

IN VIVO STUDIES CONCERNING TOXIC EFFECTS OF SODIUM FLUORIDE ON HEPATIC FUNCTION IN RABBITS

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SUMMARY: This study was designed to evaluate the role of fluoride in inducing hepatotoxicity. The experimental model comprised albino rabbits treated with 5, 10, 20, and 50 mg NaF/kg-bw/day s.c. for fifteen weeks. Parameters of hepatotoxicity were proteins, DNA, RNA, free amino acids, and cholesterol. The data indicate significant reduction in acidic proteins, basic proteins, total proteins, RNA, and cholesterol in the liver of experimental animals of both sexes. Hepatic free amino acids were highly elevated in the exposed rabbits, suggesting impairment of protein synthesis as well as reduced incorporation of amino acids into proteins. Hepatic DNA synthesis increased in rabbits treated with 5 mg NaF/kg-bw/day and then decreased at higher doses. These hepatotoxic disturbances induced by fluoride reflect functional and structural alterations in the liver in experimental fluorosis.

Keywords: Albino rabbits; Cholesterol; DNA synthesis; Fluorosis; Free amino acids; Hepatic function; Protein synthesis; RNA synthesis; Sodium fluoride.

INTRODUCTION

Fluorides affect body systems either by activation or inhibition.¹ Inhibitory effects of fluoride on protein and DNA synthesis in cultured cells have been described.² Geeraerts *et al*³ reported that fluoride interferes with tryptophan metabolism. Metabolic disturbances in hepatic lipid metabolism reveal hypolipidemia, hypophospholipidemia and hypotriglyceridemia in rabbits.⁴ In patients with Gilbert's disease, Lee⁵ recorded Hyperbilirubinemia due to fluoride. An enzyme inhibiting action of fluoride was considered to be the most likely mechanism involved. Fluoride has also been linked to bone and oral cancers in animals and humans,⁶ inhibition of DNA repair enzyme systems, genetic damage in a number of different cell lines,⁷ induction of hemorrhages, and hepatocellular necrosis.⁸

The objective of the present study was to monitor the hepatotoxic effects of fluoride on proteins, nucleic acids, amino acids, and cholesterol in experimental fluorosis.

MATERIALS AND METHODS

Albino rabbits (100) of both sexes (400-600 g) were housed in wire mesh cages with solid bottoms, and were fed pellet diet obtained from Hindustan Lever Laboratory Feed, India. Diet and water were available *ad libitum*. All animals were acclimatized for one week before being dosed.

Animals were divided into five experimental groups of 20 (10 male and 10 female) each. Prior to exposure, the animals were weighed and weighing

was done every week. Separate syringes, needles, and bottles were kept for each group.

Rabbits of one group served as the control and received vehicle solution only. They were given subcutaneous injections of 1 mL of double distilled water /kg bw/day. The remaining experimental groups of animals (Group II, III, IV and V) were administered subcutaneous injections of solution containing 5, 10, 20, and 50 mg NaF dissolved in 1 mL of double distilled water/kg bw/day.

These doses were given daily for fifteen weeks. All the animals were sacrificed under ether anesthesia. Livers were quickly dissected out, cleaned blood free in 0.9% (w/v) ice-cold normal saline, and processed for biochemical estimations. They were kept in a deep freezer at -20°C to prevent any degradation.

Biochemical Analyses: Liver samples were assayed for proteins, nucleic acid, free amino acids, and cholesterol. All samples were determined during one assay.

Proteins: The estimation of both acidic as well as basic proteins was carried out with the help of folin-phenol reagent using bovine serum albumin as standard.⁹ The intensity of blue color developed was measured at 660 μm on a spectrophotometer.

Nucleic acids: Nucleic acids were extracted by the method of Webb and Levy.¹⁰ DNA was determined by Dische's diphenylamine reaction as modified by Burton.¹¹ RNA was assayed using yeast as standard.¹²

Free amino acids: Free amino acids contents were measured by the method of Troll and Cannon¹³ using ninhydrin coloring reagent.

Cholesterol: The cholesterol content in the liver of control and fluoridated animals was determined as described by Stadtman.¹⁴ Cholesterol in the homogenate reacts with acetic anhydride in the presence of concentrated sulfuric acid to give a brownish green colored complex which was measured on a spectrophotometer.

The concentration of all biochemical components was expressed as mg/g fresh tissue weight.

Statistical evaluation: Means and standard error of individual groups were calculated. Student's t-test was used for comparisons between a single treatment and its corresponding control. $P < 0.05$ was considered to be significant.

RESULTS

The effects of fluoride on hepatic protein synthesis are given in Table 1. Acidic proteins in the liver of rabbits of both sexes decreased significantly in

all test groups after NaF treatment. In females this decrease in acidic proteins was significant in the 5 mg vs 10 mg NaF group, the 10 mg vs 20 mg NaF group and the 20 mg vs 50 mg NaF group. A dose-dependent significant depletion in basic proteins of liver was observed in rabbits of both sexes. The magnitude of decrease was 15.2% to 61.3% in males and 21.7% to 71.7% in females in fluoride-exposed groups in comparison to the control.

