FLUORIDE INDUCED BIOCHEMICAL CHANGES IN REPRODUCTIVE ORGANS OF MALE MICE

by

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SUMMARY: Adult male albino mice were given 10 mg and 20 mg/kg body weight of NaF for 30 days. NaF caused a decrease in body weight, but no change in organ weight, except for the prostate gland and seminal vesicles. No significant change in testis cholesterol and serum testosterone levels occurred. However, in the testis succinic dehydrogenase levels decreased, in the epididymides sialic acid and ATPase levels decreased; in the vas deferens glycogen levels increased, seminal vesicles fructose levels increased and in the prostate glands acid phosphatase and total protein levels increased. After withdrawal of treatment for a period of two months the levels of these substances returned to normal.

KEY WORDS: ACP; ATPase; Cholesterol; Fluoride; Fructose; Glycogen; Mice; Protein; Serum testosterone; Sialic acid; Succinate dehydrogenase.

Introduction

Fluoride is one of the elements in the earth's crust and is distributed ubiquitously throughout nature. It was reported earlier from our laboratory that ingestion of 10 and 20 mg sodium fluoride (NaF) per kg body weight by mice for 30 days caused alterations in the histology of testis, epididymides and vas deferens. The cauda epididymal spermatozoa were rendered non-motile leading to loss of fertility. The sperm density was also reduced. The sperm acrosomal integrity and morphology was altered and some were deflagellated (1,2). NaF-induced effects were tansient and reversible. A microdose of NaF when directly injected in retrograde direction in distal vas deferens of rats also caused alterations in reproductive organ structure and metabolism as well as reduction in fertility (3). The present study is an attempt to investigate the effects of fluoride ingestion for 30 days in the same doses as used earlier on the metabolism and function of reproductive organs of mice. The reversibility and recovery of these organs was also investigated.

Materials and Methods

Adult male albino mice (20-30 gm) of Swiss strain were maintained on standard chow and water was given ad libitum. The first group of 40 mice was given the control diet. The second and third group of animals (40 in each group) were fed sodium fluoride (NaF) at doses of 10 and 20 mg/kg body weight/day respectively for 30 days. In the fourth and fifth groups, treatment (10 mg NaF/kg body weight) was then withdrawn for one and two months respectively. The control and treated mice were weighed and were sacrificed. The testis, caput and cauda epididymides, vas deferens, seminal

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vesicles and prostate gland were carefully dissected out, blotted free of blood and weighed on a torsion balance to the nearest milligram and utilized for various determinations as follows.

Cholesterol: The estimation of cholesterol in testis of control and treated mice, was carried out by the method of Pearson et al., (4) and expressed as mg/100 mg fresh tissue weight.

Succinate dehydrogenase (SDH): The activity of SDH was assayed by the method of Beatty et al. (5) and expressed as µg formazan/100 mg fresh tissue weight.

Sialic Acid: Sialic acid in the epididymis of control as well as treated animals was determined by the method of Jourdian et al. (6) and was expressed as µg/mg fresh tissue weight.

Adenosine Triphosphatase (ATPase): The ATPase activity in epididymides was assayed following the method of Quinn and White (7). The enzyme ATPase hydrolyzes the substrate adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and inorganic phosphate (Pi). The Pi formed at the end of incubation was assayed to determine the rate of reaction.

Glycogen: Glycogen levels were determined in vas deferens of control, treated and withdrawal groups of animals by the method of Seifter et al. (8). The concentration was expressed as µg/100mg fresh tissue weight.

Acid Phosphatase (ACP): Prostate gland ACP activity was assayed by employing the method of Bessey et al. (9) and was expressed as µmoles of p-nitro phenol liberated/mg of fresh tissue weight/30 mins.

Protein: Protein estimation in prostate gland of control, treated and withdrawal group of mice was carried out by the method of Lowry et al. (10) and expressed as mg/100 mg fresh tissue weight.

Table 1

Summary of Treatment

Group	Treatment	Duration (days)	Day of Autopsy
Control	——	—	along with treated
NaF treated	10 mg/kg b.w. (eqiv. to) 230 ppm/animal/day	30	31
NaF treated	20 mg/kg b.w. (equiv. to) 400 ppm/animal/day	30	31
NaF treated	treatment withdrawn for one month	30	31
NaF treated	treatment withdrawn for two months	60	61

Fructose: Fructose was determined in the seminal vesicles by the modified method of Foreman et al. (11) and its concentration was expressed as µg/mg fresh tissue weight.

Testosterone levels from blood serum of control and treated animals were determined by radioimmunoassay (RIA) using RIA kits from Serono Laboratory (Italy).

Results

The body weight decreased after the treatment for 30 days in comparison to control mice, but recovery occurred after withdrawal (Table 2).

Organ Weights: The weights of testis, epididymis and vas deferens were not altered by treatment but those of seminal vesicle and prostrate were increased significantly ($p < 0.001$). Withdrawal of treatment resulted in only partial recovery (Table 2).

