Effect of vitamin E on sperm number and testis histopathology of sodium arsenite-treated rats

Hamid Reza Momeni1,2, Shahrbanoo Oryan3, Najmeh Eskandari3
2Department of Biology, Faculty of Sciences, Arak University, Arak;
3Department of Biology, Faculty of Sciences, Tarbiat Moallem University, Tehran, Iran

Received: 29 May 2011; accepted: 10 April 2012

SUMMARY

The aims of this study were to investigate the adverse effects of sodium arsenite on the reproductive system of male rats as well as to examine whether vitamin E is able to ameliorate these effects. Adult rats were divided into four groups: 1/ control, 2/ sodium arsenite (8 mg/kg/day), 3/ vitamin E (100 mg/kg/day), and 4/ sodium arsenite + vitamin E group. Treatments were administered orally by gavage for eight weeks. After treatment, body and left testis weights were recorded and the testis was used for the histological analysis. Left cauda epididymis was used to count sperm number. Body and testis weight did not differ among the groups (p>0.05). A significant decrease (p<0.001) in sperm number and mean diameter of seminiferous tubules as well as a significant increase (p<0.001) in the mean diameter of seminiferous tubules’ lumen were
found in sodium arsenite group compared to those of controls. Sodium arsenite did not affect the morphology and diameter of spermatogonial nucleus (p>0.05). In the sodium arsenite + vitamin E group, vitamin E ameliorated (p<0.001) the adverse effects of sodium arsenite on sperm number as well as the diameters of tubule and lumen. In addition, the treatment of rats with vitamin E alone significantly (p<0.001) increased the diameter of seminiferous tubules and significantly (p<0.001) decreased seminiferous tubules’ lumen compared to the control group. Vitamin E appeared to ameliorate the adverse effects of sodium arsenite on epididymal sperm number and some morphometrical parameters of the adult rat testis. Reproductive Biology 2012 12 2: 171–181.

**Key words:** rat, sodium arsenite, sperm number, vitamin E

**INTRODUCTION**

Arsenic is a toxic metal, abundant in the earth crust (1.5–2 ppm; [16]) and contaminating drinking water [2]. Arsenic is used in foods preservatives, herbicides, insecticides, rodenticides [3] and arsenic-containing drugs [9, 15]. Arsenic contamination may cause wide variety of diseases such as cancers [23], diabetes mellitus [30] and cardiovascular disorders [10, 32]. Arsenic-induced reproductive toxicity has also been documented. A dose-dependent decrease in the weight of the testis and accessory sex organs [1], reduction in sperm number [12, 17], sperm viability and motility [22] as well as abnormal sperm morphology [26] are examples of the toxic effects of this pollutant. In addition, massive degeneration of germ cells [27], necrotic changes in testicular tissue [22], Leydig cell atrophy [27], reduction in testicular protein level [7] and alterations in the level of luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone [17, 28] were reported as effects of arsenic toxicity.

Oxidative stress was reported to mediate the toxic effects of arsenic [29]. Arsenic-induced generation of reactive oxygen species (ROS) and subsequent oxidative stress affected testicular function [20]. Vitamin E is known as a major antioxidant [34] preventing membrane damage mediated by free
radicals [14]. The vitamin was reported to reduce oxidative stress in the testis [18]. Adverse effects of sodium arsenite were demonstrated in the adult male reproductive tract [26, 28]. To our knowledge, the effect of vitamin E on sodium arsenite-mediated toxicity was not examined in spermatozoa and testes of the rat. The present study was performed to investigate the effect of vitamin E on epididymal sperm count and testicular tissue in sodium arsenite treated adult rats.

MATERIALS AND METHODS

Adult male Wistar rats (250±20 g) were purchased from Pasture’s Institute, Iran. The animals were housed in plastic cages at 12 h light/dark cycle, (24±2°C) with water and food ad libitum. Adult rats (n=24) were divided into four groups: 1/ control rats receiving distilled water; 2/ sodium arsenite (8 mg/kg/day, Merck, Germany)-treated rats, 3/ vitamin E (100 mg/kg/day, Sigma, St. Louis, MO, USA)-treated rats, and 4/ (sodium arsenite + vitamin E)-treated rats. The treatments were administered by oral gavage, daily for eight weeks [13]. The experiments were approved by the local ethical committee at Arak University. At the end of the treatment, the animals were weighed, anesthetized by pentobarbital injection (60 mg/kg) and sacrificed. The left testis and cauda epididymis of the animals were dissected, and then the testis was cleared from fat tissue and its weight was recorded.

