Quantitative Assay of Arsenic in Experimentally Intoxicated Guinea Pigs

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Abstract

The present investigation was undertaken with an attempt to generate information pertaining to the assessment of arsenic residues in the vital organs like liver, lungs kidneys along with blood and hair as biomarker of chronic arsenic exposure using guinea pigs as experimental animal. For this purpose the guinea pigs were divided into two groups having 5 animals in each group. Group I animals were fed 1% of Arsenic trioxide @ 1 mg/kg body weight through oral gavages daily for 90 days to produce chronic toxicity. Estimation of arsenic residue was carried out on 90th day post administration. In the present study chronic exposure to arsenic resulted in significant enhancement of arsenic residues in the blood, hair, liver, lungs and kidneys with mean values of 57.18, 333.71, 331.96, 95.8 ppb and 272.95 in guinea pigs of chronic toxicity as compared to 3.47, 14.02, 12.94, 2.56 and 5.56 ppb in control, respectively.

Key words: Arsenic, Biomarker, Wet digestion, Tissue Arsenic concentration.

Introduction

Arsenic is a ubiquitous element, being present virtually in all rocks, soil and water. Compounds of arsenic have been used in agriculture and forestry as pesticide, herbicide, molluscicide, wood preservative, fungicide and debarking of trees (Warner and Solomon, 1990)22. All these source of arsenic contamination posses a serious threat of unreckonable dimensions to human and animal kingdom due to its deleterious health effects. Extensive epidemiological studies have been carried out in human population of arsenic endemic areas. However, such studies in animal in the affected areas are lacking to assess and address the health hazards due to chronic arsenic exposure. Chronic exposure as a result of arsenic contaminated environment causes cumulative effects in hair, nail, hoof and urine (Ranft et al., 2003)18. Arsenic accumulates in hair for a long period due to its affinity of the tissue thus can be used as biomarker of chronic arsenicosis in cattle (Francesconi et al.4, 2002 and Mitranescu et al.17, 2003) and human beings (Bencko et al.1, 1971 and Hindmarsh6, 2002). However, blood and urine do not serve as index because it is readily eliminated from blood and excreted in the urine within hours of day (Mealey et al.16, 1959). The present study was, therefore, taken to gain information about arsenic accumulation in biological samples viz. blood, hair, liver, lungs and kidneys in experimentally fed arsenic in guinea pigs.

Materials and Methods

The experiment was conducted in guinea pigs aged 6-8 weeks weighing 250-300 gm. The animals were fed standard ration and fresh tap water ad lib. The guinea pigs were divided into two groups having 5 animals in each. In Group I, the animals were fed 1% of Arsenic trioxide @ 1 mg/kg body weight through oral gavages daily for 90 days to produce chronic toxicity. The animals of Group II were provided with plain drinking water for the same period which acted as control.

Collection of Sample for Arsenic Estimation

Estimation of arsenic residue in the hair, blood, liver, lungs and kidneys was carried out on 90th day of post feeding in the animals of Group I & II. Hair samples about (1 gm) were collected without using preservative from body coat of guinea pigs in polythene packets at room temperature. The blood samples were collected by cardiac puncture before anaesthetization in clean dry, sterilized plastic vial coated with heparin. Liver, kidneys, and lungs were quickly excised from scarified guinea pigs and a portion of organ specimens weighing one gram were collected in clean, dry and sterilized McCartney vial. The samples were stored at -20oC under ice pack and transported to IVRI, Izatnagar for arsenic estimation.

Analysis of Arsenic

Arsenic concentration in hair, blood, liver, lungs and kidneys were measured following wet digestion of

Table 1: Tissue arsenic concentration in different specimens of guinea pigs of group

Specimens	No. of Animals	Arsenic (ppb)	
		Group I	Group II
Blood	5	57.18 ±1.186***	3.47 ±0.496
Liver	5	331.96 ±1.799***	12.94 ±1.148
Lung	5	95.80 ±3.677***	2.96 ±0.074
Kidney	5	272.95±2.779***	5.560±0.277
Hair	5	333.71 ±8.913***	14.02 ±1.170

*** = Highly significant at P<0.001.

individual sample following the method of Hershey and Oostdyk (1988)5. First of all the hair samples were washed properly as per the method described by IAEA (1978)7 which involved sequential washing with acetone, distilled water and acetone and dried in hot air oven.