Cholesterol level in the testis was not affected by NaF treatment but activity of succinate dehydrogenase (SDH) was significantly ($p < 0.001$) reduced (Table 3). However, recovery was noted after withdrawal of treatment for 60 days (Table 3).

ATPase activity was reduced in both caput and cauda epididymides but recovered significantly after withdrawal (Table 3). The levels of sialic acid were also decreased in both regions of epididymides and subsequently recovery occurred upon withdrawal of treatment (Table 3).

The prostatic protein and acid prosphatase were significantly increased ($p < 0.001$) after treatment. However, recovery was noted after the withdrawal of treatment (Table 4). Similarly, seminal vesicle fructose and vas deferens

Table 2

Body and Organ Weights of Control, NaF Treated and Withdrawn Groups of Mice

Parameter	Control	NaF Treated		NaF Withdrawn	
		10 mg/kg body weight	20 mg/kg body weight	30 days	60 days
Body weight (gm)	25.7 ±0.8	20.5 ±0.4	21.4 ±0.82	24.0 ±0.6	25.9 ±0.6
Testis (mg)	84.0 ±8.0	85.0 ±7.0	84.0 ±1.5	73.3 ±6.0	82.6 ±2.0
Caput Epididymis	14.0 ±0.4	14.8 ±1.0	14.3 ±0.3	15.6 ±0.8	14.6 ±0.6
Cauda Epididymis	10.0 ±1.0	10.0 ±1.0	10.0 ±0.2	10.0 ±0.0	10.6 ±0.6
Vas Deferens	12.0 ±1.0	13.5 ±0.9	13.5 ±0.4	14.3 ±0.8	14.0 ±1.1
Seminal Vesicles	54.0 ±2.0	71.0 ±6.0	74.0 ±4.7	73.6 ±0.8	68.0 ±3.0
Prostate	13.7 ±0.3	20.6 ±1.1	22.6 ±0.8	15.6 ±0.4	17.3 ±0.6

Values are mean ±S.E.

Table 3

Cholesterol; SDH in Testis; Protein, ACP in Prostate; ATPase, Sialic Acid in Epididymis in Control, NaF Treated and NaF Withdrawn Groups of Mice

Tissue	Parameter	Control	NaF Treated		NaF Withdrawn	
			10 mg/kg body wt.	20 mg/kg body wt.	30 days	60 days
Testis*	Cholesterol (mg/100 mg tissue wt.)	0.41 ±0.01	0.43 ±0.01	0.43 ±0.02	—	—
Testis*	Succinate dehydrogenase (µg/100 mg tissue wt.)	836 ±16	400 ±20	415 ±20	560 ±36	761 ±16
Caput Epididymis	ATPase**	13.9 ±0.86	9.26 ±0.47	5.2 ±0.32	—	11.4 ±1.01
Cauda Epididymis	ATPase**	13.94 ±0.23	8.95 ±0.25	6.75 ±0.39	—	12.2 ±0.4
Caput Epididymis	Sialic Acid (µg/mg tissue wt.	4.35 ±0.16	3.83 ±0.15	2.79 ±0.08	—	3.9 ±0.08
Cauda Epididymis	Sialic Acid (µg/mg tissue wt.)	5.77 ±0.1	4.29 ±0.21	3.21 ±0.15	—	5.12 ±0.14

Values are mean ±S.E.
* Data on NaF treatment alone taken from Sequeira and Chinoy (34).
** µmoles ip/30 min/100 mg tissue.

Table 4

Fructose in Seminal Vesicles; Protein, ACP in Prostate and Glycogen in Vas Deferens in Control, NaF Treated and NaF Withdrawn Groups of Mice.

Tissue	Parameter	Control	NaF Treated		NaF Withdrawn	
			10 mg/kg body wt.	20 mg/kg body wt.	30 days	60 Days
Prostate	Protein (mg/100 mg tissue wt.)	4.03 ±0.21	6.16 ±0.2	6.43 ±0.3	5.01 ±0.19	4.39 ±0.02
Prostate	ACP*	0.14 ±0.01	0.24 ±0.07	0.25 ±0.005	0.23 ±0.02	0.18 ±0.02
Seminal	Fructose (µg/mg tissue wt.)	43.00 ±2.7	50.00 ±1.5	57.00 ±2.6	48.1 ±1.0	44.0 ±0.8
Vas Deferens	Glycogen (µg/100 mg tissue wt.)	573 ±9	932 ±9.7	943 ±22	901 ±11	661 ±24

Values are Mean ±S.E.
* µmoles of p-nitro phenol released/mg fresh tissue weight/30 min.

glycogen were also increased after NaF treatments with both doses. The increase was more significant ($p < 0.001$) in case of glycogen. Recovery was obtained on withdrawal of treatment.

The serum testosterone level of treated group was about 40% lower than that of the control group (Table 5).

Table 5

RIA of Serum Testosterone

Tissues	Parameter	Control	NaF treated 10 mg/kg b.w.
Serum	Testosterone (ng/mL)	1.5 ±0.41	0.884 ±0.072*

Values are mean ±S.E.
* Data from Sequeira and Chinoy (34).

