Lipid peroxidation and antioxidant systems in the blood of young rats subjected to chronic fluoride toxicity

Y M Shivrajashankara, A R Shivashankara
Department of Biochemistry, M.R. Medical College, Gulbarga 585105, India

P Gopalakrishna Bhat
Department of Biochemistry, Kasturba Medical College, Manipal 576119, India

S Hanumant Rao
Department of Biochemistry, K.B.N. Institute of Medical Sciences, Gulbarga 585104, India

Received 5 March 2003; revised 1 May 2003

Wistar albino rats were exposed to 30 or 100 ppm fluoride in drinking water during their fetal, weaning and post-weaning stages of life up to puberty. Extent of lipid peroxidation and response of the antioxidant systems in red blood cells and plasma to prolonged fluoride exposure were assessed in these rats in comparison to the control rats fed with permissible level (0.5 ppm) of fluoride. Rats treated with 100 ppm fluoride showed enhanced lipid peroxidation as evidenced by elevated malondialdehyde (MDA) levels in red blood cells but, 30 ppm fluoride did not cause any appreciable change in RBC MDA level. 30 ppm fluoride-intake resulted in increased levels of total and reduced glutathione in red blood cells and ascorbic acid in plasma while 100 ppm fluoride resulted in decreases in these levels. The activity of RBC glutathione peroxidase was elevated in both the fluoride-treated groups, more pronounced increase was seen with 100 ppm. Reduced to total glutathione ratio in RBC and uric acid levels in plasma decreased in both the groups. RBC superoxide dismutase activity decreased significantly on high-fluoride treatment. These results suggest that long-term high-fluoride intake at the early developing stages of life enhances oxidative stress in the blood, thereby disturbing the antioxidant defense of rats. Increased oxidative stress could be one of the mediating factors in the pathogenesis of toxic manifestations of fluoride.

Keywords: Antioxidant system, Fluoride toxicity, Fluoride in water, Lipid peroxidation

Fluorides are among the abundant compounds of earth's crust. Fluoride not only has notable physical and chemical properties but also, physiological properties of great interest. The effects of fluoride on humans and other animals stem largely from dissolved fluorides present in ground and surface water. Fluoride has beneficial effects in the form of prevention of dental caries and stabilization of skeleton but, long term intake of high levels of fluoride leads to toxic manifestations. Although the obvious toxic effects of fluoride are manifested in bones and teeth, the soft tissues are not spared. There are wide reports of structural and biochemical changes in vital tissues of experimental animals subjected to chronic fluoride intoxication.

Increased generation of reactive oxygen species (ROS) and lipid peroxidation have been found to be involved in the pathogenesis of many diseases and in the toxic actions of many compounds. Studies have implicated role of ROS in mediating the toxic effects of fluoride in humans and experimental animals. However, there is paucity of studies to assess the oxidant-antioxidant status of animals on exposure to high levels of fluoride during the early developing stages of life. In the present study, we have made an attempt to assess the extent of lipid peroxidation and response of the antioxidant defense systems in the red blood cells and plasma of rats exposed to 30 ppm and 100 ppm fluoride in drinking water during the early developing stages of life up to puberty.

Materials and Methods

Chemicals — Sodium fluoride was obtained from s.d. Fine Chem. Ltd., Mumbai, India. Glutathione reductase (from baker’s yeast, type III), nicotinamide adenine dinucleotide phosphate reduced (NADPH), oxidized glutathione (GSSG), reduced glutathione...
(GSH) and 2-thiobarbituric acid were obtained from Sigma Chemical Co., USA. Nitroblue tetrazolium-chloride, riboflavin, 2,4-dinitrophenylhydrazine and thiourea were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. L-ascorbic acid, 1-chloro-2,4-dinitrobenzene, 5,5'-dithiobis-[2-nitrobenzoic acid], metaphosphoric acid and sodium dodecyl sulphate were purchased from s.d. Fine Chem. Ltd., Mumbai, India. Diagnostic reagent kit for plasma uric acid estimation was purchased from CDR Diagnostics, Hyderabad, India.

Experimental animals — Wistar albino rats were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India. The rats were maintained in the Central animal house of M.R. Medical College, Gulbarga (Registration number of the animal house-142/1999/ CPCSEA) under proper temperature, light, moisture and hygienic conditions. They were fed on commercial standard pelleted diet and drinking water ad libitum.

