiency (Se-methylselenocysteine > dimethyl selenoxide > trimethylselenonium), suggesting that complete demethylation to inorganic selenium is a normal process of selenium metabolism. However, the degree to which this occurs under chemoprevention conditions would argue against the involvement of selenoproteins in the anticarcinogenic action of these selenium compounds.

INTRODUCTION

There is much evidence that powerful anticarcinogenic effects are produced in animals given selenium (1). The chemical form and the dose of selenium administered are important factors in determining its biological activities. It is reasonable to believe that intermediary metabolism of the administered selenium compound ultimately produces the critical effects that are responsible for cancer protection. Methylated selenides are prominent metabolites at the dietary levels used for obtaining anticarcinogenic effects with selenium; dimethyl selenide and trimethylselenonium ion are well-established excretory metabolites (2), and monomethylated forms are likely to be present in urine as well (3). In accordance with the metabolic scheme shown in Fig. 1, entry of selenium at a point below the hydrogen selenide pool is expected to generate relatively large quantities of methylated metabolites and bypass the conventional assimilatory pathway in forming selenocysteine for incorporation into glutathione peroxidase (4). We have previously investigated the anticarcinogenic activity of a prototype synthetic selenium compound, selenobetaine, which has methyl groups on the selenium and is able to generate the methylated metabolites directly in vivo (5). Selenobetaine was found to be slightly more active than selenite in the inhibition of mammary tumorigenesis in the rat (6). This particular study provided the first indication that methylated metabolites of selenium might be active in cancer prevention.

We have examined additional mono- and dimethylated organoselenium compounds that are direct precursors of methylated selenides in vivo. The present report examines the chemopreventive activities of Se-methylselenocysteine and dimethyl selenoxide in the rat DMBA\(^{\text{a}}\)-induced mammary carcinogenesis model. Se-Methylselenocysteine was selected because it is an excellent precursor of methylated selenides (7), cannot be incorporated into proteins like other selenoamino acids, and occurs naturally in plants (8). Dimethyl selenoxide was tested because it is a nonvolatile compound that is expected to undergo rapid reduction to dimethyl selenide. Fig. 1 illustrates the major sites where Se-methylselenocysteine and dimethyl selenoxide enter the selenium metabolic pathway. Because of our prior observation that co-administration of arsenite increases the anticarcinogenic activity of selenobetaine (6) and trimethylselenonium (9), the current experiments were carried out in the presence and absence of arsenite to determine its modifying effects.

Before new strategies for generating critical metabolites for chemoprevention can be developed, it is essential to understand how different forms of selenium are metabolized with respect to the assimilatory and detoxification pathways as well. The possibility of demethylation is emerging as an important concept in selenium metabolism (3, 5). Inorganic selenium is generally regarded as the precursor for supplying selenium in an active form to be inserted co-translationally during the synthesis of selenoproteins such as glutathione peroxidase (10). Complete demethylation would channel selenium from methylated forms into the assimilatory pathway. We therefore undertook a separate study using Se-methylselenocysteine, dimethyl selenoxide, and trimethylselenonium as the starting compounds for delivering selenium with 1, 2, or 3 methyl groups, and measured the ability of these compounds to restore glutathione peroxidase activity in selenium-depleted animals.

MATERIALS AND METHODS

Diet and Selenium Supplementation. Female Sprague-Dawley rats that were 40 days of age were purchased from Charles River Breeding Laboratories, Wilmington, MA. They were maintained on the AIN-76A diet (substituting dextrose for sucrose) as described previously (11).