Table 1. Effect of NaF after 15 weeks on acidic proteins, basic proteins, and total proteins (mg/g) in the liver of rabbits (Data are Mean \pm SD.)

Parameter	Treatment mg NaF/kg bw/day	Male (n=10)	Percent change	Female (n=10)	Percent change
Acidic proteins	0 (Control)	3.57 \pm 0.041		4.44 \pm 0.190	
	5	2.76 \pm 0.085 [†]	-22.7	3.17 \pm 0.112 [†]	-28.6
	10	1.68 \pm 0.041 [†]	-52.9	1.50 \pm 0.051 [†]	-66.2
	20	1.73 \pm 0.061 [†]	-51.5	1.25 \pm 0.051 [†]	-71.8
	50	0.65 \pm 0.141 [†]	-81.8	0.90 \pm 0.045 [†]	-79.7
Basic proteins	0 (Control)	2.17 \pm 0.085		3.32 \pm 0.056	
	5	1.84 \pm 0.220 [†]	-15.2	2.60 \pm 0.116 [†]	-21.7
	10	1.56 \pm 0.150 [†]	-28.1	1.82 \pm 0.031 [†]	-45.2
	20	1.46 \pm 0.061 [†]	-32.7	1.06 \pm 0.071 [†]	-68.1
	50	0.84 \pm 0.064 [†]	-61.3	0.94 \pm 0.081 [†]	-71.7
Total proteins	0 (Control)	5.74 \pm 0.126		7.76 \pm 0.246	
	5	4.60 \pm 0.305 [†]	-19.9	5.77 \pm 0.228 [†]	-25.6
	10	3.24 \pm 0.191 [†]	-43.6	3.32 \pm 0.082 [†]	-57.2
	20	3.19 \pm 0.122 [†]	-44.4	2.31 \pm 0.122 [†]	-70.2
	50	1.49 \pm 0.205 [†]	-74.0	1.84 \pm 0.126 [†]	-76.3

P values compared to the control: [†]P<0.01, [‡]P<0.001.
n = number of animals in each dosage group.

The total proteins in the liver of both sexes were depleted in response to fluoride treatment. Total proteins exhibited a large decline in the liver of females as compared to males in all fluoridated groups.

The data in Table 2 demonstrate the levels of DNA, RNA and free amino acids in the liver of rabbits of both sexes during fluoride intoxication. An enhanced level of DNA was observed in the liver of male (334.9%) and female (215.5%) rabbits treated with NaF 5 mg/kg bw as compared to the control. In males of highest dose group (50 mg/kg NaF) a significant decline in DNA content (23.3%) occurred. In females, the concentration of DNA exhibited significant depletion in all test groups.

Table 2. Effect of NaF after 15 weeks on DNA, RNA, and free amino acids (mg/g) in the liver of rabbits (Data are Mean \pm S.D.)

Parameter	Treatment mg NaF/kg bw/day	Male (n=10)	Percent change	Female (n=10)	Percent change
DNA	0 (Control)	0.43 \pm 0.041		0.58 \pm 0.021	
	5	1.87 \pm 0.285 [§]	+334.9	1.83 \pm 0.061 [§]	+215.5
	10	0.42 \pm 0.120	-2.3	0.52 \pm 0.003 ^{§,}	-10.3
	20	0.40 \pm 0.002	-7.0	0.43 \pm 0.008 ^{§,}	-25.9
	50	0.33 \pm 0.006 ^{§,}	-23.3	0.27 \pm 0.004 ^{§,}	-53.4
RNA	0 (Control)	6.75 \pm 1.340		6.30 \pm 0.071	
	5	5.20 \pm 0.021 [†]	-23.0	5.43 \pm 0.560 [‡]	-13.8
	10	5.05 \pm 0.976 [*]	-25.2	5.58 \pm 0.031 [§]	-11.4
	20	4.94 \pm 1.400 [*]	-26.8	3.81 \pm 0.034 ^{§,}	-39.5
	50	3.47 \pm 0.033 ^{§,**,††}	-48.6	3.45 \pm 0.021 ^{§,}	-45.2
Free amino acids	0 (Control)	0.82 \pm 0.258		0.81 \pm 0.036	
	5	0.96 \pm 0.059	+17.1	3.28 \pm 0.016 [§]	+304.0
	10	1.49 \pm 0.013 ^{§,}	+81.7	1.18 \pm 0.071 ^{§,}	+45.7
	20	1.55 \pm 0.044 ^{§,††}	+89.0	7.51 \pm 2.271 ^{‡,}	+827.2
	50	6.46 \pm 1.905 ^{§,}	+687.8	9.21 \pm 0.166 ^{§,}	+1025.9

P values compared to the control: *P<0.05, †P<0.02, ‡P<0.01, §P<0.001.

Significant values in 5mg vs 10mg NaF group, † 10 mg vs 20mg NaF group, and 20 mg vs 50 mg NaF group are: ||P<0.001, ††P<0.02, **P<0.05.

n = number of animals in each dosage group.

