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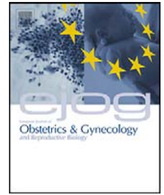

The effects of acrylamide on sperm parameters and membrane integrity of epididymal spermatozoa in mice

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ABSTRACT

Objective: Acrylamide is a chemically reactive substance used in various industries. Recently, the discovery of acrylamide in a variety of human foods including heat-processed starchy foods such as potato chips and bread has been reported. Acrylamide is also known as a carcinogen and cytotoxic material. The aim of this study was to evaluate the detrimental effects of acrylamide on membrane integrity and sperm parameters in mice.

Study design: This experimental study was conducted on thirty male NMRI mice, aged 8–10 weeks and weighing 25–30 g. They were randomly allotted into three equal groups. Group I (low dose) and group II (high dose) were fed on water solutions containing acrylamide 5 and 10 mg/kg/day, respectively, for 2 months, while the third group received fresh water as the control group. Sperm analysis was done for parameters as well as evaluation of membrane integrity by Hypoosmotic Swelling Test (HOS-test) for sperm tails and Eosin-Y staining for sperm heads.

Results: Total sperm motility and progressive motility (fast and slow) in both groups, I and II, decreased significantly ($P = 0.00$), but no significant change was observed in non-progressive motility ($P > 0.05$). The total motile sperm percentage decreased significantly only in group II ($P = 0.01$). Sperm morphology did not significantly change in the experimental groups compared to the controls ($P > 0.05$). In sperm membrane integrity evaluation, functional intact membrane of sperm tail in both groups I and II had a significant decrease ($P = 0.00$), but membrane integrity of the sperm head decreased significantly only in group II ($P = 0.00$).

Conclusion: These results indicate that acrylamide, through effects on membrane integrity, decreased sperm vitality as well as causing abnormal sperm parameters in progressive motility and total motility.

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1. Introduction

Acrylamide is a reactive, highly water-soluble monomer frequently used in the polymer industry, the cosmetic, paper and textile industries, wastewater treatment, and laboratory gels [1,2]. Exposure to acrylamide can occur in workplaces or in the environment through air, water, land, and groundwater during its production or use [3,4]. Recently, the presence of acrylamide in heat-treated food products such as potato chips, French fries, and crisp breads has been reported by Swedish Food Administration [5]. The formation of acrylamide is associated with high-temperature (>200 °C) cooking process in certain carbohydrate-rich foods, particularly when asparagines react with sugar [6,7].

Several studies have been focused on acrylamide as a cytotoxic material; for example exposure to monomeric acrylamide results in peripheral neuropathy, with accompanying weakness of the limbs [8]. Acrylamide has been classified as a probable carcinogenic compound in human-based or animal experiments, because it has been shown that acrylamide can induce an increase in tumor incidences including tunica vaginalis mesotheliomas, mammary gland fibroadenomas, adenocarcinomas, thyroid follicular adenomas and carcinomas in rats [9]. In fact it is believed that acrylamide is clearly genotoxic both in somatic and germ cells [10,11].

Acrylamide has been tested for its effects on reproductive parameters, including decreased sperm count and increased abnormal sperm morphology [12]. Song et al. observed that subchronic exposure to acrylamide could affect the normal development of spermatozoa, especially decreased sperm vitality, and increased the percentage of abnormal sperm. They also found that acrylamide can directly damage Leydig cells and affects the endocrine function of the testis [13]. It is demonstrated that acrylamide has toxic effects on seminiferous tubules and it

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decreases the production of sperm in male rats [14]. The results of Yang et al. indicate that acrylamide induces histopathological lesions such as formation of multinucleated giant cells, vacuolation and production of high numbers of apoptotic cells in the seminiferous tubules of the rat [15]. They also observed the dose-dependent effect of acrylamide on reducing serum testosterone level and Leydig cell viability, which resulted in diminished spermatogenesis; and the high doses of acrylamide can reduce the body weight, testis weight and epididymides weight [16]. These studies suggest that acrylamide has the potential to adversely affect male reproductive capacity.

