Original Article

Lipid Peroxidation in Brain Tissue Following Administration of Low and High Doses of Arsenite and L-Ascorbate in Wistar Strain Rats

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ABSTRACT

This study aimed at investigating the mechanism by which sodium arsenite induces brain injury and the role of L-ascorbate. Thirty adult (n=5) Wistar rats weighing between 140 and 160 g were used. Group 1 neither received sodium arsenite nor L-ascorbate (control), group 2 was administered low dose of arsenite only, group 3 received high dose of arsenite only, group 4 was administered L-ascorbate only, group 5 was administered low dose of arsenite and L-ascorbate, and group 6 received high dose of arsenite and L-ascorbate. Malon dialdehyde, MDA, levels were significantly increased in rats treated with high dose of arsenite when compared with those treated with low dose of arsenite. However, all treated groups except those treated with L-ascorbate only showed significant increase in MDA levels when compared with the control group. Rats treated with high dose of arsenite and L-ascorbate showed a significantly higher MDA level than those treated with low dose of arsenite and L-ascorbate. However, catalase activity, body weight gain, brain weight and mean food consumption were comparable across all groups. Brain tissue total protein was similar in all groups except in both groups treated with high dose of arsenite, where they were significantly reduced when compared with the control group. In conclusion, sodium arsenite treatment induces brain injury via a mechanism associated with lipid peroxidation, but not catalase-dependent. However, L-ascorbate ameliorates arsenite-induced oxidative injury in the brain. L-ascorbate antioxidative potential in alleviating arsenite-induced brain injury is dependent on the concentration of arsenite.

Key words: Arsenite, brain, lipid peroxidation, L-ascorbate, weight

INTRODUCTION

Arsenical compounds are environmental toxins with multiple effects in animals and humans.[1,2] They are commonly used as herbicides, fungicides and rodenticides and may cause air, soil and water pollution.[3-5] The main source of environmental arsenic exposure is oral consumption of contaminated water, food and drugs.[6,7]

Exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion, tumors in a variety of tissues including skin, lung, liver and bladder,[2,8,9] inhibition of ovarian steroidogenic function and gonadotrophins secretion,[10] severe metabolic disorders

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such as diabetes,[11] gastrointestinal tract disorders,[12] sperm toxicity[2,13,14] and brain injury.[15,16]

On the other hand, L-ascorbate (Vitamin C) has been reported to have antioxidative properties,[17,18] but its role in brain injury has not been documented.

Hence, this study aimed at investigating the mechanism by which sodium arsenite induces brain injury and the role of L-ascorbate.

MATERIALS AND METHODS

Animals and treatment

Thirty adult Wistar rats weighing between 140 and 160 g were maintained in a 12h light and 12h dark cycle at 25°C±2. They were fed standard rat chow and tap water *ad libitum*. Rats were allowed to acclimatize for 2 weeks and then divided into six groups (n=5). Sodium arsenite and L-ascorbate were dissolved in water to achieve specific doses and orally administered for 21 days.

The doses were as follows:

Group 1 : Rats given neither sodium arsenite nor L-ascorbate; control
Group 2 : Rats administered low dose of arsenite only (0.5 mg/100 kgBW/rat day); LDA
Group 3 : Rats administered high dose of arsenite only (1 mg/100 kgBW/rat day); HDA
Group 4 : Rats administered L-ascorbate only (25 mg/100 gBW/rat day); Vit.C
Group 5 : Rats administered low dose of arsenite and L-ascorbate; LDAV
Group 6 : Rats administered high dose of arsenite and L-ascorbate; HDAV

The *NIH* Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23 revised 1985: US Department of Health, Education and Welfare, Bethesda, MD, USA) was followed throughout the experimental duration. The experimental protocol also met the American Physiological Society's "Guiding Principles for Research Involving Animals".

Amount of food consumed were recorded daily throughout the experiment and the mean food consumption in g/rat/day was calculated.

After the experimental period, rats were fasted overnight and sacrificed the following day.