\(^{\text{a}}\) The abbreviation used is: DMBA, dimethylbenzaanthracene.
Selenite or Selenomethylselenocysteine

\[
\begin{align*}
\text{NH}_2 & \quad \text{HSe}\quad \text{hydroxyl selenide} \\
\text{CH}_3\text{Se}-\text{CH}_2\text{-CH-COOH} & \quad \text{Se-methylselenocysteine}
\end{align*}
\]

Incorporation into selenoproteins as selenocysteine specified by TGA codon

\[
\begin{align*}
\text{CH}_3\text{SeH} & \quad \text{methylselenol} \\
\text{CH}_3\text{SeO(\text{OH})}_2 & \quad \text{dimethyl selenoxide}
\end{align*}
\]

\[
\begin{align*}
\text{CH}_3\text{SeCH}_3 & \quad \text{dimethylselenide} \\
\text{(CH}_3\text{)}_3\text{Se}^+ & \quad \text{trimethylselenonium}
\end{align*}
\]

Fig. 1. Main sites where Se-methylselenocysteine and dimethylselenoxide enter the selenium metabolic pathway. Center of the diagram, metabolism of selenite or selenomethionine to the methylated products. The conversion of selenite to H2Se involves the reductive steps through glutathione selenonitri sulfide and glutathione selenoperoxidase, whereas selenomethionine is metabolized via the transulfuration mechanism to selenocysteine first, and then through the enzymatic lyase reaction to H2Se. Hydrogen selenide is regarded as a possible precursor for incorporation of selenium into selenoproteins. Evidence is provided in “Results” to support demethylation of trimethylselenonium and other methylated forms of Se (broken arrows).

for the entire duration of the experiment. The AIN-76 mineral mix used in the diet provided 0.1 ppm Se as sodium selenite. For the mammary cancer chemoprevention studies, additional selenium compound was given to the animals starting 1 week before DMBA administration and continued until sacrifice. With the exception of dimethyl selenoxide, which was given in the drinking water, all other selenium compounds (Se-methylselenocysteine, selenocysteine, and selenite) were added directly to the basal diet. Some diets also contained 5 ppm arsenic in the form of sodium arsenite. Water bottles containing dimethyl selenoxide were changed every other day; fresh food with or without supplemented selenium compounds was offered to the animals on the same schedule. Sodium selenite and DL-selenocysteine were purchased from Sigma and were mixed in the diet at different concentrations as indicated in the text without further purification. Se-Methylselenocysteine and dimethyl selenoxide were synthesized as described below.

Synthesis of Se-Methylselenocysteine and Dimethyl Selenoxide. DL-Selenocysteine was reduced to selenocysteine with sodium borohydride (40 moles/mole selenocysteine), then reacted with iodomethane (20 moles/mole selenocysteine) under anaerobic conditions at pH 7. The reaction mixture was adjusted to pH 2 and applied to a column of SP-Sephadex (H+). After washing the column with water, Se-methylselenocysteine was eluted with dilute HCl (pH 1.2); selenocystine is retained after hydrogen peroxide and other reagents. The fractions containing selenocystine were concentrated by rotary evaporation and stored at 4°C. Thin-layer chromatography with n-butanol:acetic acid:H2O (120:30:50) on cellulose plates was used to monitor chromatographic separations and assess purity of the compounds. After development, the plates were dried and sprayed with ninhydrin (0.2% in ethanol) or iodoplatinate reagent (150 mg of K3PtCl4, 3 g of KI, and 1 ml of 1 N HCl dissolved in 99 ml of water). Se-Methylselenocysteine showed a single ninhydrin-positive spot (Rf = 0.55), and moved slightly faster in comparison to S-methylcysteine. Dimethylselenoxide showed a single iodoplatinate-positive (reddish brown) spot (Rf = 0.67), and moved slightly slower in comparison with dimethyl sulfoxide.

Fig. 1

Mammary Tumor Induction. Mammary tumors were induced by i.g. administration of 10 mg of DMBA (Sigma) at approximately 55 days of age (13). Rats were palpated weekly to determine the appearance and location of tumors and were killed between 24 and 25 weeks after DMBA treatment. At autopsy, the mammary gland was exposed for the detection of nonpalpable tumors. Only confirmed adenocarcinomas were reported in the results. Tumor incidences at the final time point were compared by \(\chi^2\) analysis and the total tumor yield compared by frequency distribution analysis as described previously (14).