Plasma membrane function activity is one of the most important aspects of sperm biology, involving metabolic exchanges with the surrounding medium which play an important role in several events during fertilization (e.g. capacitation, acrosome reaction and sperm-oocyte fusion) [17]. Investigation of membrane integrity seems to offer more information about sperm fertility potential than sperm parameters such as count, motility and morphology [18,19], and probably there is a relationship between sperm membrane integrity and sperm parameters. There are two rapid and simple tests for the evaluation of sperm membrane integrity and the rate of necrospermia: Eosin-Y staining, for assessing the membrane of the head and Hypoosmotic Swelling Test (HOS-test) for the tail of spermatozoa [20–22]. The aims of this study are to determine the toxic effects of acrylamide on epididymal sperm membrane integrity and parameters in the mouse as an experimental model.

2. Materials and methods

2.1. Chemicals

Acrylamide monomer ($CH_2{=}CHCONH_2$) is solid, white, dry and odorless crystals with chemical purity >99% (Merk Chemical Co., Germany).

2.2. Animal

Thirty male NMRI mice (Pasteur Institute, Tehran, Iran) were preserved in the animal house of the Research and Clinical Center for Infertility (Yazd, Iran) with controlled temperature (22–24 °C) and a light cycle of 12-h light/dark. First, mice were divided into three groups including two experimental groups, I (n = 10), II (n = 10), and one control group (n = 10). The control group got usual mouse food and water ad libitum, but the experimental groups (group I or low dose, and group II or high dose) were fed on water solutions containing acrylamide 5 mg/kg/day (low dose) and 10 mg/kg/day (high dose) for 2 months (about two durations of spermatogenesis in mice) respectively. The proposal of experiments was approved by the University Ethics and Animal Use and Care Committee.

2.3. Epididymal sperm preparation

After 2 months, the mice were deeply anesthetized by i.m. injection of ketamine (80 mg/kg) and xylazine (10 mg/kg). A small piece of the cauda epididymis was dissected and placed in 1 ml of pre-warmed T6 culture medium. Gentle agitation along with tearing of the tissue was applied to make spermatozoa swim out into the medium placed in a Falcon culture dish [22,23]. Each sample was briefly incubated at 37 °C, 5% CO_2 for 1 h in order for further sperm analysis.

2.4. Sperm analysis

Sperm parameters including total sperm count (10^6/ml), sperm motility (%) and normal morphology (%) were evaluated in each of the animals. Sperm motion analysis was performed using Makler chamber (Sefi Medical Co., Haifa, Israel) and phase-contrast microscopy (Olympus Co., Tokyo, Japan) at X200. Motility was expressed as percentage of progressive (fast and slow) and non-progressive sperm cells according to WHO guidelines (1999) [20]. Total sperm count analysis was also performed using Makler chamber and phase-contrast microscopy at X200 according to WHO criteria [20]. Moreover, the percentage of normal morphology of 200 spermatozoa per sample was assessed using Giemsa staining and light microscopy (Nikon Co., Japan) at X400 [24]. All the analyses were performed by one experienced technician blinded to the study.

2.5. Evaluation of membrane integrity (necrospermia)

For evaluation of necrospermia, two different assays were applied.

2.5.1. Eosin-Y staining

This staining was done by mixing 10 μl of sperm sample with 10 μl of dye (0.5% wt/vol; Merck Chemical Co., Germany) on a microscope slide and covered with a coverslip. A total of 200 sperm cells were counted within a few minutes after the addition of the dye [20–22]. Evaluation of live (unstained) and dead (red stained) spermatozoa was done using light microscopy at X400.

