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Arsenic-induced biochemical and genotoxic effects and distribution in tissues of Sprague–Dawley rats ☆

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ABSTRACT

Arsenic (As) is a well documented human carcinogen. However, its mechanisms of toxic action and carcinogenic potential in animals have not been conclusive. In this research, we investigated the biochemical and genotoxic effects of As and studied its distribution in selected tissues of Sprague–Dawley rats. Four groups of six male rats, each weighing approximately 60±2 g, were injected intraperitoneally, once a day for 5 days with doses of 5, 10, 15, 20 mg/kg BW of arsenic trioxide. A control group was also made of 6 animals injected with distilled water. Following anaesthetization, blood was collected and enzyme analysis was performed by spectrophotometry following standard protocols. At the end of experimentation, the animals were sacrificed, and the lung, liver, brain and kidney were collected 24 h after the fifth day treatment. Chromosome and micronuclei preparation was obtained from bone marrow cells. Arsenic exposure significantly increased ($p<0.05$) the activities of plasma alanine aminotransferase–glutamate pyruvate transaminase (ALT/GPT), and aspartate aminotransferase–glutamate oxaloacetate transaminase (AST/GOT), as well as the number of structural chromosomal aberrations (SCA) and frequency of micronuclei (MN) in the bone marrow cells. In contrast, the mitotic index in these cells was significantly reduced ($p<0.05$). These findings indicate that aminotransferases are candidate biomarkers for arsenic-induced hepatotoxicity. Our results also demonstrate that As has a strong genotoxic potential, as measured by the bone marrow SCA and MN tests in Sprague–Dawley rats. Total arsenic concentrations in tissues were measured by inductively coupled plasma mass spectrometry (ICP-MS). A dynamic reaction cell (DRC) with hydrogen gas was used to eliminate the ArCl interference at mass 75, in the measurement of total As. Total As doses in tissues tended to correlate with specific exposure levels.

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1. Introduction

Arsenic is a ubiquitous element present in food, soil, water and air, and it is released into the environment from both natural and man-made sources [1,2]. The major inorganic forms of arsenic include the trivalent meta arsenite As^{+3} and the pentavalent arsenate As^{+5}. Trivalent arsenic form has a higher affinity for thiol groups [3] and is more cytotoxic and genotoxic than As^{+5} [4]. Individuals who accumulate the trivalent intermediates are thought to be greater risk of arsenic-induced diseases [4]. Some of the organic forms include the methylated metabolites — monomethylarsonic acid (MMA), dimethylarsenic acid (DMA) and trimethylarsine oxide (TMAO) as well as arsenobetaine (AsB), arsenocholine and arsenosugars. More than 80% of commercially utilized arsenic compounds are used to manufacture products with agricultural applications such as insecticides, herbicides, fungicides, algicides, sheep dips, wood preservatives, dye-stuffs, and medicines for the eradication of tapeworms in sheep and cattle. Arsenic compounds have been used for at least a century in the treatment of syphilis, yaws, amoebic dysentery, and trypanosomiasis [5]. Despite the well known toxicity of arsenic, arsenic trioxide has long been of biomedical interest, dating to traditional Chinese medicine, where it is known as *Pi Shuang* and is still used to treat cancer and other conditions [6], and to homeopathy, where it is called arsenicum album. Some discredited patent medicines, e.g., Fowler's solution, contained derivatives of arsenic oxide. Arsenic trioxide under the trade name Trisenox (manufacturer: Cephalon) is a chemotherapeutic agent of idiopathic function used to treat leukemia that is unresponsive to "first line" agents. It is

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suspected that arsenic trioxide induces cancer cells to undergo apoptosis. Due to the toxic nature of arsenic, this drug carries significant risks. It has been used as a cytostatic drug in the treatment of refractory promyelocytic (M3) subtype of acute myeloid leukemia [7,8]. The combination therapy of arsenic trioxide and all-trans retinoic acid (ATRA) has been approved by the U.S. Food and Drug Administration (FDA) for treatment of certain leukemias [9] and its therapeutic action has been attributed to the induction of programmed cell death (apoptosis) in leukemia cells [10].

Occupational sources of arsenic to human workers include vineyards, ceramics, glass making, smelting and refining of metallic ores, during production and use of arsenic containing agricultural products like pesticides and herbicides. Exposure to arsenic occurs via the oral route (ingestion), inhalation, dermal contact, and the parenteral route to some extent. Humans can be exposed to arsenic through the intake of air, food and water [11]. Epidemiological and clinical studies indicate that arsenic is a paradoxical human carcinogen that does not easily induce cancer in animal models [12].

The toxicity of arsenic depends on its chemical state. Inorganic arsenic in its trivalent form is more toxic than pentavalent arsenic. The toxicity of arsenic also depends on the exposure dose, frequency and duration, the biological species, age, and gender, as well as on individual susceptibilities, genetic and nutritional factors [13,14]. By binding to thiol or sulfhydryl groups on proteins, As (III) can inactivate over 200 enzymes. This is the likely mechanism responsible for arsenic's widespread effects on different organ system. As (V) can replace phosphate, which is involved in many biochemical pathways [15–17]. The major metabolic pathway for inorganic arsenic in humans is methylation. Arsenic trioxide is methylated to two major metabolites via a non-enzymatic process to MMA, which is further methylated enzymatically to DMA before excretion in the urine [18–20]. Hepatic cancer and other hepatic disorders are considered to be the major causes of arsenic-related mortality. Hepatic function, liver diseases and drug-induced liver injury can be assessed by various routinely ordered liver function tests, i.e., clinical investigations that measure the levels of various biomarkers (proteins or enzymes) in the blood. These proteins/enzymes reflect different aspects of a normal functioning liver. For example, ALT and AST indicate hepatocellular integrity [21,22].

