Clastogenic effect of sodium arsenite in experimental rats and ameliorative effects of antioxidant vitamins C and E

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Abstract

Arsenic, an element ubiquitous in the environment, causes serious health problems in humans. The general population is more exposed to arsenic through drinking water than through air and food. Sodium arsenite, capable of inducing genotoxic effects through oxidative stress, is evaluated for its clastogenic effect by the formation of chromosomal aberrations in the bone marrow cells of Wistar rats. We also investigated whether the oral supplementation of α-tocopherol (400 mg/kg body weight) and ascorbic acid (200 mg/kg body weight) to arsenic-intoxicated rats (100 ppm in drinking water) for 30 days, ameliorates arsenic-induced toxicity. We report that the dietary supplementation of antioxidants such as vitamins C and E could prevent sodium arsenite induced toxicity in rats under experimental conditions.

Keywords: Arsenic, genotoxicity, ascorbic acid, α-tocopherol, chromosomal aberrations, vitamins, rat, bone marrow.

Introduction

Pollution at various levels has modified the global natural events. Of all the types of pollution, contamination due to heavy metals and metalloids, including the toxic non-essential elements like Arsenic, Mercury, Cadmium and Lead is wide spread (Roy and Saha, 2002). Among these metals, arsenic is possibly the most abundant pollutant having a complex metabolism and a classified potential human carcinogen. Arsenic is widely distributed in nature and principally occurs in the form of inorganic or organic compounds. An inorganic arsenical compound consists of arsenite, considered to be the most toxic form, and arsenate the less toxic form, and organic forms the least toxic ones (WHO, 1993). As a result of wide occurrence of arsenic in the environment, human exposure to the metalloid becomes almost universal. The most common pathway for an elevated environmental exposure to inorganic arsenic worldwide is through drinking water. Chronic exposure to arsenic causes a wide range of toxic effects and thus this metalloid is classified as Group I carcinogen in humans (IARC, 1987). Based on substantial evidence on cancer risk associated with chronic exposure to relatively low concentrations of arsenic in drinking water, the international agencies like National Research Council, (NRC, 1999) has suggested 50 μg/l as the Maximum Contaminant Level (MCL) with an increase in risk for cancer. The United States Environment Protection Agency has revised the maximum containment level for arsenic in drinking water as 10 μg/l (USEPA, 2001) and the WHO, has suggested a provisional guide line value of 10 μg/l of arsenic in drinking water and the European union has set 50 μg/l and 10 μg/l as limit and guide values of arsenic in drinking water respectively (WHO, 1981). Chronic arsenicism has been observed in workers and in groups of the general population living in some areas of The United States (Welch et al., 1998; Shaw et al., 2005), United Kingdom (Goyer et al., 1995), China (Luo et al., 1997), Taiwan (Chen et al., 2005; Tseng et al., 2006), Mexico (Cebrian et al., 1993), Chile (Smith et al., 1998), Argentina (Rich et al., 1998), India (Mazumdar, 1988; Rahman et al., 2005) and Bangladesh (Gamble et al., 2005). Note worthy among them are India and Bangladesh where the arsenic concentration in drinking water is hundred times more than the recommended safety level prescribed by the international agencies.

