Food and Chemical Toxicology 56 (2013) 297–303

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Effects of sodium fluoride on reproductive function in female rats

Yongjiang Zhou, Hailing Zhang, Junlin He, Xuemei Chen, Yubing Ding, Yingxiong Wang, Xueqing Liu *

Laboratory of Reproductive Biology, School of Public Health, Chongqing Medical University, Chongqing, PR China

ARTICLE INFO

Article history:
Received 3 December 2012
Accepted 14 February 2013
Available online 28 February 2013

Keywords:
Sodium fluoride
Exposure
Female reproductive function
Rats

ABSTRACT

The aim of this study was to investigate the effects of sodium fluoride (NaF) on female reproductive function and examine the morphology of the ovaries and uteri of rats exposed to NaF. Eighty female Sprague-Dawley (SD) rats were divided randomly into four groups of 20: one control group and three NaF treated groups. The three NaF treated groups received 100, 150, and 200 ppm, respectively, of NaF for 6 months via their drinking water, while the control group (GC) received distilled water. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), progesterone (P) and estradiol (E_2) were measured using an enzyme-linked immunosorbent assay. Pathomorphological evaluation of the uteri and ovaries was conducted after staining with hematoxylin-eosin and immunohistochemistry. The rate of successful pregnancy in the NaF-treated groups declined in a dose-dependent manner. The concentration of reproductive hormones was significantly lower in the three NaF-treated groups, and the endometrium was damaged. The maturation of follicles was inhibited. In addition, the total number of follicles of all types was significantly lower in the NaF-treated groups. These results suggest that female reproductive function is inhibited by NaF and that exposure to NaF causes ovarian and uterine structural damage. NaF may thus significantly reduce the fertility of female rats.

1. Introduction

Fluoride naturally exists in water, soil, and food. Ingesting water that contains high concentrations of fluoride, primarily from natural sources, is the main source of human environmental exposure worldwide, especially in China and India. Fluoridation is a safe and effective method for the prevention of dental caries. In several countries, water fluoridation is used for this purpose. However, the beneficial range is narrow, and health may be influenced adversely if excessive fluoride is absorbed. The literature indicates that NaF may have toxic effects on the brains of suckling mice (Bouaziz et al., 2010), may impair learning and memory in rats (Basha et al., 2011; Pereira et al., 2011), and may impair hepatic function (Shashi and Bhardwaj, 2011). Increasing exposure to environmental pollutants and chemicals is a major contributor to reproductive health problems (Akinloye et al., 2006; Hansen et al., 2010; Toft et al., 2006).

Fluoride is a widespread natural pollutant with established toxic effects, and the potential relationship between long-term fluoride exposure and fertility impairment has attracted concern (Long et al., 2009; Ortiz-Perez et al., 2003; Spittle, 2009). The importance of reproductive health on offspring development has also prompted an epidemiological investigation into the apparent connections between excessive fluoride exposure and male infertility and low birth rates (Freni, 1994; Ortiz-Perez et al., 2003). Additionally, there are a number of studies in the literature regarding the toxic effects of NaF on the male reproductive system (Chinoy and Narayana, 1994; Sun et al., 2010; Wang et al., 2009).

However, the toxic effects of fluoride on the female reproductive system have rarely been reported. The reproductive tract is susceptible to disruption by fluoride at concentrations that are sufficient to produce other manifestations of toxicity (Spittlea and Meiersb, 2007). A number of animal studies have indicated that adverse reproductive and developmental outcomes occur in individuals exposed to relatively high concentrations of fluoride (Dhar and Bhatnagar, 2009). Most of these investigations, which were conducted with a number of different animal species, including rats, mice and rabbits, found alterations in the levels of reproductive hormones, fertility, histological structures and developmental outcomes (Collins et al., 2001; Elbetieha et al., 2000; Kumar and Susheela, 1994).

