Role of co-administration of antioxidants in prevention of oxidative injury following sub-chronic exposure to arsenic in rats

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ABSTRACT

In this experiment thirty rats were exposed to 0 (Gr. I, healthy controls) or 10 ppm arsenic (sodium arsenite) through drinking water ad lib for eight weeks after dividing them into five groups of six rats each. Rats in Gr. III, IV and V were administered a daily oral dose of cysteine, methionine or ascorbic acid 25 mg/kg body mass respectively, while those in Gr. II served as the treated control. At the end of the experimental period oxidative stress indices viz. lipid peroxides level (LPO), superoxide dismutase (SOD) and catalase (CAT) activities were estimated in blood, liver and kidneys from sacrificed rats. Arsenic exposure resulted in a significant (P<0.05) rise in LPO levels and activities of SOD and CAT in erythrocytes, liver and kidneys. All the different treatments reduced lipid peroxides levels and restored activities of antioxidant enzymes to variable extents in various tissues. However, arsenic exposure did not show any significant (P>0.05) effects on the body mass of rats of different groups over time. It is concluded from the present study that prophylactic co-administration of cysteine, methionine and ascorbic acid could provide tissue specific protection from oxidative injury during sub-chronic exposure to arsenic.

Key words: antioxidants, arsenic, ascorbic acid, cysteine, methionine, rat

Introduction

Arsenic is ubiquitously distributed in the environment in a number of organic and inorganic forms and thus exposure to this metalloid has become inevitable for both man and animals (Al RAMALLI et al., 2005). Chronic exposure to arsenic due to consumption of contaminated water gives rise to several ill health effects in man and animals raising public health concern globally (JIN et al., 2004; NANDI et al., 2005a). Suffering of a large human
population from arsenicosis, particularly arsenic related skin lesions due to consumption of contaminated drinking water for a prolonged period has been reported from many countries of the world, including Argentina, Bangladesh, India, Mexico, Thailand and Taiwan (TCHOUNWOU et al., 1999). The generation of reactive oxygen species and development of oxidative stress in the target organs is one of the several mechanisms through which arsenic exerts its toxicity (RAMOS et al., 1995; LIU et al., 2001; NANDI et al., 2006). Oxidative stress due to acute arsenic toxicity in rats has been ameliorated by therapeutic supplementation of nutritional antioxidants like ascorbic acid, \( \alpha \)-tocopherol or cysteine (FLORA, 1999; RAMANATHAN et al., 2002). Deficiency of methionine, a precursor amino acid needed for glutathione synthesis, in the normal diet of rabbits makes them more susceptible to arsenic toxicity (VAHTER and MARAFANTE, 1987). In the practical environment too, arsenic toxicity related skin lesions and other abnormalities are notably more predominant among persons from low socio-economic status or those suffering from malnutrition (ANONYM., 1999), and a diet rich in B-vitamins, antioxidants or micronutrients was found to provide adequate protection from arsenic toxicity in human beings (MITRA et al., 2004). We have earlier reported oxidative stress in different organs of rats chronically exposed to low doses of arsenic via drinking water and their protection by simultaneous treatment with various nutritional antioxidants, including cysteine, methionine, ascorbic acid and thiamine (NANDI et al., 2005b). However, their effects in prevention of toxicity in the case of sub-chronic exposure have not been reported. Therefore, the present study was carried out to assess the role of co-administration with cysteine, methionine or ascorbic acid to arsenic exposed rats in protection of the target organs from oxidative damage.

**Materials and methods**

*Animals and experimental design.* Growing male Wistar albino rats (~ 80 g) used in the study (after obtaining permission from the Institute Animal Ethics Committee, IAEC) were housed in plastic cages in a 12 h dark-light cycle with temperature ranging between 16-30 °C, and were provided with standard laboratory animal feed and water. The humidity of the laboratory house ranged between 75 and 85%. Rice bran was used as bedding material, which was changed every alternate day. After fifteen days of acclimatization, the rats were randomly assigned into five groups of six rats in each group. Group I served as a negative control and received only tap water without addition of arsenic, whereas, animals in groups II to V were given arsenic (sodium-m-arsenite, Sigma Chemicals, USA, 98% purity) 10 ppm in drinking water *ad lib* for 8 weeks. Group III, IV and V animals, simultaneously with arsenic exposure, received prophylactic daily oral administration of cysteine (L-cysteine hydrochloride), methionine (N-acetyl DL-methionine) or ascorbic acid (L-ascorbic acid) freshly dissolved in distilled water 25
mg/kg body mass, respectively. Body masses of the rats were taken every alternate week starting from day 0 of exposure. Rats were sacrificed under light chloroform anesthesia after 8 wks of exposure to arsenic, and blood (through cardiac puncture and kept in heparinized vials), liver and kidneys were collected immediately and stored at -20 °C for biochemical analysis.

