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Effects of low dose acrylamide on the rat reproductive organs structure, fertility and gene integrity

Saleh ALKarim[1,2], Sufyan ElAssouli[1,2], Soad Ali[3], Nasra Ayuob[3,4*], Zaki ElAssouli[2]

[1]*Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia*

[2]*King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia*

[3]*Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia*

[4]*Histology Department, Faculty of Medicine, Mansoura University, Egypt*

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ABSTRACT

Objectives: To assesses the effects of long term exposure to low dose of acrylamide (0.4 μg/g) in post-weaning Sprague–Dawley rats on the structure of the reproductive organ as well as DNA integrity.
Methods: The histological changes in the male and female reproductive organs the morphological changes in sperms as well as the genotoxic effect of acrylamide were assessed. The effect acrylamide on pregnancy outcome was evaluated.
Results: Testes of acrylamide-fed rats showed decreased number of seminiferous tubules containing mature sperms and degenerative changes in sperm germ cell layers. Some sperms of epididymal cauda showed head deformity. In female, acrylamide included cystic ovarian changes, degenerative changes of zona pelluida, granulosa cells and oocytes. Post implantation loss and decrease in the number of full term fetuses were detected. Resorption sites showed necrotic fetal tissue with vacuolation of amniotic cells.
Conclusion: Acrylamide cause harmful effect on the reproductive organ structure, fertility and cause extensive DNA damage in peripheral blood lymphocytes.

1. Introduction

Acrylamide (ACR) is carcinogenic in animal and probably carcinogenic to humans. It is carcinogenic in multiple organs in both sexes of several rodents. Glycidamide which is ACR metabolite is believed to be the cancer-risk agent in ACR exposure[1]. In rats, tumorigenesis occurs in several hormonally regulated tissues[2,3]. Some previous studies showed that ACR is capable of inducing genotoxic, carcinogenic, developmental, and reproductive effects in tested animals. Since there is sufficient evidence of carcinogenicity in experimental animals as outlined under the U.S. environmental Protection Agency (EPA) proposed guidelines for carcinogen risk assessment, ACR is categorized as a 'B2' carcinogen and therefore be considered a 'probable' human carcinogen[4].

Acrylamide is able to cross the placenta, reach significant concentrations in the conceptus and produce direct developmental and post-natal effects in rodent offspring. Acrylamide has an adverse effect on reproduction as evidenced by dominant lethal effects, degeneration of testicular epithelial tissue, and sperm-head abnormalities[5]. The finding that ACR is formed in carbohydrate rich food during preparation at high temperatures raised concern about cancer risks associated with the dietary intake of fried or backed carbohydrate food. Acrylamide is formed when frying, roasting, grilling or baking carbohydrate-rich foods at temperatures above 120 °C through interactions of amino acids with reducing sugar[6]. Acrylamide is thus found in a number of foods, such as bread, crisps, French fries and coffee. Tobacco smoking also generates substantial amounts of ACR. But it is the incidental formation during cooking of common starchy foods that leads to pervasive human exposure, typically in the range of 1 μg/kg body weight)/day [7]. Acrylamide neurotoxicity was reported in humans[8] and experimental animals[9]. Other toxic effects of ACR were reported such as chromosomal damage in somatic cells and

*Corresponding author: Dr. Nasra Ayoub, MD, JMHPE, Department of Medical Education, Faculty of Medicine, King Abdulaziz University, Jeddah, Saudi Arabia.
Tel: +966 530112205
Fax: +966 6400855
E-mails: nasraayuob@gmail.com, nayuob@kau.edu.sa

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mutagenesis[10] and disturbance in genomic imprinting during spermatogenesis[11], and recently male reproductive toxicity, prenatal lethality, and endocrine-related tumors in rodents[12–14]. The metabolism of ACR to its epoxide metabolite, glycidamide, which is mediated by cytochrome P450 2E1, is thought to be the active metabolite which plays a central role in ACR genotoxicity in experimental animals and humans[15].

The European Commission on acrylamide call for establishing an international network "Acrylamide in Food" inviting all interested parties to share relevant data as well as ongoing investigations[16]. The committee noted that the high levels of ACR formed by heat processing of food calls for urgent research to lower this formation and for research to understand the implications for human health[17]. Not enough information is available about levels of this chemical in different foods and the potential risk from dietary exposure. There is some consensus that low levels of ACR in the diet are not a concern for neurotoxicity or reproductive toxicity in humans. However, further research is needed to study the long-term, low-level cumulative effects of ACR exposure.