Experimental design — Pregnant rats were divided into three groups: control, 30F and 100F. Control group received drinking water containing 0.5 ppm fluoride, 30 and 100F groups received 30 and 100 ppm fluoride respectively (from sodium fluoride) in drinking water, during the last (39th) week of pregnancy and throughout the lactation period. The litters were separated from their mothers on weaning and were then exposed to the respective levels of fluoride (0.5, 30 and 100 ppm) in drinking water up to the age of ten weeks. The ten week-old rats of control (n=15), 30F (n=13) and 100F (n=9) groups were sacrificed after light ether anaesthesia and the blood was collected by cardiac puncture in acid-citrate-dextrose solution (1.0 ml solution per 4.0 ml of blood). Collected blood samples were centrifuged at 3500 rpm for 15 min to separate plasma and red blood cells. The packed red blood cells were washed with buffered saline (0.9% saline in 0.01 M potassium phosphate buffer, pH 7.4) thrice and then suspended in an equal volume of the buffered saline.

Assays in RBC lysates — Malondialdehyde (MDA) in RBC was estimated as thiobarbituric acid reactive substance (TBARS)13. Levels of total glutathione (GSH + GSSG) and reduced glutathione (GSH) were estimated by the spectrophotometric methods of Akberoom and Sies15 and Beutler et al.16 respectively. Activity of glutathione peroxidase (GSH-Px) was assayed by the kinetic spectrophotometric method of Pagilia and Valentine17. Activity of superoxide dismutase (SOD) was assayed by the method of Beauchamp and Fridovich18. In the RBC lysates, hemoglobin (Hb) level was estimated19 and the levels or activities of above parameters were expressed per gram hemoglobin.

Assays in plasma — Levels of ascorbic acid20 and uric acid21 were estimated by spectrophotometric methods.

Statistical analysis — All values are expressed as mean with standard deviation. Significance of the results was evaluated by Student’s t-test.

Results and Discussion

Results of the present study are presented in Tables 1 and 2.

MDA levels of control and 30F rats did not differ significantly. 100F rats showed significantly higher RBC MDA levels compared to control group (Table 1).

Table 1 — Effect of fluoride on levels of MDA and antioxidants in red blood cells

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n=15)</th>
<th>30F (n=13)</th>
<th>100F (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g Hb)</td>
<td>194.2 ± 4.74</td>
<td>187.7 ± 2.8</td>
<td>258.0 ± 7.36*</td>
</tr>
<tr>
<td>Total glutathione (nmol/g Hb)</td>
<td>9.78 ± 0.31</td>
<td>11.87 ± 1.23*</td>
<td>6.82 ± 0.70*</td>
</tr>
<tr>
<td>GSH (nmol/g Hb)</td>
<td>9.58 ± 0.19</td>
<td>11.04 ± 0.99*</td>
<td>6.14 ± 0.39*</td>
</tr>
<tr>
<td>GSSG (nmol/g Hb)</td>
<td>0.2 ± 0.001</td>
<td>0.83 ± 0.005*</td>
<td>0.68 ± 0.004*</td>
</tr>
<tr>
<td>Ratio of GSH/total glutathione</td>
<td>0.98 ± 0.005</td>
<td>0.93 ± 0.006*</td>
<td>0.90 ± 0.005*</td>
</tr>
<tr>
<td>GSH-Px (U/g Hb)</td>
<td>50.74 ± 2.2</td>
<td>55.46 ± 2.57</td>
<td>68.34 ± 2.41*</td>
</tr>
<tr>
<td>SOD (U/g Hb)</td>
<td>1292.1 ± 24.01</td>
<td>1071.4 ± 29.07*</td>
<td>888.1 ± 18.78*</td>
</tr>
</tbody>
</table>

U (GSH-Px) → μmol of NADPH oxidised per min
U (SOD) → Enzyme concentration required to inhibit the O2− mediated reduction of nitroblue tetrazolium by 50%.

*Value significantly differing from control at P<0.001.
MDA is a sensitive and convenient indicator of extent of lipid peroxidation. Red blood cells are more commonly employed in the evaluation of oxidative stress as they are prone to oxidative reactions because of relatively high oxygen tension and the presence of polyunsaturated lipid-rich plasma membrane. Elevated RBC MDA in 100F rats could be due to fluoride-induced generation of ROS. Fluoride has been demonstrated to cause increased lipid peroxidation in erythrocytes of humans and in tissues of experimental animals.

