EFFECT OF FLUORIDE INTOXICATION ON LIPID PEROXIDATION AND REDUCED GLUTATHIONE IN TUJ SHEEP

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SUMMARY: The effect of fluorosis on blood malondialdehyde (MDA) and reduced glutathione (GSH) levels was studied in 20 initially healthy yearling Tuj ewe-lambs with a mean body weight of 31±2 kg. The sheep were divided into two equal groups provided with hay and water *ad libitum* throughout the study. The water for the control group contained 0.49 mg F/L, whereas the water for the experimental group contained 13.8 mg F/L, equivalent for the latter group to an intake of ca. 1.8 mg F/kg bw/day. Although relatively unchanged from 0.9 ppm up to the 4th week, the urinary fluoride concentration in the experimental group increased by the 12th week to 1.78 ppm. By the 24th week it reached 8 ppm and 17.2 ppm by the 48th week. Throughout the study there was no significant change in the 0.9 ppm urine fluoride concentration of the control group. By the 12th week, lipid peroxidation and GSH levels in the treatment group were significantly higher (MDA $p<0.01$, GSH $p<0.05$) and remained elevated at the end of 48 weeks. These results indicate that fluorine may cause an increase in lipid peroxidation in cases of fluorosis.

Keywords: Fluorosis; Lipid peroxidation; Malondialdehyde (MDA); Reduced glutathione (GSH); Tuj sheep.

INTRODUCTION

Fluorosis is a well-defined clinical entity characterized by toxic effects of elevated fluoride intake on teeth, bones and soft tissues.[1-3] In the blood, brain, and liver of animals, various changes occur after chronic administration of fluoride. These include abnormal behaviour patterns,[1,4] altered neuronal and cerebrovascular integrity, and metabolic lesions.[5]

Generation of free radicals, lipid peroxidation, and altered antioxidant defence systems are thought to play an important role in the toxic effects of fluoride.[6-8] Increased oxygen radical generation and lipid peroxidation have been implicated in the pathogenesis of many diseases and the toxic action of a wide range of compounds.[9,10] This process has even been proposed to be an important mediating factor in the causation of the detrimental effects of chronic fluoride toxicity.[2,4]

In Turkey, water, soil, and plant samples obtained from the villages of Kızılcaören in Beylikova-Eskişehir and Bayımdır in Kaman-Kırşehir have particularly high levels of fluoride.[11] In Van in Muradiye, water levels of 5.7–15.2 ppm F are on record, and in Ağrı levels of 12.5 ppm F have been reported.[12] In Isparta more than 50 percent of the districts have high fluoride levels (4.03 ppm) in their drinking water.[13]

In this study, we assessed the effect of chronic fluoride intoxication on lipid peroxidation and reduced glutathione in the blood of Tuj sheep. In a parallel study on the same sheep, several of our colleagues also determined the serum

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level of total protein, albumin, uric acid, and nitric oxide and the activities of lactate dehydrogenase and leucine aminopeptidase.[14]

MATERIALS AND METHODS

Animals and treatments: Twenty healthy yearling Tuj ewe-lambs with a mean body weight of 31±2 kg were used. They were divided into two equal groups (n=10) and in located separate sections of the farm. Each animal was identified with an ear tag, and prior to the study the ewes were kept in their respective sections for 30 days to adapt the farm conditions. The local water of the control group contained 0.49±0.03 ppm F. For the experimental group the F concentration of 42 L batches of this water was raised to 13.8 ppm by addition of 1.24 g NaF, giving an estimated daily intake of ca. 4.0 mg NaF (= 1.8 mg F)/kg bw from drinking an average of 4.0 L of water/day. Daily urine fluoride concentrations were determined by using an EDT Micro2 pH/ION meter to determine the timing of chronic fluorosis.[15,16] The study was terminated after 48 weeks, when urine fluoride levels reached 17.2 ppm. At this point blood samples were taken from the jugular vein, and serum was separated and stored at –24 ºC.

