STUDIES ON ALTERATIONS IN BRAIN LIPID METABOLISM FOLLOWING EXPERIMENTAL FLUOROSIS

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SUMMARY: The neurotoxic effect of fluoride on lipid content of brain was assessed in rabbits during experimental fluorosis. Sodium fluoride at 5, 10, 20 and 50 mg/kg body weight/day was injected subcutaneously for 100 days into 60 rabbits of both sexes. The control animals were given 1 cc distilled water/kg body weight/day for the same period. Biochemical studies showed hyperlipidemia, hyperphospholipidemia and hypertriglyceridemia in the brain of treated animals of both sexes. The maximum increase in total lipids, phospholipids and triglycerides of brain occurred in animals treated with 50 mg NaF/kg. In male rabbits, the cholesterol content of brain rose suddenly ($p<0.001$) in the 5 mg fluoride group, followed by gradual decline in 10, 20 and 50 mg fluoride groups. In females, the cholesterol level rose ($p<0.001$) in animals of the 5, 10 and 20 mg fluoride groups and fell suddenly in the 50 mg fluoride group. Fluoride exerts an inhibitory effect on the free fatty acids in brain of both sexes. The relevance of these results in experimental fluorosis is discussed.

Key words: Brain; Cholesterol; Fluoride; Free fatty acids; Phospholipids; Rabbit; Total lipids; Triglycerides.

Introduction

The manifestations of the initial phase of fluorosis indicate injury to the central nervous system and the spinal cord. In humans, the neurological complications in advanced fluorosis in the form of partial and complete paralysis of arms and legs, headache, vertigo, spasticity in the extremities, visual disturbances and impaired mental acuity have been reported (1).

Fluoride is known to enter the brain and the blood brain barrier fails to exclude it from the nervous tissue (2). Accumulation of fluoride may induce a wide variety of changes in physiological and biochemical parameters. But due to lack of precise experimental data it is difficult to draw conclusions concerning the effect of fluoride on the nervous system.

The present investigation is aimed at elucidating alterations in lipid metabolism in brain of rabbit in experimental fluorosis.

Materials and Methods

Experimental Design: Sixty albino rabbits of both sexes weighing 400-650 gm were divided into 5 groups of 12. They were given fluoride subcutaneously at 5, 10, 20 and 50 mg/kg body weight/day for 100 days. The control animals were given 1 cc distilled water/kg body weight/day for the same period.

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All the animals were maintained on standard laboratory chow and water was supplied *ad libitum*. After 100 days, the control and F^- treated animals were sacrificed and the brains immediately removed for various biochemical studies. Extraction of total lipids was done by the method of Folch *et al* (3). Total lipids were estimated gravimetrically.

Separation of neutral lipids: Silica gel G thin layer plates (20x20 cm) were prepared for thin layer chromatography (4). Dried plates activated at 100°C for 90 minutes, were developed in a solvent system consisting of n-hexane:diethyl ether: glacial acetic acid (90:10;1 v/v). The chromatograms were air dried and stained with iodine vapours in sealed chambers. The resultant yellow spots are identified, marked and taken into extracting solvent: n-hexane:diethyl ether, 1:1 v/v. Pooled extracts evaporated to dryness were used for spectrophotometric analysis.

Determination of various lipid constituents: Triglycerides were determined by the method of VanHandle and Zilversmit (5). Estimation of phospholipids was done as described by Ames (6). Quantitative analysis of free fatty acids was done by the method of Chakrabarty *et al* (7) and cholesterol was assessed by the method of Stadtman (8).

Significance of the resulting data was determined by Student's t-test.

Results

Changes in the levels of total lipids and their various components are shown in Tables 1 and 2.

Differences in the level of total lipids in the brain of fluoridated and control rabbits of both sexes were significant ($p<0.05$ - 0.001). The female rabbits showed a higher percent increase in total lipid content of brain as compared to the males (Figure 1).

The concentration of phospholipids in the brain showed a significant ($p<0.001$) increase in the experimental animals of both sexes compared with the controls (Figure 2).

The level of neutral lipids in the brain showed a 16% increase in male rabbits treated with 5 mg fluoride. The level was slightly decreased (5%) in animals treated with 10 mg fluoride. Again 16% decrease in neutral lipid content of brain was observed in animals of the 20 mg fluoride group. The level of neutral lipids returned to the control values in animals treated with 50 mg fluoride. In females the amount of neutral lipids in the brain showed a slight to moderate elevation in all F^- treated groups compared with the control (Figure 3).

