Journal of Reproduction and Infertility 2 (3): 41-45, 2011
ISSN 2079-2166

Effect of Transplacental and Lactational Exposure to Arsenic on Male Reproduction in Mice

[1]M. Vijaya Bhaskar Reddy, [2]S.D. Sudheer, [2]P. Sasikala,
[2]P. Sreenivasula Reddy, [3]S. Hemadri Reddy and [3]A. Karthik

[1]Department of Veterinary Bio-Chemistry, C.V.Sc, Proddutur, Kadapa Andhra Pradesh, India
[2]Department of Livestock Production Management, C.V.Sc, Tirupati Andhra Pradesh, India
[3]Department of Microbiology, C.V.Sc, Tirupati Andhra Pradesh, India

Abstract: Sodium arsenite was orally administered to mice during pregnancy and lactation at a dose level of 0.4 ppm and spermatogenesis of next generation in adult male mice was analyzed. A significant depletion in sperm count, sperm motility, sperm viability and HOS-coiling was observed in mice exposed to arsenic during early stages of development with an increase in sperm abnormalities. These results indicated that exposure to arsenic during early stages of development suppresses the male reproduction in adults. Thus, it was concluded 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 % Sperm Analysis % Male Reproduction % Gestation and Lactation

INTRODUCTION

Arsenic, a nonessential 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 [1-4]. Epidemiological data indicated 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 [5].

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 [6-11]. Epidemiological reports from Ukraine, Taiwan and Bangladesh revealed that the intake of arsenic contaminated food and water caused reproductive disturbances in woman [12], adverse pregnancy outcomes [13] and also spontaneous abortions [14]. Arsenic has been suspected to be the cause for reproductive failure in male workers at a copper smelter in Sweden [15].

There is a lack of literature 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) [16]. 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 reproduction 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 23±1°C. Animals were given food and water *ad libitum*.

Corresponding Author: M. Vijaya Bhaskar Reddy, Department of Veterinary Bio-Chemistry, C.V.Sc, Proddutur, Kadapa Andhra Pradesh, India. Tel: +91-9705497317.

The mice feed was purchased from Kamadhenu Agencies, Bangalore, India. Healthy mice of 90 days age were selected for the present study.

Experimental Design: Pregnant mice were randomly divided 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 these pups, after weaning, were grown on normal diet and tap water (with out sodium arsenite) up to 60 days and used for experimentation. All animal procedures were approved by the Institutional Animal Ethics Committee at S.V. University.

Epididymal Sperm Count: Epididymis was excised at autopsy and placed in 10 ml vial containing 1.0 ml physiological saline at 37°C. A suspension of spermatozoa was prepared and the resulting sperm suspension was used for the analysis of total sperm count. The sperms were counted using Neubaeur chamber [17].

Sperm Motility: Cauda epididymal tissue was teased in to a 2.0 ml physiological saline to release the spermatozoa. Percent motility was determined at 37°C using Neubaeur chamber [17] within 5 minutes following their isolation from cauda epididymis.

Sperm Viability: The ratio of live and dead spermatozoa was determined using 1% trypan blue [18]. Undiluted sperm sample (0.2 ml) was incubated with 0.2 ml of trypan blue stain for 15 min at 37°C. A drop of the suspension was placed on Neubaeur haemocytometer under cover slip. The sperm was allowed to settle for 1 minute and observed under Olympus microscope (Model BX41). The number of stained and unstained spermatozoa was scored in 20 separate fields. The spermatozoa which were not stained with trypan blue were considered as viable and the per cent of stained sperms were determined.

Hypo-Osmotic Swelling Test: Sperm functional test was carried out by the method of Jeyendran *et al.* [19]. This test evaluates the response of spermatozoa to hypo-osmotic stress. The basis of this assay is when viable sperms are exposed to hypo-osmotic medium, there will be an influx of fluid causing the tail to coil, which can be seen under phase contrast microscope. The sperms were exposed to hypo-osmotic medium and observed for coiled tails under the microscope and the percent of coiling was estimated.

