EFFECT OF FLUORIDE ON RAT TESTICULAR STEROIDOGENESIS

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SUMMARY: In view of reports of infertility among human populations in fluorosis prevailing regions, we investigated the effect of fluoride ingestion on testicular steroidogenesis in rats. Sodium fluoride (NaF) was administered to the rats orally at a daily dose of 10 mg/kg bodyweight for 50 days. The treatment did not cause significant change in testicular cholesterol levels, indicating that metabolism was not altered and that there was no hypo/hypercholesterolemic effect. In addition, activities of the intermediary enzymes in androgenesis, *viz.*, 3β- and 17β-hydroxysteroid dehydrogenase were only modestly decreased by NaF ingestion. Subsequently, the determination of circulating androgen levels was similar in NaF-treated rats showed a downward trend compared to those of the control group, suggesting alteration in testosterone concentration. The histomorphometric studies revealed significant change in the Leydig cell diameter in correlation with the androgen levels. These results indicate that fluoride does interfere with steroidogenesis in short-term low-dose exposures in rats.

Key words: Androgen levels; Fluoride, Leydig cells; Rats, Steroidogenesis, Testis.

Introduction

In recent years the effects of fluoride on reproductive functions have been investigated. Schulz and Lamb (1) and Udall and Kellers (2) reported a positive relationship between fluoride toxicity and reduction in fertility in animals. Vogel (3) observed a slight decrease in fertility of *Drosophila melanogaster* exposed to sodium fluoride. Tokar and Savchenko (4) obtained low testosterone levels with high FSH and LH in individuals afflicted with fluorosis. On the other hand, Li *et al* (5) reported that fluoride does not have adverse effects on spermatogenesis, indicating that fluoride plays no mutagenic role. However, recent experimental studies performed in mice and rats revealed arrest of spermatogenesis due to extensive damage to the seminiferous tubules leading to denudation of cells, vacuolization in their cytoplasm, and nuclear pyknosis (6-7). Furthermore, the target organ functions (epididymides, vas deferens, and accessory sex organs) were impaired adversely, despite the fact that the serum testosterone levels were close to normal after NaF treatment for 30 days at a dose of 10 mg/kg body weight (8-9). Thus, the impact of fluoride on rat testicular steroidogenesis, along with the impairment of target organ function, is not clearly understood, a situation which led to the present investigation in experimental rats.

Material and Methods

Mature, healthy, pathogen-free albino rats (*Rattus norvegicus*) of Charles Foster strain, 50-55 days old and weighing 200-250 g, were obtained from the National Institute of Occupational Health, Ahmedabad. The animals were acclimatized to laboratory conditions for one week.

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The rats were divided into two groups and caged separately. Those in the first group (the controls) were given food and water *ad libitum*. Those in the second group were given NaF just before feeding so as to avoid contact with food.

Sodium fluoride (Loba Chemie, Bombay; 99% purity) was dissolved in distilled water and administered daily to the second group of animals at a dose of 10 mg/kg body weight orally for 50 days. The dose used was mainly based on the LD 50 value for male rats, which is 250 mg/kg body weight (10); the present dose was 1/25 of the LD 50 value. The duration was selected on the basis of the kinetics of spermatogenesis in rat. The experimental protocol is summarized in Table 1

TABLE 1. Summary of Treatment

Group	Treatment	Duration (Days)	Day of autopsy	Number of animals used
I	Control	–	sacrificed along with treated	10
II	NaF (10mg/kg body weight).	50	51	10

At the end of the treatment period, control and treated groups of rats were sacrificed by cervical dislocation. The testes were dissected out, blotted free of blood, and used freshly for carrying out different parameters, *i.e.*, cholesterol levels and 3β- and 17-β-hydroxysteroid dehydrogenase (HSD) activities. Blood was collected by cardiac puncture and allowed to clot; serum was separated by centrifugation for testosterone assay.

Histocytometric studies of Leydig cells and their nuclear diameter were also carried out. Testes of control and treated rats were excised and fixed in Bouin's fixative for 18 hours, then transferred to 70% alcohol and processed until the colour of the fixative was removed by adding lithium carbonate. Sections of 5 μ thickness were cut on a microtome and stained with haematoxylin-eosin. The morphometric analyses of Leydig cells and their nuclear diameter were determined by means of an ocular eye-piece and micrometer scale.

Cholesterol: The testicular cholesterol levels in the control and treated rats were estimated by the method of Pearson *et al* (11) and expressed as μg/mg fresh tissue weight.

3β- *and* 17β- *hydroxysteroid dehydrogenase* (HSD): The activities of testicular 3β- and 17β-HSDs were determined by the method of Talalay (12) and expressed as 5α-diol formed/mg protein/30 minutes.

Testosterone: The serum testosterone levels of control and treated rats were assayed by the double antibody technique of Peterson and Swerdloff (13) and the concentrations were expressed as ng/ml serum.

Statistics: For each biochemical parameter a minimum of 10 replicates were taken, and the data were statistically analysed by the Student's 't' test.

Results

Cholesterol: The cholesterol levels in testes of NaF-treated rats showed an insignificant increase compared to the controls (Table 2).