Biochemical Analysis. Selenium concentrations in blood, liver, and mammary gland from rats in the DMBA-carcinogenesis experiments (8 rats/group) were determined by the fluorometric procedure of Olson et al. (15). The ability of selenite, Se-methylselenocysteine, dimethyl selenoxide, and trimethylselenonium to maintain liver selenium-dependent glutathione peroxidase activity was evaluated in a selenium depletion/repletion protocol. Weanling rats were fed the AIN-76A diet without selenium in the mineral mix for 3 weeks. Our analysis indicated that this selenium-deficient diet contained approximately 0.03–0.04 ppm Se. The animals were then supplemented with various concentrations of the selenium compounds added to the diet or drinking water for an additional 3 weeks. Liver glutathione peroxidase activity was measured by the coupled assay procedure of Paglia and Valentine (16) using hydrogen peroxide as the substrate.

RESULTS

Anticarcinogenic Activity of Se-Methylselenocysteine. In an initial 40-day toxicological study, we had already ascertained that the growth rate of rats fed 2 ppm Se as Se-methylselenocysteine in the diet was identical to that of controls given the basal regimen containing 0.1 ppm Se as selenite. Thus, the DMBA-mammary carcinogenesis experiment was carried out with this compound supplemented chronically at 1 or 2 ppm Se. Fig. 2 illustrates the cumulative appearance of palpable mammary tumors as a function of time after DMBA administration. In addition to the Se-methylselenocysteine-treated groups with or without arsenite added to the diet, other treatment groups were supplemented with either selenite or selenocysteine for comparative purposes. The experiments shown in Fig. 2, A to D, were carried out concurrently. Fig. 2A shows the results from the 2 control groups given just the basal diet (containing 0.1 ppm Se) plus or minus 5 ppm As as arsenite. The rate of tumor appearance was quite similar between these 2 groups, suggesting that arsenite by itself had little effect on mammary carcinogenesis. The data from 3 levels of selenite supplementation (1, 2, and 3 ppm Se) are shown in Fig. 2B. The dose-response relationship and the magnitude of suppression of tumorigenesis by selenium (for 2 and 3 ppm Se, \(P < 0.05\)) were within our expectation, although in this case, the difference between 2 ppm Se and 3 ppm Se was less pronounced compared with our historical data (we normally observed a better graded response between 2 and 3 ppm selenite Se). The results with selenite are presented as a positive control and also served as a standard in comparing the efficacy of Se-methylselenocysteine. As shown in Fig. 2D, Se-methylselenocysteine was just as effective, if not more so, as selenite. Supplementation with 2 ppm Se as Se-methylselenocysteine produced an inhibitory response (\(P < 0.05\)) similar to that resulting from supplementation with 3 ppm Se as selenite. The data in Fig. 2D also indicated that co-administration with arsenite further enhanced the anticarcinogenic activity of Se-methylselenocysteine at each of the 2 doses. In contrast to Se-methylselenocysteine, selenocysteine was found to be much less active than selenite, and the inhibitory effect due to 2 ppm selenocysteine Se was not significant. Overall, the results in Fig. 2 suggest that...
the chemical form of the parent selenium compound is important in determining its anticancer activity.

The complete mammary tumor data at autopsy are summarized in Table 1. Nonpalpable tumors found at the time of killing the animals were included in all of the calculations. Overall, the tumor incidence data paralleled closely the tumor yield, although the latter represented a more sensitive marker of inhibitory responses. The growth curves of all 11 groups of rats were very similar to each other (results not shown). As suggested by the mean final body weight data (Table 1, Column 1), chronic feeding of Se-methylselenocysteine at these doses was well accepted by the animals. Thus, the suppression of tumorigenesis by Se-methylselenocysteine, in the presence or absence of arsenite co-administration, was independent of selenium toxicity. There were no differences in the weight of liver, kidney, and spleen in any of the selenium-treated rats compared with the control group (data not shown).