**Biochemical estimation.** Lipid peroxide level in 10% RBC hemolysate was determined as per PLACER et al. (1966) and was expressed as nmol malondialdehyde (MDA) /mg of hemoglobin (Hb) using 1.56 x 105 as extinction coefficient (UTLEY et al., 1967). For the preparation of 10% RBC hemolysate, blood samples were centrifuged at 2000 rpm for 10 minutes and supernatant plasma were separated out. The sedimented cells were washed with sterile 0.85% NaCl solution three times. Washed erythrocytes were hemolysed with 9-fold volume of distilled water to prepare 10% RBC hemolysate. Hemoglobin in the hemolysate was estimated by the cyano-methaemoglobin method (VAN KAMPEN and ZIGLSTRA, 1961). Lipid peroxides in 10% crude tissue homogenate of liver and kidneys from rats was estimated following OKHAWA et al. (1979) and was expressed in nmol of MDA/mg of protein. The tissue homogenate was prepared in 1.15% Tris-KCl buffer. The protein in 10% tissue homogenate was measured by the method of LOWRY et al. (1952). Superoxide dismutase activity in 10% supernatant tissues and RBC hemolysate was estimated as per MARKLUND and MARKLUND (1974) with certain modifications suggested by MENAMI and YOSHIKAWA (1979). Each unit of SOD activity is defined as the quantity of enzyme that inhibits auto-oxidation of pyrogallol by 50% under suitable experimental conditions. Catalase activity in tissues and RBC hemolysate was estimated as per COHEN et al. (1970). Tissue and blood samples were wet digested (HERSHEY and OOSTDYK, 1988) and concentration of arsenic in the digested samples was measured using a hydride generation atomic absorption spectrophotometer (AAS, ECIL-4141, India) at 193.7 nm wave length and 10 mA current and the values were expressed in µg/mL or µg/mg for blood and wet tissues, respectively.

**Statistical analysis.** The data were analyzed statistically using analysis of variance to compare the means of different treatment groups with that of the negative and positive control groups (SNEDECOR and COCHRAN, 1994).

**Results and discussion**

Eight weeks of arsenic exposure did not produce visible clinical signs of toxicity in the exposed animals. There was no significant (P>0.05) change on the steady gain in body mass of rats in all the groups recorded during the entire period of the experiment, except in the ascorbic acid treated group (Table 1). This could be due to the dose or duration of the arsenic exposure, which failed to produce significant differences in body mass gain pattern in those rats. Rats from the ascorbic acid treated group (Gr. V) revealed
Table 1. Effect of arsenic on body mass (g) of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>% gain in body mass</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>81.66 ± 2.77</td>
<td>93.44 ± 3.82</td>
<td>101.78 ± 4.38</td>
<td>106.25 ± 3.45</td>
<td>113.08 ± 4.30&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>138.47&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>II</td>
<td>80.89 ± 2.57</td>
<td>91.83 ± 3.58</td>
<td>97.66 ± 3.32</td>
<td>101.92 ± 2.93</td>
<td>108.83 ± 3.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>134.54&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>III</td>
<td>83.89 ± 2.71</td>
<td>91.55 ± 2.27</td>
<td>98.55 ± 2.24</td>
<td>101.83 ± 3.33</td>
<td>113.66 ± 3.94&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>135.48&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>82.55 ± 1.93</td>
<td>89.50 ± 2.01</td>
<td>97.22 ± 1.96</td>
<td>104.40 ± 1.98</td>
<td>119.25 ± 2.79&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>144.45&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>V</td>
<td>81.67 ± 2.23</td>
<td>87.72 ± 2.69</td>
<td>92.88 ± 3.12</td>
<td>109.00 ± 4.46</td>
<td>122.66 ± 5.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>150.19&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Body masses of rat were taken every alternate weeks starting from the day 0 of experiment. Mean (± SE) values with different superscripts (small letters column wise) differ significantly at P<0.05. I-Control, II-As exposed, III-As + cysteine, IV-As + methionine, V-As + ascorbic acid. Arsenic was given 10 ppm in drinking water <i>ad libitum</i> for eight weeks.

