

BRAIN LIPID PEROXIDATION AND ANTIOXIDANT SYSTEMS OF YOUNG RATS IN CHRONIC FLUORIDE INTOXICATION

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SUMMARY: A study was made of the effect of fluoride on oxidative stress in rats during their early stages in life. Wistar albino rats were exposed to 30 ppm and 100 ppm fluoride (from sodium fluoride) in drinking water during the last one week of intrauterine life and then up to ten weeks after birth. Oxidative stress was evaluated by the assays of malondialdehyde and antioxidants in brain homogenates. Malondialdehyde (MDA), the marker of extent of lipid peroxidation, was elevated in the brain of rats treated with 100 ppm fluoride but was without change in rats treated with 30 ppm fluoride. Levels of total glutathione, reduced glutathione (GSH), and ascorbic acid (vitamin C) were elevated in 30 ppm fluoride-treated rats, while these levels decreased in 100 ppm fluoride-treated rats. The activity of glutathione peroxidase (GSH-P_x) was elevated significantly in both 30 ppm and 100 ppm fluoride-treated rats. Glutathione S-transferase (GST) activity in the brain increased with 30 ppm and 100 ppm fluoride, and greater elevation occurred at 30 ppm. These results suggest that fluoride enhances oxidative stress in the brain, thereby disturbing the antioxidant defense of rats. Increased oxidative stress could be one of the mediating factors in the pathogenesis of fluoride toxicity in the brain.

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

INTRODUCTION

Fluorosis, caused by long-term intake of high levels of fluoride, is characterized by clinical manifestations in bones and teeth.¹ However, detrimental effects of high-fluoride intake are also observed in soft tissues.^{2,3} In advanced stages of fluorosis, neurological manifestations such as paralysis of limbs, vertigo, spasticity in extremities, and impaired mental acuity, are observed in human beings.⁴ Fluoride accumulation was observed in the brain of rats exposed to chronic high-fluoride intake through drinking water.⁵ Intake of high levels of fluoride is known to cause structural changes,^{2,6} altered activities of enzymes,⁷ and metabolic lesions^{8,9} in the brain of experimental animals.

Increased free radical generation and lipid peroxidation are proposed to mediate the toxic effects of fluoride on soft tissues.¹⁰⁻¹² Earlier, we reported increased lipid peroxidation and disturbed antioxidant defense systems in brain, erythrocytes and liver of rats exposed to high-fluoride intake during the stages of life after weaning.¹³ There is, however, a paucity of studies on the effect of fluoride intoxication during the early developing stages of life

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on oxidative stress. Recently, we observed increased lipid peroxidation and altered levels of antioxidants in the blood of children with endemic skeletal fluorosis¹⁴ and in the liver of young rats exposed to high levels of fluoride in drinking water during the early stages of life.¹⁵ In the present study, we attempt to assess the effect of intake of high-fluoride levels in drinking water during fetal, weanling, and post-weaning stages of life on oxidative stress in the brain of rats.

MATERIALS AND METHODS

Adult (4-6 month-old) Wistar albino rats were used in the study. The rats were fed a standard pelleted diet (Hindustan Lever Ltd., India) and were given water *ad libitum*. The animals were maintained under proper temperature (25-30°C), ventilation, and hygienic conditions. They were exposed to 12 hours each of light and dark.

Pregnant rats were divided into three groups: control, 30F, and 100F. Control rats received drinking water with 0.5 ppm fluoride, while the 30F and 100F rats received 30 ppm and 100 ppm fluoride (from NaF), respectively, in their drinking water during the last (3rd) week of pregnancy and throughout the lactation period. The litters were separated from the mother rats on weaning and were then exposed to the respective levels of fluoride (0.5 ppm, 30 ppm, and 100 ppm) in drinking water up to the age of 10 weeks. The ten-week-old rats of control (n = 15), 30F (n = 13), and 100F (n = 9) groups were sacrificed after light ether anaesthesia. They were perfused transcardially with 0.9% saline, and the brains were removed.

