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Lipid Peroxidation and Antioxidant Defense Systems in Liver of Rats in Chronic Fluoride Toxicity

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Fluoride is of great importance to the health and well being of humans and animals. The major source of fluoride in nature is drinking water. Long-term intake of drinking water with fluoride content above the permissible level (0.5-1.0 ppm) leads to chronic fluoride toxicity, the manifestations of which are together referred to as "fluorosis" (Bhussry et al. 1970). Although the most obvious toxic effects of high fluoride intake are manifested in bones and teeth, soft tissues are also affected (Monsour and Kruger 1985). Fluoride accumulation in liver is reported to cause structural and functional disturbances in experimental animals (Monsour and Kruger 1985; Chinoy et al. 1993).

Increased generation of reactive oxygen species (ROS), enhanced lipid peroxidation and disturbed antioxidant defense systems have been proposed to mediate the pathogenesis of chronic fluoride toxicity in the tissues (Zhi-Zhong et al. 1989; Saralakumari and Ramakrishna Rao 1991; Sharma and Chinoy 1998; Patel and Chinoy 1998; Vani and Reddy 2000). But, there is paucity of *in vivo* studies on the effect of chronic high-fluoride intake through drinking water during early stages of life on oxidative stress in the tissues of experimental animals. In the present study, we assess the extent of lipid peroxidation and response of antioxidant defense systems in the liver of rat litters exposed to 30 ppm and 100 ppm fluoride through drinking water during the early stages of life till puberty.

MATERIALS AND METHODS

Wistar albino rats were used in the study. The rats were maintained under proper housing conditions, and were fed standard pelleted diet (Hindustan Lever Ltd., India), and were given water *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee of the institution and met the guidelines of Committee for the Purpose of Supervision of Experiments on animals, Ministry of Social Justice and Empowerment, Government of India.

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Pregnant rats were divided into three groups: control, 30F and 100F. Control group received drinking water containing 0.5 ppm fluoride, 30F and 100F groups received 30 ppm and 100 ppm fluoride (as NaF) respectively in drinking water, from the end of 2nd week of pregnancy up to weaning. The litters from the respective groups received the respective doses from weaning up to the age of ten weeks. These litters were used for the assessment of oxidative stress in liver. The ten week old litters of the control, 30F and 100F groups were sacrificed after light ether anaesthesia, perfused transcardially with 0.9% saline and the livers were removed.

Livers were homogenized 1:40 (w/v) in 0.1M potassium phosphate buffer, pH 7.4, containing 1 mM EDTA. In the homogenates, malondialdehyde (MDA), the marker of extent of lipid peroxidation was estimated as thiobarbituric acid reactive substance (Ohkawa et al. 1979). Level of total glutathione (GSH+GSSG) in the liver homogenates was assayed by the method of Akerboom and Sies (1981), while the level of reduced glutathione (GSH) was estimated by the method of Beutler et al. (1963). The activities of glutathione peroxidase (GSH-P_X) and glutathione S-transferase (GST) were assayed by the methods of Wendel (1981) and Habig et al. (1974) respectively. One unit of enzyme activity was defined as the amount of enzyme that catalyses the formation of one micromole of product per min under the assay conditions. Protein content of the homogenates was determined (Lowry et al. 1951), and the activities of GSH-P_X and GST were expressed in terms of units/gram protein. To assay ascorbic acid, livers were homogenized 1:9 w/v in ice cold 5% trichloroacetic acid, the homogenates were centrifuged at 3500 rpm, and ascorbic acid was estimated in the supernatants (Omaye et al. 1979).

Statistical significance of the results was evaluated by Student's t test.

RESULTS AND DISCUSSION

The fluoride concentrations we used in this study (30 and 100 ppm) are much higher than the fluoride concentrations in natural water sources and the fluoride concentrations found in the water sources of the areas with endemic fluorosis. The present study revealed increased lipid peroxidation in the liver of rats treated with 30 ppm and 100 ppm fluoride as reflected by increased MDA levels (Table 1). Earlier studies have revealed increased lipid peroxidation in the red blood cells of fluorotic humans (Saralakumari and Ramakrishna Rao 1991), and in the red blood cells and tissues of experimental animals subjected to chronic fluoride toxicity (Zhi-Zhong et al. 1989; Patel and Chinoy 1998; Sharma and Chinoy 1998). Increased lipid peroxidation in chronic fluoride toxicity might be due to the generation of ROS by high levels of fluoride.