Table 2. Lipid peroxides levels (nmol MDA/mg of Hb) and activities of superoxide dismutase (units/mg of Hb) and catalase (units/mg of Hb) in erythrocytes of rats given different treatments<sup>c</sup>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>LPO</td>
<td>3.23 ± 0.20</td>
</tr>
<tr>
<td>SOD</td>
<td>2.83 ± 0.18</td>
</tr>
<tr>
<td>CAT</td>
<td>6.02 ± 0.44</td>
</tr>
</tbody>
</table>

<sup>c</sup>All values are expressed in mean ± SE, n = 6 rats. *P<0.05 compared with control values and, <sup>b</sup>P<0.05 compared with arsenic exposed group. I-Control, II-As exposed, III-As + cysteine, IV-As + methionine, V-As + ascorbic acid. Arsenic was given 10 ppm in drinking water <i>ad libitum</i> for eight weeks.

Table 3. Lipid peroxides levels (nmol MDA/mg of protein) and activities of superoxide dismutase (units/mg of protein) and catalase (units/mg of protein) in liver of rats given different treatments<sup>c</sup>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group I</td>
</tr>
<tr>
<td>LPO</td>
<td>3.05 ± 0.14</td>
</tr>
<tr>
<td>SOD</td>
<td>3.18 ± 0.18</td>
</tr>
<tr>
<td>CAT</td>
<td>22.13 ± 1.29</td>
</tr>
</tbody>
</table>

<sup>c</sup>All values are expressed in mean ± SE, n = 6 rats. *P<0.05 compared with control values and, <sup>b</sup>P<0.05 compared with arsenic exposed group. I-Control, II-As exposed, III-As + cysteine, IV-As + methionine, V-As + ascorbic acid Arsenic was given 10 ppm in drinking water <i>ad libitum</i> for eight weeks.
significantly (P<0.05) higher gain in mean body mass (150.19%) compared to the treated control (134.54%), which could either be due to the effect of this vitamin on body mass gain or the comparatively higher initial body mass of rats (81.67 Vs 80.89) belonging to this group than the arsenic treated rats. However, arsenic exposure for eight weeks resulted in significantly (P<0.05) increased LPO levels and activities of SOD and CAT in all the tissues, except CAT activity in the erythrocytes, which decreased non-significantly (P>0.05) in this tissue (Table 2-4). This could be due to accumulation of arsenic in these organs, which is evident from a significant (P<0.05) rise in arsenic burden in the liver (5.45 ± 0.35 Vs 0.32 ± 0.02 µg/gm), kidneys (4.87 ± 0.17 Vs 0.31 ± 0.04 µg/gm) and blood (3.49 ± 0.10 Vs 0.31 ± 0.02 µg/mL), compared to controls at the termination of the experimental period (NANDI et al., 2005b). The simultaneous administration of cysteine, methionine or ascorbic acid significantly (P<0.05) reduced lipid peroxidation in most of these tissues and restored SOD and CAT activities towards near normalcy. However, reduction in lipid peroxidation did not reach statistical significance (P>0.05) in the kidneys of the cysteine treated rats, in the liver of the methionine treated rats and in erythrocytes from rats given either of these treatments. Activity of SOD was highest in the liver of the ascorbic acid and the kidneys of the cysteine treated groups, suggesting better ameliorative potential of these two antioxidants in these tissues. All the treatments prevented CAT activity to rise significantly in the liver and kidneys of treated rats as compared to control rats. However, no significant (P>0.05) changes were noticed in the activities of CAT in erythrocytes from rats co-administered with any of the three antioxidants.

Oxidative stress due to arsenic involves either direct promotion of free radical generation (LIU et al., 2001) or inhibition of antioxidant enzymes which are responsible for scavenging and neutralizing free radicals (RAMOS et al., 1995). In the present study,

Table 4. Lipid peroxides levels (nmol MDA/mg of protein) and activities of superoxide dismutase (units/mg of protein) and catalase (units /mg of protein) in kidneys of rats given different treatments<sup>c</sup>

