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Research Article

Transplacental and Lactational Exposure of Arsenic to Mice: Effect on Steroidogenic Enzymes and Hormones of Male Reproduction

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ABSTRACT

The present study aimed to assess the possible interference of sodium arsenite in F1 generation male mice with special reference to steroidogenic marker enzymes. Mice were devided in to two groups. The mice in first were served as control and recived normal tap water. Sodium arsenite administered orally to mice in the second group during pregnancy and lactation at a dose level of 0.4 ppm and analyzed for spermatogenesis and steroidogenesis in next generation adult male mice. The activity levels of selected steroidogenic marker enzymes (3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase) decreased significantly in mice exposed to sodium arsenite. The circulatory levels of testosterone decreased significantly in experimental mice with an increase in follicle stimulating hormone. The decreased levels of testosterone with elevated follicle stimulating hormone and lutenizing hormone levels in mice exposed to arsenic during early stages of development are indicative of intact pituitary-testicular axis. The results indicate that exposure to arsenic during early stages of development suppresses the male reproduction in adults. Thus, we conclude that the potential of reproduction is programmed, to some extent, in the early stages of development and hence any toxic insult during embryonic development and lactation suppresses male reproductive potential in adulthood.

Key words: sodium arsenite, hormones,enzymes, male reproduction, testes, gestation and lactation

INTRODUCTION

Arsenic, a non-essential trace element and a potent toxic metalloid has drawn increasing attention in recent years as a major pollutant of drinking water. Higher levels of inorganic arsenic occurs naturally in ground water of many parts of the world including India and millions of people are exposed worldwide to the drinking water containing this known carcinogen in excess amount [5]. Epidemiological data indicates that more than six million people residing in different areas of West Bengal, India are exposed to arsenic contaminated drinking water and more than 300,000 people were reported with signs of arsenic toxicity [4].

Arsenic exposure has been associated with an increased risk of dermatitis along with hyperkeratosis, gangrene, and tumors of skin, bladder, liver, kidney, lung, prostate and other tissues [7]. Epidemiological reports from Ukraine, Thaiwan and Bangladesh revealed that the intake of arsenic contaminated food and water caused reproductive disturbances in woman [21], adverse pregnancy outcomes[20] and also spontaneous abortions [1]. Arsenic has been suspected to be the cause for reproductive failure in male workers at a copper smelter in Sweden. Arsenic intoxication in experimental animals has been associated with inhibition of steroidogenesis [17,6] spermatogenesis as well as an elevation of adrenocortical steroidogenesis [8].

There is a lack of literature and data related to the exposure to arsenic during prenatal and neonatal period on reproduction in adults, particularly at the dose levels occurring in drinking water in wide areas of India and in other countries where this element is present in the range above the admissible limit (0.01 ppm according to the World Health Organization) [14]. The present study was conducted to assess the effect of exposure to 0.4 ppm of sodium arsenite through drinking water during embryonic development and lactation on reproductive hormones and enzymes of adult male mice (F1 generation).

MATERIALS AND METHODS

Animals

Swiss Albino mice were bred at Department of Biotechnology, S.V. University, Tirupati. Animals were maintained in polypropylene cages lined with paddy husk under a well regulated light and dark (12h:12h) schedule at 27 □ 1ºC wirh relative humidity of 75%. Animals were given food and water ad libitum. The mice pellet feed was purchased from Kamadhenu Agencies, Bangalore, India. Healthy mice of 90 days age were selected for present study.

Test chemical

Sodium Arsenite was purchased from S.D fine chemicals (Mumbai, India) used as test chemical. This copmound was dissolved in normal water to obtaine the final concentrationof the 0.4 ppm/ kg body weight of the animal.

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Experimental design

Pregnant mice were divided randomly into two groups consisting of ten animals in each group. The animals in group 1 were allowed ad libitum access to tap water without sodium arsenite while the animals in group 2 were allowed *ad libitum* access to tap water containing 0.4 ppm of sodium arsenite during gestation and lactation periods. Sodium arsenite purchased from S.D. fine chemicals (Mumbai, India) was used as a test chemical. The mice were allowed to deliver the pups and the pups, after weaning, were grown on normal diet and tap water (with out sodium arsenite) up to 60 days and used for experimentation. The experimental animals were sacrificed by cervical dislocation. All animal procedures were approved by the Institutional Animal Ethics Committee at S.V. University.

Assay of testicular steroidogenic enzymes

The 3β-hydroxysteroid dehydrogenase (E.C. 1.1.1.51; conversion of NAD to NADH) and 17β-hydroxysteroid dehydrogenase (E.C. 1.1.1.61; conversion of NADPH to NADP) activities in the testis were assayed using the method described [2]. The enzyme assays were made under the conditions following zero order kinetics after preliminary standardization regarding linearity with respect to substrate concentration, time of incubation and enzyme concentration.

The reaction mixture in a volume of 2.0 ml contained : 100 µmoles of sodium pyrophosphate buffer (pH 9.0), 0.5 µmol of cofactor NAD for 3β-HSD and NADPH for 17β-HSD, 0.08 µmol of substrate (dehydroepiandrosterone for 3β-HSD and androstenedione for 17β-HSD) and 20 mg equivalent of microsomal protein as enzyme source. The reactions were carried out in a quartz cuvette of 1.0 cm path at 23ºC. The change in the absorbance was measured at 340 nm at 20s intervals for 5 min in a UV-Vis spectrophotometer (Hitachi model U-2001) against controls. Protein content in the enzyme source was estimated by the method [12] using bovine serum albumin as standard.