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 average sperm count in cauda epididymal tissue was found to be 35.23 ± 2.22 millions/ml in control mice. A significant ($p<0.001$) depletion (-60.54%) of sperm count was observed in experimental mice (Table 1 and Fig. 1A). A significant decrease in sperm motility (-43.93%), sperm viability (-37.09%) and HOS-coiling (-37.25%) was also observed in mice exposed to arsenic during early stages of development when compared with control mice (Table 1 and Fig. 1B, C and D). All the sperms were apparently normal in control mice. Whereas in mice exposed to arsenic, many morphologically altered sperms were observed. Double headed sperms, banana shaped head sperms, long-tailed sperms and two tailed sperms were the common abnormalities in experimental mice (Table 1).

Table 1: Effect of gestational and lactational exposure to sodium arsenite on sperm parameters in adult mice

Parameters	Control	Arsenite	T-test
Sperm Count (millions/ml semen)	35.23±2.22	13.90±1.78 (-60.54)	F1,10 =1.555 P=0.3198
Motile sperm (%)	41.29±3.19	23.15±2.45 (-43.93)	F1,10 =1.695 P=0.2882
Viable sperm (%)	56.4±4.98	35.48±3.02 (-37.09)	F1,10 =2.719 P=0.1482
HOS coiled sperm (%)	58.9±3.64	36.96±2.14 (-37.25)	F1,10 =2.893 P=0.1343

Values are Mean ± SEM of 8 animals

Values in parentheses are % change from the control

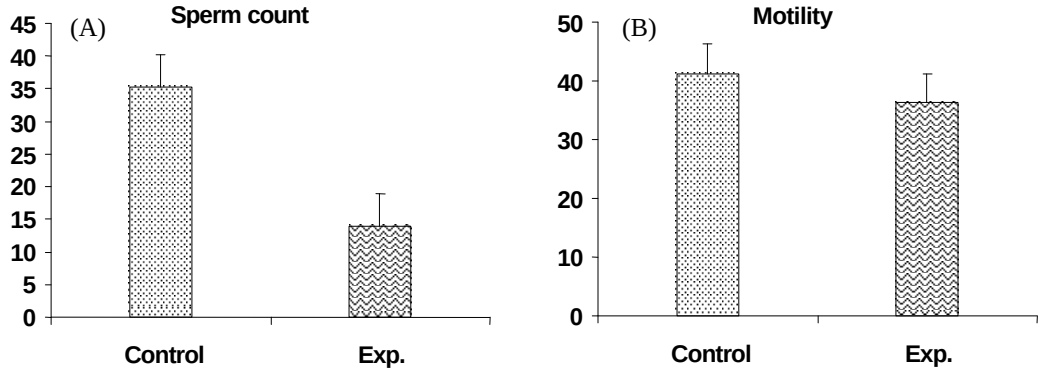

Fig. 1A,B: Effect of transplacental and Lactational Exposure to 0.4 ppm Sodium Arsenite on Sperm count (Millions / ml) an motility of adult mice

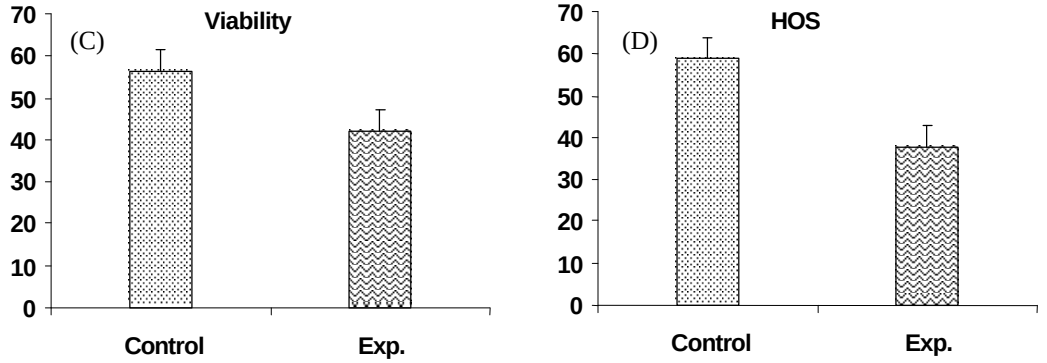

Fig. 1C,D: Effect of transplacental and Lactational Exposure to 0.4 ppm Sodium Arsenite on Sperm viability and HOS coiling of adult mice.