2.5.2. HOS-test

The HOS-test was done by mixing 10 μl of sperm sample and 100 μl of warmed hypoosmotic solution. The solution was prepared by dissolving 7.35 g of sodium citrate hydrate and 1.351 g fructose in 100 ml distilled water and then was frozen in 1 ml aliquots for use as required [20–22]. The mixture was incubated for 45 min at 37 °C before it was examined with light microscopy at X400. Two hundred spermatozoa were evaluated and the percentage of live sperm cells (with coiled tail) was calculated accordingly.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 15.0 software (SPSS Inc., Chicago, IL). Data are presented as mean ± standard deviation. Statistical significance was set at $P < 0.05$. Two-way ANOVA was considered for comparison of the results between the three groups.

3. Results

The results of sperm parameters in different doses of acrylamide are reported in Table 1. The sperm total motility in both case groups was the same but in comparison to control group, it decreased significantly ($P = 0.00$). The progressive motility (fast and slow motility) of sperm cells in both case groups in comparison to the controls decreased significantly ($P < 0.00$). However, the non-progressive motility in the case groups increased, but it was not significant in comparison to the control group ($P > 0.05$). The difference between the percentage of normal sperm morphology in the case groups was not significant with respect to the control group ($P > 0.05$).

The mean percentages of sperm concentration decreased in both case groups when compared with the control group, but it was significant only in case group II ($P = 0.012$).

In the evaluation of membrane integrity using both Eosin-Y staining and HOS-test, (Fig. 1), the rate of necrospermia increased in the case groups in comparison to control mice. However, it should be noticed that in the Eosin-Y test, it was significant only in case group II ($P = 0.007$).

Table 1
The differences in mouse sperm parameters between control and case groups presented as mean ± standard.

Variable	Case group (I), 5 mg/kg/day (n = 10)	Case group (II), 10 mg/kg/day (n = 10)	Control group (n = 10)	P value
Concentration (106/ml)	14.70 ± 3.43	12.10 ± 4.84	17.30 ± 2.54	0.280[a] 0.012[b] 0.280[c]
Fast motility (%)	9.00 ± 5.35	3.50 ± 3.95	20.00 ± 4.71	0.000[a] 0.000[b] 0.037[c]
Slow motility (%)	11.30 ± 4.24	12.20 ± 7.09	18.00 ± 3.49	0.020[a] 0.048[b] 0.921[c]
Non-progressive motility (%)	20.70 ± 8.84	24.30 ± 5.31	19.00 ± 6.99	0.858[a] 0.244[b] 0.511[c]
Immotile (%)	60.00 ± 8.16	60.00 ± 8.16	43.00 ± 7.14	0.000[a] 0.000[b] 1.000[c]
Normal morphology (%)	90.70 ± 6.20	90.50 ± 4.45	94.70 ± 2.40	0.148[a] 0.124[b] 0.995[c]

[a] Difference between case groups (I) and control group.
[b] Difference between case groups (II) and control group.
[c] Difference between case groups.

4. Comment

Semen analysis is the main part of the routine assessment of male infertility. The transportation of different materials across the sperm membrane is an important biochemical process essential for sperm viability and fertilizing capabilities [25]. So, membrane integrity assessment could be a useful complementary test in addition to standard semen analysis.

There are several studies indicating the detrimental effects of acrylamide on male fertility indices in mice and rats, but to our knowledge there are few surveys on sperm viability [13]. In fact, our results involve novel information regarding the detrimental effects of acrylamide on sperm viability in mice. Tyl et al. observed that acrylamide had no significant effect on the epididymal sperm motility or concentration in the rat testis [26] but more recently, under the same experimental design, Yang et al. found reduced sperm concentration in the cauda epididymis, as well as increased morphological abnormalities of spermatozoa [15,16]. In addition, it was found that testosterone concentration decreased in the serum of acrylamide-treated rats. Finally, they concluded that acrylamide can reduce the viability of Leydig cells, which in turn, diminishes spermatogenesis in the rat testis [15]. Also, hyperplasia of Leydig cells associated with germ cell loss was reported in mice exposed to acrylamide [27]. Leydig cells regulate tubular function and produce the high local concentrations of testosterone and control spermatogenesis [28].

