EFFECTS OF FLUORIDE ON LIPID PEROXIDATION IN RABBITS

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SUMMARY: Twenty-one 6-month-old male New Zealand rabbits, weighing 3.5±0.3 kg, were divided equally into a control group and two trial groups. For 70 days, the control group (Group I) received drinking water containing 0.07 mg F^-/L, and the second and third groups (Groups II and III) were given water containing 10 and 40 mg F^-/L, respectively. On days 0, 21, and 70, blood samples were taken from each rabbit in each group into heparinised tubes. Erythrocyte antioxidant enzyme activities of superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) were determined in each blood sample, along with the level of malondialdehyde (MDA). In Group III, by day 70, a significant decrease was observed in SOD activity (74.14% of the control group) and a significant increase in CAT activity (23.92% greater than in the control group). On days 21 and 70, significant increases compared to the control group were found, respectively, in GPx activity (14.85% and 39.29%) and the level of MDA (23.56% and 89.12%). These results indicate that fluoride causes oxidative damage *in vivo*. When considered in conjunction with other parameters such as urinary fluoride, these changes in erythrocyte antioxidant enzymes may prove useful for diagnosing fluoride poisoning and determining its severity, especially in cases of elevated fluoride exposure levels as studied here.

Keywords: Catalase; Fluoride intoxication; Glutathione peroxidase; Lipid peroxidation; Malondialdehyde; Rabbits; Superoxide dismutase.

INTRODUCTION

Fluoride (F^-) can block or induce various activities in blood cells.[1] When its mechanism of action on a molecular basis is considered, fluoride affects cellular enzymes, especially antioxidants.[2,3] Enzymes in red blood cells in particular are very sensitive to fluoride, causing various changes in the membrane function of erythrocytes in relation to structural and biochemical parameters.[2-5] The aim of the present study was to determine whether fluoride ingested by rabbits at elevated levels in their drinking water for 70 days caused any significant changes in erythrocyte antioxidant enzymes and lipid peroxidation.

MATERIALS AND METHODS

In this study, 21 New Zealand male rabbits, six months old, weighing 3.5±0.3 kg, were used. All the rabbits received drinking water (0.07 mg F^-/L) and commercial rabbit feed *ad libitum* for 15 days prior to the experiment. After 15 days, the animals were divided evenly into a control and two trial groups. The control group (Group I) was given drinking water containing 0.07 mg F^-/L, and the trial groups received water containing 10 mg F^-/L (Group II) and 40 mg F^-/L (Group

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III). Prior to the experiments, on day 0, blood was taken from each rabbit to determine and compare the MDA (malondialdehyde) levels and the activities of SOD (superoxide dismutase), CAT (catalase), and GPx (glutathione peroxidase) among all three groups. Subsequently, on days 21 and 70 of the experiment, blood was again taken from each animal. The blood samples were placed in heparinised tubes and centrifuged (3000 rpm, 10 min; 4 ºC) to separate the erythrocytes from the plasma. The erythrocytes were washed three times with 140 nM NaCl and 40 nM phosphate buffer (pH 7.4) and hemolyzed.[6] The washed erythrocyte suspension was hemolyzed with mercaptoethanol containing 2.7 nM EDTA at pH 7.0 and then used to measure MDA and the hemoglobin levels and the antioxidant enzymes activities. The activities of SOD, CAT, and GPx, and the levels of MDA and haemoglobin were determined spectrophotometrically, according to the methods, reported, respectively, by Woolliams *et al*,[7] Aebi,[8] Paglia and Valentina,[9] Draper and Hadley,[10] and Fairbanks and Klee.[11] The "SPSS 9.05 for Windows" statistical package software was used for statistical analysis. The data are expressed as arithmetical means and standard deviation. The statistical significance of results was analyzed by the Mann-Whitney U test.

RESULTS

As seen in the Table, there were essentially no differences in the four biochemical parameters among the three groups of rabbits at the beginning of the study. By day 21, in Group II, there were indications of nonsignificant decreases in SOD activity and small increases in GPx and CAT activity and possibly in the level of MDA. However, by day 21 in Group III, there was a 14.85% ($p<0.05$) increase in GPx activity and a 23.56% ($p<0.05$) higher level of MDA compared to the controls. By day 70, these trends were even more evident. The SOD activity in Group III was then only 74.14% ($p<0.05$) of the control, while the increases in the activities of PGx and CAT were 39.29% ($p<0.05$) and 23.92% ($p<0.05$), respectively, in this group. Especially striking was the 89.12% ($p<0.01$) increase in the level of MDA in Group III by day 70.

