TOXIC EFFECTS OF FLUORIDE ON REPRODUCTIVE ABILITY IN MALE RATS: SPERM MOTILITY, OXIDATIVE STRESS, CELL CYCLE, AND TESTICULAR APOPTOSIS

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SUMMARY: To investigate the effects of sodium fluoride (NaF) on sperm motility, oxidative stress, and apoptosis in the testes, male Wistar rats were exposed to 1.0, 2.0, and 3.0 mg NaF/kg bw/day by intragastric gavage for 90 days. Sperm motility was significantly inhibited, especially at the lower F intake level. Significant increases in oxidative stress with elevated malondialdehyde occurred in the 1.0 mg NaF group and increased hydrogen peroxide in the 2.0 mg NaF group. Compared with the control group without NaF, the cell percentage in G0/G1 phase increased significantly, whereas it decreased significantly in S phase. On the other hand, the percentage of cells in G2/M phase was similar to that of the control. In the 2.0 and 3.0 mg NaF groups, a significant increase in testicular cell apoptosis was observed. Thus, especially at a comparatively lower level of exposure, F exhibits toxic effects on reproductive function in the form of decreased sperm motility, enhanced oxidative stress, and increased apoptosis, although the latter does not appear to be directly connected with the increased level of oxidative stress.

Keywords: Fluoride and reproduction; Male rat reproductivity; Oxidative stress; Rat testis; Sperm motility; Testicular apoptosis.

INTRODUCTION

Excess exposure to fluoride (F) has been found to interfere with the reproductive system of animals.[1,2] Previous studies in testes of rats showed that F induced increased production of reactive oxygen species, causing decreased activity in certain antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) and inducing testicular cell apoptosis.[2,3] Moreover, an increase in oxidative stress can seriously compromise the ability of spermatozoa to engage in sperm-oocyte fusion,[4] and it appears to play a role as a common mediator of apoptosis.[5] However, Chouhan and Flora[6] reported that F at a relatively low concentration creates a condition of oxidative stress whereas at higher concentration it acts as an inhibitor of free radical production and does not generate signs of apoptosis.

Although several studies of various levels of F on male reproduction have been reported,[1-6] the results are somewhat mixed. The present study therefore aimed to explore the effects of different concentrations of NaF on the reproductive ability in male rats by studying sperm motility, testicle oxidative stress, and testicular cell apoptosis.

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MATERIALS AND METHODS

Rats: Thirty-two adult male Wistar-albino rats weighing 170–180 g were obtained from the Experimental Animal Center of Lanzhou University and were acclimated in the laboratory for 2 weeks before the experiments. The rats were housed in groups in stainless steel cages on a 12 hr/12 hr light/dark cycle at 22±2ºC and 50% relative humidity with a standard chow diet and *ad libitum* access to tap water containing <0.3 mg F/L. All rats received humane care in compliance with the Institution Animal Care and Use Committee of China.

Establishment of rat model: The rats were divided randomly into four equal groups as follows: the control group was administered normal saline (NS) and the rats in the three experimental groups received 1.0, 2.0, and 3.0 mg NaF/kg bw/day, respectively, by intragastric gavage. After 90 days, all rats were sacrificed by cervical dislocation, and the testes were quickly excised, weighed, and the related index calculated.

Sperm motility: The epididymis was excised at autopsy and placed on flat plate containing 1.0 mL of normal saline (0.86%) at 37ºC. A suspension of spermatozoa was prepared by mincing with a sterilized razor blade on three glass slides and then placed in a water bath at 37°C for 50 min to release the spermatozoa. Ten microliter suspensions were used for the analysis. Sperm motion parameters measured by the WLJY-9000 color-detection system of sperm quality [computer-aided sperm analysis (CASA) systems, WeiLi, China] included average path velocity (VAP), curvilinear velocity (VCL), linearity (LIN), straightness (STR), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), mean angular deviation (MAD), and density of spermatozoa (ρ).