Following ingestion, inorganic arsenic appears rapidly in the circulation, where it binds primarily to hemoglobin (Axelson, 1980). Skin, bone and muscle represent the major storage organs (Osborne, 1925). Inorganic arsenic does not appear to cross the blood brain barrier; however transplacental transfer of arsenic in humans (Gibson and Gage, 1982) and mice (Hood et al., 1987) occurs. The metabolism of arsenic like other toxic metals is associated with the conversion of the most potent toxic form of this element to the less toxic form, followed by cellular accumulation or excretion. Biomethylation of arsenic is considered as the primary detoxification mechanism, since the inorganic arsenic is more toxic to the living organisms (Yamauchi and Fowler, 1994). Arsenic toxicity differs in a fundamental fashion from that of other “Protoplasmic Poison”, which acts by denaturing and precipitating the cellular proteins. It has been assumed that the effect of arsenic must depend on a functional activity rather than on structural integrity (Stocken and Thompson, 1946). Arsenicals can cause cellular damage through the generation of free radicals (Barchowsky et al., 1996). Several studies suggest that arsenic compounds may also exert their toxicity through the generation of reactive oxygen species such as
superoxide, hydroxyl radicals, hydrogen peroxide and nitric oxide during their metabolism in the cells (Hei and Filipic, 2004; Liu et al., 2005). Though Arsenicals are unable to induce gene mutation in cultured cells, arsenite has been shown to enhance the cytotoxicity, mutagenicity and clastogenicity of UV-radiation, alkylating and DNA crosslinking agents in rodents and human cells (Lee et al., 1986; Okui and Fujiwara, 1986). Genotoxic studies of arsenic have largely yielded negative findings for gene mutations but positive results for chromosomal aberrations (Lee et al., 1996). Therefore the present study was aimed to study whether supplementation of antioxidants such as vitamin C and E, prevent sodium arsenite induced clastogenicity in experimental rats.

Material and Methods
Male albino rats of Wistar strain (120-150 g) were used in this study. The animals were obtained from The King Institute of Preventive Medicine, Chennai. The animals were housed in large spacious cages and were given food and water ad libitum. The animal room was well ventilated with a 12 h light/dark cycle, throughout the period of the experiment. The animals were maintained on a commercial rat-feed manufactured by Hindustan Lever Ltd., Mumbai under the trade name "gold mohur rat feed". The feed contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre with adequate mineral and vitamin contents (Anusuyadevi et al., 2008).

Grouping of animals
The animals were divided into five groups, namely, Group I: Rats that received vehicles alone, (served as control).
Group II: Rats that received arsenic as sodium arsenite in drinking water at a concentration of 100 ppm.
Group III: Rats that were treated with arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) given by oral gavage once a day.
Group IV: Rats that were given arsenic along with α-tocopherol (400 mg/kgbody wt. dissolved in mineral oil) by oral gavage once a day.
Group V: Rats that were administered arsenic along with ascorbic acid (200 mg/kg body wt. dissolved in water) and α-tocopherol (400 mg/kg body wt. dissolved in mineral oil) by oral gavage once a day.

Food and water intake and body weight of the animals were monitored throughout the 30 days of the period of the experiment.

Estimation of arsenic
Tissue/blood/urine samples were digested according to the method of Ballentine and Burford (1957). To 100 mg of tissues/1 ml of blood or urine, 1 ml of concentrated nitric acid was added, followed by 1 ml of perchloric acid. The sample was then digested over a sand bath until the solution turned yellow in colour. If the colour of the digest was brown, more nitric acid and perchloric acid were added and the oxidation was repeated. The digest was made up to known volume with deionized water. Aliquots of this were used to estimate arsenic by using the atomic absorption spectrophotometer. The concentration of arsenic was expressed as μg/ dl blood or μg/g tissue.

Assessment of chromosomal aberration
Chromosomal aberrations were assessed by the method of Sharma and Sharma (1994). After 30 days of the experimental period, colchicine (0.15 ml) was administered intra-peritoneal to the animals 90 min before sacrifice. The animals were killed by cervical dislocation. Bone marrow cells from control and experimental animals were processed for analysis of chromosomal aberrations. The bone marrow from the femurs was flushed into a centrifuge tube containing 0.9% saline and centrifuged at 500 g for 5 min. The supernatant was removed and hypotonic KCl was added to the sediment. After incubation for 20 min at 37°C, the contents were centrifuged for 5 min and the sediment was fixed in methanol-acetic acid (3:1 v/v). Three changes of fixative were given prior to slide preparation. The slides were air-dried, stained with Giemsa solution. This stock was prepared from 1 g of Giemsa dissolved in 56 ml of glycerol, kept on a magnetic stirrer for an overnight at 50°C. On cooling to room temperature, 84 ml of methanol was added and kept again on the magnetic stirrer for 60 min. The filtrate was then stored in dark colour bottles and stored at 0-4°C. Working stain solution was prepared by mixing 2 ml of 10% di-sodium hydrogen phosphate and 2 ml of stock and made up to 50 ml with double distilled water. The slides were then scored blindly with oil-immersion magnification in a light microscope.