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPO</td>
<td>2.73±0.32</td>
<td>4.89±0.62*</td>
<td>3.66±0.36</td>
<td>2.31±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.21±0.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SOD</td>
<td>2.88±0.13</td>
<td>5.21±0.29*</td>
<td>3.06±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.95±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.62±0.14&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CAT</td>
<td>9.83±1.51</td>
<td>19.40±1.12*</td>
<td>12.50±0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11.60±1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.51±0.65&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>c</sup>All values are expressed in mean ± SE, n=6 rats. *P<0.05 compared with control values and, <sup>b</sup>P<0.05 compared with arsenic exposed group. I-Control, II- As exposed, III- As+ cysteine, IV-As + methionine, Gr .V- As+ ascorbic acid. Arsenic was given 10 ppm in drinking water ad libitum for eight weeks.
increase in SOD and CAT activities following arsenic treatment may be associated with overproduction of reactive oxygen species (ROS) or their accumulation due to dysfunction of the antioxidases-antioxidant system (PIE et al., 2002). Up regulation in the synthesis of SOD and CAT during arsenic exposure could also be attributable to activation of NF-k beta through production of ROS, the process which is inhibited by ascorbic acid, thus preventing oxidative injury to the cells (RAMANATHAN et al., 2003). In contrast to the present experiment, significantly decreased SOD and CAT activities with increased LPO levels has been documented in the blood, liver and kidneys of arsenic treated rats (RAMANATHAN et al., 2002; FLORA, 1999). Increased lipid peroxidation and alterations in antioxidant enzymes in different organs, including the brain, have been reported even at very low doses of arsenic treatment in rats (NAG CHOWDHURY et al., 1999). The results from this study indicate that arsenic induced oxidative stress in different organs is prevented variably by different co-treatments. Ascorbic acid, a low molecular mass water-soluble antioxidant, interacts directly with oxidizing radicals and prevents oxidative damage to the cell membrane induced by radicals in the aqueous environment (JONES et al., 1995). Ascorbic acid has earlier been reported as a possible chelator of lead with similar potency as that of EDTA (BRATTON et al., 1981) but its propensity to chelate arsenic and help in easy excretion of the metalloid has not been evaluated properly. The therapeutic role of ascorbic acid, along with chelator, is on record in acute arsenic toxicity in rats (RAMANATHAN et al., 2002). In the present study, the chemo-prophylactic potential of ascorbic acid during arsenic exposure could be due to both of its metal chelation and antioxidant properties. However, methionine and cysteine treatment produced tissue specific protection from oxidative damage in rats with more reactivity of methionine in the liver than cysteine, which may be due to the fact that the former is readily taken up by the hepatocytes for the synthesis of glutathione, a low molecular mass antioxidant (REED and ORRENIUS, 1997) and thereby protects this organ from impending damage by free radicals. Being sulfur containing amino acid methionine also provides a binding site for sulfhydryl reactive metals such as arsenic, facilitating their removal from different parts of body (TANDON et al., 1994). Cysteine is an important structural component of glutathione and thus could help in the detoxification of arsenic (BOEBEL and BACKER, 1983). In addition to that, the thiol group in cysteine chelates arsenic from the tissue and facilitates removal of the arsenic-sulfur complex from the liver through bile (QUIG, 1998). This may be the reason for its higher efficacy in the liver and kidneys than any other tissues. Therefore, it is concluded from the present study that arsenic exposure led to varying degree of lipid peroxidation and changes in the activities of antioxidant enzymes in different tissues of rats, and prophylactic supplementation of cysteine, methionine or ascorbic acid resulted in tissue specific protection from oxidative damage due to arsenic.
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References


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SAŽETAK
U istraživanju su štakori bili izloženi natrijevom arsenitu (10 ppm arsena) primijenjenom u vodi za piće tijekom 8 tjedana nakon čega su bili podijeljeni u 5 skupina po 6 štakora. Štakorima u skupinama 3, 4 i 5 oralnim je putem dnevno davan cistein, metionin ili askorbinska kiselina u količini od 25 mg/kg tjelesne mase. Štakori 2. skupine poslužili su kao kontrola. Na kraju pokusa određeni su pokazatelji oksidativnog stresa i to razina lipidnog peroksida, superoksidne dismutaze i katalaze u krvi, jetri i bubrezima. Izloženost arsenu očitovala se značajnim (P<0,05) porastom razine lipidnih peroksida kao i aktivnosti superoksidne dismutaze i katalaze u eritrocitima, jetri i bubrezima. Zabilježene su niže razine lipidnih peroksida kao i povratak aktivnosti protuoksidacijskih enzima na različitim razinama i u različitim tkivima. Izloženost arsenu nije imala značajni učinak (P>0,05) na tjelesnu masu štakora različitih skupina. Zaključeno je da istodobna profilaktička primjena cisteina, metionina ili askorbinske kiseline pruža specifičnu zaštitu tkiva od oksidacijskih oštećenja uzrokovanih supkroničnim izlaganjem arsenu.

Ključne riječi: antioksidansi, arsen, askorbinska kiselina, cistein, metionin, štakor

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