Brain triglyceride levels were highly elevated in fluorotic animals of both sexes (Figure 4). The females showed a greater increase in all of the F^- treated groups compared with the control. The highest percent increase was seen in animals of the 50 mg fluoride group (150.7% in males *vs* 265.5% in females).

Table 1. Effect of fluoride on total lipids, phospholipids and neutral lipids in the brain of rabbits (Data are means ± SD)

Constituent	Treatment F mg/kg b.w.	Male	Percentage of control	Female	Percentage of control
Total lipids (mg/g w.w.)	0(control)	69.81 ±3.372		60.76 ±9.418	
	5	78.28 ±7.187*	+12.1	90.61 ±9.164***	+49.1
	10	81.82 ±9.200*	+17.2	95.07 ±8.058***	+56.4
	20	79.28 ±5.610**	+13.5	102.22 ±7.952***	+68.2
	50	253.36 ±21.346***	+262.9	245.91 ±10.539***	+304.7
Phospho-lipids (mg/g w.w.)	0(control)	30.36 ±0.550		28.36 ±0.130	
	5	38.99 ±0.250***	+28.4	40.84 ±0.350***	+44.0
	10	36.44 ±0.240***	+20.0	46.44 ±0.210***	+63.7
	20	41.04 ±0.510***	+35.1	44.31 ±0.325***	+56.2
	50	39.86 ±0.260***	+31.2	42.87 ±0.296***	+51.1
Neutral lipids (mg/g w.w.)	0(control)	41.83		42.60	
	5	48.55	+16.0	56.28	+33.8
	10	39.35	-5.92	50.16	+19.2
	20	35.02	-16.2	43.77	+4.0
	50	40.83	-2.3	50.06	+19.0

P values as compared with control: * p<0.05 ** p<0.01 *** p<0.001

Table 2. Effect of fluoride on triglycerides, free fatty acids and cholesterol in the brain of rabbits (Data are means ± SD)

Constituent	Treatment F mg/kg b.w.	Male	Percentage of control	Female	Percentage of control
Triglycerides (mg/g w.w.)	0(control)	12.17 ±1.091		11.12 ±0.015	
	5	19.12 ±0.550**	+57.1	21.44 ±0.220**	+92.8
	10	19.23 ±0.150**	+58.0	31.53 ±0.180**	+183.5
	20	17.66 ±0.160**	+45.1	29.19 ±0.190**	+162.5
	50	30.52 ±0.166**	+150.7	40.65 ±0.226**	+265.5
Free fatty acids (mg/g w.w.)	0(control)	22.94 ±0.230		25.15 ±0.110	
	5	9.96 ±0.109**	-56.5	20.57 ±0.140**	-18.2
	10	14.34 ±2.842**	-37.4	8.92 ±0.169**	-64.5
	20	12.93 ±0.120**	-43.6	7.52 ±0.220**	-70.0
	50	6.62 ±0.155**	-71.1	5.46 ±0.190**	-78.2
Cholesterol (mg/g w.w.)	0(control)	6.72 ±0.306		5.79 ±0.061	
	5	19.47 ±0.310**	+18.0	14.27 ±0.440**	+146.4
	10	5.78 ±0.336*	-13.9	9.71 ±0.169**	+67.7
	20	4.43 ±0.046**	-34.0	7.06 ±0.094	+21.9
	50	3.69 ±0.052**	-45.0	3.95 ±0.370**	-31.7

