European Review for Medical and Pharmacological Sciences 2013; 17(Suppl 1): 34-38

Myocardial toxicity of acute promyelocytic leukaemia drug-arsenic trioxide

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Abstract. – BACKGROUND: Arsenic trioxide (As_2O_3) is an environmental toxicant as well as an effective anti cancer agent against many types of cancers. It is a promising drug for patients with relapsed acute promyelocytic leukaemia (APL), but its clinical efficacy is burdened by the serious cardiac toxicities.

AIM: The present study was designed to investigate the toxic mechanism of arsenic in cardiac tissue at its clinically relevant concentrations.

MATERIALS AND METHODS: Experimental rats were administered with As_2O_3 2, 4 and 8 mg/kg body weight, orally for a period of 45 days. Cardiac toxicities were recorded by lipid peroxidation, activities of glutathione dependent antioxidant and antiperoxidative enzymes, cardiac arsenic accumulation and histopathological changes.

RESULTS: *In vivo* studies revealed a significant rise in lipid peroxidation, decline in reduced glutathione, glutathione dependent antioxidant enzymes and antiperoxidative enzymes in the cardiac tissue of arsenic treated rats. The extent of free radical production was found increased with periodic rise in the arsenic concentration. The experimental group which received 8 mg/kg body weight of arsenic exhibited the highest deposition of arsenic in cardiac tissue. Light microscopic examination of cardiac tissues in arsenic treated rats has showed increased structural abnormalities like myocardial fibre swelling, capillary congestion and micro-haemorrhages.

CONCLUSIONS: The study concludes that the mechanism of arsenic induced cardiac toxicity is associated with the accumulation of arsenic in tissue and the extent of free radical production.

Key Words:
Arsenic trioxide, Cardiac toxicity, Lipid peroxidation, Arsenic accumulation.

Introduction

Arsenic is one of the most dangerous occupational and environmental toxins. The drinking water sources of south-east Asia, especially West Bengal and Bangladesh are heavily contaminated by arsenic poisoning. A population of about 40 million is at risk in West Bengal alone[1]. Even though arsenic is an environmental pollutant, trivalent arsenic is being used as a drug for the treatment of chronic and acute leukaemia.

Arsenic trioxide (As_2O_3) is an effective chemotherapeutic agent for relapsed acute promyelocytic leukaemia (APL). The use of As_2O_3 to treat APL began at the Harbin Medical University in the 1970s[2]. However, the therapeutic use of As_2O_3 has been limited by its dose-dependent toxicity. Unfortunately, the clinical usefulness of As_2O_3 has been limited by its toxicity. Cardiac toxicity, including QT prolongation, torsades de pointes and sudden cardiac death, has been reported in many studies[3,4]. Due to these limitations, some patients are precluded from receiving a highly effective treatment.

Ventricular tachy-arrhythmias were reported in about 30% of patients treated with intravenous As_2O_3[5]. According to Siu et al[6], oral As_2O_3 is convenient and safe for prolonged arsenic treatment in patients with APL. About 80-90% of the ingested arsenic is absorbed from the gastrointestinal tract and gets methylated to monomethylarsonous acid and dimethylarsinic acid. Methylated species from inorganic arsenic metabolism are considered as relevant agents during arsenic carcinogenicity, especially through induction of oxidative stress and impairing DNA repair processes[7].

The exposure to arsenic can be through ingestion of arsenic-containing water, food and drugs (such as Fowler's solution containing 1% of potassium arsenite used to treat psoriasis; and arsenic trioxide used to treat leukaemia). Clinical application of anticancer agents has been often hampered by toxicity against normal cells, so the achievement of their cancer-specific action is still one of the major challenges to be addressed. One primary issue for cancer chemotherapy is how to kill cancer cells selectively without damaging

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normal cells. Experimentally, the selective cell death is partially achieved through various approaches. Clinically, however, poor selectivity of anticancer drugs can cause damage to normal cells, resulting in severe side effects and, thus, limits their clinical efficacy[8].

The present study was performed *in vivo* to understand the toxicological mechanism of As_2O_3 using it's clinically relevant concentrations.