Serum analysis: Lipid peroxidation activity in the blood plasma was assessed by estimation of malondialdehyde (MDA) according to the method of Ohkawa et al.[17] Reduced glutathione (GSH) was measured in the red cell lysates by the method of Beutler et al.[18]

Statistical Analysis: The observed MDA and GSH values as means ± SE were statistically analysed (Student's t test; IFFC, 1987) and evaluated using the SPSS 6.0 (1993) software program. P values <0.01 and <0.05 were considered significant.[19]

RESULTS

Horizontal lines on the tooth surfaces and a decrease in body weight gain ($p<0.001$) were observed as clinical manifestation of fluorosis. Up to the 4th week there was no significant increase in the urine fluoride concentrations. By the 12th week the urinary fluoride concentration in the experimental group had doubled from 0.9 ppm at the start to 1.78 ppm, and by the 24th week it reached 8 ppm and 17.2 ppm by the 48th week. Throughout the study there was no significant change in the 0.9 ppm urine fluoride concentration of the control group. Although no significant changes were observed at the end of the first month, by the 12th week MDA levels in the plasma and GSH levels in the erythrocytes showed significant increases (Table).

DISCUSSION

A close association between chronic fluoride intoxication and increased oxidative stress has been reported in experimental animals.[3,8] Erythrocytes are more commonly employed in the evaluation of oxidative stress, since they are prone to oxidative reactions because of relatively high oxygen tension and the presence of polyunsaturated lipid-rich plasma membranes.[20,21] Fluoride has been demon-

strated *in vivo* and *in vitro* to cause increased lipid peroxidation in the blood of humans.[22]

Table. Malondialdehyde (MDA) in plasma and reduced glutathione (GSH) in red blood cells of the sheep (values are means±SD)

	Control group (n=10)		Experimental group (n=10)	
	MDA (nmol/mL)	GSH (nmol/mL)	MDA (nmol/mL)	GSH (nmol/mL)
4th week	3.23±0.67	8.36±1.12	3.32±0.18	8.57±0.76
12th week	3.40±0.12	7.76±1.17	5.28±0.54*	9.68±1.28†
48th week	3.65±0.19	8.23±1.22	4.94±0.75*	9.94±1.03*

*p<0.01; †p<0.05.

The increased MDA levels in fluorotic sheep observed in this study are in accord with earlier findings. Oxidative stress produced by free radicals and H_2O_2 is greater if fluoride impairs the production of free radical scavengers such as GSH, CAT (catylase), GSH-Px (GSH peroxidase), SOD (superoxide dismutase), and GST (glutathione S-transferase).[23] Studies have shown a decrease in the activity of GST and increased levels of GSH in animals.[22] The decrease in the levels of GSH in the red blood cells observed in our study may be due to increased utilization of GSH-GSH-Px in detoxification of H_2O_2 generated by fluoride-induced oxidative stress. Various authors have investigated relationships between fluoride and free radical reactions. [2,3,24,25] Yur et al[25] reported changes in oxidative stress parameters of erythrocytes in twenty 3–4 year-old fluorotic sheep. They found that plasma MDA level in fluorosed sheep increased from a baseline of 2.51±0.16 nmol/mL.

As already noted, in our study we found that plasma MDA and erythrocyte GSH levels were increased. This could be associated with peroxidation of membrane phospholipids and the accumulation of MDA. On the other hand, high fluoride concentrations are likely to inhibit GSH. A decrease in the activity of free radical-scavenging enzymes also occurs in people living in areas of endemic fluorosis,[26] and a similar inhibitory effect of fluoride on seed germination has been observed by Wild and Yu.[27]

In conclusion, we found that chronic fluoride toxicity in sheep increased lipid peroxidation associated with free radical mediated oxidative stress as demonstrated by increased serum levels of MDA and red cell levels of GSH.

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