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Full Length Article

Effect of arsenic acid withdrawal on hepatotoxicity and disruption of erythrocyte antioxidant defense system

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ABSTRACT

We investigated the effects of withdrawal from Sodium arsenite ($NaAsO_2$) on the hepatic and antioxidant defense system in male Wistar rats using a before and after toxicant design. Rats were orally gavaged daily with varying doses of $NaAsO_2$ for a period of 4 weeks. One half of the population was sacrificed and the remaining half had the toxicant withdrawn for another further 4 weeks. Biochemical and immunohistochemical techniques were used to assess the impact of withdrawal on the erythrocyte and hepatic systems. Exposure of Wistar rats to $NaASO_2$ led to a significant ($p < 0.05$) increase in hepatic and erythrocyte markers of oxidative stress (malondialdehyde, thiol contents and hydrogen peroxide generation). Concurrently, there was a significant ($p < 0.05$) increase in hepatic and erythrocyte antioxidant enzymes (glutathione-S-transferase, glutathione peroxidase and superoxide dismutase) following exposure. Withdrawal from $NaAsO_2$ exposure led to a decline in both erythrocyte and hepatic markers of oxidative stress and together with a significant improvement in antioxidant defense system. Histopathology and immunohistochemistry revealed varying degrees of recovery in hepatocyte ultrastructure alongside increased expression of the pro-survival protein Kinase B (Akt/PKB) after 4 weeks of $NaAsO_2$ withdrawal. Conclusively, withdrawal from exposure led to a partial recovery from oxidative stress-mediated hepatotoxicity and derangements in erythrocyte antioxidant system through Akt/PKB pathway.

1. Introduction

Of the many naturally occurring elements found abundantly distributed in the earth's crust, arsenic has found its way into prominence as a toxicant of significant public health risk [1]. A heavy metal, specifically classed as a metalloid [2], it is naturally produced during processes such as volcanic eruptions and the biodegradation of other organic minerals and rocks [3]. Increased dependency on arsenic among other heavy metals for anthropogenic causes has resulted in widespread release of arsenic by-products into the environment [4]. Human exposure is then unavoidable, not just from occupational causes, but also from atmospheric pollution, ingestion of contaminated food and water sources and from contact with certain finished industrial products [5].

Of the routes to exposure, the most important source, by far appears to be dietary. Tchounwou et al. [1] report that each individual has an average intake of 50 μg per day. Recent studies show a rise in the levels of inorganic arsenic in food items, especially rice, a staple of third world and underdeveloped countries [6–8]. Arsenic was found to accumulate more in the liver than other tissues after one month of exposure, when administered orally and subcutaneously. However, after 3 months of exposure, was found to accumulate more in the kidney than in the liver or other tissues. [9]. With a high correlation established between exposure and increased health risks, and an uptick in the incidence of health-related conditions among affected populations, it is no wonder arsenic is listed as one of the high malignancy causing elements today [10,11].

Certain heavy metals as cobalt and copper have been classified as essentials due to their absolute requirements in trace quantities for normal biochemical and physiological functions in the human body [12]. Arsenic, on the other hand, has been found to have no beneficial physiologic role in the human body and is classified as a non-essential

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metal [13]. Even at low exposure levels, it has been shown to cause multiple organ damage by generating reactive oxygen species (ROS) and promoting oxidative stress: a redox deficiency or imbalance that can cause bimolecular damage [2,14], disrupting cellular structures and components [15], affecting detoxification and repair enzymes [16], causing DNA damage, disrupting normal cell cycle regulation and in extreme cases, cancer and cell death [17].

Free radicals are produced in living organisms during normal metabolism (e.g., the reactions of mitochondrial respiratory chain and cytochrome P450), inflammation, phagocytosis and other physiological processes. The most important category of free radicals is constituted by ROS, such as superoxide radical anion ($O_2 \cdot -$), hydroxyl radical ($OH \cdot$), peroxyl radical ($RO \cdot_2$) and hydrogen peroxide (H_2O_2) [18]. The mechanisms of liver toxicity and damage in chronic arsenic toxicity are primarily a consequence of increased free radical activity and oxidative stress [19].

From this point, two possible routes to hepatic damage have been reported: The activation of kinase signaling molecules such as C-Jun N-terminal kinases (JNK), p38 Mitogen activated protein kinase (p38 MAPK) and cytochrome P450, leading to cell lysis, apoptosis and accumulation of bile acids [20,21], or increased peroxidation of lipids which also cause hepatocyte damage [22]. It has been reported that in erythrocytes, $NaAsO_2$ can absorb into blood circulation and bind to hemoglobin. This might lead to the oxidation of sulfhydryl groups, which are critical for heme synthesis, with a concomitant reduction in oxygen uptake capabilities [19]. Also, significant decreases are triggered in erythrocyte reduced glutathione levels, coupled with increases in malondialdehyde and protein carbonyl levels, all indicative of oxidative damage to erythrocyte membranes. Grossly, this is evident as distorted, misshapen erythrocytes [23].