Brain tissues were homogenized 1:40 (w/v) in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA. In the tissue homogenates the levels of MDA,¹⁶ total glutathione (GSH+GSSG)¹⁷ and reduced glutathione (GSH),¹⁸ and the activities of GSH-P_x¹⁹ and GST²⁰ 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*,²¹ and the GSH-P_x and GST activities were 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.²²

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

RESULTS

MDA levels in the brain homogenates of control and 30F rats did not differ appreciably, whereas the 100F rats showed significantly higher MDA levels compared to the controls. Levels of total glutathione and GSH were

increased in the 30F rats but were decreased in the 100F rats when compared to the controls. In both the 30F and 100F rats, the ratio of total to reduced glutathione decreased significantly. The activities of GSH-P_x and GST were markedly increased in the 30F and 100F groups compared to the control group. Increase in GSH-P_x activity was more in the 100F rats while increase in GST activity was more in the 30F rats. Ascorbic acid levels were markedly increased in the 30F rats but were decreased in the 100F rats (Table).

Table. Malondialdehyde (MDA) and antioxidants in brain homogenates of young rats

Parameter (per g protein)	Control	30 ppm F	100 ppm F	% change from control	
MDA (nmol/g)	1287 ± 52.82	1226 ± 81.62	1790 ± 156.5*	- 4.7	+ 39.1
GSH (µmol/g)	6.06 ± 0.54	7.07 ± 0.45*	4.93 ± 0.40*	+ 16.7	- 18.6
Total glutathione (µmol/g)	6.19 ± 0.46	7.60 ± 0.38*	5.55 ± 0.22*	+ 22.8	- 10.3
Ratio of GSH to total glutathione	0.98 ± 0.007	0.93 ± 0.006*	0.89 ± 0.006*	- 5.1	- 9.2
GSH-P _x (units/g)	16.44 ± 1.31	35.39 ± 1.98*	63.33 ± 4.0*	+ 115.3	+ 285.2
GST (units/g)	74.25 ± 9.62	180.7 ± 10.95*	90.12 ± 9.29*	+ 143.4	+ 21.4
Ascorbic acid (µg/g wet tissue)	154.2 ± 6.1	176.9 ± 4.2*	139.0 ± 3.1*	+ 14.7	- 9.8

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

DISCUSSION

Increased generation of reactive oxygen species (ROS) is implicated in the pathogenesis of many diseases and in the toxicity of a wide range of compounds.²³ Brain tissue is rich in polyunsaturated lipids, has high iron content, and is critically dependent on aerobic metabolism and thus highly vulnerable to ROS mediated oxidative damage.²⁴ The present study revealed increased levels of MDA, the marker of extent of lipid peroxidation, in the brain of 100 ppm fluoride-treated rats. Earlier studies have recorded increased MDA levels in the erythrocytes of fluorotic humans,^{14,25} and in the erythrocytes, liver, kidney, and ovary of experimental animals.^{10,12,13,15,26}

The present study also revealed alterations in the levels of antioxidants of brain as a response to high-fluoride intake. Levels of total and reduced glutathione increased with 30 ppm fluoride but decreased with 100 ppm fluoride, whereas GSH-P_x activity increased with both concentrations. An increased glutathione level at 30 ppm fluoride, where the degree of lipid per-

oxidation is comparable with that of controls, indicates that glutathione might have effectively combated small increments of fluoride in the cells and thus exerted a protective effect against oxidative stress. The observed increase in glutathione level also tempts us to speculate that fluoride-induced oxidative stress might have enhanced the rate of synthesis of GSH. The decrease in glutathione level with an increase in MDA level and GSH-P_x activity at 100 ppm fluoride indicates utilization of GSH for GSH-P_x catalyzed scavenging of H₂O₂ or lipid hydroperoxides generated. Thus, this degree of toxicity of fluoride resulted in reduction in the level of the free radical scavenger glutathione. The decreased ratio of GSH to total glutathione along with an increased activity of GSH-P_x in both the fluoride-treated groups (30F and 100F) suggests increased conversion of GSH to GSSG to combat lipid hydroperoxides or H₂O₂.