Measurements of SOD, MDA, CAT and H_2O_2: Testis tissue was immediately weighed and then homogenized with 1:9 (w/v) saline solution at –4ºC. SOD activity, CAT activity, MDA content, and H_2O_2 content in the testis tissue were determined with the reagent kit provided by the Nanjing Jianchen Biological Institute (China).

Measurements of cell cycle and cell apoptosis: After removal of the tunical albuginea, parts of the left testis were first minced with surgical scissors, filtered through nylon mesh and centrifuged for 5 min at 1000 rpm. The supernatant layers were decanted and washed three times with phosphate buffered saline (PBS: NaCl 8.0 g, KCl 0.2 g, $Na_2HPO_4 \cdot 12H_2O$ 2.8 g, KH_2PO_4 0.2g/L) before being fixed at 4ºC for 24 hr with 95% ethanol pre-chilled at –20ºC. The cell suspension was then adjusted to approximately 10^6 cells per mL in PBS. For each test, 1 mL of cell suspension was stained with 50 µg/mL of propidium iodide (PI) for 30 min in the dark at 37ºC and used for study by flow cytometry (FCM, EpicsXL-4, Beckman, Germany) equipped with a 488-nm argon ion laser.

Statistical analysis: Data were expressed as means ± standard deviation (SD). Statistical significance was assessed by one-way analysis of variance (ANOVA) using SPSS (13.0). $P<0.05$ or $P<0.01$ was considered significant.

RESULTS

Effects of NaF on testes and body weights: During the 90-day period of treatment and observation, there were no significant changes in body and relative testes weights between the control and all three NaF-treated groups, and no deaths occurred in any of the groups.

Effects of NaF on sperm motility: As summarized in Table 1, there were no significant differences in VCL between the control and each of the NaF-treated groups. Significant increases in VAP and BCF occurred in the 3.0 mg NaF group, and decreases in STR and ALH were observed in the 1.0 mg NaF group. Table 1 also indicates that all NaF-treated groups displayed significant decreases in MAD and LIN compared with the control group. For the most part, the levels of these parameters were higher in the 3.0 mg NaF group than in the other two NaF groups. In the 1.0 and 2.0 mg NaF groups, ρ was significantly lower compared with the control and the 3.0 mg NaF groups.

Table1. Effects of NaF on sperm motility of rats (n=8; mean±SD)

Sperm motility parameters	Control	1.0 mg NaF/kg bw/day	2.0 mg NaF/kg bw/day	3.0 mg NaF/kg bw/day
VAP (µm/s)	20.22 ± 3.29	21.07 ± 2.97	23.95 ± 4.00	25.04 ± 1.59 [*]
VCL (µm/s)	56.10 ± 7.29	55.08 ± 1.98	58.36 ± 7.34	57.14 ± 3.55
LIN (%)	33.28 ± 4.00	23.84 ± 1.58[†]	24.99 ± 3.36[†]	26.75 ± 5.06 [*]
STR (%)	65.46 ± 5.18	57.37 ± 6.88[*]	62.06 ± 4.71	63.90 ± 4.33
ALH (µm)	1.04 ± 0.70	0.47 ± 0.19[*]	0.75 ± 0.34	0.74 ± 0.29
BCF (Hz)	9.49 ± 0.33	10.47 ± 0.60	10.66 ± 1.66	11.47 ± 0.61[†]
MAD (radians)	81.57 ± 8.44	68.29 ± 5.71[†]	70.61 ± 7.73[*]	71.09 ± 4.96[*]
ρ (10^6/mL)	3.50 ± 0.56	1.25 ± 0.24 [†, ‡]	1.64 ± 0.57[*, †]	4.37 ± 0.51

*p<0.05, [†]p<0.01 compared with the control group; [‡]p<0.01 compared with the 3.0 mg NaF/kg group.

Effects of NaF on MDA, SOD, CAT and H_2O_2 in testis: As seen in Table 2, no significant changes in SOD and CAT activity were observed in any of the NaF-treated groups. There were, however, significant increases in the level of MDA in the 1.0 mg NaF group and of H_2O_2 in the 2.0 mg NaF group.