The aim of this study was to investigate the effects of NaF on female reproductive function and to examine the morphology of the ovaries and uteri in rats that had been exposed to NaF.

2. Materials and methods

2.1. Animal model and fluoride administration

All experimental procedures involving animal care were carried out in accordance with the Guiding Principles for the Use of Animals in Toxicology, adopted by the Chinese Society of Toxicology. The rats used in this study were maintained

* Corresponding author. Address: Laboratory of Reproductive Biology, School of Public Health, Chongqing Medical University, No. 1 Yixueyuan Road, Yuzhong District, Chongqing 400016, PR China. Tel.: +86 13368113542; fax: +86 23 68485008.
E-mail address: a68733172@online.cq.cn (X. Liu).

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http://dx.doi.org/10.1016/j.fct.2013.02.026

Table 1
Experimental protocol.

Group	Dose of NaF (mg/L)	Duration of exposure (days)	No. of animals		Day of treatment	Treatment
GC	_	180	20	10	181st	Mating to detect the pregnancy rate
				10	181st	Examination of the ovaries and uteri
GL	100	180	20	10	181st	Mating to detect the pregnancy rate
				10	181st	Examination of the ovaries and uteri
GM	150	180	20	10	181st	Mating to detect the pregnancy rate
				10	181st	Examination of the ovaries and uteri
GH	200	180	20	10	181st	Mating to detect the pregnancy rate
				10	181st	Examination of the ovaries and uteri

in the experimental animal center of ChongQing medical university, in accordance with the institutional animal welfare policy [certificate No.: SCXK (YU) 20050002]. Adequate measures were taken to minimize the pain, discomfort and stress of the animals. The rats were housed in plastic cages containing shavings as bedding material and were exposed to 12 h light/dark cycles at a constant temperature (22 ± 2 °C) and humidity (50%), with access to food and water ad libitum. As shown in Table 1, eighty sexually mature female SD rats, with weights ranging from 160 to 250 g (8–10 weeks old), were used in this investigation. During the experimental period, the animals were randomly assigned to one of four groups of twenty rats: the control group (GC) or one of three fluoride-treated groups (low-dose (GL), medium-dose (GM), or high-dose (GH). Sodium fluoride (NaF, 99% pure, Tianjin Guangfu Fine Chemical Research Institute, Tianjin, China) was used as the source of F^-. The GC received distilled water. The three F^- treated groups had ad libitum access to distilled water containing 100, 150, or 200 ppm NaF, respectively, for 6 months, until the completion of the study. The duration and dose of F^- exposure used in this study were based upon previous studies that were performed in rats (Basha et al., 2011; Sun et al., 2010). At the end of the NaF exposure period, ten rats from each group were used for histopathological examinations of the ovaries and uteri, and another ten rats in each group were chosen for mating. During mating, female and male rats were housed at a 3:1 ratio at the end of a 6-month period. One male (weighing 200–250 g) and three females were housed in a cage for one night, and the following day, the vaginal plugs of each female were examined to confirm pregnancy. After mating, all rats were sacrificed, and the uteri and ovaries were removed for examination.

2.2. Fertility assessment

To evaluate fertility at the end of the NaF exposure period, each female rat was housed with virgin untreated males of the same strain for one night to ensure a successful pregnancy. After the removal of the males, all of the females were killed by cervical dislocation. The numbers of pregnant females were immediately recorded after cesarean sections of tissue were performed.

2.3. Serum sampling and processing

After the administration of NaF for 6 months, the rats were sacrificed. Blood samples were then collected from the hearts of 10 rats and centrifuged at 3000 rpm for 10 min to obtain the serum. The serum samples were used for further analyses of the F^-, E_2, P, LH and FSH levels.

2.4. Relative weights of reproductive organs

Before being sacrificed by cervical dislocation, ten females from each of the four groups were weighed separately using an electronic balance. The reproductive organs, including the ovaries and uteri, were then weighed on an electronic balance. Organ coefficients of the uteri and ovaries were measured (organ coefficient = organ weight/body weight × 100) (Wang et al., 2012).

