Arsenic Increased Lipid Peroxidation in Rat Tissues by a Mechanism Independent of Glutathione Levels

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The role of lipid peroxidation in the mechanism of arsenic toxicity was investigated in female rats pretreated with N-acetylcysteine (NAC, a glutathione (GSH) inducer) or with buthionine sulfoximine (BSO, a GSH depletor). Rats were challenged with sodium arsenite, and sacrificed 1 hr after this treatment. Results showed that arsenic decreased GSH levels and increased lipid peroxidation in liver, kidney, and heart, with a larger effect at 18.2 mg/kg than at 14.8 mg/kg for lipid peroxidation induction. In the liver of rats treated with arsenic, pretreatment with NAC increased the levels of GSH and decreased lipid peroxidation. In kidney and heart, NAC pretreatment protected the tissues against arsenic-induced depletion of GSH levels, but the same degree of protection was not found for lipid peroxidation induction. In its turn, BSO had an additive effect with arsenic in lowering the levels of GSH in the liver and kidney, but an inverse correlation between GSH levels and lipid peroxidation was found only in liver. Arsenic content in tissues of rats pretreated with NAC was lower than in rats treated only with arsenic. In rats with depleted levels of GSH (BSO-pretreated rats), a shift in arsenic tissue distribution was found, with higher levels in skin and lower levels in kidney. A clear tendency for a positive correlation between arsenic concentration and lipid peroxidation levels was found in liver, kidney, and heart. — Environ Health Perspect 103(Suppl 1):85–88 (1995)

Key words: arsenic, buthionine sulfoximine, glutathione, lipid peroxidation, metals, N-acetylcysteine

Introduction

Arsenic is a naturally occurring element that has been recognized as a human poison since ancient times. Inorganic arsenic is known to cause skin cancer after ingestion and lung cancer after inhalation (1). Recently, it has been suggested that ingested arsenic also causes cancers of the bladder, kidney, lung, and liver (2). In the environment near copper and zinc smelters, arsenic occurs together with other metals, such as cadmium and lead (3). Therefore, it becomes important to study the coexposure to these metals, including the cellular defense mechanisms used against them.

Previously, we have shown that the mixture of As+ Cd is more toxic than either arsenic or cadmium alone (4,5). However, in testes of rats treated with As + Cd, a 37% increase in glutathione (GSH) content was related to protection against cadmium-induced morphologic changes (4). Furthermore, in heart tissue of rats treated with As + Cd, an increase in arsenic content was related to an increase in GSH (5). In searching for a possible mechanism of the arsenic–cadmium interaction in rats, these results prompted us to study further the relationship between GSH and arsenic toxicity.

Studies in vivo and in vitro have concluded that GSH may play a role in arsenic detoxification through different mechanisms: facilitation of its uptake by the cells (6), modulation of the first methylation reaction (7), and stimulation of the excretion of dimethylarsinic acid (6). Furthermore, it has been shown that GSH participates in the biliary excretion of arsenic (8). In agreement with these results, it has also been shown that GSH levels and GSH–S-transferase activity were increased in arsenic-resistant Chinese hamster ovary cells (9), and that in asynchronous human fibroblasts G1 phase (10), in asynchronous Chinese hamster ovary cells (10), or in endothelial cells pretreated with GSH (11), the intracellular level of GSH is inversely correlated with the cytotoxic effect of arsenic.

Conversely, it has been reported that arsenic toxicity was potentiated after pretreatment with drugs that produced GSH depletion (12). This enhancement of arsenic-induced injury in GSH-depleted animals, may result from impaired methylation. Pretreatment with depletors of GSH levels in hamsters (12), rat (13), and rat tissue slices (6), impaired the methylation of inorganic arsenic. It has been shown that methylation renders arsenic less reactive with tissue and therefore facilitates its elimination from the body (14). Arsenic tissue accumulation has been demonstrated in blood, liver, and kidney of hamsters pretreated with BSO (12), and in liver of rats pretreated with phorone (a depleter of GSH concentrations) (13).