P values as compared with control: • p<0.05 ** p<0.001

Figure 1. Total Lipids in Brain during Experimental Fluorosis

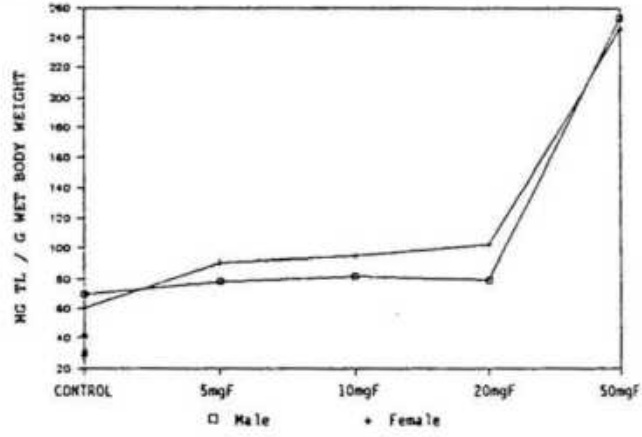


Figure 2. Phospholipid Content of Brain in Experimental Fluorosis

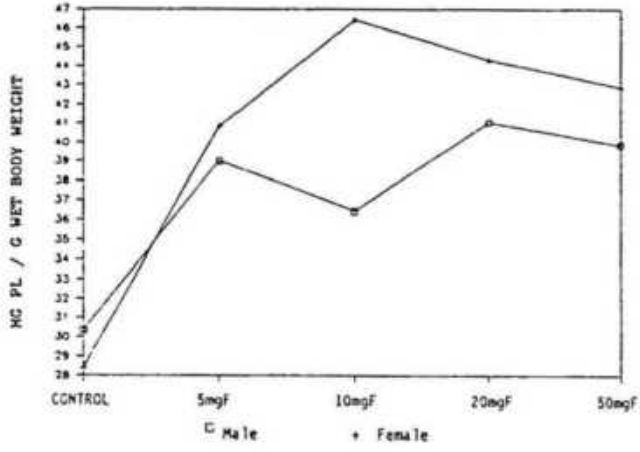


Figure 3. Neutral Lipids in Brain in Experimental Fluorosis

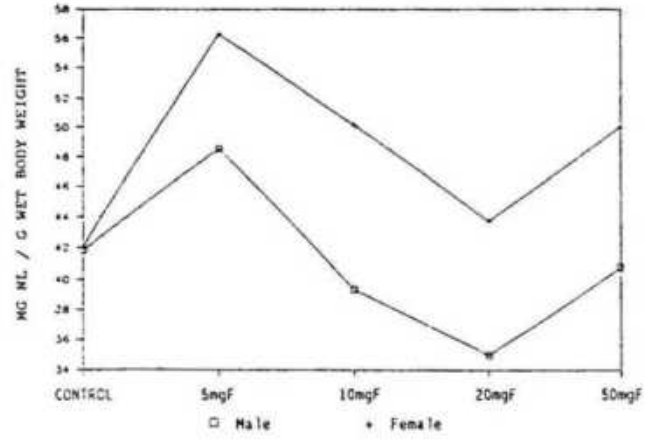


Figure 4. Triglyceride Levels in Brain of Rabbit in Fluoride Intoxication

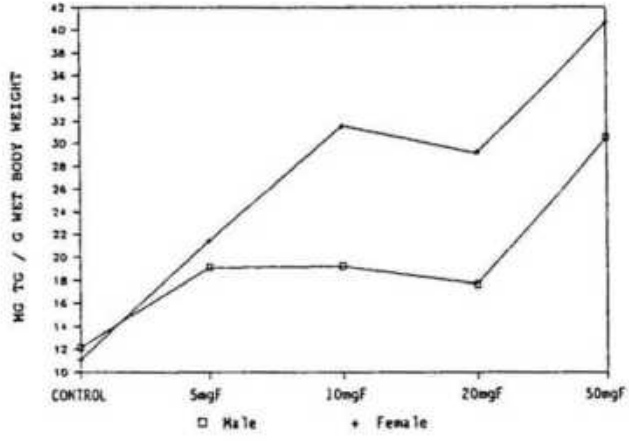


Figure 5. Free Fatty Acid Levels in Brain in Experimental Fluorosis

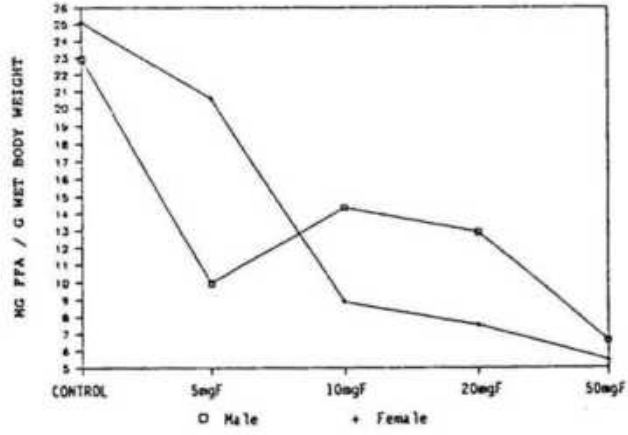
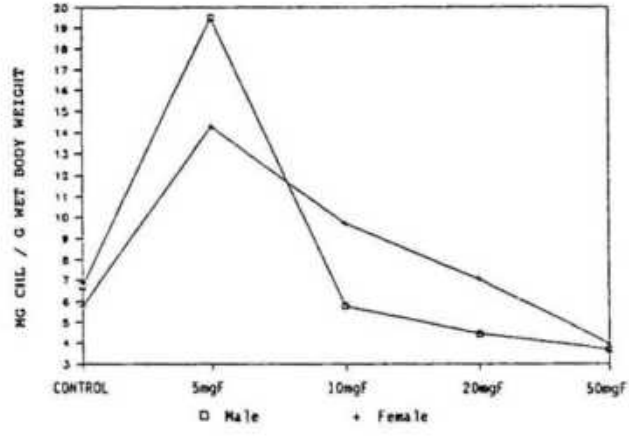


Figure 6. Cholesterol Levels in Brain during Experimental Fluorosis



Fluoride caused a decrease in brain free fatty acids in the experimental animals of both sexes (Figure 5).

