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Toxicology

Suppression of the brain-pituitary-testicular axis function following acute arsenic and manganese co-exposure and withdrawal in rats

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ABSTRACT

Despite the fact that most environmental exposures to metals do not occur in isolation, the combined effects of metal mixtures on brain-pituitary-gonadal axis are poorly known. The present study investigated the impacts of co-exposure to arsenic (As) and manganese (Mn) on sperm characteristics, reproductive hormones and selected oxidative stress indices in the brain, testes and epididymis of rats following exposure for 15 consecutive days to 60 mg/L of AsO_2Na and 30 mg/L of $MnCl_2$ in drinking water. The results showed that while the brain weight remained unaffected, the fluid intake and the weights of testes and epididymis significantly ($p < 0.05$) decreased in all the treatment groups. A significant decrease in the body weight gain when compared with control was noted only in the co-exposed rats. Moreover, the significant decreases in the antioxidant status in brain, testes and epididymis as well as in the circulatory concentrations of follicle-stimulating hormone, luteinizing hormone and testosterone were similar following separate or combined exposure of rats to As and Mn. The marked oxidative damage in the investigated tissues was accompanied by a significant decrease in the sperm quantity and quality in all the treated rats when compared with the control. Interestingly, most of the parameters determined immediately after the exposure period persisted in rats from the withdrawal experiment. Collectively, co-exposure to As and Mn suppressed the brain-pituitary-testicular axis function and the post-testicular events such as sperm function possibly via a mechanism involving persistent oxidative stress and endocrine disruption in the exposed rats.

1. Introduction

Arsenic (As) is a crystalline "metalloid" with characteristics intermediate between metals and non-metals. It was ranked as the 20th most occurring trace element in the earth's crust, 14th in seawater, and 12th in the human body [1]. Manganese (Mn) is a ubiquitous, naturally occurring trace metal [2]. There is a growing body of evidence indicating that environmental exposure to As and Mn and their mixtures poses public health threats to animals and humans [3,4]. The enrichment and pollution of surface sediments and groundwater by arsenic (As) and manganese (Mn) in several countries including West Bengal in India, Bangladesh, Italy, China and Nigeria have been reported [5–11]. The elevated levels of As and Mn in sediments and groundwater have been attributed to the intense anthropogenic activities in agriculture and unregulated discharge of industrial wastes into rivers [12]. Both As and Mn coexist in occupational contexts and the populations living in these contaminated areas are exposed to higher levels of this mixture through water, soil and food contamination [13,14]. Recent study conducted in Rio Branco, Acre, Brazil revealed higher blood levels of As and Mn in individuals living near industrial facilities, working in a glass factory, a compost plant or in metal mining activities than in other non-occupationally exposed populations [15]. Thus, humans are most often exposed to harmful chemical mixtures than one chemical.

Moreover, since exposure to environmental contaminants elicits different sorts of adverse effects in reproductive systems, no single parameter is reliably the most sensitive indicator to predict reproductive health risks [16]. The male reproduction in vertebrates is tightly regulated by the brain-pituitary-gonadal axis. Generally, the brain secretes the gonadotropin-releasing hormone (GnRH) which stimulates the pituitary to produce follicle-stimulating hormone (FSH) and luteinizing hormone (LH) which, in turn, controls Leydig cells to produce and secrete testosterone necessary for spermatogenesis and regulation of the male phenotype [17,18]. Previous investigations showed that chronic exposure to either As or Mn induced oxidative stress and impaired testicular function of spermatogenesis and steroidogenesis in laboratory

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animals [16,19–23]. However, the severity of oxidative damage depends on the degree of perturbation in normal redox status within the cells. While a cell can regain its original functional state after overcoming small perturbations, more severe oxidative stress can cause cell death and necrosis [24]. Although As and Mn often occur in mixtures, there is no detail information in literature on the effects of their co-exposure on reproductive endpoints. Toxicological effects due to chemical-chemical interaction may result in a stronger effect i.e. additive or synergistic, or a weaker effect i.e. antagonism or inhibition [25]. In addition, considering the antioxidant defense capacity of the brain and reproductive system, there is a need to investigate whether testicular damage resulting from co-exposure to As and Mn is transient or permanent. Data on toxicants withdrawal effects are significant for extrapolating exposure effects from animals to humans [24].

The present investigation therefore sought to evaluate the nature, the mechanism and the possible reversibility of the brain-pituitary-testicular axis toxicity induced by As and Mn co-exposure in rats using an array of endpoint markers of toxicity to provide a more comprehensive understanding of the noxious effects of As and Mn mixture on the reproductive system. In addition, the influence of As and Mn co-exposure on the epididymis which is responsible for the transport and storage of sperm cells was investigated.