Tissue selenium levels in these DMBA-treated rats are also shown in Table 1. Ingestion of 2 ppm Se in the diet as selenite, selenocystine, or Se-methylselenocysteine resulted in comparable increases in selenium concentrations in blood, liver, and mammary gland; the magnitude of the increase was more pronounced in the latter 2 organs compared with the increase in the blood. Co-administration of arsenite resulted in minimal changes in tissue selenium levels in rats given Se-methylselenocysteine. Thus, even though tissue selenium level is clearly dependent on intake, it is not a reliable marker for predicting host protection against tumorigenesis.

Anticarcinogenic Activity of Dimethyl Selenoxide. In preliminary short-term toxicity studies, dimethyl selenoxide was found to be much less toxic than selenite or Se-methylselenocysteine. When supplemented in the drinking water, rats could tolerate up to 10 ppm Se as dimethyl selenoxide with no adverse effect on growth. Even at concentrations of 20, 50, and 75 ppm Se, the growth rate was reduced only by 12, 20, and 23%, respectively, when compared with the controls. To avoid any confounding effect on growth of the animals, the chemopreventive activity of dimethyl selenoxide was evaluated in the DMBA-mammary carcinogenesis experiment with 3 different doses (1, 5, and 10 ppm Se) in the nontoxic range. Results of this study are summarized in Fig. 3. The basal diet control group and the basal diet plus arsenite control group are shown in Fig. 3A, together with the 3 ppm selenite Se-treated group (positive control). Compared with selenite, dimethyl selenoxide was found to be much less efficacious in chemoprevention, even at a concentration of 10 ppm Se (Fig. 3B). There was a suggestion
Selenoxide, even at a level of 10 ppm Se in the drinking water, had no overt toxicity in the animals, as evidenced by the final body weight (Table 2, Column 1). Tissue selenium levels in rats given 5 or 10 ppm Se as dimethyl selenoxide were much lower than would otherwise be expected based on increases observed in rats given 3 ppm Se as selenite.

Repletion of Hepatic Glutathione Peroxidase Activity by Se-Methylselenocysteine, Dimethyl Selenoxide, and Trimethylselenonium. The ability of the different selenium compounds to restore hepatic glutathione peroxidase activity following selenium deprivation was evaluated in a selenium depletion/repletion protocol as described in “Materials and Methods.” The results shown in Table 3 are expressed as percentages of control activity in rats that were maintained throughout on the basal diet containing 0.1 ppm Se. The activity in rats that were fed continuously the casein-based selenium-deficient diet dropped to about 28% of the control value. As expected, supplementation with selenite was very effective in repleting glutathione peroxidase activity; full restoration was achieved at a dietary concentration of 0.1 ppm Se. The same was true also for Se-methylselenocysteine. Dimethyl selenoxide, on the other hand, was much less active; supplementation at 1 ppm Se only produced a partial restoration, but nearly full restoration was achieved at levels of 5 to 10 ppm Se in the drinking water. Trimethylselenonium was by far the least active in terms of nutritional potency. Nonetheless, repletion with this compound in the range of 5 to 40 ppm Se led to gradual increases in glutathione peroxidase activity, with complete recovery detected at the highest dose level. The results of this experiment support the conclusion that trimethylselenonium can be metabolized to form the precursor for glutathione peroxidase, presumably a fully demethylated form of selenium such as H₂Se.

Table 2 summarizes the complete mammary tumor data at autopsy, final body weights, and tissue selenium levels of DMBA-treated rats. Long-term supplementation of dimethyl selenoxide, even at a level of 10 ppm Se in the drinking water, produced no overt toxicity in the animals, as evidenced by the final body weight (Table 2, Column 1). Tissue selenium levels in rats given 5 or 10 ppm Se as dimethyl selenoxide were much lower than would otherwise be expected based on increases observed in rats given 3 ppm Se as selenite.