The relationship between arsenic tissue levels and GSH levels seems to be clear. However, because glutathione is also a part of a cellular defense mechanism against peroxidative attacks, the role of lipid peroxidation as a mechanism of arsenic toxicity remains to be investigated. In the present work, this subject was investigated in female rats pretreated with N-acetylcysteine (a GSH inducer) or with buthionine sulfoximine (a GSH depletor), and challenged with different concentrations of sodium arsenite.
Materials and Methods

Animals

Wistar strain female rats (200–225 g) were allowed free access to food (Laboratory rodent diet, St. Louis, MO) and water.

Buthionine Sulfoximine or N-Acetyl-L-cysteine Administration

Buthionine sulfoximine (BSO) is an agent that depletes GSH by inhibiting the enzyme glutamylcysteine synthetase (15), whereas N-acetyl-L-cysteine (NAC) stimulates GSH synthesis by providing higher than normal concentrations of L-cysteine (16). Animals were injected sc with BSO (0.89 g/kg), and NAC was administered po (1.2 g/kg). At different times posttreatment, groups of six animals each were sacrificed and GSH levels were quantified in the liver. When compared to a saline-treated group, the maximum depletion of GSH by BSO was found to be 60% at 4 hr posttreatment (p < 0.001) and remained at this level during 2 hr. When compared to a saline-treated group, the maximum induction of GSH by NAC was found to be 40% at 60 min posttreatment (p < 0.001) and remained at this level during 1 hr.

Arsenic Administration

It was of interest to examine if the arsenic toxicity could be enhanced by GSH depletion. The effect of decreased GSH concentrations on arsenic-induced injury was studied in rats pretreated with BSO and challenged with sodium arsenite ip at a nonlethal dose (LD100). Also, to investigate the possible attenuation of arsenic toxicity by increased levels of GSH, rats were pretreated with NAC and challenged with sodium arsenite ip at a lethal dose (LD100).

To calculate the LD0 and the LD100, a dose–response determination was performed. Determinations were carried out using five doses of sodium arsenite injected intraperitoneally. Groups of eight animals were employed for each dose. Animals were observed frequently during normal working hours. For the LD50 and LD100 calculations, the numbers of deaths in 24 hr, 48 hr, and 72 hr were recorded, data were evaluated by probit analysis, and confidence limits were calculated (17).

For all experiments, 14.8 mg/kg of arsenic (As0), which is a dose within the 95% confidence limits for the LD100, was given 1 hr after NAC treatment.

In order to observe the effect of arsenic during the time of maximum effect either of NAC or BSO, animals were sacrificed by cervical dislocation 1 hr after the arsenic challenge. For studying the effects of administering BSO or NAC alone, saline was given in place of arsenic. For studying the effects of arsenic alone, saline was given in place of BSO or NAC.

Biochemical Analysis

All operations were done at 4°C. Animals were sacrificed by cervical dislocation 1 hr after the challenge either with arsenic or saline. Tissues were immediately perfused with cooled buffer that contained 0.9% KCl, pH 7.2. They were homogenized in 9 vol of 1.15% KCl, 125 mM sucrose, pH 7.2. The homogenates were employed for the assays. Lipid peroxidation was determined by measuring the amounts of thiobarbiturate-reactive substances (TBA-RS) according to the method of Ohkawa et al. (18). Using 1,1,3,3-tetramethoxy propane (TMP) as an external standard, the levels of the TBA-RS were expressed as nmole of TMP/g of tissue. Glutathione levels were determined as nonprotein soluble thiols, as previously described (4).

Arsenic Analysis

Arsenic analyses were performed using a Perkin-Elmer model 2380 atomic absorption spectrophotometer. Tissue samples were placed in acid-washed glass test tubes and solubilized with a mixture of nitric, perchloric, and sulfuric acids, for at least 9 hr. Arsenic was determined by hydride evolution–atomic absorption technique. As an internal quality control, blind random samples of NIST standard reference material SRM-1577a (bovine liver) were analyzed. For arsenic, recovery was 94%.