Antioxidant defense systems of RBC and plasma responded to fluoride-induced oxidative stress by increase or decrease in their levels (Tables 1 and 2). 30 ppm fluoride caused increased levels of total reduced glutathione, while 100 ppm fluoride caused decreased levels of these parameters in RBC. Both the fluoride-treated groups showed higher activity of RBC GSH-PX compared to control group, pronounced change being found with 100 ppm fluoride. An increase in total glutathione and GSH levels at 30 ppm fluoride where the degree of lipid peroxidation is comparable with that of controls, indicates that glutathione might have effectively combated oxidative stress induced by small increments of fluoride in the cells and thus exerted a protective effect. The observed increase in glutathione level also tempts us to speculate that small amount of fluoride might have enhanced the rate of synthesis of GSH. The decrease in glutathione level with an increase in MDA and GSH-PX at 100 ppm fluoride indicates utilization of GSH for GSH-PX catalyzed scavenging of H₂O₂ or lipid hydroperoxides generated. The decreased ratio of GSH to total glutathione along with an increased activity of GSH-PX and increased level of GSSG in both the fluoride-treated groups (30F and 100F) suggests increased conversion of GSH to GSSG to combat lipid hydroperoxides or H₂O₂.

Our previous study revealed increased total glutathione, GSH and GSH-PX in brain, liver and RBC of rats exposed to 100 ppm fluoride for four months after weaning. Thus, 100 ppm fluoride had different effects on glutathione antioxidant system when ingested at early and later stages of life. High-fluoride intake has more pronounced toxic effect on the growing young ones than on the adults. In children with endemic skeletal fluorosis, we observed decreased GSH levels and increased GSH-PX activity in red blood cells. Other workers have reported decreased GSH and GSH-PX in various tissues of experimental animals subjected to chronic fluoride toxicity. These differences in the response of the glutathione antioxidant system to fluoride intoxication in animals might be attributed to variations in dose, duration and mode of fluoride administration, the stage of life at which fluoride was administered, animal species used, individual tissue response and the degree of toxicity.

RBC GSH-PX activity increased in fluoride-treated rats. At 30 ppm fluoride where the extent of lipid peroxidation was comparable with that of controls, increase in GSH-PX activity was marginal. At 100 ppm fluoride level, enhanced lipid peroxidation was associated with significant elevation of GSH-PX. Observed increase in GSH-PX might be an adaptive response of the cells to the oxidant challenge due to prolonged exposure to fluoride. Studies have revealed elevation of GSH-PX activity in the tissues of animals exposed to high, prolonged oxidative stress.

Lower RBC SOD activity in 30F and 100F rats suggests decreased ability of the cells to handle O₂⁻ radicals. Similar findings on SOD have been reported in the tissues of mice exposed to high-fluoride intake.

Ascorbic acid is an important antioxidant of plasma and in the aqueous phase of the cells. 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. The present study revealed increased plasma ascorbic acid levels in 30F rats but decreased levels in 100F rats (Table 2). Our findings indicate a definite role for ascorbic acid as an antioxidant and an anti-stress factor in fluoride intoxication. Uric acid acts as an
antioxidant by virtue of its ability to tightly bind iron and copper. Present study revealed lower plasma uric acid levels of 30F and 100F rats when compared to controls.

In conclusion, long-term high-fluoride intake at the early developing stages of life resulted in increased oxidative stress in rats as evidenced by enhanced lipid peroxidation in RBC. The antioxidant systems of blood showed varied response to fluoride-induced oxidative stress. Adaptive mechanisms of the cells appear to operate on prolonged exposure to fluoride. ROS generation and impaired functioning of antioxidant systems could be the mediating factors in the pathogenesis of toxic manifestations of fluoride.

Acknowledgement

The authors are grateful to Dr.B.Mallikarjun, Dean, M.R. Medical College, Gulbarga for his kind support and encouragement.

References

23. Chow C K & Tappel A L, An enzymatic protective mechanism against lipid peroxidation damage to lungs of oxygen exposed rats, LIPIDS, 7 (1972) 518.