

EFFECT OF FLUORIDE INTOXICATION ON LIPID PEROXIDATION AND ANTIOXIDANT SYSTEMS IN RATS

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SUMMARY: The effect of fluoride intoxication on lipid peroxidation and antioxidant systems in the blood, brain, and liver of rats was studied. Twelve one-month-old albino rats were administered 100-ppm fluoride (as NaF) in their drinking water for four months. In the red blood cells the levels of malondialdehyde (MDA) and glutathione (GSH) increased, along with the activity of glutathione peroxidase (GSH-P_x), but the activity of superoxide dismutase (SOD) decreased. In the plasma the level of ascorbic acid increased while that of uric acid decreased. In the brain and liver, MDA and GSH levels increased, as did the activities of GSH-P_x and glutathione S-transferase (GST). The level of ascorbic acid increased in the brain, but it decreased in the liver. These results suggest that fluoride enhances lipid peroxidation in the red blood cells, brain and liver of rats and causes increased or decreased enzyme activity associated with free radical metabolism.

Keywords: Albino rats, Antioxidants, Ascorbic acid, Fluoride intoxication, Glutathione, Glutathione peroxidase, Glutathione S-transferase, Lipid peroxidation, Sodium fluoride, Superoxide dismutase.

INTRODUCTION

The most obvious early toxic effects of fluoride in humans are dental and skeletal fluorosis, which are endemic in areas with elevated exposure to fluoride.¹ Fluoride is also known to cross the cell membranes and to enter soft tissues.^{2,3} Impairment of soft-tissue function has been demonstrated in fluoride-intoxicated animals.⁴⁻¹¹

In blood, brain, and liver of animals, various changes occur after chronic administration of fluoride. These include abnormal behaviour patterns,^{4,5} altered neuronal and cerebrovascular integrity,⁵ and metabolic lesions.⁶⁻⁸ Generation of free radicals, lipid peroxidation, and altered antioxidant defense systems are considered to play an important role in the toxic effects of fluoride.⁹⁻¹¹ In this study we assess the effect of chronic fluoride toxicity on lipid peroxidation and antioxidants in blood, brain, and liver of rats.

MATERIALS AND METHODS

Wistar albino rats were obtained from the National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India, and were maintained in the central animal house of MR Medical College, Gulbarga. They were housed in polypropylene cages with stainless steel grill tops, fed

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a standard pelleted diet (Hindustan Lever Ltd, India), and were given water *ad libitum*.

One-month-old albino rats, each weighing about 50 grams, were chosen for the study and divided into two groups: Control and Test. Control rats (14) received drinking water containing 0.5 ppm fluoride. Test rats (12) received 100 ppm fluoride (as NaF) in their drinking water. After four months, the rats of both groups were sacrificed under light ether anaesthesia. Blood samples were collected by cardiac puncture in acid-citrate-dextrose solution (1.0 mL per 4.0 mL of blood), the rats were perfused transcardially with 0.9% saline, and the brains and livers were removed for further study.

Blood samples were centrifuged at 3000 rpm for 15 min to remove the plasma and buffy coat (consisting of leukocytes and platelets). The erythrocytes were washed three times in buffered saline (0.9% saline in 0.01M phosphate buffer, pH 7.4), and the packed cells were suspended in equal volume of the buffered saline. By the procedures here cited, malondialdehyde (MDA)¹² and glutathione (GSH)¹³ levels along with glutathione peroxidase (GSH-P_x)¹⁴ and superoxide dismutase (SOD)¹⁵ activities were estimated in the red cell lysates. Hemoglobin (Hb) in the red cell lysates was determined by cyanmethemoglobin method.¹⁶ Ascorbic acid¹⁷ and uric acid¹⁸ were estimated in the plasma.

Brain and liver tissues were homogenized 1:40 w/v in 0.1M phosphate buffer, pH 7.4, containing 1 mM EDTA. In the tissue homogenates the levels of MDA¹² and total GSH¹⁹ and the activities of GSH-P_x²⁰ and glutathione S-transferase (GST)²¹ were assayed. One unit of enzyme activity is defined as the amount of enzyme that catalyses the formation of one micromole of product per minute under the assay conditions. Protein content of the homogenates was determined by the method of Lowry *et al*,²² and the GSH-P_x and GST activities are expressed in terms of units/gram protein.

For estimation of ascorbic acid, the tissues were homogenized 1:9 w/v in ice-cold 5% trichloroacetic acid. Ascorbic acid assay was based on oxidation of ascorbic acid to dehydroascorbic acid followed by treatment with 2,4-dinitrophenylhydrazine to form the bis-dinitrophenylhydrazone.²³

The statistical significance of the results was analyzed by Student's t test.

RESULTS

In the red blood cells of the fluoride-treated (test) rats, MDA and GSH levels and the GSH-P_x activity were significantly elevated while the SOD activity was significantly lower (Table 1). Levels of plasma ascorbic acid were markedly higher, and those of uric acid were lower in the fluoride-treated group than the controls (Table 2).

In the brain and liver homogenates of the test rats, MDA and GSH levels and GSH-P_x and GST activities were significantly increased compared with

those of the controls. Ascorbic acid levels were markedly increased in the brain but were lower in the liver of test rats (Table 3).