In poor resource regions of the world where government regulatory mechanisms are weakened or virtually non-existent, and where indiscriminate dumping of heavy metals and their metabolites occur on a daily basis, the dangers to health from arsenic exposure persist. We explore to see the effects of withdrawal from exposure on the known disease states arising from the multi-organ damage associated with this toxicant.

2. Materials and methods

2.1. Chemicals

Epinephrine, $NaAsO_2$, Hydrogen peroxide (H_2O_2), hydrochloric acid, sulphuric acid, xylenol orange, sodium hydroxide, potassium iodide, reduced glutathione (GSH), potassium dichromate, O-dianisidine, sodium potassium tartarate, copper sulphate, glacial acetic acid, ethanol, sodium azide, 2-dichloro-4-nitrobenzene (CDNB), thiobarbituric acid (TBA), Trichloroacetic acid, Ellman's reagent (DTNB), ammonium ferrous sulphate, sorbitol was purchased from Sigma (St Louis, MO, USA). Normal goat serum, Biotinylated antibody and Horse Radish Peroxidase (HRP) System was purchased from (KPL, Inc., Gaithersburg, Maryland, USA). Akt/PKB antibody was purchased from (Bioss Inc. Woburn, Massachusetts, USA) while 3, 3′-Diaminobenzidine (DAB) tablets were purchased from (AMRESCO LLC. Ohio, USA). All other chemicals used were of analytical grade and obtained from British Drug Houses (Poole, Dorset, UK).

2.2. Experiment and design

80 healthy adult male Wistar rats (200–250 g) obtained from the Experimental animal unit of the Faculty of Veterinary Medicine, University of Ibadan were used for this study. The animals were handled humanely according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Ethical regulations were followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments [24].

All animals were kept in wire mesh cages under controlled light cycle (12 h light/12 h dark) and temperature of 32 ± 3 °C. The rats were fed with commercial rat chow (Ladokun Feeds Nigeria Limited) *ad libitum* and liberally supplied with clean tap water. Randomly, they were assigned into four groups comprising twenty animals per group. Group A was provided tap water with no test substance added. Group B was treated with tap water into which $NaAsO_2$ was dissolved at 10 mg/kg/day; Group C was treated with tap water into which $NaAsO_2$ was dissolved at 20 mg/kg/day; Group D was treated with tap water into which $NaAsO_2$ was dissolved at 40 mg/kg/day.

One half of the population was sacrificed at the end of 4 weeks of $NaAsO_2$ exposure. $NaAsO_2$ was withdrawn from the remaining rats and they were administered clean tap water only for another 4 weeks. The rats were monitored weekly for clinical signs of toxicity. After four weeks, the remaining animals were sacrificed. For each phase of sacrifice, the rats were starved overnight and the following procedure was observed. Blood was drawn from the retro-orbital venous plexus of the animals into vials containing heparin as an anticoagulant. The animals were then sacrificed by cervical dislocation. The liver was removed, rinsed in 1.15% KCl and homogenized in aqueous potassium phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000*g* for 20 min to obtain the supernatant fraction stored at 4 °C till use.

2.3. Preparation of erythrocytes for biochemical assays

The preparation of erythrocytes was described according to the method of Steck and Kant [25]. The erythrocytes were washed three times with ice-cold phosphate buffer saline at pH 7.4, and centrifuged. The erythrocyte membrane was lysed and the pellets were resuspended in PBS at 1:109 dilutions until the time of use. The pellets thus obtained were washed repeatedly in the same buffer to obtain haemoglobin-free white membranes.

2.4. Biochemical assays and hematological parameters

Protein concentration was carried out as described by Gornall et al. [26]. Hydrogen peroxide generation was evaluated as described by Wolff [27]. The malondialdehyde concentration was determined according to Farombi et al. [28]. Nitric oxide was quantified as described by Olaleye et al. [29], while the thiol contents were estimated by the method of Ellman [30].

The erythrocyte and hepatic reduced glutathione (GSH) concentration was determined using the method of Jollow et al. [31]. The superoxide dismutase (SOD) activity was evaluated by the method of Misra and Fridovich [32] with slight modification [33]. Glutathione peroxidase (GPx) and Glutathione-S-transferase (GST) activities were measured by the method of Rotruck et al. [34] and Habig et al. [35], respectively. The packed cell volume (PCV) was determined by microhaematocrit method. The haemoglobin (Hb) concentration was determined by Cyanmethaemoglobin method while red blood cell (RBC) and white blood cell (WBC) counts were determined using an haemocytometer.

2.5. Histopathology

Small pieces of liver tissues were collected in 10% buffered formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Sections of 5–6 μm in thickness were made and stained with haematoxylin and eosin for histopathological examination [36].

2.6. Immunohistochemistry of protein kinase B

Immunohistochemistry of paraffin embedded tissue of the liver was performed after the tissues were obtained from buffered formalin

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