In both sexes, RNA content fell significantly ($P < 0.05$ – 0.001) in all fluoridated groups of animals compared with the control.

The hepatic free amino acids exhibited abnormal accumulation in fluorotic animals of both sexes. The highest percent increase was seen in animals exposed to 50 mg NaF/kg bw (687.8% in males and 1025.9% in females).

Table 3 shows the effects of NaF on hepatic cholesterol. A significant dose-dependent decrease in liver cholesterol occurred in both male and female rabbits at all levels of exposure to NaF.

Table 3. Effect of NaF after 15 weeks on the cholesterol level (mg/g) in the liver of rabbits

Sex	Treatment mg NaF/kg bw/day	Mean \pm S.D.	Percent change
Male n=10	0 (Control)	3.76 \pm 0.130	
	5	2.50 \pm 0.087*	-33.5
	10	2.17 \pm 0.051*†	-42.3
	20	1.24 \pm 0.031*†	-67.0
	50	0.94 \pm 0.012*†	-75.0
Female n=10	0 (Control)	3.52 \pm 0.061	
	5	1.73 \pm 0.021*	-50.9
	10	0.58 \pm 0.029*†	-83.5
	20	1.98 \pm 0.042*†	-43.8
	50	2.02 \pm 0.046*	-42.6

P values compared to the control are: *P < 0.001. Significant values in 5 mg vs 10 mg NaF group, 10 mg vs 20 mg NaF group, and 20 mg vs 50 mg NaF group are: †P < 0.001. n = number of animals in each dosage group.

DISCUSSION

The weakening of protein metabolism by fluoride is due to repression of the activity of a number of enzymes responsible for protein synthesis. NaF is a well known inhibitor of protein phosphatases.¹⁵ Nucleoside triphosphatase (Mg^{2+} dependent adenosine triphosphatase) is present in the liver nuclear envelope¹⁶ and is involved in nucleo-cytoplasmic translocation of RNA.¹⁷ NaF inhibits this enzyme,¹⁸ which might be responsible for decrease in hepatic protein synthesis. In rats, fluoride-induced fluctuations in liver protein biosynthesis have been attributed to decrease in RNA transcription and inhibition of the methionin-activating enzyme of the liver, catalyzing certain stages of protein synthesis.¹⁹ In mice, decrease in protein levels in the liver were due to alteration in metabolism and change in osmotic balance.²⁰ The results obtained in the present study also revealed that the concentrations of acidic proteins, basic proteins, and total proteins in the liver were reduced after NaF treatment. A similar decrease in the protein content of the liver of fluoride intoxicated experimental animals has also been reported.²¹ Ravel *et al*²² claim that NaF acts as a specific inhibitor of protein synthesis by interfering with a reaction associated with new peptide chains on ribosomes.

Fluoride intoxication produces specific metabolic alterations in nucleic acid synthesis in the liver of experimental rabbits. An inhibitory effect of fluoride on DNA synthesis in liver cells has been reported.²³ Fluoride causes an increase in cAMP production by stimulating adenylate cyclase,²⁴ and the enzyme itself catalyzes the production of cAMP from ATP.²⁵ In the present

experiments, the decrease in hepatic DNA during experimental fluorosis is attributable to increased cAMP production from ATP. Kleiner and Allman²⁶ reported elevated levels of cAMP in liver of rats given fluoride in drinking water. Agutter²⁷ found that fluoride releases RNA from isolated rat liver nuclei. The decrease in hepatic RNA synthesis observed here may be due to reduced protein synthesis.

The increase in concentration of amino acids reflects a decreased shunting of amino acids into the tricarboxylic acid cycle for energy production as fluoride also inhibits enzyme enolase.¹ Fluoride affects the mechanism of glutamine synthesis, a stage in the deamination process and in Na⁺- and K⁺-activated ATPase which is essential for active uptake of amino acids.²⁸ The abnormal increase in hepatic amino acids may also be due to reduced incorporation of amino acids into proteins.² In addition, fluoride affects the amino acid sequence of newly synthesized proteins in rat liver.²⁹

A significant decrease in hepatic total lipids, triglycerides, free fatty acids, phospholipids,⁴ and cholesterol has been reported earlier.³⁰ The decline in cholesterol content of the liver in the present study may be due to inhibition of acetyl CoA by fluoride which results in decreased synthesis of cholesterol from acetyl CoA. Fluoride decreases the absorption of cholesterol and bile salts from plasma and intestine which could result in an increased conversion of bile acids in the liver, and bile acids are known to inhibit cholesterol synthesis in the intestine. This is indicative of hepato-biliary disturbances in fluoride intoxication.³¹

In conclusion, the results of the present investigation indicate fluoride-induced hepatotoxicity and metabolic disturbances of energy metabolism in the liver of rabbits. Structural changes in the form of hepatocellular necrosis, hepatic hyperplasia, extensive vacuolization in hepatocytes, and centrilobular necrosis in the liver caused by fluoride in rabbits³² and in patients³³ afflicted with fluorosis have also been reported earlier.

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