Table 1. Malondialdehyde (MDA) and antioxidants in red cell lysates

	Control (n = 14)	Test (n = 12)	% Change
MDA (nanomoles/g Hb)	228.1 ± 6.35	291.4 ± 11.84*	+27.7
GSH (micromoles/g Hb)	10.19 ± 0.44	13.22 ± 0.28*	+29.7
GSH-Px (units/g Hb)	53.52 ± 3.89	85.28 ± 2.64*	+59.3
SOD (units/g Hb)	1360 ± 35.19	1153 ± 47.21*	-15.2

Values: Mean ± SD. Significance of the results: *p < 0.001.

Table 2. Ascorbic acid and Uric acid levels in plasma

	Control (n = 14)	Test (n = 12)	% Change
Ascorbic acid (mg/dL)	0.77 ± 0.09	1.18 ± 0.11*	+53.2
Uric acid (mg/dL)	1.29 ± 0.30	1.01 ± 0.16†	-21.7

Values: Mean ± SD. Significance of the results: *p < 0.001; †p < 0.01.

Table 3. Malondialdehyde (MDA) and Antioxidants in brain and liver homogenates

	Control (n = 14)	Test (n = 12)	% Change
<i>MDA (nanomoles/g protein)</i>			
Brain	1188 ± 62.83	2108 ± 151.3*	+77.4
Liver	794.9 ± 27.54	1631 ± 56.31*	+105.2
<i>Total GSH (micromoles/g protein)</i>			
Brain	6.42 ± 1.25	10.42 ± 1.60*	+62.3
Liver	11.42 ± 3.14	20.43 ± 2.13*	+78.9
<i>GSH-Px (units/g protein)</i>			
Brain	21.53 ± 2.64	83.51 ± 10.07*	+287.9
Liver	95.60 ± 10.86	172.1 ± 23.60*	+80.0
<i>GST (units/g protein)</i>			
Brain	85.27 ± 6.82	117.9 ± 10.17*	+38.3
Liver	182.3 ± 11.10	440.6 ± 13.43*	+141.7
<i>Ascorbic acid (µg/g wet tissue)</i>			
Brain	160.8 ± 10.83	215.6 ± 17.78*	+34.1
Liver	139.7 ± 12.31	98.68 ± 8.78*	-29.4

Values: Mean ± SD. Significance of the results: *p < 0.001.

DISCUSSION

Reactive oxygen species (ROS) are implicated as important pathologic mediators in many disorders. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of a wide range of compounds.²⁴⁻²⁶ Various authors have investigated relationships between fluoride and free radical reactions.^{8-11,27-29,31} Earlier studies reported increased lipid peroxidation,²⁷ increased²⁸ or unaltered levels of GSH,²⁹ decreased activity of GSH-P_x,²⁹ and unaltered activity of SOD²⁹ in the erythrocytes of fluorotic humans. We recorded increased lipid peroxidation, decreased GSH levels, increased GSH-P_x activity and decreased SOD activity in the erythrocytes of children with skeletal fluorosis.³⁰ Enhanced lipid peroxidation, decreased GSH levels, lower GSH-P_x activity, and unaltered SOD activity have been observed in the erythrocytes of fluoride-treated experimental animals.¹¹ Likewise, enhanced lipid peroxidation and decreased activities of antioxidant enzymes have been recorded in soft tissues of fluoride-treated mice.^{8,10,31} Our study revealed increased lipid peroxidation in the red blood cells, brain, and liver of 100-ppm fluoride-treated rats.

In our work, antioxidant glutathione levels and GSH-P_x activities were elevated in red blood cells, brain, and liver. Thus, our results differ from those of earlier studies on effect of fluoride on these antioxidants in experimental animals.^{10,11,31} The discrepancy might be due to differences in the route of fluoride administration, time duration, dose, and individual animal response. Increased glutathione levels and GSH-P_x activities may be attributed to adaptive response of the tissues to the oxidant challenge due to prolonged exposure (4 months) to high fluoride. Earlier, Edes *et al*,³² and Chow and Tappel³³ independently observed an increase in GSH-P_x activity in rats exposed to high oxidant stress. An increase in GST activity in brain and liver was also observed in the current study. Studies on hepatoma cells in culture have demonstrated induction of GST on exposure to sodium fluoride in a dose-dependent manner.³⁴ Thus it appears that prolonged exposure to oxidant stress can lead to adaptive response in the form of increased antioxidant defense. Decreased SOD levels in red blood cells of rats in our study indicate decreased ability of the tissues to handle O₂⁻ radicals. Similar findings on SOD have been reported in the tissues of mice exposed to high-fluoride intake.^{8,10,31}

Ascorbic acid is an important antioxidant in plasma³⁵ and acts in tissues, involving ROS in aqueous phase. In the brain, ascorbic acid has a dual effect – at low concentrations promoting lipid peroxidation and at higher concentrations acting as an antioxidant.²⁶ It is also an anti-stress factor. Augmented synthesis and mobilization of ascorbic acid were observed in rats exposed to prolonged fluoride intake, thereby implicating its role in the amelioration of fluoride-induced stress.^{36,37} We observed increased levels of ascorbic acid in

plasma and brain of our rats. Plasma ascorbic acid levels of children with skeletal fluorosis were increased.³⁰ These findings suggest a definite role for ascorbic acid as an antioxidant and anti-stress factor in fluoride intoxication. However, the decline in liver ascorbic acid level that we observed does not appear to be consistent with this interpretation.

In conclusion, we see that chronic fluoride intoxication markedly enhanced lipid peroxidation in rats. Adaptive changes apparently occurred in antioxidant systems in the blood, brain, and liver of rats to counteract fluoride-induced oxidative stress on prolonged exposure.

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