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High fat diet aggravates arsenic induced oxidative stress in rat heart and liver

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ABSTRACT

Arsenic is a well known global groundwater contaminant. Exposure of human body to arsenic causes various hazardous effects via oxidative stress. Nutrition is an important susceptible factor which can affect arsenic toxicity by several plausible mechanisms. Development of modern civilization led to alteration in the lifestyle as well as food habits of the people both in urban and rural areas which led to increased use of junk food containing high level of fat. The present study was aimed at investigating the effect of high fat diet on heart and liver tissues of rats when they were co-treated with arsenic. This study was established by elucidating heart weight to body weight ratio as well as analysis of the various functional markers, oxidative stress biomarkers and also the activity of the antioxidant enzymes. Histological analysis confirmed the biochemical investigations. From this study it can be concluded that high fat diet increased arsenic induced oxidative stress.

1. Introduction

It has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential elements like arsenic (As) (Clarkson, 1995). Among these metals, arsenic exhibits a complex metabolism and is possibly the most abundant and potential carcinogen (El-Demerdash et al., 2009). Arsenic is present in nature in stable form as As^{5+} species, and As^{3+} species.

Exposure of animals and humans to different metal components through contaminated drinking water can result in a wide range of adverse clinical conditions. Arsenic pollution in the environment is becoming a major concern for environmental and occupational health, owing to its widespread toxic and multidimensional effects on humans and aquatic life and plants through polluted ground water and food chains (Chowdhury et al., 2000). Groundwater contamination with arsenic in West Bengal, India, is reported to be the largest arsenic calamity in the world (Chowdhury et al., 2000). Arsenic toxicity involves oxidative damage that plays a vital role for biochemical alteration (Nandi et al., 2006). Decreased level of antioxidants and increased levels of oxidation products in blood were reported in human population exposed to arsenic (Wu et al., 2001). Various studies reported that arsenic could participate in the cellular oxidation–reduction reactions resulting in the formation of excess reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$) and free radicals such as hydroxyl radical ($OH^{\cdot}$) via a chain reaction (Valko et al., 2005). The potential role of oxidative stress in the injury associated with arsenic poisoning suggests that antioxidants may enhance the efficacy of treatment protocols designed to mitigate arsenic induced toxicity. In particular, arsenic induces oxidative DNA damage and lipid peroxidation (Pineda-Zavaleta et al., 2004). In contrast to the extensive fetal exposure in women exposed to arsenic during pregnancy, the breast-fed infant is protected against arsenic exposure because the excretion of arsenic in breast milk is limited (Bhattacharya et al., 2012). There is wide variation in susceptibility to arsenic-induced toxicity, and

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there is reason to believe that nutrition is an important susceptibility factor. A number of studies have shown associations between the prevalence or severity of arsenic related health effects and indicators of food and nutritional status (Guha Mazumder et al., 1998), suggesting that people with poor nutrition are particularly susceptible. Although these studies mainly concern health effects in adult life, it seems likely that nutrition also may modify the arsenic induced effects of early life.

A number of studies have shown arsenic-induced formation of reactive oxygen and nitrogen species as well as elevated DNA oxidation (Pineda-Zavaleta et al., 2004). The toxic effects of such events are highly dependent on defense mechanisms in the body (Rana et al., 2010), i.e., the status and dietary intake of antioxidants. It is becoming increasingly evident that arsenic not only induces reactive oxygen and nitrogen species but also affects the defense against those species. Inorganic arsenic has been shown to inhibit several of the antioxidant systems in the body, such as glutathione, glutathione peroxidase, thioredoxin reductase, and superoxide dismutase (Mazumder, 2005). Antioxidant supplementation such as Vitamin C supplementation can mitigate the arsenic induced oxidative stress in goat (Das et al., 2012). Chronic inorganic arsenic exposure through drinking water in mice produced liver pathology, and a high fat diet greatly enhanced arsenic induced liver fibrosis (Wu et al., 2008).

There is a wide variation in susceptibility to arsenic toxicity, which is likely to be related to factors such as variation in arsenic metabolism, nutrition, host-related defense mechanisms and genetic predispositions. The main mechanisms of arsenic–nutrition interactions include arsenic-induced oxidative stress, which involves nutrient-dependent defense systems and arsenic metabolism via 1-carbon metabolism, which requires methyl groups, folic acid, vitamin B-12, and betaine for the remethylation step. This step seems to be most critical from a toxicological point of view. A second mode of arsenic-nutrition interaction involves epigenetic effects and fetal programming via DNA methylation (Vahter, 2007).

Till date, there is no evidence of effect of high fat diet on arsenic induced toxicity in other organs than the liver. Development of modern civilization and improvement of technologies caused changes in lifestyle of the people which is not confined to urban area but also in rural area. One of the adverse impacts of this is excess use of junk food containing high level of fat. So, our present study has been carried out to test the causal relationship between arsenic generated oxidative stress and high fat diet. The results indicate increased levels of oxidative stress and tissue damage in rat heart and liver when the animals were co-treated with arsenic and high fat diet (HFD).

2. Materials and methods

2.1. Chemicals

Sodium arsenite was purchased from Sigma Aldrich, USA. All the other chemicals used including the solvents, were of analytical grade and obtained from Sisco Research Laboratories (SRL), Mumbai, India, Qualigens (India/Germany), SD fine chemicals (India), Merck Limited, Delhi, India.