Although many previous researches have been conducted to study the impact of acrylamide on the different body organs, further research in this area focusing on better understand of the dose-response of health effects corresponding to dietary intake is recommended in recent studies[18].

The present work was designed to assess the cumulative effect of the exposure to low dose of ACR (0.4 μg acrylamide/gram of regular rodent food) *via* oral route early in post weaning period on the developing reproductive system of both male and female rat. The ACR-induced changes on the DNA integrity of lymphocytes were also assessed.

2. Materials and methods

2.1. Animals and acrylamide administration

This study was approved by the biomedical research ethics committee at King Fahed Medical research Center (KFMRC). Forty male and female Sprague–Dawley rats aged 3 weeks with an average weight of 30–35 g were used in this study. Animals were maintained on a 12 h light/dark cycle with food and sterile distilled water available ad libitum, the temperature range was 20 °C–24 °C, and the humidity range was 60%–70%. After one week of acclimatization, animals were randomized into two main groups; control (n = 10) and ACR-fed groups (n = 30; 15 male and 15 female).

Acrylamide ultra-pure electrophoresis grade was used in this study. The experimental ACR-fed group received diet containing ACR at a dose of 0.4 μg/g, while the control group received regular diet. The diet was prepared weekly. The total amount of acrylamide taken by the animals was equal to 60 μg/kg of body weight/daily. Exposure to ACR-contained diet was continued daily for the age of 60 and 90 days. The ACR dose and regimen adopted in the present study was according to the previous studies[19,20].

2.2. Assessment of ACR-induced changes in reproductive organs

For sperm morphology assessment, sperms were collected from cauda in normal saline, killed by incubating the sperm suspension at 80 °C for 30 min. For morphological abnormalities a total of 300 sperms were smeared on glass slide examined under light microscopy at 400×. Sperms lacking hook, banana-like head, and twin headed, and twin-tailed sperms were considered abnormal[21].

At age of 60 and 90 days, ten animals of each group were anesthetized, the chest was opened then pericardial perfusion with saline followed by 10% neutral buffered formalin was done to ensure in situ perfusion and avoid postmortem changes. The whole male and female reproductive system was removed as one block, re-fixed in 10% neutral buffered formalin. Testes, seminal vesicles, ovaries, fallopian and uterine horns were dissected out weighted and processed to obtain paraffin blocks for histological examination. The paraffin sections were stained with haematoxlin and eosin (H&E).

2.3. Assessment of ACR-induced changes on fertility outcome

Fifteen adult female rats (90 days) fed normal diets were housed with 5 ACR-fed male partner (3 female with one male in each cage). The females were checked every 24 h for appearance of vaginal plug or the positive vaginal smear for sperm. If any appear, then this day was considered the day one of pregnancy. The number of pregnant animals was recorded. Pregnant rats were individually caged. At the day 18 of pregnancy animals were sacrificed by decapitation. The abdomen was opened and pregnant uterine horns were removed and the numbers of full mature fetuses, site of resorption were recorded. The ovaries were inspected for presence of corpora lutea then processed for histological studies.

2.4. Assessment of ACR-induced genotoxicity and mutagenicity

To assess the genotoxicity of pure ACR, the Single Cell Gel Electrophoresis (SCGE) or alkaline comet assay was performed according to the protocol of Hartmann and Speit[22], and was analyzed using Loat's comet assay software with extended dynamic range imaging (EDRI). A total of 2 of whole blood taken from the rats, initially collected in EDTA tubes, were used. Analysis of the comet tail was carried out using Loat's single gel comet assay software with EDRI and observed using a fluorescent microscope (Olympus BX-51; Japan). The positive control used in this assay was glycidamide (from LKT laboratories, St Paul, Minnesota, USA). It was reconstituted with distilled water. For the preparation of a negative control, control rat whole blood was used, the alkaline comet assay was performed, and the average tail moment was calculated. For the preparation of different glycidamide dilutions, glycidamide was incubated with the whole rat blood for 4 h at 37 °C, and then the alkaline comet assay and analysis of comet tail were carried out. Slides were observed at 40× magnifications using a fluorescence microscope equipped with an excitation filter of BP 546 nm and a barrier filter of 590 nm. Images of 200 lymphocytes were randomly selected (100 cells from each of 2 replicate slides) and analyzed from each sample. Cells were automatically analyzed by Loats comet assay software.

To assess the genotoxicity of pure ACR, Salmonella typhimurium strains TA98, TA100 & TA1535 have been used. These strains are histidine requiring mutants and have been tested for