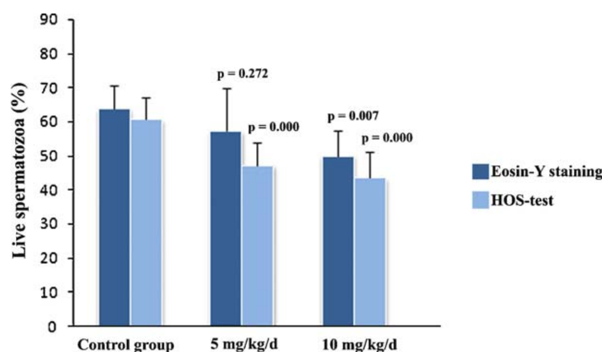

Fig. 1. Comparison of sperm membrane integrity, evaluated by Eosin-Y staining and HOS-test between three groups presented as mean ± SD.

Therefore, the hyperplasia of Leydig cells may affect male reproductive function.

Wang et al. evaluated the effect of acrylamide on development and reproductive performance of male rats and showed that acrylamide can induce histopathological lesions in the testis, has toxic effects on the seminiferous tubules and decreases the production of viable sperm cells in male rats [14]. In another study acrylamide induced histopathological changes in the testis such as vacuolation and swelling of the round spermatids, necrosis of the late elongated spermatids, numerous apoptotic cells and formation of multinucleated giant cells in the seminiferous tubules which can affect the sperm parameters [15]. Song et al. observed that subchronic exposure to acrylamide could affect the normal development of sperm, especially decreased sperm vitality, and increased the rates of abnormal morphology in sperm; also, acrylamide directly damages Leydig cells and affects the endocrine function of the testis and spermatogenesis [13,29].

There has been extensive study on the induction of dominant lethal mutations by acrylamide. It is known as a germ cell mutagen which induces clastogenic effects predominantly in spermatids of mice and rats [30,31]. Acrylamide is metabolized by hepatic P450 CYP2E1 to glycidamide [32]: approximately 50% of orally administered acrylamide is metabolized via this pathway and glycidamide binds to DNA and induces DNA adducts and mutations [33]. In addition, Sega and Generoso observed DNA breakage during specific stages of germ cell development in male mice exposed to acrylamide [34].

Our findings are in agreement with the above-mentioned studies and indicate that acrylamide has a dose-dependent effect on mouse sperm parameters which can decrease the concentration and progressive motility of sperm cells. In sperm analysis in the case groups (low dose and high dose of acrylamide) fast and slow motilities decreased significantly and non-progressive motility increased, though it was not significant. On the other hand, the sperm count decreased significantly only in the high-dose group and morphology had no significant changes in the experimental groups. Also, evaluation of membrane integrity showed that the viability of sperm cells decreased in the experimental groups. In sperm membrane integrity evaluation, functional intact membrane of the sperm tail in both low- and high-dose groups had a significant decrease, but membrane integrity of the sperm head decreased significantly only in the high-dose group.

To explain the etiology of the reproductive toxicity of acrylamide, it should be noticed that acrylamide, by its effects on Leydig cells and seminiferous tubules and by increasing perturbed gene expression level, causes histopathological changes in the testis and decreases in serum testosterone and spermatogenesis which affect sperm parameters including decreased count, motility and normal morphology and an increased rate of necrospermia. Another explanation for these effects has been shown by Tyl and Friedman [35]. According to their findings, acrylamide and/or glycidamide bind to spermatid protamines and cause dominant lethality and effects on sperm morphology. This chemical compound also binds to the motor proteins, kinesin and dyneine, resulting in reduction of sperm motility by interference with flagellar function.

5. Conclusion

Our study showed that acrylamide has detrimental effects on parameters of mouse sperm, including decrease in count, total motility, progressive motility (fast and slow motility) and increase in non-progressive motility, but it probably cannot produce any changes in sperm morphology. On the other hand, membrane integrity in the head and the tail of sperm cells is interrupted and the viability of sperm will be decreased in acrylamide-treated animals.

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