2.2. Animals

Male Wistar rats, weighing 140–200 g, were obtained from a CPCSEA registered animal supplier. The animals were handled as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Empowerment, Government of India. The experimental protocols had the approval of the Institutional Animal Ethics Committee (IAEC) of the Department of Physiology, University of Calcutta. Prof. P.K. Samanta, M.Sc. (Vet.), Ph.D., Professor and Veterinary Surgeon and CPCSEA Nominee to Department of Physiology, University of Calcutta, acted as the advisor for animal care and handling and continuously monitored animal experimentations.

2.3. Experimental design

2.3.1. Induction of myocardial and hepatic damages with arsenic: a dose–response study

During experiment, the animal room was maintained at a temperature of 25 ± 1 °C, humidity 50 ± 10% and a 12-h light/dark cycle and the rats were allowed to access standard diet containing 18% protein (casein) and water *ad libitum* for 7 days (quarantine period). The 18% protein diet was considered as an adequate dietary protein level, which was used on earlier occasions (Nayak and Chatterjee, 2002).

Myocardial and hepatic damages were induced in rats by intraperitoneal (i.p.) injection of sodium arsenite. Briefly, male rats were divided into four groups. Each group of animals comprised of 6 rats. The rats of the first group constituted the vehicle-treated control. The rats of the second, third and the fourth group were injected i.p., respectively, with different doses of sodium arsenite (0.325, 0.75, 1.5 mg/kg body weight, i.e. 2.5%, 5% and 10% of LD_{50}) for 8 consecutive days where water was used as the vehicle. After the completion of treatment the animals were sacrificed by cervical dislocation following mild ether anesthesia. The heart and the liver were surgically extirpated after carefully opening the thoracic and abdominal cavity and the tissues were washed thoroughly in cold saline. The saline was soaked properly with a piece of blotting paper from the tissues and the tissues were stored at −20 °C for further biochemical analyses. Prior to sacrifice, the blood was collected by cardiac puncture for the preparation of the serum. The cardiac and liver damages following arsenic treatment were confirmed by measurement of the level of activities of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) and also by measurement of oxidative stress biomarkers (i.e. the levels of lipid peroxidation and reduced glutathione content) in cardiac and hepatic tissues.

2.3.2. Induction of myocardial and hepatic damages with arsenic: a time dependent study

Myocardial and hepatic damages were induced in rats by intraperitoneal (i.p.) injection of sodium arsenite. The rats were divided into sixteen groups. Each group of animals comprised of 6 rats. The rats of the first four groups constituted the vehicle-treated controls for 2 days, 4 days, 8 days and 12 days, respectively. The rats of the fifth, sixth, seventh and the eight group were injected i.p., respectively for 2 days, 4 days, 8 days and 12 days with sodium arsenite at a dose of 0.75 mg/kg body weight, i.e. 5% of LD_{50} where water was used as the vehicle. Four groups of animals were treated with high fat diet only for 2 days, 4 days, 8 days and 12 days, respectively. The last four groups were co-treated with sodium arsenite (0.75 mg/kg body weight, i.p.) for 2 days, 4 days, 8 days and 12 days and high fat diet, respectively. After the completion of treatment, the animals were sacrificed by cervical dislocation following mild ether anesthesia. The heart and the liver were surgically extirpated after carefully opening the thoracic and abdominal cavity and the tissues were washed thoroughly in cold saline. The saline was soaked properly with a piece of blotting paper from the tissues and the tissues were stored at −20 °C for further biochemical analyses. Prior to sacrifice, blood was collected by cardiac puncture for the preparation of serum. Pre-experimental body weights of rats before treatment and at the end of the treatment period, i.e., the post-experimental body weights of rats of all the groups were noted. Heart weight of every animal was measured after sacrifice of the animals and heart weight to body weight ratio was determined using the body weight of every animal on the third day (i.e., the day of sacrifice of the two days treatment group), fifth day (i.e., the day of sacrifice of the four days treatment group), ninth day (i.e., the day of sacrifice of the eight days treatment group) and thirteenth day (i.e., the day of sacrifice of the twelve days treatment group), respectively. The cardiac and liver damages following arsenic treatment were confirmed by measurement of the level of activities of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) and also by measurement of oxidative stress biomarkers (i.e. the levels of lipid peroxidation and reduced glutathione content) in cardiac and hepatic tissues. A portion of the extirpated rat heart and liver were fixed immediately in 10% formalin and embedded in paraffin following routine procedure as used earlier by Mukherjee et al. (2012) and Mitra et al. (2012). Sections of heart and liver tissues (5 μm thick) were prepared. Heart tissues were stained with hematoxylin–eosin stain and the liver tissue sections were stained separately with hematoxylin–eosin stain and periodic acid Schiff (PAS) stain. The stained tissue sections were examined under Leica microscope and the images were captured with a digital camera attached to it.

2.3.3. Co-treatment of rats with high fat diet and arsenic

In a separate experiment, the animal room was maintained at a temperature of 25 ± 1 °C, humidity 50 ± 10% and a 12-h light/dark cycle and the rats were allowed to access standard diet containing 18% protein (casein) and water *ad libitum* for 7 days (quarantine period). The 18% protein diet was considered as an adequate dietary protein level, which was used on earlier occasions (Nayak and Chatterjee, 2002).

Initially the animals were divided in such a way that the average weight of control and treated rats remained within a range of 145–170 g. The animals were divided into four groups. Each group of animals comprised of 6 rats:-

Group I: Control Animals (Control)
Group II: Animals fed on high fat diet (HFD)
Group III: Animals treated with arsenic (As)
Group IV: Arsenic treated animals fed on high fat diet (As + HFD.)