3β- *and* 17β-HSD: The activity of both 3β- and 17β-HSDs were lowered after 50 days of treatment compared to controls (Table 2).

Testosterone: The serum testosterone levels also showed a downward trend ($P < 0.02$) with NaF treament (Table 2).

Histocytochemistry: The Leydig cell diameter of NaF-treated rats showed a significant ($P < 0.01$) decline compared to control (Table 3). The Leydig cell nuclear diameter also showed a significant ($P < 0.01$) change after NaF treatment (Table 3).

TABLE 2. Testicular cholesterol, 3β- and 17β-hydroxysteroid dehydrogenase (HSD) and serum testosterone levels of control and after 50 days in NaF-treated rats

Parameter	Control	NaF Treatment
Cholesterol (μg/mg fresh tissue wt.)	0.46 ± 0.01	0.48 ± 0.01
3β- HSD (5∝-diol formed/mg protein/30 minutes)	5.8 ± 0.92	4.78 ± 0.83
17β- HSD (5∝-diol formed/mg protein/30 minutes)	5.5 ± 0.63	4.65 ± 0.38
Testosterone	0.46 ± 0.01	0.42 ± 0.01*

Values are mean ± S.E. * $P < 0.02$

TABLE 3. Histocytometry: Leydig cell and nuclear diameter (μm) of control and after 50 days in NaF-treated rats

Parameter	Control	NaF Treatment
Leydig cell diameter	8.4 ± 0.28	6.7 ± 0.12*
Leydig cell nuclear diameter	4.9 ± 0.25	3.1 ± 0.08*

Values are mean ± S.E. * $P < 0.01$

Discussion

The present study was undertaken to explore the effect of fluoride on testicular steroidogenesis in rats because that effect is not clearly understood. Also in view was the wide prevalence of infertility in the fluorosis-afflicted human population in India and other parts of the globe (4,14).

The sodium fluoride treatment, a daily dose of 10 mg/kg body weight administered orally for 50 days, caused no significant alterations in testicular cholesterol levels. Saralakumari *et al* (15) also reported normal serum cholesterol concentration in rats that were fluoride intoxicated for two months by a dose of 100 ppm. In corroboration, Chinoy and Sequeira (8) also obtained unaltered cholesterol in testes of mice fed with 10 mg fluoride/kg body weight for 30 days. A single microdose vasal injection of sodium fluoride (50 µg/50 µL) also caused no change in cholesterol levels (9). Our subsequent studies in human populations afflicted with fluorosis found cholesterol levels to be within the normal range (16). These results indicate that fluoride does not interfere with cholesterol metabolism in mammals and imply no hypo/hypercholesterolemia leading to atherosclerosis - at least after short-term low-dose exposure.

However, fluoride treatment of rabbits with 100 mg/kg body weight for 100 days resulted in a 2- to 3-fold increase in cholesterol and triglycerides (17). Ectopic calcification of rabbit aorta after chronic fluoride intoxication has also been reported (18). Therefore, effects of chronic exposure to fluoride on human populations in endemic areas should be examined closely because of the possibility of cardiovascular problems.

The treatment investigated here brought about a small change in serum testosterone levels indicating that there is a downward trend of circulating androgen levels caused by fluoride. In accordance with these results, Chinoy *et al* (16) found that the circulating testosterone levels of human populations in endemic areas of Gujarat, India, exposed to 3-4 ppm water fluoride for about 5-7 years, were only slightly affected. However, Tokar and Savchenko (4) reported low serum testosterone levels with high FSH and LH in fluoride-afflicted patients. This discrepancy is difficult to interpret as the duration and concentration of fluoride exposure were not known in their investigations.

In the present investigation, further exploration of intermediary enzymes in androgenesis showed, in testes after fluoride ingestion for 50 days, small reductions in 3β-hydroxysteroid dehydrogenase (HSD), which converts dehydroepiandrosterone into androstenedione, and in 17β-HSD, which converts androstenedione into testosterone. Furthermore, the histomorphometric studies revealed a significant change in Leydig cell diameter and its nuclear diameter. In support of these findings, Zahvoronkov and Strochkova (19) reported a decrease in dry weight of Leydig cells together with cytochemical alterations as evident by disturbances in protein synthesizing system. Hence, hormonal imbalance would occur as observed in the present study.

In conclusion, fluoride seems to interfere with androgenesis. The target organ structures and functions (epididymis and other accessory sex organs), which are dependent on circulating androgen levels, were adversely impaired after fluoride intoxication, leading to failure of fertility in experimental animals. This failure can affect endemic populations, as mentioned elsewhere (4,8,9,14). The possible impact

of fluoride may be on the receptor sites, *viz.*, by altering the concentration or configuration of the receptor, thus inhibiting the action of testosterone on target organs. This interpretation was further supported by alterations in phospholipid concentration, especially reduction in phosphatidyl inositol in the sperm membrane after fluoride treatment, which is important for hormone receptor transduction (19). Furthermore, the conversion of testosterone into its potent metabolite, dihydrotestosterone, is probably impaired by fluoride. Hence, it is important to investigate these aspects in order to understand the exact mechanism of action of fluoride at the receptor level, especially in the target sites.

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