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Synergistic Toxicity Between Arsenic and Methylated Selenium Compounds

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ABSTRACT

Arsenite has been known for half a century to have a protective effect against selenium poisoning. Paradoxically, arsenite inhibits the conversion of inorganic selenium salts to methylated excretory products, although methylation has long been regarded as a detoxification mechanism for selenium. Moreover, there is evidence for a pronounced synergistic toxicity between arsenite and methylated selenium metabolites. We investigated the effect of arsenite on the acute toxicity of a variety of methylated or nonmethylated selenium compounds, as well as methylated forms of sulfur and tellurium. Adult male rats were injected with sodium arsenite (4 mg As/kg bw, s.c.) 10 min prior to injection of the test compounds; at the doses employed, none of the test compounds caused mortality, nor did arsenite, when given alone. When given with arsenite, the following methylated compounds produced toxic signs and high morality at the indicated dosages (mg Se/kg): Methylseleninic acid (2), dimethylselenoxide (2), trimethylselenonium chloride (3), selenobetaine (2), selenobetaine methylester (2, also 1 and 0.5), and Se-methylselenocysteine (2). Toxic signs but no mortality occurred when arsenite was given with selenomethionine (2 mg Se/kg). No enhancement of toxic signs or mortality occurred when arsenite was given with sulfobetaine (0.8 mg S/kg), dimethylsulfide (320 mg S/kg), or the following (nonmethylated) forms of selenium: sodium selenite (2), selenocystine (2), and phenylselenol (2). Arsenite also increased the toxicity of trimethyltelluronium chloride (4.8 mg Te/kg). Like arsenite, periodate-oxidized adenosine (100 μmoles/kg), which is known to inhibit the formation of dimethylselenide and trimethylselenonium ion in vivo, caused in-

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creased 24 h mortality when given with various methylated selenium compounds. The results show that arsenite markedly enhances the toxicity of methylated selenium compounds, in contrast to the effect of arsenite on inorganic selenium salts. Arsenite appears to have a number of different, sometimes opposing, effects on selenium metabolism. It may enhance the toxicity of partially-methylated forms by blocking some process in their detoxification, such as methylation.

Index Entries: Selenium; arsenic; sulfur; tellurium; methylation; selenium toxicity; periodate-oxidized adenosine.

INTRODUCTION

Numerous interactions involving arsenic and selenium toxicity and metabolism are known (*1*). Moxon was the first to report that arsenic, given as sodium arsenite, was protective against toxic levels of inorganic selenium and naturally occurring selenium in grains (*2*), and later reports confirmed this observation (*3*). Injected arsenite (3–4.5 mg As/kg) prevented death in rats given single lethal injections of selenite (4 mg Se/kg) (*4*). However, Obermeyer et al. (*5*) reported enhanced toxicity when arsenite was injected together with dimethylselenide (DMSe) or trimethylselenonium chloride (TMSeCl), two relatively nontoxic forms of selenium in rats. While investigating the effect of arsenite on DMSe production from injected selenobetaine (*6*), we found that the combination of these two substances was extremely toxic. Consequently, we have investigated the effect of arsenite on the toxicity of selenobetaine and other methylated selenium compounds in comparison to nonmethylated selenium compounds, as well as methylated forms of sulfur and tellurium.

MATERIALS AND METHODS

Materials

Selenobetaine and its methyl ester (*see* Tables 1 and 2 for structures of all compounds) were prepared as described by Foster el al. (*6*).

Dimethylthetin chloride (sulfobetaine) was purchased from ICN Pharmaceuticals (Plainview, NY). Trimethylselenonium iodide and trimethyltelluronium chloride were purchased from Organometallics, Inc., East Hampstead, NH; trimethylselenonium chloride was prepared from the iodide salt by anion exchange chromatography on Sephadex QAE (chloride form). Dimethylselenide, selenium dioxide, and phenylselenol were purchased from Alpha Products, Danver, MA. Sodium selenite was prepared by neutralizing an aqueous solution of SeO_2. Dimethylsulfide was obtained from Aldrich Chemical Co., Milwaukee, WI. Periodate-oxidized adenosine and selenocystine were purchased from Sigma Chemical Co., St. Louis, MO. Methylseleninic acid was kindly provided by Barry Sharpless.

