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Arsenic induced apoptosis in rat liver following repeated 60 days exposure

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Abstract

Background: Accumulation of the wide spread environmental toxin arsenic in liver results in hepatotoxcity. Exposure to arsenite and other arsenicals has been previously shown to induce apoptosis in certain tumor cell lines at low (1–3 μM) concentration.
Aim: The present study was focused to elucidate the role of free radicals in arsenic toxicity and to investigate the nature of in vivo sodium arsenite induced cell death in liver.
Methods: Male wistar rats were exposed to arsenite at three different doses of 0.05, 2.5 and 5 mg/l for 60 days. Oxidative stress in liver was measured by estimating pro-oxidant and antioxidant activity in liver. Histopathological examination of liver was carried out by light and transmission electron microscopy. Analysis of DNA fragmentation by gel electrophoresis was used to identify apoptosis after the exposure. Terminal deoxy-nucleotidyl transferase mediated dUTP Nick end-labeling (TUNEL) assay was used to qualify and quantify apoptosis.
Results: A significant increase in cytochrome-P450 and lipid peroxidation accompanied with a significant alteration in the activity of many of the antioxidants was observed, all suggestive of arsenic induced oxidative stress. Histopathological examination under light and transmission electron microscope suggested a combination of ongoing necrosis and apoptosis. DNA-TUNEL showed an increase in apoptotic cells in liver. Agarose gel electrophoresis of DNA of hepatocytes resulted in a characteristic ladder pattern.
Conclusion: Chronic arsenic administration induces a specific pattern of apoptosis called post-mitotic apoptosis.

Keywords: Arsenic; Apoptosis; Oxidative stress; Hepato-toxicity

1. Introduction

Arsenic is a naturally occurring metal that is present in food, soil and water; it is released in the environment from both natural and man-made sources (Tchounwou et al., 1999). Chronic exposure to arsenic contaminated water and food causes cancer of skin, liver, lung and bladder (Chiou et al., 2001; Smith et al., 1998; Steinmaus et al., 2000; Ferreccio et al., 1998; Schwartz, 1997). Toxicity of arsenic may be mediated by its methylated products (Yamanaka et al., 2000; Hughes et al., 2000; Li et al., 1998; Hayashi et al., 1998). Arsenic is also said to exert

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Table 1
Effect of 60-day oral administration of sodium arsenite on weight of body and liver of rat

Parameters	Group I (Control)	Group II (0.05 mg/l)	Group III (2.5 mg/l)	Group IV (5 mg/l)
Weight of body (g)	163.13 ± 0.009	157.66 ± 0.01	142.16 ± 0.008*	123.66 ± 0.015*
Weight of liver (g)	3.90 ± 0.036	4.16 ± 0.028	4.48 ± 0.01*	4.76 ± 0.015*

Data represent mean ± S.E. of six individual values.
* $p < 0.05$ denotes significant when compared with controls. The initial weight of animal was 160 ± 20 g in all groups.

its toxicity through oxidative stress by generating reactive oxygen species. Free radicals have been detected in cells treated with arsenite (Iwama et al., 2001; Lynn et al., 2000; Liu et al., 2001). Arsenic induced apoptosis and the mechanisms behind the process have been studied in vitro using cell cultures (Marisa et al., 2003, Table 1) but to the best of our knowledge, this has not been studied in vivo in rat liver. The objective of the present study is to elucidate the role of free radicals in arsenic toxicity and to investigate the nature of in vivo sodium arsenite induced cell death.

2. Materials and method

2.1. Chemicals

Sodium arsenite obtained from *Merck limited* was used for the study. All other chemicals used were of analytical grade.

2.2. Study animals

Male Wistar rats, aged 20–21 week and weighing 160 ± 20 g, were selected from an inbred colony maintained in the animal house of All India Institute of Medical Sciences (under the supervision of the Animal committee) under controlled conditions of 25 ± 2 °C temperature, 50 ± 15% relative humidity and normal photoperiod (12 h dark and light). The animals were given sterile food pellets supplied by *Ashirwad India* (protein 24%, fat 5%, fiber 4%, carbohydrates 55%, calcium 0.6%, moisture10% and ash 9%) and water ad libitum. Throughout the experiment, the animals were housed in polypropylene cages with each cage housing six animals. Locally procured sterile paddy husk was used as bedding in the cages. The animals were allowed to acclimatize to the laboratory environment for 7 days prior to the study.

2.2.1. Experimental design

Rats were randomly divided into four groups each containing six animals. Route of arsenic administration selected was oral. Group I (control group) animals were fed pure distilled water, while groups II, III and IV animals were fed distilled water containing sodium arsenite at concentrations of 0.05, 2.5 and 5 mg/l, respectively. Animals from all the experimental groups took comparably equal amount of water as the control animals.

All the groups were fed food. During the period of exposure, body weight and water intake of rats were measured. The animals were sacrificed after 60 days just after the last dose of arsenic, under light ether anesthesia.

2.2.2. Sample collection

Liver samples were removed from the experimental animals. Small representative tissue slices were taken for histopathological examinations. A portion of the liver (120–150 mg) was used for estimating the tissue arsenic concentration. The remainder of the liver was washed with ice-cold saline and was homogenized in 4 volumes of ice-cold 0.1 M phosphate buffer (pH of 7.4) containing 0.15 M potassium chloride. The homogenate was centrifuged at 9000 × *g* at 4 °C for 20 min. The supernatant was pipetted into clean centrifuge tubes and centrifuged further at 104,000 × *g* at 4 °C in high-speed ultracentrifuge. Microsomal and cytosolic fractions obtained were kept for use in our study.

2.2.2.1. Tissue arsenic estimation. A portion of the liver (120–150 mg) was digested in microwave digester system. Total arsenic, which would include inorganic and organic forms, was determined using vapour generator coupled atomic absorption spectrophotometer (Electronics Corporation of India (ECIL) Model AAS-4136) and results were expressed as arsenic μg/g tissue.

2.2.2.2. Assay systems. Cytochrome P450 was assayed in hepatic microsomes according to the method of Omura and Sato (1964) and its activity was expressed as nmol/mg microsomal protein.

Lipid peroxidation (LPO) was measured in hepatic tissue homogenates according to the method of Ohkawa et al. (1979) based on the formation of thiobarbituric acid reactive substances (TBARs) and expressed as the extent of malondialdehyde (MDA) production.

Glutathione (GSH) levels in homogenates were quantified by the method of Beutlar et al. (1967) and expressed as μmol/ml protein. *Glutathione reductase* (GR) (E.C.1.6.4.2) activity in liver was determined by monitoring the oxidation of NADPH in the presence of GSSG using the method of Carlberg and Mannervik (1985) and expressed as μmol NADPH oxidized/min/mg protein. *Glucose-6-phosphate dehydrogenase* (G6PD) (EC1.1.1.49) activity was assayed by the method of Ells and Krickman (1961) and expressed as nmol/min/mg protein. *Glutathione-S-transferase* (GST) (EC 2.5.1.18) activity assayed by the method of Habig et al. (1974) and expressed as