Materials and Methods

Chemicals and Reagents

Arsenic trioxide, sodium pyruvate, thiobarbituric acid and triton X-100, phenazine methosulphate, nitroblue tetrazolium (NBT) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich, Bangalore, India. L-aspartate, α-oxoglutarate, 2,4-dinitro phenyl hydrazine, nicotinamide adenine dinucleotide (reduced), thiobarbituric acid, 5,5'-dithiobis-nitro benzoic acid, nicotinamide adenine dinucleotide phosphate (NADPH), reduced glutathione, 1-chloro-2,4 dinitro benzene (CDNB) were obtained from Merck Specialties Pvt. Ltd., Mumbai, India. All other chemicals were purchased from Sisco Research Laboratories (SRL), Mumbai, India.

Animals

Wistar male albino rats 180-200 g of body weight were used and standard laboratory food and water were provided. Experiments were conducted in accordance with the guidelines of Institutional Animal Ethical Committee, School of Biosciences, Mahatma Gandhi University.

Experimental Protocol

Rats were randomly divided into four groups; group I: – Normal control, Groups II-IV were treated with As_2O_3 (2, 4 and 8 mg/kg body weight) via oral intubation for a period of 45 days respectively. At the end of the experimental period, rats were decapitated; heart was removed immediately, washed in ice cold 0.15M NaCl and blotted on a filter paper. Then, the tissue was weighed and homogenized by using Teflon glass homogenizer ($1/10^{th}$ weight/volume) in ice cold tris-HCl buffer (0.2M, pH 7.4). The homogenate was centrifuged at 10,000 g for 20 min at 4°C and the supernatant was used for the estimation of lipid peroxidation and various enzymatic and non enzymatic assays.

Detection of Arsenic Accumulation in Cardiac Tissue

Heart tissue was digested by thermal acid microwave digestion and diluted with double distilled water. Total arsenic deposition in heart tissue was analyzed by standard inductively coupled plasma-optical emission spectroscopy (Optima 2000 DV ICP-OES, Perkin Elmer, Inc., Waltham, Massachusetts, USA).

Tissue Analysis

Tissue catalase (CAT) was determined from the rate of decomposition of H_2O_2[9]. Glutathione peroxidase (GPx) was determined by measuring the decrease in reduced glutathione (GSH) content after incubating the sample in the presence of H_2O_2 and sodium nitrite[10]. Glutathione reductase (GR) was assayed by the method of Goldberg and Spooner[11]. The amount of NADPH consumed during GSSG (glutathione oxidised) reduction was used as the index of enzyme activity. Superoxide dismutase (SOD) was measured by the method of Kakkar et al[12]. One unit was taken as the amount of enzyme that gave 50% inhibition of nitroblue tetrazolium (NBT) reduction/mg protein. Glutathione-S-transferase (GST) was determined from the rate of increase in conjugate formation among reduced glutathione and 1-chloro-2,4-dimitrobenzene (CDNB)[13]. Reduced GSH was determined according to the method of Ellman[14] based on the formation of a yellow coloured complex with β-dystrobrevin (DTNB). The lipid peroxidation was measured as malondialdehyde (MDA), a thiobarbituric acid reacting substance (TBARS), using 1'1'3'3' tetramethoxypropane as standard[15]. Protein content in the tissue was determined[16] using BSA as the standard.

Histopathology

Small sections of heart were fixed in 10% buffered formalin and processed for embedding in paraffin. Sections of 5-6 μm were stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Wetzlar, Germany). The microphotographs were taken using Moticam-1000 camera at original magnification of 100X.

Statistical Analysis

Data were analyzed by one way analysis of variance, followed by LSD post hoc multiple comparison test to determine significant difference among groups. Effects with a probability of $p < 0.05$ was considered to be significant.

Results

Arsenic Accumulation in Heart

The arsenic concentration in cardiac tissue was found increased with the increasing concentration of As_2O_3. The deposition of arsenic in treated groups showed significant variations ($p < 0.05$) with the control group. Accumulation of arsenic was very high in 8 mg/kg body weight arsenic treated rats (Table I).