Table 1
Effect of Arsenite on Toxicity of Methylated Selenium Compounds

Compound	Se (mg/kg)	Mortality[a]		Toxic signs[b]	
		− As	+ As[c]	− As	+ As
Selenomethionine $CH_3SeCH_2CH_2CH(NH_2)COOH$	2.	0/4	0/5	−	+
Se-methylselenocysteine $CH_3SeCH_2CH(NH_2)COOH$	2.	0/4	2/4	−	+
Methylseleninic acid CH_3SeO_2H	2.	0/4	3/5	−	+
Selenobetaine $(CH_3)_2Se^+CH_2COOH$	2.	0/5	3/4	−	+
Selenobetaine methyl ester $(CH_3)_2Se^+CH_2COOCH_3$	2.	0/5	3/4	−	+
Trimethylselenonium chloride $(CH_3)_3Se^+Cl^-$	3.	0/5	2/5	−	+
Dimethylselenoxide $(CH_3)_2SeO$	2.	0/4	1/4	−	+

[a]24 h.
[b]*See text* for description of toxic signs. In a column,
+ signifies that all animals in group showed toxic signs;
− signifies that none showed toxic signs.
[c]4 mg/kg as $NaAsO_2$, 10 min before Se; this dose of As alone produced no signs of toxicity.

Table 2
Effect of Arsenite
on Toxicity of Other Selenium, Sulfur, and Tellurium Compounds

Compound	S, Se, Te (mg/kg)	Mortality		Toxic signs[b]	
		− As	+ As[a]	− As	+ As
Selenocystine $[-SeCH_2CH(NH_2)COOH]_2$	2.	0/4	0/4	−	−
Sodium selenite Na_2SeO_3	2.	0/5	0/5	−	−
Phenylselenol C_6H_5SeH	2.	0/5	0/5	−	−
Sulfobetaine $(CH_3)_2S^+CH_2COOH$	0.80	0/5	0/5	−	−
Dimethylsulfide $(CH_3)_2S$	320.	0/4	0/4	−	−
Trimethyltelluronium chloride $(CH_3)_3Te^+Cl^-$	4.8	0/4	1/5	−	+

[a]4 mg/kg as $NaAsO_2$, 10 min before other compounds.
[b]*See* Table 1.

Dimethylselenoxide was prepared by the careful addition of 0.7 g of dimethylselenide to 8 mL of precooled (−18°) 16 N HNO_3 in an ice–salt mixture. After overnight reaction at room temperature the solution was evaporated to dryness at 37° using a rotary evaporator, and the residue treated with 50 mL of chloroform plus 10 g of anhydrous sodium carbonate. The sodium carbonate was removed by filtration, washed with chloroform, and the chloroform solutions evaporated to dryness. The residue was dissolved in chloroform and the entire sodium carbonate treatment repeated. The crystalline product was stored at −20°C. Analysis by direct probe mass spectrometry showed two selenium-containing peaks corresponding to dimethylselenoxide (m/e = 126) and dimethylselenide.

Toxicity Studies

Male rats (175–200 g) purchased from SASCO/King (Oregon, WI) were maintained on a crude stock diet for 2 d prior to toxicity studies. With the exception of dimethylsulfide and phenylselenol, all compounds were dissolved in 0.9% NaCl and injected sc in the scapular region (0.3 mL/rat; dimethylselenide and phenolselenol were dissolved in absolute ethanol (0.2 mL) and injected sc. Sodium arsenite or periodate-oxidized adenosine were injected sc in the back 10–15' prior to the other compounds.

RESULTS

When rats were injected subcutaneously with sodium arsenite (4 mg As/kg), then 10 min later given the various selenium compounds shown in Table 1, increased morbidity or mortality occurred with every methylated selenium compound when compared to rats treated with the selenium compound alone. The initial signs of poisoning were lethargic behavior and loss of balance, followed by severe dyspnea, then convulsions. Death occurred shortly after the appearance of the convulsions and usually within 2–4 h after injection at the levels of arsenic and selenium used in Table 1. These signs were practically identical to those reported previously for selenium toxicity (5). Selenomethionine, at 2 mg Se/kg, did not produce mortality when given with arsenite, but the rats showed signs of toxicity within 1 h. All the other methylated selenium compounds produced mortality when given with arsenite, but no deaths occurred in the absence of arsenite.