The diploid number of chromosomes in a rat is 42. The chromosomes are classified into three major groups and one sex pair. Group A consists of four pairs, each pair is morphologically distinct, group B consists of nine pairs in a gradation of length, group C consists of meta and sub-metacentrics constituting seven pairs. All these pairs are relatively short and are similar in morphology and the sex pair consists of a medium sized acrocentric X-chromosome and an acrocentric Y chromosome, which is slightly shorter than chromosome no. 9 (Miller et al., 1972; Unakul and Hsu,1977). One hundred well scattered metaphase plates were scored for each animal, giving a total of 1000 metaphases per group. Aberrations like breaks, gaps, deletions, fragments are recorded.

Statistical analysis
Values are expressed as mean ± SD for six rats in each group, and significance of the differences between mean values were determined by one way analysis of variance (ANOVA) followed by Duncan test for multiple comparison using statistical package for social sciences (SPSSS) version 11.0 package. Values of p<0.05 were considered to be significant. Correlation analysis was done using statistical package Statistica/w 5.0 for windows.
Table 1. Chromosomal aberrations

<table>
<thead>
<tr>
<th>Group</th>
<th>Chromatid gap</th>
<th>Chromosome gap</th>
<th>Chromatid breaks</th>
<th>Chromosome breaks</th>
<th>Deletions</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>55.98 ± 4.4</td>
<td>14.8 ± 1.47</td>
<td>26.6 ± 1.63</td>
<td>5.5 ± 1.04</td>
<td>37.66 ±2.6</td>
<td>5.0 ± 1.41</td>
</tr>
<tr>
<td>Group II</td>
<td>176.16 ± 24.6</td>
<td>56.33 ± 7.65</td>
<td>192.0 ±27.24</td>
<td>28.16 ± 3.6</td>
<td>154.0 ±21.4</td>
<td>13.6 ± 2.2</td>
</tr>
<tr>
<td>Group III</td>
<td>130.16 ± 10.26</td>
<td>23.6 ± 1.6</td>
<td>84.0 ± 6.3</td>
<td>30.0 ± 2.3</td>
<td>78.66 ± 6.3</td>
<td>11.5 ± 1.04</td>
</tr>
<tr>
<td>Group IV</td>
<td>126.0 ± 9.4</td>
<td>30.0 ± 2.3</td>
<td>98.16 ± 7.8</td>
<td>36.0 ± 2.3</td>
<td>116.0 ± 9.7</td>
<td>5.83 ± 0.75</td>
</tr>
<tr>
<td>Group V</td>
<td>83.82 ± 6.9</td>
<td>22.3 ± 1.9</td>
<td>56.16 ± 4.7</td>
<td>14.0 ± 1.5</td>
<td>62.0 ±4.9</td>
<td>7.5 ± 1.04</td>
</tr>
</tbody>
</table>

Each value is expressed as mean ± SD for six rats in each group. *As compared with group I;  a As compared with group II;  b As compared with group III;  c As compared with group IV.  a, b, c, d represent p<0.05
Results
Chromosomal aberration as observed by metaphase preparation revealed an increase in chromosome gap by 214%, chromatid gap 280%, chromatid break 621%, chromosome break 412%, deletions 308% and fragments 172% in group-II animals (rats) treated with arsenic alone. Whereas group-V animals supplemented with antioxidant vitamin C and vitamin E showed a remarkable reduction in the types of aberration to 52.41%, 60.41%, 70.75%, 50.28%, 59.74% and 44.85% compared to that of group-II (Table 1 & Fig.1). Moreover a positive correlation was observed between the types of aberrations and the blood concentration level of arsenic. The correlation coefficient ‘r’ being 0.97 for chromosome gap, 0.56 for chromosome break, 0.93 for chromatid gap, 0.98 for chromatid break, 0.91 for deletions and 0.83 for fragments. The types of aberrations induced by arsenic have been shown in Fig. 2, 3 & 4.