Body and organ weight

The body weights were recorded on the first day of treatment (initial weight) and on the day of sacrifice (final weight). Brain of each rat was dissected out and weighed. The brain was homogenized and malon dialdehyde level (MDA), and catalase activity (CAT) were assayed using the homogenate as described by Varshney and Kate,[19] and Beers and Sizer,[20] respectively. Total brain tissue protein was assayed using a standard kit (Randox).

Statistical analysis

Results of the experiment are expressed as mean±standard error of mean. The differences between the mean values were evaluated by ANOVA followed by multiple Student's *t*-test. The values $P<0.05$ were considered significant.

RESULTS

In all treated groups, the body weight gain was not significantly different from that of the control. Similarly, the brain weight was also comparable across all the groups. No differences in the mean food consumption were seen in all the groups during the experimental period [Table 1 and Figure 1].

Brain tissue total protein was similar in all groups except in HDA and HDAV, where they were significantly reduced when compared with the control group [Figure 2].

MDA levels were significantly increased in rats treated with HDAV and HDA when compared with those treated with LDAV and LDA, though HDAV and LDAV showed a significantly lower MDA levels when compared with HDA and LDA, respectively. However, all treated groups except those treated with L-ascorbate only showed significant increase in MDA levels when compared with the control group. CAT activity was comparable in all groups.

Table 1: Weight changes and lipid peroxidation index in brain tissue following administration of low and high doses of arsenite and L-ascorbate (Vitamin C) in rats

Variable	Control	LDA	HAD	Vit.C	LDAV	HDAV
BWG	39.5 ± 0.99^{a}	38.9 ± 0.86^{a}	37.9 ± 0.92^{a}	39.55 ± 0.79^{a}	39.2 ± 1.02^{a}	39.0 ± 0.97^{a}
BW	7.2 ± 1.5^{a}	6.82 ± 0.8^{a}	6.5 ± 1.7^{a}	7.0 ± 1.2^{a}	6.9 ± 1.1^{a}	7.1 ± 0.7^{a}
MDA	86.7 ± 4.7^{a}	378.16 ± 9.6^{b}	638.16 ± 13.1^{c}	76.50 ± 2.79^{a}	326.88 ± 18.31^{d}	485.86 ± 9.62^{e}
CAT	0.06 ± 0.15^{a}	0.05 ± 0.11^{a}	0.03 ± 0.13^{a}	0.05 ± 0.15^{a}	0.05 ± 0.13^{a}	0.06 ± 0.11^{a}

Values with different superscripts $^{a, b, c,d,e}$ are significantly different ($P<0.05$). BWG: Body weight gain (g); BW: Brain weight (g); MDA: Brain malon dialdehyde (unit/kg/protein); CAT: Brain catalase activity (µmoles H_2O_2 consumed $min^{-1}mg^{-1}$ protein)

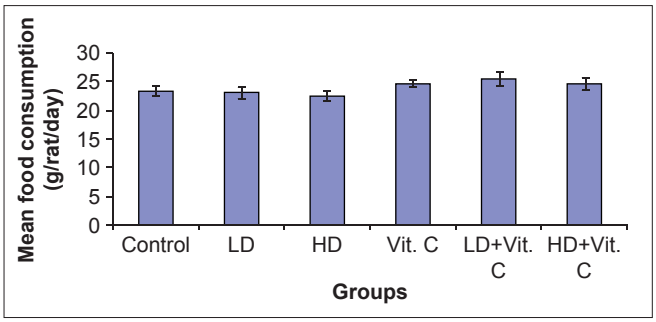

Figure 1: Mean food consumption following administration of low and high doses of sodium arsenite and L-Ascorbate (Vitamin C) in rats