Discussion

Treatment with 10, 20 mg NaF/kg body wt. for 30 days resulted in a decrease in the body weight. Schwartz and Milne (12) reported that much lower levels of fluoride (1-2 µg F/gm of diet) stimulates their growth when fed a highly purified amino acid diet and maintained in trace element controlled isolators. However, other workers were not able to confirm these findings (13,14). Saralakumari et al. (15) observed that supplementation of drinking water with 100 ppm of fluoride for two months resulted in reduction in growth rate.

Fluoride is known to disturb carbohydrate metabolism (16). In fluorotic rats the levels of glucose-6-phosphate dehydrogenase was decreased and glycogen turnover depressed (17,18). Rats consuming 450 ppm F in the diet were unable to metabolize glycogen normally due to some effect at the liver enzyme level (19,20). These observations are in agreement with the data of the present study wherein a signifcant increase in glycogen levels in vas deferens was obtained in 10, 20 mg/kg body weight NaF treated mice. This might be related to increase in activity of some enzymes of carbohydrate metabolism as reported by Strochkova and Zhavoronkov (21), or to reduced utilization of glycogen in vas deferens related to the decline in sperm density (2). Macuch et al. (22) have reported that fluoride interferes with binding of amino-acyl-t RNA adducts to the ribosomal RNA template, which is responsible for the impaired polypeptide formation. In the present study, the prostatic protein levels were increased significantly. Tsunoda et al. (23) observed that the total protein in serum in the control goats and those subjected to air-borne fluoride were about the same.

Underwood (16) has reviewed enzyme changes in chronic fluorosis in animals. The testis succinate dehydrogenase (SDH) activity decreased significantly with both doses of NaF treatment which suggests that the oxidative metabolism of testis was affected. Similar data has been obtained for muscle SDH (24). The decrease in SDH might be similar to that of isocitrate dehydrogenase (25), another tricarboxylic acid cycle enzyme which leads to accumulation of citric acid. Bogin et al. (20) reported declines in LDH and isocitrate dehydrogenase levels in the livers, kidneys, hearts, and skeletal muscles of mice treated with 100 ppm NaF. Similarly, Chitra et al. (26) have found decreased LDH in muscle and liver of NaF-treated Channa punctatus. Sullivan

(27) found that animals receiving drinking water supplemented with fluoride (F^-) (around 100 ppm) showed a marked lowering of hepatic SDH activity after continued administration. Fasske (28) and Androsov (29) also reported interference of fluoride with enzymes like SDH in heart and in the liver.

Distinct hypercholesterolemic effects in the serum were observed in the animals after exposure to fluoride (24,25,30,31). Fluoride and zinc increase blood cholesterol and may constitute a predispostion to atherosclerosis (32). However, testis cholesterol levels remained constant throughout treatment. It has been reported elsewhere (1) that Leydig cell and nuclear diameter were not affected by NaF ingestion in mice. Hence it follows that androgenesis by testis might not be altered. Levels of ATPase, sialic acid in epididymides, ACP, protein in prostate, fructose in seminal vesicles, glycogen in vas deferens as well as the histolgy of these organs was altered by NaF treatment. It is also likely that these changes might be the outcome of reduced end-organ response to androgens. According to Hodge and Smith (33) NaF toxicity involves inhibition of enzyme activity particularly those in which divalent metal cations act as cofactors. In the present study too, the alterations especially in ATPase, SDH and ACP activities might be due to the fact that they either Mg^{2+}, Ca^{2+}, or Zn^{2+} metalo-proteins. The altered membrane integrity of spermatozoa by NaF ingestion in mouse as reported earlier (2) might result from a decrease in ATPase in epididymis leading to sperm structural and functional changes and finally to infertility.

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MONITORING OCCUPATIONAL FLUORIDE EXPOSURE THROUGH URINARY AND SALIVARY TESTS

by

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SUMMARY: To monitor individual fluoride exposure urine and saliva of workers exposed to fluoride contamination at their work place in an enamel factory in Kecskemét (Hungary) were analyzed. The authors recommend testing of renal function and determination of salivary fluoride concentration to complement the currently accepted determination of urinary fluoride and fluoride creatinine ratio.

KEY WORDS: Hungary; Occupational fluoride exposure; Urinary, salivary fluoride.

Introduction

To monitor fluoride metabolism, also in case of occupational fluoride exposure, the most widely accepted test – indeed almost the only test used for this purpose all over the world – is still the determination of the urinary fluoride concentration.

In recent years several papers (1,2,3) have drawn attention to the determination of plasma fluoride level because it gave more adequate information about fluoride exposure, even when renal function was normal. However, obtaining blood samples is difficult on ethical and technical grounds.

Fluoride excretion may significantly decrease when renal function is impaired (diabetes, pregnancy, nephritis, nephrosis, etc.) (4). In other words, urinary fluoride values themselves of persons with impaired renal function fail to provide information with any degree of precision of actual fluoride exposure.

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