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 sperm parameters like motility, viability, count and hypo osmotic test as biological parameters.

The arsenic dose selected in the present study did not result 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 demonstrated the adverse effect of sodium arsenite by significantly decreasing the sperm count and sperm motility which were observed in mice exposed to arsenic during embryonic development and lactation. Decrease in sperm viability and impaired reproduction was observed in rats after exposure to arsenic [20]. Low doses of arsenic via drinking water for 35 days did not produce any effect on sperm count, motility and morphological abnormalities [21]. However at high doses significant decrease in sperm count, motility and increase in number of abnormal sperm were observed [22].

Significant decrease in sperm counts was also observed in arsenic exposed in rats [23]. Mammalian sperm contain large amounts of thiol-rich proteins in the flagellum which maintains sperm motility and stability. Arsenic is a well known thiol-inhibiting metalloid [24]. The decrease in sperm motility in the present study may be due to accumulation of arsenic in epididymis where the sperm matures and acquires motility. Although the exact mechanism of toxicity is not under stood, because of electrophilic nature of the arsenic it binds to sulphydryl groups on proteins and thereby inhibits enzyme activity [25].

In conclusion, these data indicated that exposure to arsenic during early stages of development resulted in decrease in sperm count, sperm motility, viability and damage in testicular architecture.

ACKNOWLEDGEMENTS

The authors are grateful to Head, Department of Biotechnology, S.V.University for providing necessary research facilities. Part of the work was submitted for the award of M. Phil degree in Biotechnology by first author.