Results

In liver, As0 and As100 increased the level of lipid peroxidation and a correlation with the doses was observed. Arsenic also depleted GSH; however, in this case the relationship to the doses was not evident because the results with 18.2 mg/kg (As100) were not significantly different from those with 14.8 mg/kg (As0) (Table 1). Rats pretreated with NAC and challenged with As100 also showed a significant increase in lipid peroxidation and a depletion in GSH levels when compared to a control group (NAC–saline treatment). Similar results were obtained in the BSO–As0 rats, compared to rats treated only with BSO (Table 1).

In the kidney, As0 and As100 increased lipid peroxidation and this increase was inversely related to GSH levels (Table 2). In rats pretreated with NAC, As100 induced lipid peroxidation; however, GSH levels were similar to those in NAC–saline-treated rats. The opposite effect, depletion of GSH levels without a change in lipid peroxidation, was observed in rats pretreated with BSO and treated with As0, compared to rats treated only with BSO (Table 2).

Results in the heart are shown in Table 3. It can be observed again that As0 and As100 increased lipid peroxidation, whereas only As100 decreased GSH levels. Rats pretreated with either NAC or BSO and challenged with arsenic did not show a decrease in GSH levels; however, in both types of pretreatment, arsenic induced an increase in lipid peroxidation.

Arsenic tissue levels were also investigated (Table 4). Rats pretreated with BSO and challenged with As0 had less arsenic in the kidney and more arsenic in the skin than rats receiving only As0. When compared to a group of rats that received only As100, pretreatment with NAC before the As100 dose markedly reduced arsenic concentration in all the tissues we studied. The effect was more evident in the liver, skin, and ovaries.

| Table 1. Lipid peroxidation and glutathione levels in liver of rats pretreated with N-acetylcysteine or buthionine sulfoximine and subsequently challenged with arsenic. |
| Treatment | Peroxidation, nmol TMP/g tissue | Glutathione, umol/g tissue |
|-----------|---------------------------------|--------------------------|
| Saline–saline | 349.15 (46.88) | 3.49 (0.13) |
| Saline–As0 | 598.56 (73.61) | 1.85 (0.14) |
| Saline–As100 | 807.06 (82.44) | 1.68 (0.20) |
| NAC–saline | 499.84 (61.25) | 5.23 (0.16) |
| NAC–As100 | 567.31 (70.34) | 2.20 (0.53) |
| BSO–saline | 528.73 (35.58) | 1.18 (0.19) |
| BSO–As0 | 692.90 (63.46) | 0.64 (0.17) |

Rats were pretreated with NAC (1.2 g/kg, po) and challenged with sodium arsenite (As100 18.2 mg/kg, ip) 1 hr after the treatment with NAC, or were pretreated with BSO (0.89 g/kg, sc) and challenged with sodium arsenite (As0 14.8 mg/kg, ip) 4 hr after the treatment with BSO. Control rats were pretreated and challenged with saline. Rats were sacrificed 1 hr after the treatment with arsenic. *Different from control p < 0.001. †Different from NAC p < 0.001. ‡Different from BSO p < 0.001. Results are mean values obtained from six animals in each treatment group. Standard deviation is given in parentheses. Data were evaluated by the Student-Newman-Keuls test.
Table 2. Lipid peroxidation and glutathione levels in kidney of rats pretreated with N-acetylcysteine or buthionine sulfoximine and subsequently challenged with arsenic.

| Treatment          | Peroxidation, nmole TMP/g tissue | Glutathione, μmole/g tissue |
|--------------------|----------------------------------|----------------------------|
| Saline−saline      | 253.78 (71.61)                   | 1.99 (0.34)                |
| Saline−AsO         | 407.44 (100.7)                   | 1.59 (0.10)                |
| Saline−AslOO       | 589.49 (73.90)                   | 1.13 (0.23)                |
| NAC−saline         | 328.83 (51.43)                   | 2.64 (0.35)                |
| NAC−AslOO          | 505.38 (42.39)                   | 2.50 (0.52)                |
| BSO−saline         | 517.11 (57.69)                   | 0.85 (0.26)                |
| BSO−AsO            | 506.64 (61.84)                   | 0.45 (0.05)                |