The cholesterol content of the brain showed a sudden rise in male animals treated with 5 mg fluoride followed by rapid decline ($p<0.001$) in subsequent groups. In females, the brain cholesterol showed a significant increase ($p<0.001$) in animals treated with 5, 10 and 20 mg fluoride, whereas it significantly declined ($p<0.001$) in animals treated with 50 mg fluoride (Figure 6).

Discussion

In this study on rabbits, there are appreciable changes in the brain lipid metabolism induced by fluoride. They are similar to the disorders known as "lipid storage diseases". Lipidosis is a disorder of lipid metabolism leading to abnormal fat accumulation in body tissues particularly in the liver and brain (9). The present data indicate hyperlipidemia in the brain of rabbits of both sexes resulting from fluoride intoxication. Hyperlipidemia may occur due to enzymatic defect, the inability of brain to degrade the lipid in the body.

Since lipids are transported in association with carrier proteins, it is possible that hyperlipidemia may result from a defect in lipoprotein metabolism. Several possible mechanisms are suggested:

a) an inhibition of the production of plasma lipoproteins;
b) a block in lipoprotein apoprotein synthesis;
c) a block in the synthesis of lipoprotein from lipid and apoprotein;
d) a failure to provide the phospholipids found in lipoproteins;
e) a deficiency in lipotropic factors.

The deficiency of a lipotropic agent causes triglycerides to accumulate. Elevation of triglycerides may lead to a decrease in the synthesis of free fatty acids during fluoride intoxication (10). The decrease in free fatty acids synthesis in soft tissues during experimental fluorosis has been reported earlier (11-13). The hyperlipidemia, hypertriglyceridemia and hyperphospholipidemia represent excessive mobilization of fat (14).

Fluoride inhibits many enzymes involved in lipid metabolism - *e g* lipases, phospholipases which are capable of hydrolyzing the fatty acids from phospholipids (15). The inhibition of these enzymes could result in elevated levels of phospholipids and decrease in free fatty acids.

In this study, the brain cholesterol was significantly elevated in the early phase of intoxication (5 mg fluoride group), but declined in subsequent fluoridated groups of male animals, whereas in females, hypercholesterolemia in the brain was found in animals treated with 5, 10 and 20 mg/kg fluoride. The cholesterol content of the brain was highly elevated in female animals of the 5 mg fluoride group (146%) and the 10 mg group (67%). In animals of the 20 mg fluoride group the cholesterol levels in the brain were moderately elevated (21%). The levels were significantly decreased (31%) in animals receiving the highest dose of fluoride (50 mg/kg body weight). Hypercholesterolemia may be due to the deficiency of liposomal lipase which hydrolyzes cholesterol esters taken up by the cell.

Several studies involving the effects of fluoride on serum lipids have been reported. Townsend and Singer (16) observed decrease in serum cholesterol in guinea pigs. On the other hand, Vatassery *et al* (17) reported an increase in serum cholesterol in guinea pigs which received deionized water containing 25 ppm fluoride for 13 weeks. According to Singer and Armstrong (18) an increase in fluoride intake did not influence serum cholesterol.

As a result of an imbalance in the synthesis and breakdown of the lipids in the brain due to fluoride intoxication, the neurons of the cerebellar cortex showed degeneration. In addition to this, there was retarded development, paraplegia and quadriplegia.

Conclusions

1. Fluoride interferes with lipid metabolism in the brain of experimental rabbits. The abnormal accumulation of lipid in rabbit brain may be due to deficiency of a lipotropic agent.

2. Hyperlipidemia, hyperphospholipidemia and hypertriglyceridemia in the brain may result from a defect in lipoprotein metabolism.

3. Depletion of cholesterol and free fatty acid in the brain of experimental rabbits may be the result of decreased lipolysis due to inhibition of lipase.

4. Hypercholesterolemia in rabbit brain may be due to the deficiency of liposomal lipase which is necessary to hydrolyze the cholesterol esters.

Whether similar changes take place in humans after excessive ingestion of sodium fluoride is yet to be ascertained.

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