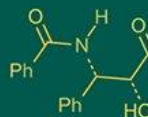


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Oxidative stress markers in relation to acute sodium arsenate (Na_3AsO_4) exposure in buffalo red blood cells *in vitro*

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Abstract

Arsenic is a toxic metalloid that disturbs the cellular oxidant-antioxidant status in buffaloes affecting their health. In the present study, buffalo erythrocytes exposed to varying concentration of sodium arsenate (Na_3AsO_4) (0.01-0.5 μg for 2hrs.) were observed with declined superoxide dismutase, glutathione peroxidase and catalase activities; with enhanced lipid peroxidation as evidenced from the generation of malondialdehyde in a concentration dependent manner compared to the corresponding controls. It was noticed that acute exposure of Na_3AsO_4 creates oxidative stress in buffalo erythrocytes through increased membrane lipid peroxidation and depletion of enzymatic antioxidants inside the cells.

Keywords: Oxidative stress, antioxidants, sodium arsenate, buffalo, RBCs

Introduction

Contamination of arsenic in the environment can be due to its natural existence and manmade industrial activities (Dash *et al.* 2016) [3]. Arsenic contaminates the water, air, feed and soil globally (Rana *et al.* 2010) [11]. Buffalo health is under threat due to arsenic exposure as well as human health due to consumption of contaminated milk. Acute arsenic contamination causes imbalances in oxidant-antioxidant system leading to different health issues in buffaloes (Flora *et al.* 2007) [6].

Red blood cells (RBC) have high oxygen carrying capacity. Hence, different biomolecules in RBCs are vulnerable to oxidative damage. Documented studies on sodium arsenate induced changes in oxidant-antioxidant status in buffalo RBCs is scanty. Keeping in view the above facts, changes in oxidant-antioxidant markers were studied in buffalo erythrocytes exposed to sodium arsenate *in vitro*.

Materials and Methods

Preparation of erythrocytes

Female healthy buffalo red blood cells were obtained from Innovative Research Inc. biotechnology company. Erythrocytes were washed thrice with phosphate-buffered saline (PBS), pH 7.4 and resuspended in PBS. Counting of erythrocytes was conducted using hemocytometer to adjust cell number to 2×10^6 cells/ml.

Exposure of Erythrocytes to sodium arsenate:

Erythrocytes (2×10^6 cells/ml) were incubated with PBS (Control) or sodium arsenate (0.01 μg to 0.05 μg) for 2 hrs (acute exposure) at 37 °C as follows:

Control group: Erythrocyte suspension incubated with PBS.

Treatment groups: Consisted of Group T₁ to T₅ in which erythrocyte suspensions were incubated with 0.01, 0.02, 0.03, 0.04 and 0.05 μg Na_3AsO_4 respectively for 2 hrs.

[Maximum Permissible Limit of arsenic in drinking water for animal health is 0.01 $\mu\text{g}/\text{ml}$ (WHO)].

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Estimation of Oxidative stress Markers:

Concentration of malondialdehyde, was estimated by the method of Placer *et al.* 1966 [9]. Assay of superoxide dismutase was carried out as per the method of Nishikimi *et al.* 1972 [8]. Glutathione peroxidase assay was carried out as per the method of Flohe and Gunzler 1984 [5]. Estimation of catalase activity was conducted as per the method of Aebi 1984 [1]. UV/VIS spectrophotometer was used for all estimations.

Statistical analysis

Independent t-test was used for the analysis of data.

Results and Discussion

Buffalo RBCs challenged with sodium arsenate (0.01-0.5 µg for 2 hrs.) showed enhanced ($p < 0.05$) generation of malondialdehyde, in a concentration dependent manner. Peak level of malondialdehyde production was at 0.5 µg sodium arsenate concentration. Increased antioxidant enzyme response e.g. superoxide dismutase (T_1 and T_2), glutathione peroxidase (in group T_1) and catalase (T_1) was noticed during initial concentrations of sodium arsenate exposure compared to the corresponding controls. However, the activities of superoxide dismutase (T_3 - T_5), catalase (T_2 - T_5) and glutathione peroxidase (T_2 - T_5) significantly ($p < 0.05$) decreased in a concentration dependent manner with increasing dose of sodium arsenate treatment to buffalo RBCs. (Tab. 1).

Acute Na_3AsO_4 exposure increased the lipid peroxidation and declined the level of antioxidant enzymes e.g. superoxide dismutase, glutathione peroxidase and catalase in buffalo erythrocytes as observed in this study. This creates the oxidative stress environment inside the RBCs. Different arsenic compounds disturbs the redox homeostasis inside the cell producing oxidative stress (Flora *et al.* 2007) [6]. Na_3AsO_4 can generate reactive oxygen species (ROS) causing oxidative stress and apoptosis in various animal cells (Lynn *et al.* 2000, Felix *et al.* 2005) [7, 4]. Exposure to arsenic at very low concentration for short duration also produces superoxide anion radical, singlet oxygen, hydroxyl radical, hydrogen peroxide etc. in various animal cells (Qian *et al.* 2003, Bashir *et al.* 2006; Flora *et al.* 2007) [10, 2, 6] causing lipid peroxidation, membrane damage and cell death. The results of this study are in line with above reports.

Cellular antioxidants modulate the oxidative stress response in the cells. Elimination of ROS was carried out by the combined action of superoxide dismutase and catalase. Superoxide radicals were converted to hydrogen peroxide and molecular oxygen by superoxide dismutase. Catalase converts hydrogen peroxide to water. In this study, decline in activities of superoxide dismutase and catalase in Na_3AsO_4 exposed buffalo erythrocytes can attributed to increased ROS production by arsenic or decrease action and synthesis of antioxidant enzymes due to toxic insult (Flora *et al.* 2007, Sinha *et al.* 2008) [6, 12].

Table 1: Oxidative stress markers (Mean±SE) in buffalo erythrocytes with acute exposure of sodium arsenate *in vitro*.

Parameters	Groups					
	Control	Group T_1	Group T_2	Group T_3	Group T_4	Group T_5
Malondialdehyde (nmol/mg Hb)	1.59±0.13	2.52±0.41	8.17±0.31*	13.17±0.31*	19.41±1.02*	27.24±1.01*
Superoxide dismutase (U/mg Hb)	2.64±0.19	3.62±0.48	10.11±0.42*	1.32±0.10*	1.13±0.10*	0.34±0.02*
Glutathione peroxidase (U/mg Hb)	12.33±1.02	13.18±1.10	9.73±1.01*	7.42±0.51*	5.54±0.63*	2.34±0.21*
Catalase (U/mg Hb)	20.53±1.14	24.06±1.02	16.54±1.11*	13.12±1.12*	9.14±1.13*	4.17±0.72*

*Indicates significant ($p < 0.05$) difference from corresponding controls.

Conclusion

Short duration exposure of sodium arsenate to buffalo RBCs creates oxidative stress as observed from the enhanced cellular lipid peroxidation and decreased enzymatic antioxidants.

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