Arsenic, BSO, and NAC were given as stated in Table 1. *Different from control p<0.005. †Different from control p<0.025. ‡Different from AsO p<0.05. §Different from AsO p<0.01. ¶Different from NAC p<0.001. *Different from BSO p<0.001. Results are mean values obtained from six animals in each treatment group. Standard deviation is given in parentheses. Data were evaluated by the Student-Newman-Keuls test.

Table 3. Lipid peroxidation and glutathione levels in heart tissue of rats pretreated with N-acetylcysteine or buthionine sulfoximine and subsequently challenged with arsenic.

| Treatment          | Peroxidation, nmole TMP/g tissue | Glutathione, μmole/g tissue |
|--------------------|----------------------------------|----------------------------|
| Saline−saline      | 217.10 (42.88)                   | 1.08 (0.11)                |
| Saline−AsO         | 399.78 (32.19)                   | 1.07 (0.10)                |
| Saline−AslOO       | 399.97 (28.41)                   | 0.75 (0.12)                |
| NAC−saline         | 185.71 (31.04)                   | 1.17 (0.15)                |
| NAC−AslOO          | 247.29 (26.37)                   | 1.00 (0.18)                |
| BSO−saline         | 217.32 (65.64)                   | 0.98 (0.39)                |
| BSO−AsO            | 364.05 (22.50)                   | 0.85 (0.16)                |

Arsenic, BSO, and NAC were given as stated in Table 1. *Different from control p<0.001. †Different from control p<0.01. ‡Different from AsO p<0.001. §Different from NAC p<0.005. ¶Different from BSO p<0.001. Results are mean values obtained from six animals in each treatment group. Standard deviation is given in parentheses. Data were evaluated by the Student-Newman-Keuls test.

Table 4. Arsenic concentration in tissues of rats pretreated with N-acetylcysteine or buthionine sulfoximine and subsequently challenged with arsenic (μg/g tissue).

| Tissue | Saline−AsO | BSO−AsO | Saline−AslOO | NAC−AslOO |
|--------|------------|---------|--------------|-----------|
| Liver  | 17.8 (2.9) | 18.6 (2.9) | 22.6 (5.7) | 11.3 (1.6) |
| Heart  | 11.2 (1.6) | 6.8 (2.8)  | 12.4 (0.9) | 10.0 (0.3) |
| Skin   | 4.3 (0.8)  | 7.1 (0.8)  | 7.4 (1.3)  | 4.4 (1.2)  |
| Kidney | 30.8 (3.5) | 17.7 (2.9) | 43.3 (5.1) | 32.4 (5.4) |
| Lung   | 5.7 (1.4)  | 6.1 (1.1)  | 7.1 (1.9)  | 5.4 (1.7)  |
| Ovary  | 17.1 (3.7) | 19.2 (4.2) | 20.9 (1.8) | 13.3 (3.0) |

Arsenic, BSO, and NAC were given as stated in Table 1. Saline−AsO versus BSO−AsO treatment: skin and kidney p<0.001, all others not significantly different (Student’s t-test). Saline−AslOO versus NAC−AslOO: heart and lung p<0.05; liver p<0.002; skin, kidney, and ovary p<0.001. Heart and liver were evaluated by the Mann-Whitney test, the others by Student’s t-test. Results are mean values obtained from six animals in each treatment group. Standard deviation is given in parentheses.

Discussion

This work showed that arsenic decreased GSH levels and increased lipid peroxidation in liver, kidney, and heart. A greater effect was observed at 18.2 mg/kg than at 14.8 mg/kg for lipid peroxidation induction in all the three tissues. Because glutathione is part of a cellular mechanism concerned with defense against peroxidative attacks (19), the arsenic-induced lipid peroxidation can be explained by the depletion of GSH. However, for these results, we have to take into account that, in the present work, glutathione levels were determined as nonprotein-soluble thiols. Therefore, we cannot distinguish between an arsenic-induced inhibition in the synthesis of glutathione, an arsenic-induced inhibition in glutathione reductase activity (20,21), or the conjugation of glutathione with arsenic (22). All these mechanisms could decrease the reduced glutathione levels in arsenic-treated rats.

