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Modulatory Effects of Ascorbic Acid and α-tocopherol on Arsenic Induced Micronuclei Formation

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Abstract: Arsenic is a well known human carcinogen and has many other toxic effects. Sodium arsenite, a compound of arsenic, capable of inducing genotoxic effects through oxidative stress, is evaluated for its genotoxic effect by the formation of micronuclei in the polychromatic erythrocytes in the bone marrow cells of Wistar rats. Further, the modulatory effects of antioxidants like ascorbic acid and α-tocopherol on arsenic intoxicated rats is investigated. Therefore, the present study was designed to determine whether the oral supplementation of α-tocopherol (400 mg kg^{-1} body weight) and ascorbic acid (200 mg kg^{-1} b.wt.) to arsenic-intoxicated rats (100 ppm in drinking water) for 30 days, modulates the genotoxicity caused by arsenic through the formation of micronuclei ($p<0.05$). The findings suggest that co-treatment of ascorbic acid and α-tocopherol to arsenic-exposed rats protects the antioxidant system and modulates arsenic induced micronuclei formation.

Key words: Arsenite, antioxidant vitamins, supplementation, genotoxicity, polychromatic erythrocytes

INTRODUCTION

Arsenic occurs very widely in the environment and is available to the human population through sources that include drinking water, food and air. Population exposed to drinking water contamination of arsenic includes those in Taiwan, China, Europe, United States, Bangladesh and India (WHO, 1997; Rahman *et al*., 2005). Exposure to arsenic compounds is associated with several human diseases and ailments including blackfoot disease (Tseng *et al*., 2006), diabetes mellitus (Lai *et al*., 1994), hypertension (Chen *et al*., 1985) and cancers of the bladder, lung, skin and liver (Chen *et al*., 1992). Several studies suggest that arsenic compounds may 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*., 1986a; Okui and Fujiwara, 1986). However, genotoxic studies of arsenic have largely yielded negative findings for gene mutations but positive results for chromosomal aberrations (Lee *et al*., 1986b). Treatment with arsenite has been reported to induce micronuclei formation in chinese hamster cells (Gurr *et al*., 1998). In mice, a significant increase in the frequency of micronucleated polychromatic erythrocytes upon treatment with sodium arsenite has been reported by Tice *et al*. (1997).

Arsenic has also been shown to alter cellular function by affecting signal transduction pathways in humans (Ardjmand *et al*., 2006). Moreover, human population exposed to arsenic has been shown to have a high cytogenetic damage as measured by micronuclei formation in oral mucosa cells by Gosh *et al*. (2006).

Arsenic exposure has been shown to depress the functions of antioxidant defense system leading to oxidative damage to cellular macromolecules (Hei and Filipic, 2004), whereas antioxidants protect the cellular machinery from peroxidative damage inflicted by reactive oxygen species (Halliwell, 1996). Ascorbic acid is the most widely cited form of water-soluble antioxidant that prevents oxidative damage to the cell membrane induced by radicals in an aqueous environment (Li *et al*., 2001). Hydrophilic ascorbic acid is not able to scavenge radicals

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within the interior of membranes, but α-tocopherol is an efficient scavenger of free radicals within the membranes (Csala *et al*., 2001). 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). It scavenges a wide array of reactive oxygen species, peroxyl and alkoxyl radicals. Vitamin E is composed of a number of derivatives of tocopherols and tocotrienols. Alpha-tocopherol (α-TOH) which is a derivative of Vitamin E possesses the greatest antioxidant activity of any other vitamin E derivative. In homogeneous solutions, α-TOH is a strong inhibitor of polyunsaturated lipid peroxidation (Samokyszyn *et al*., 1990). Both these antioxidants can act synergistically against reactive oxygen species in a cell. Therefore the aim of this study was to evaluate the frequency of micronuclei formation by sodium arsenite and the effects of ascorbic acid and α-tocopherol on arsenite-induced micronuclei formation.

MATERIALS 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 in the years 2002 to 2005, 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 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.

This research on Wistar rats was sanctioned and approved by the institutional animal ethical committee.

Source of chemicals: Sodium arsenite, ascorbic acid and α-tocopherol, were purchased from Sigma Chemical Company, St. Louis, USA in the year 2002.

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^{-1} b.wt. dissolved in water) given by oral gavage once a day

Group IV: Rats that were given arsenic along with α-tocopherol (400 mg kg^{-1} b.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^{-1} b.wt. dissolved in water) and α-tocopherol (400 mg kg^{-1} b.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 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^{-1} blood or μg g^{-1} tissue.

Assessment of micronucleus formation: The micronucleus test was carried out according to the method described by Schmidt (1975):

The bone marrow from the femurs was flushed in the form of a fine cell suspension into a centrifuge tube containing fetal calf serum. The cell suspension was centrifuged at 500 g for 10 min and the supernatant was discarded. The pellet was resuspended in a drop of serum and used for preparing slides. The air-dried slides were stained with May-Grunwald and Giemsa. A total of 1000 polychromatic erythrocytes were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (Mn-PCE).