Our long-term objective is to correlate the chemical form and metabolism of selenium compounds with their anticarcinogenic activity. The present study provides additional evidence that the chemical form of selenium fed to animals is an important determinant of such activity. With the four compounds evaluated here, the relative efficacy was Se-methylselenocysteine > selenite > selenocystine > dimethyl selenoxide. Our previous studies with the same tumor model system indicated that selenobetaine and its methyl ester are comparable in activity to Se-methylselenocysteine, whereas trimethylselenonium is less active than dimethyl selenoxide (6, 9). Overall, our results with these 7 selenium compounds indicate that the degree of methylation is an important factor affecting the anticarcinogenic activity of selenium. The completely methylated form, trimethylselenonium, is relatively inactive, probably because it is rapidly excreted and has a modest spectrum of chemical or biochemical reactivity. On the other hand, some monomethyl- or dimethylated selenium compounds such as Se-methylselenocy-

**DISCUSSION**

Table 2. Autopsy mammary tumor data and tissue selenium levels of DMBA-treated rats given dimethylselenoxide

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Final body wt (g)</th>
<th>Incidence (%)</th>
<th>Yield (%)</th>
<th>Multiplicity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>307 ± 6*</td>
<td>76.7</td>
<td>83</td>
<td>2.8</td>
</tr>
<tr>
<td>Arsinite (5 ppm As)</td>
<td>302 ± 5</td>
<td>70.0</td>
<td>77</td>
<td>2.6</td>
</tr>
<tr>
<td>Selenite (3 ppm Se)</td>
<td>301 ± 7</td>
<td>40.0*</td>
<td>40/</td>
<td>1.3/</td>
</tr>
<tr>
<td>Dimethylselenoxide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ppm Se</td>
<td>305 ± 5</td>
<td>73.3</td>
<td>79</td>
<td>2.6</td>
</tr>
<tr>
<td>1 ppm Se + As</td>
<td>304 ± 6</td>
<td>66.7</td>
<td>72</td>
<td>2.4</td>
</tr>
<tr>
<td>5 ppm Se</td>
<td>304 ± 7</td>
<td>63.3</td>
<td>68</td>
<td>2.3</td>
</tr>
<tr>
<td>5 ppm Se + As</td>
<td>301 ± 7</td>
<td>63.3</td>
<td>63</td>
<td>2.1</td>
</tr>
<tr>
<td>10 ppm Se</td>
<td>300 ± 7</td>
<td>60.0</td>
<td>61</td>
<td>2.0</td>
</tr>
<tr>
<td>10 ppm Se + As</td>
<td>297 ± 8</td>
<td>56.7</td>
<td>55</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**Table 2. Autopsy mammary tumor data and tissue selenium levels of DMBA-treated rats given dimethylselenoxide**