Other studies have revealed varied response of the glutathione antioxidant system to fluoride-induced oxidative stress. Our study with the same protocol as the present one revealed decreased levels of total and reduced glutathione with an increase in GSH-P_x activity in the liver of both the 30F and 100F rats.¹⁵ In the brain, erythrocytes and liver of rats exposed to 100 ppm fluoride for 4 months after weaning, total glutathione and GSH levels and the GSH-P_x activity were increased.¹³ Previous studies in this field reported decreased GSH and GSH-P_x in various tissues of experimental animals subjected to chronic fluoride toxicity.^{10,12,26} In children with endemic skeletal fluorosis, we observed decreased GSH levels and an increase in GSH-P_x activity in erythrocytes.¹⁴ These differences in the response of the glutathione antioxidant system to fluoride intoxication in animals might be due to variations in dose, duration and route of fluoride administration, the stage of life at which fluoride was administered, animal species used, and individual tissue response. The observed increase in GSH-P_x activity in brain, erythrocytes, and liver in our studies might be an adaptive response of the tissues to the oxidant challenge due to prolonged exposure to fluoride. Earlier, Chow and Tappel,²⁷ and Edes *et al*²⁸ independently observed an increase in GSH-P_x activity in the tissues of rats exposed to high oxidative stress.

GST activity increased in the brain of the fluoride-treated (30 and 100 ppm) rats of our study. Elevated GST activity was also observed in the liver of young rats exposed to 30 and 100 ppm fluoride during early stages of life¹⁵ and in brain and liver of rats exposed to 100 ppm fluoride at later stages of life.¹³ Earlier, Dierickx²⁹ reported dose dependent elevation of GST activity in the cultured rat hepatoma cells on exposure to sodium fluoride (0.4 – 1.2 mM) for 72 hr. GST synthesis is known to be induced by exposure of the cells to xenobiotics.³⁰ Significant induction of GST activity observed in the present study suggests an adaptive mechanism in response to exposure to high fluoride levels. However, at 100 ppm fluoride, the toxic effect of

fluoride appears to override the adaptive response of the brain tissue as indicated by a smaller increment in GST activity than at 30 ppm fluoride.

Ascorbic acid is an important antioxidant of plasma and in the aqueous phase of the cells.³¹ In the brain, it has a dual effect – at low concentrations promoting lipid peroxidation and at higher concentrations acting as an antioxidant.²³ It is also an anti-stress factor.³² 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.^{32,33} Earlier, we reported increased ascorbic acid levels in the plasma of children with endemic skeletal fluorosis¹⁴ and in brain and plasma of rats exposed to 100 ppm fluoride after weaning.¹³ The present study revealed increased brain ascorbic acid levels in the 30F rats but decreased levels in the 100F rats. Decreased levels of ascorbic acid and glutathione at 100 ppm fluoride exposure during the early developing stages of life suggest utilization and depletion of these intrinsic free radical scavengers in combating fluoride-induced oxidative stress. All the above findings indicate a definite role for ascorbic acid as an antioxidant and anti-stress factor in fluoride intoxication.

In conclusion, we see that chronic fluoride intoxication at the early stages of life markedly enhanced lipid peroxidation in rats. This was associated with increased or decreased levels or activities of antioxidants in the brain. Antioxidant defense systems showed either adaptive response or were depleted depending on the degree of fluoride intoxication. High-fluoride intake in the early stages of life appears to have a more pronounced toxic effect on the antioxidant defense systems of the body compared to high-fluoride intake in the later stages of life.

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