

HYPOMETHYLATION OF DEOXYRIBONUCLEIC ACID IN TESTICULAR TISSUE DUE TO ARSENIC EXPOSURE IN MICE

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Received: 28 August 2016, Revised and Accepted: 14 September 2016

ABSTRACT

Objective: Among various environmental carcinogens, arsenic is highly sensitive and possesses potential to cause several diseases including cancer. Nevertheless, arsenic has not been observed to induce mutation directly but is involved in epigenetic changes. Hypomethylation of oncogenes and hypermethylation of tumor suppressor genes are reported to be associated with accumulation of arsenic. The present investigation demonstrates a direct correlation arsenic and deoxyribonucleic acid (DNA) methylation.

Methods: Swiss albino mice were grouped as control and arsenic treated for 12 weeks. Arsenic concentration in blood and testes was analyzed by atomic absorption spectrometer. Furthermore, DNA was extracted from the testes of mice by DNA purification kit and used for determining global methylation in mice genome with the help of MethylFlash Methylated DNA Quantification Kit.

Results: Arsenic concentration in arsenic-treated mice was significantly higher than the control group in both blood and testes. Interestingly, arsenic concentration in blood was recorded to be higher than testes in the arsenic-treated group with significance ($p < 0.0001$). Moreover, a lower percentage of cytosine of mice genome was found to be methylated in arsenic-treated mice group than control group ($p < 0.0001$).

Conclusion: Greater concentration of arsenic in mice leads to hypomethylation of mice genome globally. Arsenic fosters deregulation of gene expression by modifying methylation of CpG island of the promoter region. Epigenetic study is of prime importance in the field of oncology. Drug development for repressing alteration of DNA methylation is imperative for cancer treatment.

Keywords: Arsenic, Cancer, Deoxyribonucleic acid, Methylation, Carcinogen.

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INTRODUCTION

Arsenic, a metalloid released from both natural and anthropogenic sources, is a major health concern in many countries and has been associated with many human cancers [1,2]. In addition, chronic exposure of arsenic is also usually linked with many other diseases including abnormalities related to the reproductive system [3]. Metals such as arsenic may affect male reproductive system directly when it targets specific reproductive organ or indirectly when it targets neuroendocrine system. The deleterious effect of arsenic on spermatogenesis can be long lasting and irreversible if the Sertoli cells are damaged. The number of motile sperm produced during adulthood is determined by the number of Sertoli cells because each Sertoli cell supports the maturation of an only limited number of germ cells to sperm. Inorganic arsenic is an endocrine disruptor which is demonstrated to jeopardize the process of spermatogenesis leading to lower sperm count, impair the stability sperm chromatin or sperm deoxyribonucleic acid (DNA) damage [4].

Inorganic arsenic is primarily metabolized in the liver and converted to the organic entity. Thereafter, the organic arsenic is distributed to the entire body by peripheral blood. The excretion of arsenic has been observed to be miniature. According to a study, the highest amount of arsenic excreted was in form of monomethylarsonic acid (MMA) followed by dimethylarsinic acid (DMA) and inorganic arsenic [5]. An autoradiographic study of ⁷⁴As-arsenite (As III) injected intravenously in mice showed the highest accumulation of arsenic in skeleton and long-term retention in hair and skin, squamous epithelium of gastrointestinal tract, the epididymis, thyroid, and lens [6]. In another study, 80% of the oral dosed arsenic was observed in gastrointestinal

tract and the highest initial retention was found in kidney, lungs, intestinal mucosa, stomach, and testes [7].

Both genetic alteration (mutation) and epigenetic changes (methylation) have been linked with environmental carcinogenesis. Chronic exposure of low dose of arsenic to the non-tumorigenic prostate epithelial cells transformed to tumorigenic cells [8]. Several mechanisms at molecular and cellular levels have been proposed to explain the carcinogenicity induced by arsenic. Global DNA hypomethylation and activation of various oncogenes have been reported in the literature [8,9]. If the arsenic, by any mean, is ingested by humans, the inorganic arsenic is converted to organic forms, i.e., MMA and DMA in liver [10]. Since methylation of inorganic arsenic is considered to be detoxification process evolved by our body because methylated arsenic has lower toxic effect as compared to that of inorganic arsenic [11,12]. The conversion of inorganic arsenic to DMA or MMA is catalyzed by an enzyme called methyltransferase. Methyltransferase is an indigenous enzyme which transfers methyl group from *s*-adenosylmethionine to cytosine of CpG island of the promoter region. However, in this context, methyltransferase transfers methyl group from inorganic arsenic to CpG island and vice versa leading to hypermethylation or hypomethylation of CpG island of various oncogenes and tumor suppressor genes. Hypermethylation and hypomethylation were linked to tumor suppressor gene inactivation and oncogene activation, respectively [13-15].