Table 1. Malondialdehyde and antioxidants in liver homogenates

	Control	30 F	100 F
MDA (nmoles / g protein)	845 ± 43 (15)	1020 ± 84[a] (13)	1459 ± 97[a] (9)
Total glutathione (μmoles / g protein)	9.2 ± 0.7 (10)	8.5 ± 0.9 (9)	7.2 ± 0.4[a] (7)
GSH (μmoles / g protein)	8.4 ± 0.6 (10)	7.3 ± 0.6[a] (9)	5.7 ± 0.3[a] (7)
Ratio of $\frac{\text{GSH}}{\text{total glutathione}}$	0.91 ± 0.005	0.85 ± 0.005[a]	0.79 ± 0.004[a]
GSH-P_X[b] (units/g protein)	87 ± 6 (15)	114 ± 8[a] (13)	202 ± 13[a] (9)
GST[c] (units/g protein)	177 ± 33 (15)	558 ± 37[a] (13)	423 ± 24[a] (9)
Ascorbic acid (μgrams/g wet tissue)	112 ± 6 (15)	96 ± 6[a] (13)	77 ± 8[a] (9)

Values are mean ± SD of the number of experiments indicated in parenthesis.
[a] The values significantly differing from controls at $p<0.001$.
Units of enzyme activities:
[b] μmoles of NADPH oxidized per min.
[c] μmoles of GSH-CDNB (1-chloro, 2,4-dinitrobenzene) conjugate formed per min.

Antioxidant systems of the liver either increased or decreased in the rat litters of the 30F and 100F groups, compared to control litters (Table 1). Decrease in the levels of total and reduced glutathione with an increase in GSH-P_X activity was observed in the liver homogenates of both 30F and 100F rats. This was also accompanied by decreased ratio of GSH to total glutathione in the fluoride-treated rats (30F and 100F). A decrease in glutathione levels and a decreased ratio of GSH to total glutathione with elevation of GSH-P_X suggests an increased utilization of GSH for the GSH-P_X catalyzed detoxification of H_2O_2 or lipid hydroperoxides generated due to increased oxidative stress. The increased GSH-P_X seems to be an adaptive mechanism of the liver cells to the oxidant challenge posed by prolonged exposure to high-fluoride intake. Exposure to high oxidative stress is reported to cause an elevation in GSH-P_x activity in the tissues of rats (Edes et al. 1986). Earlier studies have reported decreased GSH and GSH-P_X in the red blood cells (Zhi-Zhong et al. 1989) and decreased GSH-P_X in the tissues (Patel and Chinoy 1998; Sharma and Chinoy 1998) of experimental animals subjected to chronic fluoride toxicity. The discrepancy with regard to GSH-P_x might be due to differences in the dose, duration and route of fluoride administration, and the animal species used.

GST, the major enzyme participating in the phase-II of metabolism of xenobiotics, was found to be elevated in the liver of both fluoride-treated groups (30F and 100F), the increase was more marked in the 30F group as shown in table 1. Vani and Reddy (2000) reported decreased GST activity in the brain and muscle of mice on high-fluoride intake. But, Dierickx (1998) had observed a dose-dependent elevation of GST in the cultured rat hepatoma cells on exposure to sodium fluoride (0.4-1.2mM F). GST synthesis is known to be induced by exposure of the cells to xenobiotics. We conclude from our study that prolonged exposure to high levels of prooxidant toxic compound sodium fluoride leads to induction of GST, as an adaptive response and protective mechanism of the liver tissue.

Ascorbic acid is considered as an important antioxidant in aqueous phase (Jacob and Buri 1996) and also as an anti-stress factor (Chinoy 1978). The present study revealed decreased ascorbic acid levels in the liver of both the 30F and 100F rats (Table 1). Chinoy et al. (1993) recorded increased ascorbic acid levels in liver and adrenals of rats, but Sharma and Chinoy (1998) reported decreased ascorbic acid levels in liver and kidney of mice, on high-fluoride exposure. Our studies point towards an increased utilization and/or mobilization of ascorbic acid as a response to fluoride-induced stress. The mechanism of interaction between fluoride and ascorbic acid needs to be studied further.

In conclusion, exposure to high fluoride levels in drinking water during early stages of life led to increased oxidative stress in the liver of rats as indicated by elevated MDA level and disturbed antioxidant defenses. Adaptive mechanisms in the form of increased activities of GSH-P_x and GST operated to combat fluoride induced oxidative stress. But, increased oxidative stress led to depletion of the intrinsic free radical scavengers glutathione and ascorbic acid.

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