EFFECT OF LONG-TERM ADMINISTRATION OF FLUORIDE ON LEVELS OF PROTEIN, FREE AMINO ACIDS AND RNA IN RABBIT BRAIN

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SUMMARY: Biochemical alterations in the brain produced during experimental fluorosis were studied. Albino rabbits of both sexes were administrated sodium fluoride solutions in the concentrations of 5, 10, 20, and 50 mg/kg body wt/day by subcutaneous injection for 100 days. The control rabbits were given 1 cc distilled water/kg body weight/day for the same length of time. In fluoride treated rabbits the brain showed significant decline ($P < 0.001$) in soluble, basic, total protein and free amino acid levels. RNA content rapidly decreased ($P < 0.001$) in the brains of experimental animals compared to the controls. However, in male animals treated with 5 and 10 mg fluoride no statistically significant differences in RNA content of brain were observed. The depletion of proteins produced degenerative changes in purkinje cells of the cerebellar cortex. These changes in the brain lead to paralysis of limbs in fluoridated animals.

Key words: Basic protein; Brain; Experimental fluorosis; Free amino acid; RNA; Soluble protein.

Introduction

Endemic fluorosis is related to a high concentration of fluoride in water. The manifestations of the initial phase of fluorosis indicate injury to the central nervous system, *i.e.* the brain and the spinal cord (1). Involvement of the nervous system in skeletal fluorosis has been reported mainly from India. Singh and Jolly (2) studied 60 cases of chronic fluorosis in patients with skeletal fluorosis. The most important symptoms in these patients were muscular wasting, referred pain along the nerve roots and fibrillation and fasciculation of the muscles. Siddiqui (3) studied 53 advanced cases of the disease with neurological manifestations and recorded a patchy type of anaesthesia, spastic paraplegia, absence of vibration sense and loss of sphincter control. The neurological signs were due to pressure on the spinal roots and the cord by bony growths into the spinal canal which in the cervical region resembled the clinical picture of cervical spondylosis. Popov *et al* (4) detected neurological symptoms in 79% of patients with occupational fluorosis, thus also suggesting direct nerve involvement. Frank *et al* (5), while studying a fatal case of industrial fluorosis, recorded that fluoride ions damage nervous tissue without physical pressure upon the spinal cord. However, the effects of acute and chronic doses of fluoride on the brain in experimental animals are almost unknown. In the present investigation the following parameters were measured in order to evaluate such effects: soluble proteins and basic proteins, free amino acids, and RNA.

Materials and Methods

Animals: Albino rabbits of both sexes weighing 400-600 gm, procured from the Kaila Scientific Corporation, Agra, India, were divided into five groups of 12. All were fed a standard pellet diet, and water was supplied *ad libitum*.

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Experimental design: The animals were weighed before the experiment. One group given 1 cc distilled water/kg body weight/day was kept as control (Group C). The remaining four test groups (F_5, F_{10}, F_{20}, and F_{50}) were administered subcutaneous injections of sodium fluoride in the dosages of 5, 10, 20, and 50 mg/kg body weight/day for 100 days, respectively. All the animals were sacrificed under ether anaesthesia, and the brains rapidly removed for quantitative analysis of proteins (soluble and basic), free amino acids, and RNA.

Analytic procedure: Protein in brain was assayed by the method of Lowry *et al* (6) using bovine serum albumin as standard. The free amino acids were estimated according to the method of Troll and Cannon (7) using KCN-pyridine solution and phenol reagent. The colour was developed by adding alcoholic ninhydrin, the intensity of which was read at 570 mμ on a spectronic-2 colorimeter. The nucleic acids were extracted accordingly to the method of Webb and Levy (8). The estimation of RNA was done as per the orcinol method described by Markham (9). The results were calculated as mg/g wet weight of tissue. However, to compare the effects of different doses of fluoride on the same biochemical parameters, the results are expressed as percent change relative to control.

Statistics: The statistical significance was assessed using student's t-test.