The effects of arsenic in liver of rats pretreated with saline, NAC, or BSO (Table 1) can be explained also by an inverse relationship between GSH levels and lipid peroxidation. In this tissue, the interaction of arsenic with GSH may have particular importance, as it has been suggested that the transport of arsenic as a GSH complex may account for the GSH dependence of biliary arsenic excretion (8).

In kidney and heart, NAC pretreatment protected the tissues against the AslOO-induced depletion of GSH. However, the same protection was not observed for the induction of lipid peroxidation. AsO depletes GSH in the kidney of BSO-pre-treated rats, but an increase in lipid peroxidation was not detected. In this case, BSO decreased the tissue concentration of arsenic, indicating that the arsenic concentration may not be high enough for the induction of lipid peroxidation.

Altogether, the results generally indicated that arsenic induced an increase in lipid peroxidation by a mechanism not directly related to a reduction in GSH levels, at least in kidney and heart (in liver, some evidence for a negative correlation between GSH levels and lipid peroxidation was found). If this were true, the peroxidation of lipids would depend on the tissue concentration of arsenic.

Using the arsenic concentration found for each kind of treatment in the three tissues (Table 4), a direct correlation was found between arsenic concentration in the tissues and lipid peroxidation levels: in liver (r=0.88, n=4), kidney (r=0.90, n=3); BSO−AsO results were not considered, and heart (r=0.84, n=4). The correlations were not statistically significant because of the small number of samples; however, a clear tendency was observed. Similar analyses were done for the tissue concentration of arsenic and GSH levels; in all tissues we did not obtain statistically significant correlations.

Arsenic may induce lipid peroxidation independently of GSH depletion, either by inhibiting the enzyme that catalyzes GSH-dependent reduction of peroxides (GSH peroxidase) or by generating higher-than-normal levels of free radicals.

Two types of GSH peroxidase activities have been described (23,24): one is affected by selenium depletion and the other is not. A mechanism for the arsenic-induced inhibition of the selenium-dependent activity can be postulated if we consider that arsenic decreased the selenium levels in rats (25). However, for the selenium-independent activity the same rationale is not applicable. This activity, which is associated with a GSH transferase (23,24), has been related to arsenic resistance in cells (26). The class of glutathione transferase involved in the resistance to arsenic belongs to the Pi family (26), and this specific class of enzyme is found in appreciable amounts in rat kidneys (24).

As an alternative working hypothesis, we can suggest that inorganic arsenic may induce oxidative stress. Whether inorganic arsenic causes cell damage through the production of oxygen radicals is a matter that deserves further research. It has been reported that oxidative damage was induced by arsenic compounds (arsine and dimethylated arsenics) (26–28) and that in cells treated with inorganic arsenic, the synthesis of heme oxygenase is increased (this enzyme is part of a general response to oxidative agents in mammalian cells) (29).

It has not escaped our attention that in rats with depleted levels of GSH (BSO-treated rats), an arsenic shift in tissue distribution was found; higher levels were observed in skin and lower levels in kidney. We can explain this result by assuming that arsenic would accumulate in tissues with available sulphhydril groups. In this case, skin keratins would provide those groups. It is worth noting that arsenic causes skin cancer and other skin diseases as a result of chronic exposure (30), although our study describes acute toxicity in rats and not chronic exposure in humans.
This work has shown that arsenic can increase lipid peroxidation in different tissues, and initial evidence was obtained for the mechanisms involved in this effect. Among those mechanisms, areas that need further research include the direct interaction between arsenic and the glutathione molecule, arsenic-induced inhibition of GSH peroxidase activity, and the possibility of a direct role of arsenic in the generation of oxygen radicals.

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