REFERENCES

1. Nordstrom, S., L. Beckman and I. Nordenson, 1978. Occupational and environmental risks in and around a smelter in northern Sweden. III. Frequencies of spontaneous abortion. Hereditas, 88: 51-54.
2. Mazumder, D.N., A. Guha, A.K. Chakraborty and A. Ghosh, 1988. Chronic arsenic toxicity from drinking tube-well water in rural West Bengal. Bull. WHO., 66: 499-506.
3. Nickson, R., J. McArthur, W. Burges, K.M. Ahamed, P. Ravenserof and M. Rahman, 1998. Arsenic poisoning of Bangladesh ground water. Nature, 395: 338-338.
4. Chatterjee, A., D. Das, B.K. Mandal, G. Samanta and P. Banerjee, 1995. The biggest arsenic calamity in the world. Analyst., 120: 643-650.
5. Chappell, W.R., B.D. Beck, K.G. Brown, R. Chaney, C.C. Richard, K.J. Irgolic and D.W. North, 1997. Inorganic arsenic: A need and an opportunity to improve risk assessment. Environ. Health. Perspect., 105: 1060-1065.
6. Chakraborti, D., M.M. Rahman, K. Paul, U.K. Chowdhury, M.K. Sengupta, D. Lodh, C.R. Chanda, K.C. Saha and S.C. Mukherjee, 2002. Arsenic calamity in the Indian subcontinent-What lessons have been leaned? Talanta, 58: 3-22.
7. Chen, C., T. Kuo and M. Wu, 1988. Arsenic and cancers. Lancet, 1: 1414-1415.
8. Chen, C. and C. Wang, 1990. Ecological correlation beween arsenic level in well water and age-adjusted mortality from malignant neoplasms. Cancer. Res., 70: 5054-5474.
9. Saha, K.C., 1995. Chronic arsenical dermatosis from tube-well water in West Bengal during 1983-87. Indian. J. Dermatol., 40: 1-12.
10. Abernathy, C.O., Y. Liu, D. Longfellow, H.V. Aposhian, B. Beck, B. Fowler, R. Goyer, R. Menzer, T.G. Rossman, G. Thomson and M.P. Waalkes, 1997. Arsenic: Health effects, mechanisms of actions and research issues. Environ. Health. Perspect., 107: 593-597.
11. Chowdhury, T.R., B.K. Mandal, G. Samanta, G.K. Basv, P.P. Chowdhury, C.R. Chanda, N.K. Karan, D. Lodh, R.K. Dhar and D. Das, 1997. Arsenic in ground water in six districts of West Bengal, India-The biggest arsenic calamity in the world: The status report up to August, 1995. In Arsenic Exposure and Health Effects (C. O. Abernathy, R.L. Calderson and W.R. Chappell, Eds.), Chapman and Hall, New York, pp: 93-111.
12. Gebel, T.W., 1999. Arsenic and drinking water contamination. Sci., 283: 1458-1459.
13. Zadorozhanaja, T.D., R.E. Little, R.K. Miller, N.A. Mendel, R.J. Taylor, B.J. Presley and B.C. Gladen, 2000. Concentrations of arsenic, cadmium, copper, lead, mercury and zinc in human placentas from two cities in Ukraine. J. Toxicol. Health A., 61: 255-263.
14. Yang, C.Y., C.C. Chang, S.S. Tsai, H.Y. Chuang, C.K. Ho and T.N. Wu, 2003. Arsenic in drinking water and adverse pregnancy outcome in an arseniasis-endemic area in northeastern Taiwan. Environ. Res., 91: 29-34.
15. Ahmad, S.A., M.H. Sayed, S. Barua, M.H. Khan, M.H. Faruquee, A. Jalil, S.A. Hadi and H.K. Talukder, 2001. Arsenic in drinking water and pregnancy outcomes. Environ. Health. Perspect., 109: 629-631.
16. Beckman, L., 1978. The Ronnaskar smelter-occupational and environmental effects in and around a polluting industry in northern Sweden. Ambio., 7: 226-231.
17. Rahman, M.M., B.K. Mandal, T.R. Chowdhury, M.K. Sengupta, U.K. Chowdhury, D. Lodh, C.R. Chanda, G.K. Basu, S.C. Mukherjee and K.C. Saha, 2003. Arsenic groundwater contamination and sufferings of people in North 24-Parganas, one of the nine arsenic affected districts of West Bengal, India. J. Environ. Sci. Health Part A Tox. Hazard Subst. Environ. Eng., 38: 25-59.
18. Belsey, M.A., K.S. Moghisis, R. Eliasson, C.A. Pau Lsen, A.J. Callgos and M.R.N. Prasad, 1980. Laboratory Manual for the Examination of Human Semen and Semen Cervical Mucus Interaction Singapore: Press Concern.
19. Talbot, P. and R.S. Chacon, 1981. A triple stain technique for evaluating normal acrosome reaction of human sperm. J. Exp. Zool., 215: 201-208.
20. Jeyandran, R.S., H.H. Vander Ven and L.I.D. Zaneveld, 1992. The hypo-osmotic swelling test: an update. Arch. Androl., 29: 105-116.

21. Danielsson, B.R.G., L. Dencker, A. Lindgreen and H. Tjalve, 1984. Accumulation of toxic metals in male reproductive organs. Arch. Toxicol., 7: 177-180.
22. Pant, N., R. Kumar, R.C. Murthy and S.P. Srivastava, 2001. Male reproductive effect of arsenic in mice. Biometals., 14: 113-117.
23. Pant, N., R.C. Murty and S.P. Srivastava, 2003. Male reproductive toxicity of sodium arsenite on spermatogenesis, plasma gonadotrophins and testosterone in rats. Asian. J. Androl., 1: 27-31.
24. Jana, K. and P.K. Samanta, 2006. Evaluation of single intratesticular injection of calcium chloride for non-surgical sterilization in adult albino rats. Cantraception, 73: 289-300.
25. Working, P.K., J.S. Bus and T.E. Hamm, 1985. Reproductive effects of inhaled methyl chloride in the male fischer rat spermatogonial toxicity and sperm quality. Toxicol. Appl. Pharmacol., 77: 144-157.
26. John, M.D., C.F. Jacobson, R.S. Anthony, H.F. Craig and F.H. Joseph, 1998. An assessment of the developmental toxicity of inorganic arsenic. Rep. Toxicol., 12: 385-433.