The dissected epididymis of each animal was transferred into 10 ml Ham’s F10 medium and cut into small slices to release the spermatozoa into medium. Ten minutes later, one ml of the sperm suspension was diluted with 9 ml of formaldehyde. The diluted sperm suspension was transferred into Neubauer hemocytometer’s chamber and sperm heads were counted under a microscope. Sperm count was performed according to WHO guidelines [33] and data were expressed as the number of sperm per ml. Left testes were fixed in Modified Davidson’s Fluid fixative (30% formaldehyde, 15% ethanol, 5% glacial acetic acid and 50% distilled water) for five days. The fixed testes were cut into small slices, washed in phosphate-buffered-
saline (PBS, 3×5 min) and incubated in 20% sucrose in PBS at 4 °C. Tissue samples were cut into 7-μm-thick sections in a cryostat (Leica, Germany) and mounted onto poly-L-lysine-coated slides.

To evaluate morphological features of spermatogonia the cryostat sections were washed in PBS (3×5 min) and stained with Hoechst 33342 (Sigma, St. Louis, MO, USA, 5 µg/ml in PBS, 30 seconds at room temperature). The sections were then washed in PBS (3×5 min), mounted in PBS/glycerol solution (1:1) and coverslipped. Photographs were taken with an Olympus camera attached to an Olympus fluorescence microscope at 400× magnification. To detect DNA fragmentation in spermatogonia, TdT-mediated nick-end labeling (TUNEL) method was performed according to the manufacturer’s protocol (Roche, Germany). To analyze the histomorphological changes of the testes, the cryostat sections were stained by Heidenhain’s Azan method [21]. The samples were then evaluated under a light microscope and photographed. Motic Image 2000 Software was used to measure the diameter of seminiferous tubules, seminiferous tubule’s lumen and spermatogonia nuclei. The results were expressed as mean±SD for six animals per group. One-way analysis of variance (ANOVA) followed by Tukey’s test was used to assess the statistical significance of data. p<0.05 was considered significant.

RESULTS

No significant differences were found in the weight of body and testis among the examined groups (tab. 1). The mean epididymal sperm number was lower (p<0.001) in the sodium arsenite group compared to that of controls and higher (p<0.001) in the sodium arsenite + vitamin E group compared to that of sodium arsenite group. The highest number of spermatozoa was observed in the vitamin E group. No significant difference (p>0.05) in the mean diameter of spermatogonial nucleus was also found between the sodium arsenite and control group (tab. 2). The morphological changes of apoptosis, i.e. cellular shrinkage as well as nuclear and chromatin condensation, were not found in spermatogonia of the sodium arsenite-
Table 1. Body and testis weight (mean±SD) of male rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Testis weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>276.5±20.1a</td>
<td>1.38±0.26a</td>
</tr>
<tr>
<td>Sodium arsenite</td>
<td>270.8±15.2a</td>
<td>1.23±0.14a</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>286.5±6.7a</td>
<td>1.41±0.24a</td>
</tr>
<tr>
<td>Sodium arsenite + vitamin E</td>
<td>271.3±22.5a</td>
<td>1.34±0.28a</td>
</tr>
</tbody>
</table>

n=6 per group; means with the same superscripts do not differ significantly (p>0.05)

Table 2. Epididymal sperm number and diameter of selected morphological parameters (mean±SD) of rat testes

<table>
<thead>
<tr>
<th>Groups</th>
<th>Sperm number (10⁶)</th>
<th>Diameter (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Seminiferous tubules</td>
<td>Seminiferous tubule’s lumen</td>
</tr>
<tr>
<td>Control</td>
<td>15.86±0.8c</td>
<td>281.7±1.8c</td>
</tr>
<tr>
<td>Sodium arsenite</td>
<td>11.46±1.5a</td>
<td>259.5±2.0a</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>16.94±1.2c</td>
<td>293.0±2.4d</td>
</tr>
<tr>
<td>Sodium arsenite + vitamin E</td>
<td>14.55±0.2b</td>
<td>271.3±1.6b</td>
</tr>
</tbody>
</table>

n=6 per group; means with the same superscripts do not differ significantly (p>0.05)

treated rats (data not shown). Similarly, TUNEL analysis did not reveal any features of apoptosis in the testis of examined rats including those from the sodium arsenite group (fig. 1).

The typical structure of seminiferous tubules was observed in the control group (fig. 2a). In the sodium arsenite group, seminiferous tubule’s wall was irregular, less compact and vacuolated, with reduced height of seminiferous tubule’s wall. In this group, a significant decrease (p<0.001) in the mean diameter of seminiferous tubules and an increase (p<0.001) in the diameter of seminiferous tubule’s lumen were observed compared to controls (tab. 2, fig. 2ab). In the sodium arsenite + vitamin E group, vitamin E ameliorated the histopatological changes induced by sodium arsenite in the testis.
Figure 1. Images of rat testicular tissue examined by TUNEL staining. a/ control group; b/ sodium arsenite group; c/ vitamin E group and d/ sodium arsenite+vitamin E group. TUNEL-positive cells were not found in the examined tissues; scale bar: 50 µm.