Determination of serum Testosterone, FSH and LH levels

Radioimmuno assay of serum testosterone was performed by the method [15]. The sensitivity of the assay was calculated as 0.002 ng. Serum FSH and LH were assayed as per the method[11]. Iodination of rFSH and rLH with I^{125} was performed by the method [9] using chloramine T as an oxidizing agent. The sensitivity of the assay was calculated as 0.004 and 0.006 respectively for FSH and LH. All the samples were run at the same time to avoid interassay variation.

Statistical analysis of the data

The data were presented as mean ± SEM. Statistical analysis was performed using analysis of variance (ANOVA) followed by Dunnett's test, using SPSS 10.0 version.

RESULTS

No mortalities were observed in control or in experimental groups. No behavioral abnormalities were observed in experimental mice.

The activity levels of 3β-hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase decreased significantly in the testis of mice exposed to arsenic during early stages of development when compared with the corresponding controls (Table 1). Serum FSH, LH and testosterone concentrations in mice exposed to arsenic during early stages of development were shown in table 1. The levels of serum testosterone decreased significantly in adult mice exposed to

Table 1. Effect of gestational and lactational exposure to sodium arsenite on testicular 3-β HSD and 17-β HSD activity levels and serum FSH, LH and testosterone levels in adult mice.

Parameters	**Control**	**Arsenite**	**Anova**
3β-HSD (µmol NAD converted to NADH/mg. protein/h)	0.0262 ± 0.0045	0.0079 ± 0.0012 (- 99.21)	$F_{2,21}$=14.063 P<0.01
17β-HSD (µmol NADPH converted to NADP/mg. Protein/h)	0.0168 ± 0.0019	0.0039 ± 0.0011 (- 99.61)	$F_{2,21}$=14.063 P=0.1277
Testosterone(ng/ml)	7.89 ± 1.21	4.25 ± 0.76 (-46.13)	$F_{2,21}$ =2.535 P=0.1652
FSH (ng /ml)	5.26 ± 0.82	11.21 ± 1.96 (+113.11)	$F_{2,21}$ =5.713 P<0.05
LH (ng/ml)	1.25 ± 0.03	3.89 ± 0.11 (+211.2)	$F_{2,21}$=13.444 P<0.01

values are Mean ± SEM of 8 animals.Values in parentheses are percent change from the control. Values are significantly different at *p<0.01.

arsenic during early stages of development when compared with the corresponding group of control animals. Where as the levels of serum FSH and LH increased significantly in experimental mice with respect to the corresponding group of control mice (Table 1).

DISCUSSION

The present study was aimed to determine the reproductive toxic effects of mice exposed to arsenic during embryonic development and lactation. The route chosen in this study for exposure was via drinking water through mothers to mimic human exposure and to reflect the impact on fertility of next generation. In the present study reproductive potential of male mice was measured using activity levels steroidogenic enzymes, circulatory levels of gonadotropins and testosterone as biological parameters.

The arsenic dose selected in the present study was not resulted in any toxic symptoms in mice. No mortality and no behavioral abnormalities were recorded in experimental mice indicating the arsenic do not exhibit any toxicity at the selected dose level. The results of the present investigation demonstrate the adverse effect of sodium arsenite on production of reproductive enzymes and hormones. Maintenance of spermatogenesis in mice depends upon adequate testosterone concentrations [18]. The levels of serum testosterone were significantly decreased in adult mice exposed to arsenic during early stages of development. The decrease in serum testosterone could be due to diminished responsiveness of Leydig cells to leutinizing hormone and/or the direct inhibition of testosterone steroidogenesis. In steroidogenesis, Δ^5, 3-βHSD and 17-βHSD are the key regulatory enzymes [10]. A significant decrease in the activity levels of these steroidogenic enzymes in testis of experimental mice indicate decreased steroidogenesis, which in turn may suppress the reproductive activities in the male mice. This is in agreement with the previous findings where arsenic treatment was associated with inhibition of testicular steroidogenesis in rat [16] . This alteration in steroidogenic enzyme activity in experimental mice may be the result of changes in the levels of plasma FSH and LH, since these are the regulators of HSD activities [13]. The elevated levels of serum FSH and LH with lowered circulatory testosterone levels in experimental mice are indicative of intact pituitary-testicular axis. Testosterone plays an important role in attachment of the germ cells in seminiferous tubules. Low levels of intra-testicular testosterone may lead to detachment of germ cells from seminiferous epithelium and may initiate cell apoptosis [3]. The increase in the levels of serum FSH could be due to the impairment of spermatogenesis by the arsenic on the spermatogenic compartment or through the inhibition of testosterone production. Thus, the increase in the levels of serum FSH reflect the germ cell loss in the spermatogenic compartment or damage to the sertoli cells, thereby affecting the feed back regulation of FSH secretion as described [19].

In summary, these data indicated that exposure to arsenic during early stages of development resulted in alterations in the activity of testicular steroidogenic enzyme activities, along with a decrease in serum testosterone levels, sperm count, sperm motility, viability and sperm function in our further studies. The study has also shown that exposure to arsenic during early stages of development produces a castration-like effect at pituitary level due to decreased production of testosterone by the testis. The decreased levels of testosterone might affect the status of reproductive potential of these mice. Though the mice were maintained on arsenic-free water after weaning, the reproductive potential was not restored in these animals. Thus, these data indicate reproductive organ continues to develop during puberty, its programming, to some extent, was shaped during early stages of development. Studies are in progress to test the reproductive potential of these mice. This study has relevance since embryos are more sensitive to arsenic toxicity and arsenic is a common toxicant in several parts of the world, including India. Though extrapolation of mice data to human is not relevant but the arsenic level in some parts of India is more than the concentration exposed to the mice in the present study.

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