Effect of Lipid Peroxidation and Antioxidant Defence System

Table I shows lipid peroxidation, reduced glutathione and glutathione dependent antioxidant enzymes and antiperoxidative enzymes in the heart tissue of normal and arsenic groups. A significant ($p < 0.05$) increase in tissue MDA was observed in As_2O_3 treated rats. The lipid peroxidation was directly proportional to the concentration of arsenic, i.e., the lipid peroxidation was found to be increased with the increase in concentration of arsenic in treated rats. A significant ($p < 0.05$) decline in GSH was noted in heart tissue of group III arsenic administered rats as compared to group I controls. Also a significant ($p < 0.05$) reduction in glutathione dependent antioxidant enzymes (GPx and GST) and antiperoxidative enzymes (SOD and CAT) were observed in group III when compared with normal control group. The As_2O_3 exposure significantly reduced GR compared to normal control ($p < 0.05$).

Histopathological Changes

In light microscopic examinations, no significant pathological changes were observed in the cardiac tissue of the control group (Figure 1A). In group II the myocardial fibres showed mild swelling more towards pericardial zone and around vessels and there was mild interstitial oedema (Figure 1B). In group III rat's myocardial fibres showed more pronounced cellular swelling, near epicardium and also near the endocardium. And also a noticeable capillary congestion and fibre separations were observed (Figure 1C). The myocardial fibres were markedly swollen in different zones. Focal lymphocytic infiltrations towards the pericardium, capillary congestion, necrosis and micro-haemorrhages were noticed in group IV rats (Figure 1D).

Discussion

In humans and several experimental animals, inorganic arsenic is enzymatically methylated in-

Table I. As_2O_3 induced arsenic accumulation and biochemical alterations in heart tissue.

Groups	Arsenic concentration in Heart (ppm)	MDA (nM/mg protein)	SOD (U/mg protein)	GPx µg of GSH (consumed/ min/mg protein)	Catalase (µ moles of H_2O_2 consumed/ min/mg protein)	GSH (µM/g tissue)	GST (µM of CDNB-GSH conjugate formed/min/ mg protein)	GR (nmol of NADPH oxidised/ min/mg protein)
Normal control	0	0.72 ± 0.02	7.36 ± 0.41	3.96 ± 0.29	20.46 ± 1.36	6.7 ± 0.93	4.1 ± 0.52	82.15 ± 3.49
As_2O_3 2 mg/kg	1.38 ± 0.09	0.84 ± 0.03	6.19 ± 0.21	3.08 ± 0.15	14.85 ± 1.0	4.82 ± 0.4	2.56 ± 0.29	76.27 ± 1.21
As_2O_3 4 mg/kg	1.57 ± 0.07*	0.9 ± 0.02*	5.37 ± 0.18*	2.12 ± 0.2*	11.7 ± 0.72*	3.72 ± 0.29*	1.56 ± 0.16*	72.04 ± 0.67*
As_2O_3 8 mg/kg	2.01 ± 0.04#	1.03 ± 0.08#	4.42 ± 0.32#	0.99 ± 0.26#	9.67 ± 0.62#	1.65 ± 0.5#	0.9 ± 0.11#	70.19 ± 0.91#

Data represented as mean ± SD, n=6. *$p < 0.05$Vs normal control, #$p < 0.05$ Vs normal control

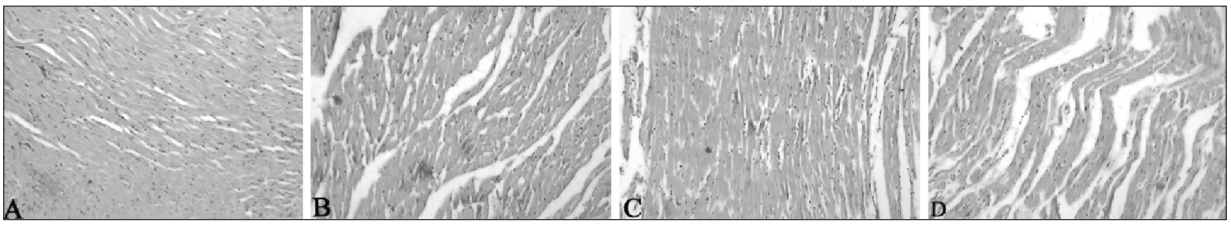

Figure 1. Histopathology of heart tissue. Histopathological changes occurred in rat's heart tissue after As_2O_3 administration (Hematoxylin and Eosin, 100 ×). ***A,*** Normal control. ***B,*** As_2O_3 (2 mg/kg body weight). ***C,*** As_2O_3 (4 mg/kg body weight). ***D,*** As_2O_3 (8 mg/kg body weight).