Figure 2. Images of rat testicular tissue stained with Heidenhain’s Azan. a/ control group: typical height with regular arrangement of seminiferous tubule’s wall; b/ sodium arsenite group: irregular and vacuolated (arrows) seminiferous tubule’s wall and increased diameter of seminiferous tubule’s lumen compared to control group; c/ vitamin E group: increased height of seminiferous tubule’s wall compared to control group; d/ sodium arsenite+vitamin E group: more regular arrangement of seminiferous tubule’s wall compared to sodium arsenite group; scale bar: 50 µm.
Moreover, the vitamin partially restored (p<0.001) the adverse changes in the mean diameter of seminiferous tubules and seminiferous tubule’s lumen caused in the testis of sodium arsenite-treated rats (tab. 2). In comparison to the control group, the application of vitamin E alone significantly (p<0.001) increased the height of seminiferous tubule’s wall (fig. 2d) and the mean diameter of seminiferous tubules as well as decreased the mean diameter of seminiferous tubule’s lumen (tab. 2).

**DISCUSSION**

In the study we examined the adverse effect of sodium arsenite on epididymal sperm counts and selected histological parameters of the adult rat testes. We also tested the protective effects exerted by vitamin E in sodium arsenite-treated rats. Our results were consistent with some previous reports on sodium arsenite effects on body and testis weight [25, 27], however, other studies reported a reduction in body and testis weight [1, 4]. This discrepancy may be caused by different doses and treatment periods [5].

In the present study a significant decrease in the total sperm number was found in sodium arsenite-treated rats compared to controls. Sodium arsenite was reported to induce apoptosis in the testis [24]. However, we were not able to demonstrate the effects of sodium arsenite on spermatogonia apoptosis by means of Hoechst staining and TUNEL assay. Moreover, no differences in spermatogonial nuclear diameter were observed among the groups. These findings suggest that the reduced sperm number in sodium arsenite-treated rats were not caused by spermatogonia damage or death.

Arsenic is reported to produce free radicals known to cause peroxidation of polyunsaturated fatty acid in spermatozoa [20, 22]. We hypothesized that toxic effects of sodium arsenite on the reduction of sperm number is due to oxidative stress. In such a case, vitamin E, a well-known antioxidant [34], should improve the adverse effects of sodium arsenite on sperm number. Indeed, we showed that vitamin E ameliorated sodium arsenite-mediated sperm number reduction. Arsenic as an endocrine disruptor [11] may also affect sexual hormone levels and spermatogenesis [17, 28]. The inhibitory
effects of sodium arsenite on the gene expression of enzymes involved in steroidogenesis have also been reported [8]. Therefore, it is possible that alterations in synthesis/secretion of reproductive hormones (testosterone, LH, FSH) are also responsible for the reduction of sperm number in sodium arsenite-treated rats.

Our results suggested that spermatogenic cells in seminiferous tubules in rats treated with sodium arsenite were damaged. Sodium arsenite via binding to sulfhydryl and carbonyl groups of proteins [20] as well as the inhibition of transcription factors [19] affected the synthesis of enzymes essential for cell metabolism. Sodium arsenite through oxidative stress exerted adverse effects on the testis [20]. Thus, it is likely that histopathological changes observed in seminiferous tubules could result from sodium arsenite-induced oxidative stress. The fact that in our study vitamin E ameliorated the histopathological changes induced by sodium arsenite in the testis is consistent with this notion.

It is of interest that vitamin E alone enhanced the mean diameter of seminiferous tubules, the height of seminiferous tubule’s wall and reduced the mean diameter of seminiferous tubule’s lumen in comparison to control animals. Traditionally, vitamin E is called an anti-sterility vitamin [31] and is associated with normal function of the male reproductive system [6]. Since vitamin E plays an important protective role against oxidative stress by reducing malondialdehyde level and improving the of antioxidant defense system activity in testicular cells [34], it is reasonable to assume that the positive effects of vitamin E observed in the present study resulted from its antioxidant property. In conclusion, our results indicate that sodium arsenite has a negative influence on sperm number and testis histological parameters in adult rats. In addition, vitamin E was able to ameliorate the adverse effects of sodium arsenite.

ACKNOWLEDGMENTS

We would like to thank Monireh Mahmoodi and Mehdi Farahani for their excellent assistance during this study.
REFERENCES