Results

During the course of the study the animals consumed normal amounts of food and had normal gains in weight. However, in the Control, F_5 and F_{10} groups the increase in body weight was more than in the F_{20} and F_{50} groups.

In the F_{10} and F_{20} groups some of the animals showed signs of paralysis at 35 days from the onset of the experiment. Most of the animals in the F_{50} group were attacked with paralysis. None of the rabbits completed the total duration of the experiment. The maximum survival was for 70 days at which stage the animals showed paralysis of both fore- and hind-limbs. The remaining animals showed signs of paralysis at 35, 40, 49, 59, 61 and 70 day intervals, and their weight fell suddenly during the experiment.

The metabolic responses of the brain from rabbits receiving different amounts of fluoride are remarkably different. The soluble and basic proteins are significantly decreased ($P < 0.001$) in brains of all fluoridated groups compared to the control (Table 1). The total proteins showed a higher percentage of degradation in males (94.5%) than in females (87.4%) of the F50 group.

The free amino acid (FAA) content of the brain (Table 2) suddenly decreased in test animals compared to controls. In female animals FAA dropped from 8.5 mg/g w.w. to 1.8 mg/g w.w., 1.2 mg/g w.w, 1.1 mg/g w.w and 0.3 mg/g w.w. in F_5, F_{10}, F_{20} and F_{50} respectively. The female rabbit brain showed a higher percent change in levels of FAA than the male (96.4% vs. 88.6%).

The concentration of RNA in male rabbit brain showed slight variations in two fluoridated groups (F_5 and F_{10}) which were not statistically significant (Table 2). However, RNA content of the brain in F20 group declined to almost half that of the control group (2.2 mg/g w.w to 4.1 mg/g w.w). In the F_{50} group RNA content in brain dropped significantly ($P < 0.001$) to 1.8 mg/g w.w. In female rabbit brains RNA declined in all the fluoridated groups. The greatest percent decrease (50%) was in the F_{50} group.

TABLE 1. Effect of different doses of fluoride on proteins of rabbit brain (Mean ± SD)

	Dose mg/kg/b.wt.	Soluble protein	Percent reduction	Basic protein	Percent reduction	Total protein	Percent reduction
Male	0 (Control)	7.6 ± 0.12		3.5 ± 0.09		11.1	
	5	3.7 ± 0.19[a]	-51.3	2.0 ± 0.41[a]	-42.8	5.7	-48.6
	10	1.6 ± 0.09[a,b]	-78.9	1.2 ± 0.06[a,b]	-65.7	2.8	-74.7
	20	1.0 ± 0.06[a,b]	-86.8	0.5 ± 0.06[a,b]	-85.7	1.5	-86.4
	50	0.4 ± 0.07[a,b]	-94.7	0.2 ± 0.01[a,b]	-94.2	0.6	-94.5
Female	0 (Control)	8.6 ± 0.33		4.1 ± 0.10		12.7	
	5	3.8 ± 0.07[a]	-55.8	2.0 ± 0.03[a]	-48.7	5.8	-54.6
	10	1.2 ± 0.04[a,b]	-86.0	1.5 ± 0.11[a,b]	-63.4	2.7	-78.7
	20	1.1 ± 0.10[a,d]	-87.2	1.3 ± 0.14[a,c]	-68.2	2.3	-81.8
	50	1.0 ± 0.12[a]	-88.3	0.6 ± 0.14[a,b]	-85.3	1.6	-87.4

Values are expressed in mg/g wet weight of tissue.
a = Difference between mean of experimental group and control was significant at $P < 0.001$.
b, c or d = Difference between the mean of the group and the one above it was significant at
b: $P < 0.001$, c: $P < 0.02$, d: $P < 0.05$.