Sodium selenite, selenocystine, and phenylselenol produced no toxic effects either with or without arsenite (Table 2). Trimethyltelluronium chloride caused toxic signs in all animals and mortality (1/5) when given with arsenite, with symptoms very similar to those seen with trimethylselenonium chloride. Methylated sulfur compounds (dimethylsulfide, sulfobetaine) did not produce toxic effects when given either with or without arsenite, even at high doses.

Table 3
Effect of Dose on Toxicity
of Selenobetaine Methyl Ester Plus Arsenite

	Dose (mg/kg)		Mortality
	Se	As[a]	(24 h)
Animals fed			
	0	4.	0/5
	0.1	4.	0/5
	0.5	4.	4/5
	1.0	4.	6/6
	0.5	2.	1/5
	1.0	2.	3/5
	1.0	0.5	0/5
	2.0	0.5	0/5
	5.0	0.5	0/5
	20.0	0	0/2
Animals fasted[b]			
	0	4.	0/4
	0.1	4.	3/5
	0.5	4.	4/5
	1.0	4.	5/5

[a]Injected sc as $NaAsO_2$ 10 min before selenobetaine methyl ester.

[b]Fasted animals were deprived of food for 24 h prior to injections, but were given water ad libitum.

The effect of lower doses of selenium on the synergistic toxicity with arsenite was studied in fed or fasted rats, using selenobetaine methyl ester as the test compound (Table 3). At 4 mg As/kg, high mortality (4/5) occurred in fed or fasted rats at 0.5 mg Se/kg; in fasted rats as little as 0.1 mg Se/kg caused high mortality (3/5). This degree of toxicity is remarkable since injection of the test compound (selenobetaine methyl ester) alone at 20 mg Se/kg produced no mortality in fed animals. Considering that the minimum lethal dose of sodium selenite in the rat is about 3–5.7 mg Se/kg (3), and higher in the presence of arsenite (4), the combination of selenobetaine methyl ester and arsenite is approximately an order of magnitude more toxic than selenite alone, and the difference is even greater when compared to selenite given with arsenite.

The synergism between arsenite and methylated selenium also was dependent on the level of arsenite (Table 3). At levels below 1 mg As/kg, essentially no enhancement of toxicity was seen with selenobetaine methyl ester. It is known that at levels below 2 mg As/kg, arsenite affords little protection against acute selenite toxicity (4). Thus, it appears that similar levels of arsenic are needed to provide protection against selenite toxicity and to produce enhanced toxicity with methylated selenium compounds.

Table 4
Mortality of Male Rats Given Methylated Selenium Compounds Plus Arsenite or Periodate-Oxidized Adenosine (PAD)

Form of selenium	mg Se/kg	Mortality (24h) Se (alone)	Se + As[a]	Se + PAD[b]
Trimethylselenonium	5.0	0/5	5/5	3/5
Dimethylselenide	5.0	0/5	5/5	3/4
Dimethylselenocysteine selenonium	0.5	—	5/5	4/5
Se-methylselenocysteine	2.0	0/4[c]	3/5	5/5
	0.5	—	2/5	1/5

[a]4 mg As/Kg as $NaAsO_2$ in 0.9% NaCl injected sc 10 min before Se; mortality was 0/5 for As given alone.
[b]100 μmole PAD/kg in 0.9% NaCl injected ip 15 min before Se; mortality for PAD given alone was 0/4 after 24 h, 1/4 after 48 h, 3/4 after 56 h.
[c]Data from Table 1.

Arsenite is known to inhibit selenium methylation in animals (*7*) and is a potent inhibitor of hydrogen selenide methylation in microsomal systems (*8*). Periodate-oxidized adenosine (PAD) also is known to inhibit the methylation of selenium in animals (*9,10*). We found that PAD, like arsenite, caused increased mortality when given with trimethylselenonium, dimethylselenide, dimethylselenocysteine selenonium, and Se-methylselenocysteine (Table 4).

DISCUSSION

It has been known for 50 years that arsenic has a protective effect against selenium poisoning, under a variety of conditions. This historical view of arsenic as an antagonist, and even as an antidote (*1*), for selenium poisoning, is complicated by the marked synergistic toxicity between arsenic and the methylated products known to arise during selenium metabolism. The present investigation confirms the contrasting effect of arsenic on the acute toxicity of selenium in different chemical forms, demonstrates enhancement by arsenic of the toxicity of a methylated tellurium compound as well, and provides additional information regarding possible mechanisms for these effects.