2.5. Histopathological examination

The uteri were removed and fixed in a 10% formaldehyde solution at room temperature (RT). The tissue samples were dehydrated and embedded in paraffin according to standard histological procedures. Serial cross-sections of 5 mm were prepared from each uterus. The sections were mounted and stained with hematoxylin-eosin. At minimum of 10 histological sections from the endometrium were assessed using 10× magnification and photographed using an Olympus BX50 (Olympus) photomicroscope. Two independent observers, who were blinded to treatment, conducted the histopathological evaluations twice (Kamble and Velhal, 2010).

2.6. Fluoride determination

The concentrations of fluoride in the serum were examined after 6 months of fluoride exposure by collecting 2 ml of venous blood from each rat. Fluoride levels were measured according to a potentiometric method using an ion selective electrode (Orion 9609) (Del Razo et al., 1993).

2.7. Detection of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), progesterone (P) and estradiol (E_2) in the serum of female rats

The levels of E_2, P, T, FSH and LH were detected using an enzyme-linked immunosorbent assay (ELISA Kit) (Yan Hui Biological Technology Co. Ltd. Shanghai, China), according to the manufacturer's recommended instructions.

2.8. Immunohistochemistry

The tissue sections were deparaffinized in xylene and rehydrated in descending concentrations of ethanol, followed by antigen retrieval in sodium citrate buffer for 10 min in a microwave oven at 100 °C. Endogenous peroxidase was inhibited by incubation with 3% hydrogen peroxide for 10 min at RT. The tissue sections were blocked in 10% normal goat serum for 30 min and incubated with a primary antibody at 4 °C overnight. The primary antibody was a rat monoclonal anti-PCNA antibody (1:1000; catalog number ab-29; Abcam Biotechnology). After incubation with the primary antibody, the tissue sections were incubated with a biotinylated goat anti-rat IgG for 30 min at 37 °C. The secondary antibody was a biotinylated goat anti-rabbit IgG. The tissue sections were later incubated with a streptavidin-conjugated horseradish peroxidase (ExtrAvidin Kit, Sigma, St. Louis, MO) for 30 min at 37 °C. The staining was developed with a diaminobenzidine (DAB, Sigma) substrate for 5 min at RT, and the sections were subsequently stained with hematoxylin.

2.9. Statistical analysis

To evaluate the influence of NaF administration, SPSS software version 13.0 (SPSS Inc, Chicago, IL, USA) was used to conduct statistical analyses of the collected data for all of the variables. Comparisons between groups were analyzed using an analysis of variance (ANOVA), according to the general linear model procedure in SPSS. Differences were considered significant if the $p < 0.05$. The data shown in all of the figures are expressed as the means ± SD

3. Results

3.1. Measurement of fluoride concentrations in the serum and fertility assessments of female rats

Fluoride concentrations in the serum of female rats were detected to estimate the toxicity of NaF in our rat model. Table 2 presents the serum fluoride concentrations in the female rats. As compared to the GC (0.987 ± 0.063 mg/L), the fluoride concentrations were increased in all of the F-treated groups, and there were significant differences between the GL (7.152 ± 1.026 mg/L), GM (8.891 ± 1.251 mg/L) and GH (10.563 ± 1.30 mg/L) (all $p < 0.05$).

Table 2
Concentrations of fluoride in the serum of female rats. GC: control group, GL: low-dose group, GM: medium-dose group, GH: high-dose group (means ± SD).

Groups	GC ($n = 10$)	GL ($n = 10$)	GM ($n = 10$)	GH ($n = 10$)
Serum (mg/L)	0.987 ± 0.063	7.152 ± 1.026*	8.891 ± 1.251*	10.563 ± 1.30*

* $p < 0.05$ Compared with the GC.