to organic arsenic, such as monomethylarsonic acid and dimethylarsinic acid. They are the major organic pentavalent arsenic metabolites in human urine after the exposure to inorganic arsenic. Dimethylarsinic acid is the ultimate metabolite in humans, while it is further methylated to trimethylarsine oxide in some rodents[17]. It has also been reported that methylated arsenic compounds accumulate during chronic arsenic poisoning in human body[18]. Inorganic arsenic deposition was found to be the most significant cause of toxicities in hepatic and neuronal cells of rats treated with arsenite[19]. The deposition of arsenic in heart was elevated with the increase in concentration of arsenic in our study.

Exposure to monomethyl arsenic or trimethyl arsenic induced significant tolerance to the acute toxicity of inorganic arsenate. GSH play a critical role in arsenic tolerance[17]. In the present study, the cellular GSH and GST level were found decreased with the increase in concentration of As_2O_3. GSH may decrease the cytolethality of arsenic through several processes, possibly through its role as an antioxidant, as a cofactor in the enzymatic methylation reaction of arsenic or by direct binding to arsenic and, thereby, reducing the toxic potential, or through enhanced efflux of an arsenic-GSH conjugate[20]. GST catalyses the formation of arsenic-GSH conjugates[21,22].

Antioxidant enzymes are considered as the first line of cellular defence that prevents cellular ingredients from oxidative damage. Among them superoxide dismutase (SOD) and catalase (CAT) mutually function as important enzymes in the elimination of reactive oxygen species (ROS). In order to remove the excess free radicals from the system, GST and GPx utilise GSH during their course of reactions[23]. In the present study, a dose dependent significant rise in the activity of lipid peroxidation with concomitant decline in the level of reduced glutathione (GSH) and in the activities of glutathione dependent antioxidant enzymes: glutathione peroxidase (GPx) and glutathione-S-transferase (GST) and antiperoxidative enzymes: SOD and CAT were observed in heart tissue of arsenic treated rats. The arsenic induced reduction in GSH concentration simultaneously reduced the activity of GSH-regenerating enzyme, GR.

Lipid peroxidation is a primary measure of oxidative damage in cardiac tissue. In the present study, arsenic induced a greater degree of lipid peroxidation in the cardiac tissue. Arsenic exposure is known to stimulate the release of free iron from ferritin[23] and the resulting free iron is believed to be one of the potent inducer of ROS formation via the Fenton-type reaction. Arsenic intoxication decreased the ability of intracellular antioxidant power and, hence, attenuated the reducing ability of Fe (III) to Fe (II)[24].

As_2O_3 treatment caused infiltration of inflammatory cells, myocardial disorganisation and interstitial oedema in the heart. As_2O_3 acts at the structural level, which results in deposition and binding of arsenic to tissues[25]. In the present study, arsenic accumulation in cardiac tissue leads to the generation of free radicals and this might be the reason for structural abnormalities. Histopathological examination reveals that arsenic treatment caused fibre swelling, necrosis and haemorrhages in the cardiac tissue. The extent of tissue damage was found sequentially increased with the increase in arsenic concentration in experimental rats.

Arsenic trioxide is a promising drug for the treatment of APL. Our findings show that even in its clinical concentration itself As_2O_3 is poisonous via the generation of ROS and arsenic accumulation. The arsenic poisoning is a dose dependent toxicity, which can result in cardiac toxicities. So we suggest further development and refinement of pharmacokinetic and pharmacodynamic swot ups for the clinical exploration of arsenic.

Acknowledgements

We thank the University Grants Commission, New Delhi for rendering financial support for the study (F. No: 39-683/2010 SR) and the award of research fellowship in sciences for meritorious student to Mr. Mathews. V. Varghese (No. F. 4-1/2006 (BSR)/11-29/2008 (BSR).

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