This study aims to investigate the effect of acute toxicity by arsenic on gene expression and attempts to establish a correlation between accumulation of arsenic in the body and its ability to modify the methylation pattern. Arsenic, a potent carcinogen, has been shown to possess the capability to alter gene expression at epigenetic level in this study.

METHODS

Animals

Swiss albino mice (*Mus musculus*) were reared in the animal house of S.S. Hospital and Research Institute, Patna. The male mice selected for the study were 12 weeks old, and their weight was measured as 30 ± 2 g. The mice were kept in the polypropylene cages with paddy husk at room temperature $28 \pm 2^\circ\text{C}$ and humidity $50 \pm 5\%$ in a controlled light (12 hrs light and 12 hrs dark). Animals were maintained in ideal conditions as per the ethical guidelines of the CPCSEA (CPCSEA Regd. No. 1840/PO/ReBi/S/5/CPCSEA), Government of India, and Institutional Animal Ethics Committee (IAEC).

Study groups and sampling

Male mice of the control group ($n=6$) were given distilled water as drinking water, and "treated group" ($n=6$) were administered 2 mg/kg body weight of endosulfan for 12 weeks. Blood samples were collected from the sacrificed mice and centrifuged to obtain serum samples for estimation of arsenic concentration. Testes from all the animals were removed and stored at -80°C for estimation of arsenic concentration and DNA methylation.

Atomic absorption spectrometer

Tissues of 6 controls and 6 arsenic-treated male mice stored at -80°C for estimation of arsenic concentration. Further, sample preparation and estimation of arsenic was done by standard protocol of atomic absorption spectrometer graphite flame (Perkin Elmer) model number AAN3160080 (PinnAAcle900T).

DNA isolation

The testes tissue of male mice was homogenized in liquid nitrogen with the help of mortar and pestle. DNA from the testes was extracted by standard protocol of DNA purification kit (Promega, Wizard Genomic DNA Purification Kit, A1120).

DNA methylation

The isolated DNA was stored at $2-4^\circ\text{C}$ overnight and processed for DNA methylation next day. The DNA methylation was assessed with the help of MethylFlash Methylated DNA Quantification Kit (EPIGENTEK).

Statistical analysis

T-test was performed to generate significance of DNA methylation and two-way ANOVA for arsenic concentration in blood and testes. $p < 0.05$ was considered significance. Statistical analysis was performed with the help of SPSS 16.

RESULTS AND DISCUSSION

Blood and testes collected after dissection of the mice were used for estimation of arsenic concentration. The mean arsenic concentration in blood of arsenic-treated mice (316.36 ± 43.607 ppb) was recorded to be higher than the mean arsenic concentration in blood of control group (1.005 ± 0.88522 ppb). Similarly, the mean value of arsenic concentration in testes of arsenic-treated mice (128.3 ± 31.423 ppb) was observed to be higher than that of arsenic concentration in testes of the control group (0.7 ± 1.274 ppb) as shown in Fig. 1. Importantly, arsenic concentration in blood was significantly greater than in testes of arsenic-treated group and both higher than the control with significance value ($*p < 0.0001$).

The isolated DNA from testes from each group was tested for the global methylation of active genes in the genome of mice. 0.457% of the mice genome was found to be methylated in the control group, and only 0.21% of genome was methylated in arsenic-treated mice group with significance value ($**p < 0.0001$) as shown in Fig. 2.

Ambiguous nature of arsenic, a deadly poison, a carcinogen, and antitumor capacity [16] makes it more interesting and hence rivets us to explore more to understand the elaborate mechanism by

which it causes toxicity. Most satisfying mechanisms proposed for its carcinogenicity were by inducing double strand break leading to chromosomal aberration [17] and its capacity to induce changes in DNA methylation [9]. We have demonstrated how arsenic affects methylation of DNA on global genome.