Table 2. Effects of NaF on MDA, SOD, CAT, and H_2O_2 in testis of rats (n=8; mean ± SD)

Group (mg NaF/kg bw/day)	SOD (U/mg prot)	MDA (nmol/mg prot)	CAT (U/mg prot)	H_2O_2 (nmol/L)
Control	135.24 ± 14.39	6.17 ± 0.25	65.80 ± 7.08	641.34 ± 79.05
1.0	139.19 ± 29.09	9.32 ± 0.32[†]	59.94 ± 5.51	707.74 ± 93.76
2.0	162.79 ± 8.85	8.35 ± 0.90	50.38 ± 1.82	877.31 ± 67.07[*]
3.0	139.69 ± 9.85	8.44 ± 0.91	59.73 ± 7.73	820.94 ± 65.37

[*]p<0.05, [†]p<0.01 compared with the control group. No significance differences were found among the three NaF-treated groups.

Effects of NaF on cell cycle and cell apoptosis: As seen in Table 3, a significant increase in the percentage of G0/G1 stage cells and a significant decrease in the percentage of S stage cells were observed in all the NaF-treated groups compared with the control group. On the other hand, the percentage of G2/M stage cells

decreased significantly in the 3.0 mg NaF group. The testes cell apoptosis ratios in all the NaF-treated groups were higher than that in the control group, but only the 2.0 and 3.0 mg NaF groups exhibited significant increases compared with the control group.

Table 3. Effects of NaF on testicular cell cycle and cell apoptosis of rats (n=8; mean±SD)

Group (mg NaF/kg bw/day)	G0/G1 (%)	S (%)	G2/M (%)	Apoptosis ratio (%)
Control	44.35±4.13	19.23±0.61	36.34±5.05	60.80±2.34
1.0	57.60±7.26[†]	10.58±2.58[†]	31.85±6.70	67.05±4.00
2.0	54.33±3.68[†]	9.35±0.37[†]	36.31±3.65	71.03±2.30[*]
3.0	73.13±4.08[†]	9.55±0.50[†]	17.18±2.21[†]	71.90±2.16[*]

[*]p<0.05, [†]p<0.01 compared with the control group. No significance differences were found among the three NaF-treated groups.

DISCUSSION

Although it is well known that toxic effects of F are seen in diminished sperm quality,[3,7-8] only a few studies appear to have addressed sperm motility induced by F using CASA. The results from this study indicated a reduction of MAD and LIN in all NaF-treated groups, which are characteristics usually exhibited by immature spermatozoa.[9] This finding is similar to the results of other investigations on sperm motility parameters.[10-11] At the same time, statistically significant differences of STR and ALH in the 1.0 mg NaF group were also observed. Therefore, it is plausible that excess F can decrease male fertility with effects that are more obvious at lower than at higher F concentration.

There is much evidence that oxidative stress is an important mediator of F-induced toxicity.[3,12-16] Wang et al.[17] reported that superoxide radicals prevail at high F concentration, whereas at low F concentration there is dominance of hydroxyl radicals generated in the Haber-Weiss reaction with the participation of Fe^{2+} as well as H_2O_2. Other previous studies revealed the same results.[6,18] In present study, the increases of H_2O_2 and MDA at lower dosages of F were also observed.

Huang et al.[3] reported that NaF affected testicular cell cycle and apoptosis in mice and postulated that oxidative stress is a sign of adverse effects on testicular function. Moreover, it has been suggested that oxidative stress plays a role in mediating apoptosis.[5] The results of our study also indicate that F disrupted normal testicular cell cycle in all NaF-treated groups and induced a significant increase in apoptosis in the 2.0 and 3.0 mg NaF groups. However, the changes in oxidative stress did not follow a similar trend. Further studies of this observation are needed to determine the possible mechanism involved.

In conclusion, excess F exposure has been found to produce adverse effects on the reproductive function of male rats by inducing oxidative stress and causing apoptosis. However, the changes of testicular cell cycle and apoptosis do not

appear to be directly connected with the increased level of oxidative stress, since some deleterious effects were more prominent at lower F intake.

ACKNOWLEDGEMENTS

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