2. Materials and methods

2.1. Chemicals

Sodium (meta) arsenite (AsO_2Na; ≥99%), manganese chloride ($MnCl_2{\cdot}4H_2O$; 99.99%), 5′, 5′-dithio-bis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), thiobarbituric acid, glutathione, epinephrine and hydrogen peroxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Experimental animals and research design

Sixty-four male Wistar rats (8 weeks old, 104 ± 8 g) obtained from the Department of Biochemistry, University of Ibadan, Ibadan, Nigeria, were used for this study. The animals were housed in plastic cages placed in a well-ventilated rat house, provided rat chow and water *ad libitum* and subjected to natural photoperiod of 12-h light: 12-h dark. All the animals received humane care according to the criteria stated in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health. The experimental protocols were carried out after approval by the University of Ibadan Ethical Committee. The ethical regulations have been followed in accordance with national and institutional guidelines for the protection of animal welfare during experiments [26]. The rats were randomly assigned to four groups of 16 rats each. The research design involves treatment of the rats with the As and Mn for 15 consecutive days as follows:

Group 1: Control rats exposed to normal drinking water
Group 2: Rats exposed to 60 mg AsO_2Na/L via drinking water
Group 3: Rats exposed to 30 mg $MnCl_2$/L via drinking water
Group 4: Rats exposed to the metal mixture (As and Mn) at the same doses and conditions as the single metal exposed groups.

The dose of AsO_2Na was chosen from previously published data [27] whereas the dose of $MnCl_2$ was chosen from the various doses (15, 30, 150 and 300 mg/L) investigated in our preliminary studies (data not shown). Although people, of both sexes and all ages, are usually exposed to environmental pollution for long periods of time, the 15 days exposure time was chosen based on the preliminary experiments conducted to determine concentrations that would result in reproductive toxicity within short-term of exposure. The duration of the present study corresponds to about a quarter (1/4) of the time required to achieve a cycle of spermatogenesis in rats [28,29]. We chose arsenite instead of arsenate because in most of the reported incidences of contaminated water As occurs as arsenite and its oxidation to arsenate is necessary for complete As removal [30].

One-half of the rats from each group was sacrificed on day 16, and the remaining half stayed for an additional 15 days without treatment before they were sacrificed. In each case, the rats were sacrificed by cervical dislocation and blood collected from retro-orbital venous plexus using heparin containing tubes. Plasma samples were separated from blood cells by centrifugation at 3000*g* for 10 min. The plasma samples were subsequently stored frozen at −20 °C until the determination of hormones concentrations using ELISA strip reader (Robonik India Private Limited, Mumbai, India). The brain, testes and epididymides were quickly removed, weighed and then processed for biochemical determinations and histology.

2.3. Determination of plasma concentrations of pituitary and testicular hormones

The commercial enzyme immunoassay kits specific for rats were used to assay the plasma concentrations of pituitary hormones namely LH (RPN 2562, Amersham, UK), FSH (RPN 2560, Amersham, UK) and plasma concentration of testosterone was assayed using EIA-5179 (DRG Diagnostics GmbH, Marburg, Germany) as per the manufacturer's instruction. The sensitivity of LH was 0.07 ng at 90% and FSH sensitivity was 0.06 ng at 95%. The intra-assay coefficients of variations were 3.5% for LH and 3.8% for FSH. The sensitivity of the testosterone assay was 0.04 ng/mL with negligible cross-reactivity with other androgen derivatives like methyl testosterone, 5α-dihydrotestosterone and androstenedione. The intra-assay coefficient of variation was 3.8%. Inter-assay variation was avoided by assaying all the samples on the same day.

2.4. Determination of antioxidant status and oxidative stress indices

The post-mitochondrial fractions of the brain, testes and epididymis of the rats were obtained by homogenizing the tissues in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride. Subsequently, the homogenates were centrifuged at 12,000*g* for 15 min at 4 °C and the supernatant collected for biochemical determinations. Protein concentration was assayed according to the method of Lowry et al. [31]. Superoxide dismutase (SOD) activity was assayed according to the method described by Misra and Fridovich [32] whereas catalase (CAT) activity was assayed using hydrogen peroxide as a substrate according to the method described by Clairborne [33]. Glutathione-S-transferase (GST) activity was assayed according to the method of Habig et al. [34]. The level of reduced glutathione (GSH) was assayed at 412 nm according to the method described by Jollow et al. [35] whereas hydrogen peroxide (H_2O_2) generation was determined according to the method described by Wolff [36]. Lipid peroxidation was quantified by measuring malondialdehyde, an end product of lipid peroxidation, according to the method described by Farombi et al. [37].

2.5. Sperm progressive motility assay

The sperm progressive motility of the experimental rats was evaluated according to the method of Zemjanis [38]. Briefly, epididymal sperm was obtained by cutting the caudal epididymis with surgical blades and released onto a sterile clean glass slide. Subsequently, the sperm was diluted with 2.9% sodium citrate dehydrate solution which had been pre-warmed to 37 °C, mixed carefully