Discussion
Our observation demonstrated that free radicals might be involved in arsenic induced chromosomal aberration. This is corroborated by a reduction in the formation of these aberrations in groups supplemented with antioxidants such as vitamin C and vitamin E (Table 1). Recent studies show that Reactive Oxygen Species (ROS) are involved in arsenite induced cell signaling and activation of transcription factor (Barchowsky et al., 1999) leading to chromosomal aberrations (Hei et al., 1998), DNA strand breakage (Lynn et al., 2000), gene mutation (Hei et al., 1998) generation of micronuclei (Wang & Huang, 1994; Gurr et al., 1998) and apoptosis (Gurr et al., 1999). Moreover metabolism of arsenic generates oxygen radicals (Kessel et al., 2002; Hei and Filipic, 2004; Valko et al., 2006) which may damage the cellular macromolecules and decrease CytP450 biotransformation enzymes involved in xenobiotic metabolism (Albores et al., 1989). Antioxidant Vitamin E is an important lipid soluble antioxidant present in cells, as it is the major chain terminating antioxidant in biological membranes (Burton et al., 1983) and scavenges a wide array of ROS including \( \cdot \text{O}_2 \), \( \cdot \text{HO} \), \( \cdot \text{O}_2 \), Peroxyl, and alkoxyl radicals. Vitamin E is composed of a number of derivatives of tocopherols and tocotrienols. The major isomer in humans is \( \alpha \)-TOH, which also possesses the greatest antioxidant activity of any vitamin E derivative. In homogenous solutions, \( \alpha \)-TOH is a strong inhibitor of polyunsaturated lipid peroxidation (Samokyszyn et al., 1990) and in vivo, most of the cellular vitamin E is concentrated in the lipid membranes (Bjorneboe et al., 1990). The primary antioxidant activity of tocopherols is to stop chain propagation of peroxyl radicals (k = 6 x 10^{-1} - 3 x 10^{6} M^{-1} sec^{-1}) (Niki and Matsuo, 1993).

Tocopherol can typically scavenge two ROS per tocopherol molecule (Horswel et al., 1966). Ascorbic acid (Vitamin C), a water soluble antioxidant, exists primarily as ascorbate at physiological pH. Ascorbate is a powerful reducing agent capable of rapidly scavenging a number of ROS including \( \cdot \text{O}_2 \) (Halliwell, 1990). Antioxidant Vitamin E contains \( \alpha \)-tocopheroxyl radical \( \alpha \)-TO•. Through this mechanism, ascorbate in the aqueous phase is able to regenerate membrane-bound \( \alpha \)-TOH, prolonging the life time of this important antioxidant in the lipid phase and effecting removal of the radical from the lipid to the aqueous phase. In its action as an antioxidant, ascorbate is rapidly oxidized to dehydroascorbate, which shows little antioxidant activity. Dehydroascorbate may be reduced back to ascorbate via GSH-dependent reductase (Sharma and Buettner, 1993). Therefore group V animals supplemented with antioxidant vitamins E and C, showed reduced chromosomal aberrations due to the free radical scavenging effects of vitamins.

The level of chromosomal aberrations, was found to be elevated in arsenic-alone treated group-II, whereas the group-V supplemented with antioxidant vitamins showed a minimal levels of alteration in the above said parameters suggesting primarily, that toxicity caused by arsenic was mediated by free radicals.

Exposure to arsenic at low doses chronically causes oxidative stress and depletion of vital antioxidants in the cells. The toxicity of arsenic comprises altered membrane properties, loss of cellular functions, macromolecular damage and finally induction of apoptosis. Co-treatment of antioxidant vitamins like vitamin C and vitamin E to arsenic exposed rats may have restored the antioxidant status of the cells and protected them from arsenic induced clastogenicity. Our study corroborated with similar findings of Ramanathan et al. (2005) that the supplementation of ascorbic acid and \( \alpha \)-tocopherol modulated arsenic induced apoptosis in rats by improving the cellular antioxidant status and scavenging of free radicals. It also supports the perspective of co-administration of antioxidant vitamins to arsenic-induced toxicity (Karafitavas et al., 2005; Wei et al., 2005; Balakumar et al., 2010). Therefore it is strongly believed that the present vitamins supplementation perspective will have a probable proactive protection to arsenical exposure.

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