DISCUSSION

Fluoride compounds have long been of interest for their biochemical properties. However, the relationship between fluoride toxicosis/fluorosis and oxidative stress has not been clarified. Enormous amounts of information about the potential toxicological and pathological effects of compounds can be derived from studies of harmful reactive oxygen species (ROS).[4,12-15]Various experiments on the animals have examined how strongly fluoride affects MDA and antioxidant enzymes, using different doses and lengths of exposure.[12-15] Moreover, similar parameters have also been evaluated in humans with fluorosis.[3-5,16] One of the most popular methods to determine whether fluoride causes lipid peroxidation and to what extent it does so has been to determine MDA levels and antioxidant enzyme activities in erythrocytes.[17] The erythrocyte cell membrane is known to be highly sensitive to free radical oxidation due to its unsaturated fatty acid content.[18]

Table. Activities of erythrocyte SOD, GPx, and CAT and MDA levels in control (n = 7) and trial groups (n = 14)

Parameter	Group	Day		
		0	21	70
SOD (U/mg Hb)	Group I (control)	2.46±0.40	2.64±0.33	2.63±0.62
	Group II (10 mg F^-/L)	2.48±0.38	2.56±0.21	2.30±0.47
	Group III (40 mg F^-/L)	2.47±0.32	2.31±0.55	1.95±0.44*
GPx (U/g Hb)	Group I (control)	14.75±3.38	15.01±2.84	14.66±2.21
	Group II (10 mg F^-/L)	14.82±2.54	15.74±4.46	17.70±1.78
	Group III (40 mg F^-/L)	14.75±2.29	17.24±2.77*	20.42±3.49*
CAT (k/mg Hb)	Group I (control)	2.43±0.37	2.35±0.52	2.55±0.44
	Group II (10 mg F^-/L)	2.47±0.41	2.63±0.36	2.86±0.31
	Group III (40 mg F^-/L)	2.45±0.32	2.74±0.51	3.16±0.47*
MDA (nmol/mg Hb)	Group I (control)	1.56±0.27	1.57±0.35	1.93±0.11
	Group II (10 mg F^-/L)	1.59±0.23	1.60±0.51	2.21±0.41
	Group III (40 mg F^-/L)	1.59±0.12	1.94±0.48*	3.65±0.82†

Values mean ± SD. Significance of results: *$p<0.05$; †$p<0.01$.

Cholesterol in erythrocyte membrane plays a key role in the membrane stability. It interacts with phospholipids and regulates the fluidity and function of the membrane. In fluorosis the cholesterol level of erythrocyte membrane changes, and the cell structure is impaired.[3] Any increase in ROS caused by fluoride may easily lead to destructive peroxidation in erythrocyte membrane, the structure of which is highly fragile.[3,4] Peroxidation occurs by the action of free radicals such as singlet oxygen ($^1O_2\cdot$), hydroxyl radicals (·OH), and the superoxide radical ($\cdot O_2^-$).[17] Their conversion into impotent or less harmful products is of critical importance for living cells. Fluoride, however, can generate high levels of free radicals. SOD, GPx, and CAT, which are involved in cellular defense, are specifically required for protection against the destructive action of ROS.[18-20]

SOD is an enzyme basically responsible for the conversion of $\cdot O_2^-$ into O_2. SOD is also a metalloprotein that facilitates elimination of the primary toxicity of $\cdot O_2^-$ and the secondary toxicity of $\cdot OH$ and H_2O_2 by decreasing the concentration of $\cdot O_2^-$.[19] Hydrogen peroxide, in turn, is decomposed by GPx and CAT.[18,20] If the cell has the enzyme capacity and sufficient amounts of other essential compounds (carotenoids, alpha-tocopherol and glutathione), it has the ability to remove ROS, and thus peroxidation may not take place or occur at a low level.[5,17-20] In our work, a decrease in SOD activity was observed in rabbit erythrocytes on days 21 and 70, compared to that in the control. The decrease was found to be statistically significant for exposure to 40 mg F^-/L fluoride by day 70, indicating that the SOD activity of erythrocytes was inhibited by high fluoride intake. An increase in GPx activity was observed in Group II and III on days 21 and 70, but this increase was statistically significant only in Group III for both periods. This increase in GPx activity might be due to fluoride-induced increase in H_2O_2 levels; however, the increase of CAT activity was significant only in Group III by day 70. This finding suggests that fluoride increased the level of free radicals as well as CAT activity. The absence of statistically significant differences in SOD, CAT, and GPx activities and MDA levels between the controls and those animals exposed to 10 ppm fluoride for both periods (days 21 and 70) indicates that at this lower dose fluoride induced very little lipid peroxidation.

Shivarajashankara *et al*[13,14] report that while fluoride caused significant increases in MDA levels and GPx activities in the erythrocytes, brain, and liver tissues of rats, the activity of erythrocyte SOD was significantly decreased. They also found that the level of erythrocyte MDA and the activity of GPx showed increases, and SOD activity was decreased in children with endemic skeletal fluorosis.[16] Kaushik *et al*[15] found that fluoride-intoxicated rats exhibited significant increases in erythrocyte lipid peroxidation levels as well. On the other hand, Vani and Reddy[12] observed significant decreases in CAT as well as SOD activities in the brain and gastrocnemius muscle of female mice receiving fluoride. Finally, Saralakumari and Ramakrishna[3] report significant increase in the levels of MDA in humans with chronic fluoride intoxication. Overall, our results on changes in MDA levels and enzyme activities, with the partial exception of CAT, are consistent with those reported by others.

In conclusion, it is obvious from the statistically significant changes in MDA levels and the significant increases in the activities of GPx and CAT, along with the decrease in SOD activities by day 70, that the ingestion of fluoride via drinking water at 40 mg F^-/L caused oxidative damage for both study periods (by days 21 and 70). It was also clear from these results that antioxidant enzyme activities and MDA levels, along with other parameters, may be determinative criteria for diagnosing fluoride poisoning caused by the ingestion of fluoride at 40 F^- mg/L in rabbits, but these parameters would not be suitable if fluoride is ingested at 10 mg F^-/L.

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