- *Rats were killed 24–25 weeks after DMBA administration.
- †There were 30 rats/group.
- ‡Includes both palpable and nonpalpable tumors.
- §Number of tumors per rat.
- ‡Mean ± SE.
- P < 0.05 compared with the corresponding control value.
control rats fed continuously with 0.1 ppm Se as selenite. These methylated precur-
sors are known to undergo facile release of their methylated selenium moiety, and eventually a large proportion of the administered compound is recovered in the form of dimethyl selenide or trimethylselenonium excretory products. Taking these facts into consideration, one could conclude that the rate of generation and elimination of dimethyl selenide is an important factor in determining the anticarcinogenic activity, and the metabolism of selenium is inferior activity compared with selenite. For selenomethionine, nonspecific incorporation into proteins may prevent the release of its monomethylated selenium moiety and thus attenuate its anticarcinogenic potential. Selenomethionine is known to be incorporated into tissue proteins in place of methionine, and intermediary metabolism studies showed that selenomethionine generated lower quantities of methylated metabolites in comparison to Se-methylselenocysteine (7). Nonspecific insertion of selenocysteine into proteins also might occur, especially when intracellular levels of the selenoamino acid are elevated following administration of selenocysteine at high levels. It should be noted that such incorporation would be distinct from the TGA codon specific, stoichiometric incorporation of selenocysteine into selenoproteins such as glutathione peroxidase (18). The lack of correlation between tissue selenium levels and inhibition of tumorgenesis as observed in the present study (Tables 1 and 2) has been reported previously (17). Compared with rats given selenite, the higher burden of tissue selenium in selenomethionine-treated rats does not appear to confer a better protection against tumorgenesis. In other words, tissue selenium level is not necessarily a reliable marker for predicting anticarcinogenic activity, and the metabolism of selenium is essential for its chemopreventive action.

An additional factor to consider with regard to selenium metabolism and anticarcinogenic activity is demethylation and the ability of organoselenium compounds to provide selenium to the inorganic pool. Methylation had been regarded as an irreversible process for selenium, but there is considerable evidence for metabolism in the “back” direction (Fig. 1, dashed arrows). Animals given trimethylselenonium exhale dimethyl selenide (7). Further metabolism to the inorganic pool is indicated by the ability of trimethylselenonium to restore glutathione peroxidase activity in selenium-depleted animals (Table 3). Since an inorganic form of selenium is believed to be the precursor for synthesis of selenocysteine in glutathione peroxidase (10), this provided a sensitive in vivo measurement of the extent to which a given organoselenium compound can be metabolized to an inorganic form. Trimethylselenonium fed at levels of 40 ppm Se fully restored glutathione peroxidase activity, and levels as low as 5 ppm had partial activity. A previous report of the nutritional unavailability of trimethylselenonium used a level of only 0.15 or 1.5 ppm Se (19). Dimethyl selenoxide also restored glutathione peroxidase activity when administered in the drinking water at levels of 5–10 ppm Se (Table 3). Moreover, a monomethylated form of selenium, Se-methylselenocysteine, was fully as active as selenite at a level of 0.1–0.5 ppm Se. We are unaware of any previous reports of its nutritional bioavailability, and the high activity of this naturally occurring compound in the selenium-depleted rat is of considerable interest.

It is clear from these studies that the administration of selenium in monomethylated or even di- or trimethylated forms does not preclude formation of inorganic selenium. This complicates the interpretation of anticancer efficacy versus selenium methylation state, at least qualitatively. Since complete demethylation does occur in vivo, is it possible that selenoproteins are involved? So far, there is no evidence to support this contention. Glutathione peroxidase (18) and plasma selenoprotein-P (20) are the only 2 known selenoproteins in mammalian tissue in which the insertion of selenium as selenocysteine is dictated by the TGA codon. The levels of these 2 proteins are sensitive to physiological but not to pharmacological levels of selenium intake. The degree to which demethylation occurs inferior activity compared with selenite. For selenomethionine, nonspecific incorporation into proteins may prevent the release of its monomethylated selenium moiety and thus attenuate its anticarcinogenic potential. Selenomethionine is known to be incorporated into tissue proteins in place of methionine, and intermediary metabolism studies showed that selenomethionine generated lower quantities of methylated metabolites in comparison to Se-methylselenocysteine (7). Nonspecific insertion of selenocysteine into proteins also might occur, especially when intracellular levels of the selenoamino acid are elevated following administration of selenocysteine at high levels. It should be noted that such incorporation would be distinct from the TGA codon specific, stoichiometric incorporation of selenocysteine into selenoproteins such as glutathione peroxidase (18). The lack of correlation between tissue selenium levels and inhibition of tumorgenesis as observed in the present study (Tables 1 and 2) has been reported previously (17). Compared with rats given selenite, the higher burden of tissue selenium in selenomethionine-treated rats does not appear to confer a better protection against tumorgenesis. In other words, tissue selenium level is not necessarily a reliable marker for predicting anticarcinogenic activity, and the metabolism of selenium is essential for its chemopreventive action.