RESULTS AND DISCUSSION

The levels of arsenic in blood, liver, kidney and urine of controlled, arsenic exposed and antioxidant vitamins treated rats are shown in Table 1. A manifold increase in the accumulation of arsenic was observed in blood, liver and kidney of arsenic exposed rats when compared to the controlled. This shows that the accumulation of arsenic is high in blood, followed by liver and kidney. Treatment

Table 1: Level of arsenic accumulation and excretion in control and experimental rats

Parameters	Group I (Control)	Group II (Arsenic)	Group III (Arsenic+ascorbic acid)	Group IV (Arsenic+α-tocopherol)	Group V (Arsenic+ascorbic acid+α-tocopherol)
Blood (μg dL^{-1})	0.29±0.04	43.6±5.76[a]	15.1±2.44[b]	12.9±1.58[bc]	5.42±0.51[bcd]
Liver (μg g^{-1} tissue)	0.26±0.27	19.1±2.10[a]	6.59±0.67[b]	6.69±0.58[b]	2.01±0.15[bcd]
Kidney (μg g^{-1} tissue)	0.11±0.01	12.31±1.15[a]	4.08±0.37[b]	3.84±0.46[b]	1.23±0.15[bcd]
Urine (μg/24 h)	0.37±0.04	346±34.1[a]	796±69.9[b]	903 ±72.3[bc]	1267±104.1[bcd]

Each value is expressed as mean±SD for six rats in each group; [a]As compared with group I; [b]As compared with group II; [c]As compared with group III; [d]As compared with group IV, [a-d] represent $p<0.05$

with ascorbic acid and α-tocopherol to arsenic-intoxicated rats significantly reduced the level of arsenic in blood, liver and kidney ($p<0.05$), whereas an increase in the urinary excretion of arsenic was observed ($p<0.05$).

The damage inflicted by free radicals to cellular macromolecules plays a crucial role in the pathogenesis of several diseases including cancer and neurodegeneration.

The formation of micronuclei as observed in polychromatic erythrocytes in bone marrow are found to be statistically significant when compared to arsenic intoxicated groups and antioxidant-vitamins supplemented groups ($p<0.05$). The frequency of micronuclei as observed between the experimental groups is graphically represented in the Fig. 1. A positive correlation was observed when compared to the blood levels of arsenic and frequency of micronuclei formation ($r = 0.92$).

Arsenic treated rats showed an increase in the level of micronuclei formation, indicating oxidative damages to lipids, proteins and DNA. Arsenic is known to stimulate the release of free iron through the activation of hemeoxygenase, the rate limiting enzyme in heme degradation (Menzel *et al*., 1998). The free iron may thereby involve in fenton type reaction leading to an enhanced lipid peroxidation; increased lipid peroxidation alters membrane stability, fluidity and membrane potential which ultimately leads to loss of cellular function and cell death (Pompella *et al*., 1991). Recent studies show that ROS are involved in arsenite induced cell signaling and activation of transcription factor (Wang and Huang, 1994; 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 *et al*., 1997; Gurr *et al*., 1998). The administration of vitamin C and vitamin E to arsenic exposed group replenishes the level of reduced glutathione and thereby regulates the redox potential of the cell and maintains DNA and proteins in native form (Ramanathan *et al*., 2002). Apart from these factors the improvement in the activities of enzymatic antioxidants by vitamin C and vitamin E in arsenic treated rats could play an additional role in eliminating the deleterious radicals. Therefore experimental animal groups treated with antioxidant ascorbic acid and α-tocopherol exhibit a reduction in the frequency of micronuclei.

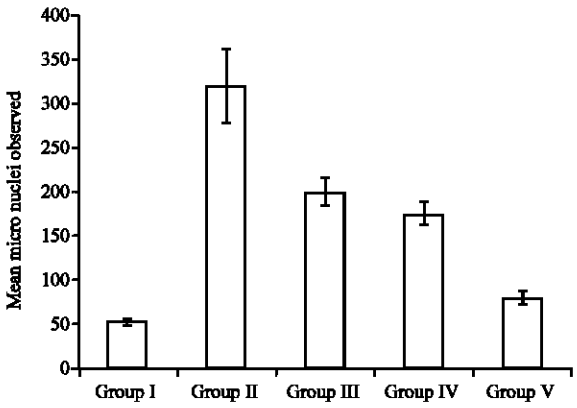

Fig. 1: Effects of ascorbic acid and α-tocopherol on arsenic induced micronuclei formation

This study corroborated with the similar findings of Ramanathan *et al*. (2005) that the supplementation of ascorbic acid and α-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 (Peraza *et al*., 1998; Wei *et al*., 2005; Karasavvas *et al*., 2005; Balakumar *et al*., 2010).

Thus our observation demonstrated that free radicals might be involved in arsenic induced micronuclei formation; and supplementation of antioxidant vitamins C and E could scavenge the free radicals generated by arsenic and improves the cellular antioxidant status. The results of this investigation advocate strongly that supplementation of antioxidant vitamins promises therapeutic and possible preventive applications.

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