Accumulation of arsenic in different organs in mice and humans is evident and has been reported several times in literature [5,6]. Exposure to arsenic has been observed to cause prostate cancer in humans and liver cancer in mice [18,19]. Mutation caused by arsenic has not been evident till date, but epigenetic changes by altering methylation of promoter of genes are superfluous in the literature [20-22]. Arsenic has also been shown to cause hypermethylation of tumor suppressor genes [18]. According to a recent report, hypomethylation of DNA was observed in the genome of mice exposed to low dose of arsenic for shorter period, and hypermethylation was recorded in mice with chronic exposure of arsenic [23].

In the current investigation, mice were exposed to arsenic contaminated water of 200,000 ppb daily for 3 months. The concentration of arsenic in testes of mice after 3 months was observed 128 ppb (Fig. 1). The accumulation of arsenic in testes is high enough to cause epigenetic changes. $< 1\%$ of genome contains exons in mammals that code for proteins and our data show only 0.21% cytosine methylation in arsenic-treated mice as compared to 0.457% in control (Fig. 2). To convert inorganic arsenic to organic one, methyltransferase transfers the excess methyl group from CpG island of promoter to pentavalent or tetravalent arsenic. If arsenic is accumulated in higher concentration, the promoter region would so become hypomethylated as a consequence leading to altered gene expression which might include several oncogenes.

CONCLUSION

In the field of epigenetic study, arsenic is a potent toxic agent and carcinogen which leads to hypomethylation of oncogenes and

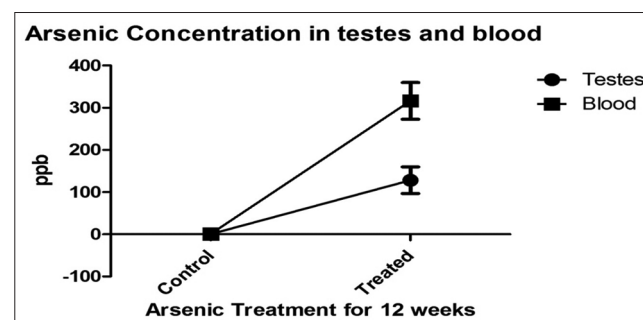


Fig. 1: Arsenic concentration in blood and testes of arsenic-treated mice and control with mean \pm standard deviation ($*p < 0.0001$)

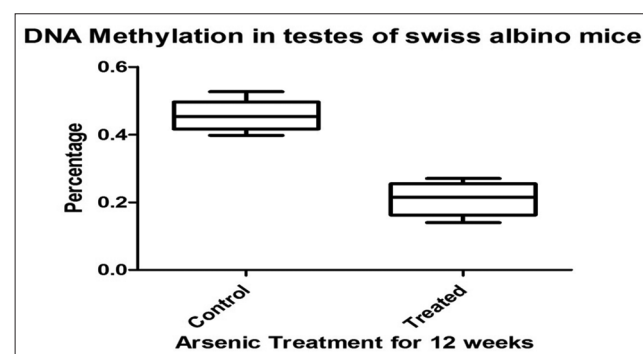


Fig. 2: Mean \pm standard deviation of percentage of global methylation in genome extracted from testes of control and arsenic-treated mice group ($**p < 0.0001$)

hypermethylation of tumor suppressor genes. Acute exposure of arsenic for 3 months leads to global hypomethylation of DNA in mice. Since arsenic is accumulated in high concentration in testes, the genome becomes vulnerable to epigenetic changes. Apart from direct or indirect mutation in oncogene or tumor suppressor genes, epigenetic modification due to toxicity has emerged as a new and yet challenging mechanism of carcinogenesis which needs to be studied extensively.

ACKNOWLEDGMENT

Authors are thankful to ICMR, New Delhi, for financial support to the study and to S. S. Hospital and Research Institute, for providing infrastructure facilities. We are also indebted to Dr. A.K. Saxena, Head, Department of Pathology and Laboratory Medicine, AIIMS, Patna, for his kind cooperation. We are also thankful to Mahavir Cancer Institute and Research Centre, Patna, for technical support.

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