TABLE 2. Effect of different doses of fluoride on free amino acids and ribonucleic acid content of rabbit brain (Mean ± SD)

	Dose mg/kg/b.wt.	Free amino acid	Percent reduction	Ribonucleic acid	Percent reduction
Male	0 (Control)	4.4 ± 0.03		4.1 ± 1.06	
	5	2.1 ± 0.26[a]	-52.2	3.6 ± 0.08	-12.1
	10	1.8 ± 0.01[a,d]	-59.0	3.3 ± 0.04[c]	-19.5
	20	0.8 ± 0.19[a,c]	-81.8	2.2 ± 0.01[b,c]	-46.3
	50	0.5 ± 0.02[a,c]	-88.6	1.8 ± 0.03[a,c]	-56.0
Female	0 (Control)	8.5 ± 1.12		3.2 ± 0.04	
	5	1.8 ± 0.04[a]	-78.8	2.8 ± 0.07[a]	-12.5
	10	1.2 ± 0.06[a,c]	-85.8	2.3 ± 0.02[a,c]	-28.1
	20	1.1 ± 0.01[a]	-87.0	1.9 ± 0.40[a]	-40.6
	50	0.3 ± 0.09[a,c]	-96.4	1.6 ± 0.01[a]	-50.0

Values are expressed in mg/g wet weight of tissue.
a or b = Difference between mean of experimental group and control was significant at
a: $P < 0.001$, b: $P < 0.01$
c or d = Difference between the mean of the group and the one above it was significant at
c: $P < 0.001$, d: $P < 0.05$.

Discussion

Protein synthesis is affected in various ways by fluoride ions. They affect the rate of cellular protein synthesis (10,11) due to impairment of peptide chain initiation (12). The present experimental data show that in treated animals there is a decreased level of soluble, basic protein and a general decrease in total protein content. The observations agree with those of Kathpalia and Susheela (13) who recorded a 10 to 46 percent decrease in total protein content of different rabbit organs including kidney, liver, testis, and brain.

Administration of sodium fluoride inactivates certain enzymes of glycolysis and of the tricarboxylic acid cycle (14). Leonard (15) reported that NaF (50 µg in 10 µl) increases brain glycolysis in albino mice after one minute of intraventricular administration.

Shearer and Suttie (16) recorded that, in female rats ingesting 450 ppm fluoride for 3 days, the concentration of plasma free amino acids was decreased. During the present investigation, a reduction in the brain free amino acids of all fluoridated rabbits of both sexes was found. Such a reduction had already been reported by Schwartz *et al* (17) who observed that fluoride progessively lowered amino acid uptake and ATP content in incubated brain slices of mice as the concentration of fluoride increased.

Fluoride is also known to inhibit nucleic acid synthesis (18) and attachment of m-RNA to ribosomes. NaF retards initiation of globin chain synthesis and the free m-RNA are rendered inactive by fluoride (10). The decrease in RNA content of rabbit brain observed during acute and chronic fluoride intoxication seems to be due to fluoride-induced inhibition of protein synthesis.

Fluoride inhibits many enzymes *in vitro* (14). The action of fluoride on most of these enzymes is magnesium dependent. The degree to which fluoride complexes with magnesium ions varies in different cell compartments causing local changes in the concentration of the activator. This in turn affects the function of the RNA-polymerase system, the free energy of ATP hydrolysis and the conformational structure of some types of RNA and proteins (19). The biochemical changes in brain levels of proteins, amino acids, and ribonucleic acids are reflected in the morphological patterns of different cell structures of the brain.

The results reported here indicate that fluoride has a specific effect on the synthesis of proteins in the brain which may lead to degenerative changes in the form of ballooning degeneration of neurons, various degrees of loss of nisal substance, and changes in the purkinje cells of the cerebellar cortex. Such changes would provide a plausible explanation for some of the diverse neurological complaints in arms and legs such as numbness, muscle spasms and pains, tetaniform convulsions, and spastic paraplegia, encountered in patients afflicted with skeletal fluorosis (20).

Conclusion

Observed alterations in the brain protein synthesizing system in experimental fluorosis may offer a partial biochemical explanation for some of the diverse neurological complaints in patients with skeletal fluorosis.

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