Tests for genotoxicity have indicated that arsenic compounds inhibit DNA repair, and induce chromosomal aberrations, sister-chromatid exchanges, and micronuclei formation in both human and rodent cells in culture [18,23–26] and in cells of exposed humans [14]. Reversion assays with Salmonella typhimurium fail to detect mutations that are induced by arsenic compounds. Although arsenic compounds are generally perceived as weak mutagens in bacterial and animal cells, they exhibit clastogenic properties in many cell types in vivo and in vitro [18,23,25–27]. In the absence of animal models, in vitro cell transformation studies become a useful means of obtaining information on the carcinogenic mechanisms of arsenic toxicity. Arsenic and arsenical compounds are toxic to and induce morphological transformations of Syrian hamster embryo (SHE) cells as well as mouse C3H10T1/2 cells and BALB/3T3 cells [28–30]. Based on the comet assay, it has been reported that arsenic trioxide induces DNA damage in human lymphophytes [31], colon cancer cells [32] and also in mice leukocytes [33]. Arsenic compounds have also been shown to induce gene amplification, arrest cells in mitosis, inhibit DNA repair, and induce expression of the c-fos gene and the oxidative stress protein heme oxygenase in mammalian cells [34,35]. They have been implicated as promoters and comutagens for a variety of toxic agents [36]. Recent studies in our laboratory have demonstrated that arsenic trioxide is cytotoxic and able to transcriptionally induce a significant number of stress genes and related proteins in human liver carcinoma cells [37], also demonstrated induction of cytotoxicity and genotoxicity in HL-60 cells [38].

Analyzing the toxic effects of arsenic is complicated because it exists in many different inorganic and organic compounds, and its toxicity varies according to its oxidation state, its solubility and many other factors including the exposure dose, frequency and duration, the biological species, age and gender, as well as individual susceptibilities, genetic and nutritional factors [39–41]. Most cases of human toxicity from arsenic have been associated with exposure to inorganic arsenic. Inorganic trivalent arsenite (As^{+3}) is 2–10 times more toxic than pentavalent arsenate (As^{+5}) [42]. Interest in the toxicity of arsenic has been heightened by recent reports of large populations in West Bengal, Bangladesh, Thailand, Inner Mongolia, Taiwan, China, Mexico, Argentina, Chile, Finland and Hungary that have been exposed to high concentrations of arsenic in their drinking water and are displaying various clinico-pathological conditions, the major effects being skin alterations and skin cancer. General health effects that are associated with arsenic exposure include cardiovascular and peripheral vascular disease, developmental anomalies, neurologic and neurobehavioral disorders, diabetes, hearing loss, portal fibrosis, hematologic disorders (anemia, leukopenia and eosinophilia) and multiple cancers: significantly higher standardized mortality rates and cumulative mortality rates for cancers of the skin, lung, liver, urinary bladder, kidney, and colon in many areas of arsenic pollution [13,43,44].

Although arsenic and arsenic containing compounds have been the subject of important toxicology research, there exists a lack of appropriate animal model for carcinogenicity assessment, as well as a scarcity of scientific data describing the tissue distribution of arsenic in relation to the biomarkers of arsenic-induced hepatotoxicity and genotoxicity in in vivo systems. Therefore, the present work was undertaken to study the distribution of arsenic in tissues, as well as the hepatotoxic and cytogenetic effects in Sprague–Dawley rats. Serum aminotransferases (ALT, AST), structural chromosomal aberrations (SCA), micronuclei (MN) formation and mitotic index (MI) in bone marrow cells were used as biomarkers of toxic effects.

Cytogenetic biomarkers (SCA, MN) play an important role in toxicological hazard evaluation as the first step towards quantification of cancers. Biomarkers serve as internal indicators of environmental or occupational exposures and have the potential for the prevention of effects of carcinogen exposure by early detection. The possible use of biomarkers representing intermediate steps in the exposure-to-disease continuum to estimate health risk in human populations has gained increasing attention.

2. Material and methods

2.1. Chemicals

Arsenic trioxide (As_2O_3) with an active ingredient of 100% arsenic in 10% nitric acid, methanol, glacial acetic acid, and superfrost microscope slides were purchased from Fischer-Scientific Houston, TX, USA. Potassium chloride solution (0.075 M) and Giemsa stain stock solution (0.4%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hanks Balanced Salt Solution was purchased from GIBCO (Grand Island, NY, USA). Fetal Bovine Serum (FBS) was obtained from Hyclone (Logan, UT).

2.2. Animal maintenance

Healthy adult male Sprague–Dawley rats (8–10 weeks of age), with average body weight (BW) of 60 ± 2 g were used in this study. They were obtained from Harlan-Sprague–Dawley Breeding laboratories in Indianapolis, Indiana, USA. The animals were randomly selected and housed in polycarbonate cages (three rats per cage) with steel wire tops and corn-cob bedding. They were maintained in a controlled atmosphere with a 12 h:12 h dark/light cycle, a temperature of 22 ± 2 °C and 50–70% humidity with free access to pelleted feed and fresh tap water. The animals were supplied with dry food pellets commercially available from PMI Feeds Inc. (St. Louis, Missouri). They were allowed to acclimate for 10 days before treatment.

The local Ethics committee for animal experiments [Institutional Animal Care and Use Committee] at Jackson State University, Jackson MS, (USA) approved this study. Procedures involving the animals and their care conformed to the institutional guidelines, in compliance