A common feature of all selenium compounds that showed synergistic toxicity with arsenite is the potential for forming dimethylselenide without the intermediary formation of hydrogen selenide. Hydrogen selenide is believed to be a key intermediate in the conversion of selenite to dimethylselenide (*8,11*). On the other hand, compounds that contain the preformed dimethylselenide moiety, such as selenobetaine and its methyl ester, are direct precursors of dimethylselenide, and can generate large amounts of methylated selenides without being metabolized to inorganic selenium (*6*). This could be an important difference because ar-

senite is capable of reacting with hydrogen selenide and trapping this intermediate (*1,8,11*), thus preventing dimethylselenide formation from inorganic forms of selenium. Arsenite might also inhibit enzymes catalyzing reduction of selenium. Selenite and selenocystine failed to show synergistic toxicity with arsenite and both are known to be enzymatically convertible to H_2Se (*11,12*). Phenylselenol is unlikely to undergo scission of the bond between selenium and the aromatic ring and thus would be precluded from forming dialkyl selenides. Enhanced toxicity by arsenite of methylseleninic acid and dimethylselenoxide, as well as dimethylselenide and dimethyldiselenide, was described previously in an abstract by Palmer and Halverson (*13*), who also noted that selenite toxicity was potentiated if arsenite injection was delayed beyond the onset of methylated selenium excretion in the breath.

In comparing other group VIA elements to selenium, it was interesting to note that methylated tellurium, like selenium, showed synergistic toxicity with arsenic, whereas sulfur did not. Obermeyer et al. (*5*) also found little or no enhancement of trimethylsulfonium chloride toxicity with arsenite, in contrast to trimethylselenonium chloride. It is known that the stability of the bond to carbon is greatest for sulfur and decreases with increasing atomic number. It should be noted that arsenic is not the only element giving a lethal interaction with methylated selenium. Parizek et al. (*14*) observed that rats were very sensitive to dimethylselenide when low doses were given together with $HgCl_2$.

At this time the mechanism of the synergistic toxicity is unknown and warrants further study. For acute selenite toxicity, it appears that arsenite is protective only if it is injected before high levels of methylated selenides are formed (*13*). It is reasonable to propose that arsenite blocks some process that is responsible for detoxification of methylated selenides, such as further methylation to the trimethylselenonium ion. We obtained evidence that inhibition of methylation may well be involved, since rats injected with PAD plus methylated selenium compounds showed enhancement of toxicity similar to that observed with arsenite; PAD is an inhibitor of transmethylation processes in general (*15*), and both it and arsenite inhibit dimethylselenide excretion in vivo (*7,9,10*). Arsenite is a potent inhibitor of a microsomal enzyme that forms dimethyl selenide (*8*) but does not inhibit a soluble methyltranferase in lung that converts dimethylselenide to trimethylselenonium (*16*). If arsenite inhibited a final phase of selenium detoxification, such as a rate-limiting methyltransferase activity, this might cause an accumulation of mono- or dimethylated forms of selenium. Such partially methylated compounds of selenium might be toxic *per se*, or might be converted to other substances having higher toxicity.

Although methylation of selenium often has been viewed as a detoxification pathway, it is clear that this is an oversimplified view. Selenium, like mercury, lead, and other elements, can undergo biomethylation that results in enhanced toxicity compared to the inorganic element. In envi-

ronments such as the Kesterson Refuge, where selenium may occur naturally in elevated levels (*17*), synergism involving methylated selenium compounds and other environmental toxicants is potentially significant.

Recently, on the basis of the evidence described here demonstrating enhancement by arsenite of the toxic effects of methylated selenides, a study was conducted with Clement Ip to determine the effect of arsenite on the anticarcinogenic activity of trimethylselenonium ion when tested in a DMBA-induced mammary tumor model (*18*); arsenite enhanced the effectiveness of this selenium compound, since arsenite plus trimethylselenonium ion was active in chemoprevention, whereas either substance alone was inactive. In contrast, arsenite decreased the chemopreventive action of selenite. Schrauzer and coworkers previously have reported the antagonism between arsenite and selenite for spontaneous mammary adenocarcinomas in C3H/St mice (*19*). Thus, there is an enhancement by arsenite of the biological activity of a methylated form of selenium measured either by acute toxicity or by chemoprevention activity, in marked contrast to the antagonism by arsenite of the biological activity of selenite in the same systems.

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