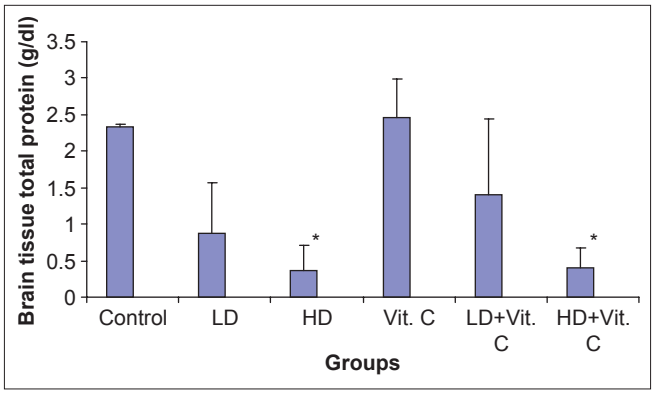

Figure 2: Total protein level in brain tissue following administration of low and high doses sodium arsenite and L-ascorbite (Vitamin C) in rats *P <0.05 vs control

DISCUSSION

This study reveals that the administration of sodium arsenite, in low and high doses, and L-ascorbate does not affect food consumption. This agrees with the study of Kuladip *et al*.,[21] which showed comparable values of food consumption in arsenite-treated and control rats. This suggests that arsenite exposure does not affect the appetite center in anyway.

This study also reveals that sodium arsenite and L-ascorbate administration does not affect body weight gain and brain weight. This does not agree with previous studies that reported though no significant differences in body weight, but significant decrease in both male and female reproductive organ weight following arsenite administration.[10,13,21] This variation might be due to higher doses and longer period of exposure of rats to arsenite in previous studies.

Previous study of Akhigbe *et al*.,[22] has reported the effect of food intake on body weight gain. High food intake is known to help in tissue repair and other anabolic processes with consequent increase weight gain besides the production of energy for daily activities. The comparable body weight gain and brain weight seen across all groups in this study could be associated with the similar food consumption also seen across all the groups. Since all groups consumed comparable amount of food throughout the experimental schedule, they consequently have similar body weight gain and brain weight.

Arsenite, like other heavy metals, has been associated with various tissue damage. Study of Lin *et al*.,[15] revealed that arsenite induces oxidative injury in the nigrostriatal dopaminergic system of rats' brain. They also documented that arsenite induces apoptosis in the central nervous system of rat. Similar arsenite-induced oxidative injury in rats' brain was also reported by Su-Feng *et al*.[16] This study shows the mechanism by which arsenite induces brain injury and the possible role of L-ascorbate in alleviating arsenite-induced brain injury.

This study shows that CAT activity was comparable in all groups. On the other hand, LDAV and LDA-treated rats showed significant increase in MDA levels when compared with the control and L-ascorbate-treated group, while HDAV and HDA-treated rats showed significant increase in MDA levels when compared with all other groups. This is consistent with previous studies[15,16] which reported increased lipid peroxidation following arsenite treatment. This study shows that L-ascorbate enhances lipid peroxidation status in rats exposed to arsenite as seen in LDAV and HDAV groups, which showed a significantly lower MDA levels when compared with LDA and HDA groups, respectively.

Lipid peroxidation, usually measured by the level of MDA, causes degenerative changes of brain cell membrane.[23] The free radicals released in association with lipid peroxidation oxidize polyunsaturated fatty acid, a main component of the cell membrane, thereby destroying the spatial arrangement of membrane and removing the biological activities impairing the functions of the bound enzymes like Na^+/K^+ ATPase which plays an important role in the functional activity of nerve cells.[24-27]

Administration of L-ascorbate alleviated arsenite-induced raise in MDA level in both LDAV and HDAV-treated rats, though HDAV-treated rats showed a significantly higher level of MDA when compared with the LDAV-treated rats. This result suggests that L-ascorbate protective potential on lipid peroxidation status is dependent on the concentration of arsenite.

L-ascorbate is a free radical scavenger and functions as an antioxidant. However, Frei *et al*.,[28] reported that L-ascorbate disappears faster than other antioxidants when plasma is exposed to reactive oxygen species. This might also explains why L-ascorbate has a better antioxidative potential in low dose of arsenite than in high dose.

CONCLUSIONS

From the present study, sodium arsenite treatment

induces brain injury via a mechanism associated with lipid peroxidation, but not catalase-dependent. L-ascorbate antioxidative potentials in alleviating arsenite-induced brain injury is, however, dependent on the concentration of arsenite.

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