Arsenic concentration in washed hair samples, blood, liver, lungs and kidneys were measured by taking 1 gm of tissue sample and 1-2 ml of blood kept in conical flask with 10 ml triple acid mixture (HNO3 : H2SO4 : HClO3 = 4:1:1) for overnight. Next day the samples were heated on a hot plate inside fume hooder chamber at temperature below 900C for 4-6 hours till perchloric acid started producing white fumes that emanated from the digestion flask. The samples were removed from hot plate when the volume reduced to about one ml. The volume was adjusted to 10 ml using triple distilled water. Each time, at least three arsenic standards of almost equal strength were run.

The concentrations of arsenic in digested samples were measured at 193.7 nm wave length and 10mA current using Atomic Absorption Spectrophotometer (ECIL-4141) equipped with arsenic lamp. Vapor generation accessory (VGA) was used to produce hydride vapors using 0.6% sodium borohydride and 10 Mm HCl. Arsenic content in hair, blood, liver, kidneys and lungs were determined by multiplying with corresponding dilution factor and the values of arsenic were expressed in ppb.

Results and Discussion

In the present study chronic exposure to arsenic @ 1mg/kg body weight through oral rout for 90 days, resulted in significant (P<0.001) enhancement of arsenic residues in the blood, hair, liver, lungs and kidneys with mean values of 57.18, 333.71, 331.96, 95.8 and 272.95 ppb in guinea pigs of chronic toxicity (Group I) as compared to 3.47, 14.02, 12.94, 2.56 and 5.56 ppb in control (Group II), respectively (Table 1). The analysis of variance revealed highly significant increase in the mean concentration of arsenic in all the tissue in Group I as compared to the Group II. The decreasing orders of arsenic residue in different specimen were hair, liver, kidneys, lungs and blood. The present finding is in the resonance with the observations made in arsenic intoxicated rats (Kannan et al., 2001)8 and goats (Biswas et al., 1998)2. Most of the arsenates are rapidly and extensively absorbed from gastrointestinal tract of rats and distributed to various organs with highest concentration being detected in liver followed by kidneys, spleen, lungs and to lesser extent it crosses the placenta and blood brain barrier (Lindgreen et al.12, 1982; Marafante et al.14, 1982; and Vahter et al.21, 1982). Klassen9, (1996) observed cumulative effects of arsenic in the liver, kidneys, heart and lungs while traceable amount present in the muscles and neural tissue.

The highest concentration of arsenic in hair in the present study may be associated to the binding affinity of arsenic to the keratin sulfhydryl groups of the tissue (Schoolmeester and White19, 1980). Some added advantages in using hair as biomarker of arsenicosis is that ingested nontoxic forms of arsenic like arsenobetaine and arsenocholine in animals are not deposited in hair in contrary to human (Vahter et al.20, 1983; Marafante et al.15, 1984 and Koons and Peters10, 1994). Biomarkers are of major significance in assessing the risk of exposure before the onset of clinical symptoms and help in establishing mechanistic relationship between exposure to any foreign substance and internal dose or adverse health effects (Chappell et al.3, 1997). Therefore, the hair sample may be considered as the prime biomarker of arsenic toxicity for long term exposure.

The level of arsenic in blood was significantly higher in the present study inspite of report that the heavy metal in blood gets cleared within 10 hours of administration (Mealey et al.16, 1959). Bencko et al.1 (1971) observed that the major part of ingested arsenic is rapidly excreted in the urine. Therefore, the blood was considered to be of little significance in assessing long term exposure. The observation of significantly increased level of arsenic in blood may be corroborated that the nontoxic forms of arsenic like arsenobetaine and arsenocholine are excreted in urine that falsely elevates urinary arsenic level (Le et al.11, 1994; Ma and Le13, 1998 and Francesconi et al.4, 2002).

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