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**Table 3** Repletion of liver selenium-dependent glutathione peroxidase activity by different selenium compounds

<table>
<thead>
<tr>
<th></th>
<th>Glutathione peroxidase (U/min/mg protein)</th>
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<tbody>
<tr>
<td>Control rats fed continuously with 0.1 ppm Se as selenite*</td>
<td>100</td>
</tr>
<tr>
<td>Rats fed continuously with selenium-deficient diet</td>
<td>28.0 ± 1.9</td>
</tr>
</tbody>
</table>

* The basal glutathione peroxidase activity in the control rats was 1.21 EU/min/mg protein.

* This compound was added to the drinking water.
under chemoprevention conditions must be kept in mind. Although a significant portion of Se-methylselenocysteine may go into the inorganic pool in a selenium-depleted animal, animals fed a selenium-adequate diet excrete a high percentage of Se-methylselenocysteine in the form of di- and trimethylated products (7). Similarly, 40 ppm Se as trimethylselenonium restores glutathione peroxidase activity fully but has no anticarcinogenic activity (9). In summary, the evidence discussed above would argue against the significance of the inorganic selenium pool in the anticarcinogenic action of these selenium compounds.

The modifying effects of arsenite observed in this and earlier studies are pertinent to the question of critical selenium metabolites and chemoprevention. A low level of arsenite (5 ppm) alone was inactive (9). Similar results were seen with selenobetaine (6) and Se-methylselenocysteine (this paper). In no case did arsenite decrease the anticarcinogenic activity of methylated selenium compounds as it did with inorganic selenite. Interestingly, arsenite had little effect on the anticarcinogenic activity of those compounds that release dimethyl selenide rapidly (dimethyl selenoxide, selenobetaine methyl ester) but enhanced activity considerably for compounds likely to release selenium mainly in monomethylated form (Se-methylselenocysteine, selenobetaine) or release dimethyl selenide slowly (trimethylselenonium). The dependence of the effects of arsenite on the chemical form of selenium also has been observed in acute studies (21).

In our carcinogenesis experiments reported here, Se-methylselenocysteine and dimethylselenoxide were given to the animals beginning 1 week after DMBA administration and continued until sacrifice. Thus the action of these selenium compounds could be exerted at either the initiation or promotion stage of carcinogenesis, or both. This design is intentional, because when the chemopreventive effect of selenite was first characterized by one of the authors a decade ago (22), the supplementation of selenite was maintained throughout the initiation and promotion phases. Subsequently it was found that the protective effect of selenite, at least in the DMBA model, was primarily expressed during the tumor progression period (23). We had no a priori knowledge of whether Se-methylselenocysteine or dimethylselenoxide would be effective in cancer prevention, and if so, how it would affect the carcinogenic process. On this basis, we decided to expose the animals to these second-generation selenium compounds before, during, and after DMBA treatment to cover all eventualities. Future experiments will be refined to delineate their role in initiation versus neoplastic progression.

Based on the information obtained so far, what characteristics should be looked for in a selenium compound to obtain good efficacy for cancer prevention? First, selenium compounds that are able to supply a steady stream of methylated metabolites, particularly the monomethylated species, are probably high-priority candidates for further investigation. Second, we should avoid compounds having an escape mechanism via random incorporation into proteins. Third, forms of selenium that can be converted rapidly to dimethyl selenide and trimethylselenonium and excreted are likely to have a lower anticarcinogenic activity and be poor choices in that respect. However, these desirable attributes for anticarcinogenic activity will have to be balanced against potential toxicity that may be associated uniquely with chemical form, as well as the administered dose.

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