

NITRIC OXIDE OXIDATION PRODUCTS AND THE ACTIVITIES OF CATALASE AND CARBONIC ANHYDRASE IN SHEEP WITH FLUOROSIS

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SUMMARY: This study was conducted to determine the levels of nitrite and nitrate and the activities of catalase and carbonic anhydrase in the blood of sheep with and without endemic fluorosis. Blood samples from 20 fluorotic and 10 healthy Morkaraman sheep 3–4 years old in the Van area of Turkey were collected and analyzed. In the fluorotic sheep the average catalase activity level in the blood was significantly lower at 1.72 vs. 11.23 mg/Hb in the healthy sheep. On the other hand, carbonic anhydrase enzyme activities were significantly higher in the fluorotic sheep at 2.13 vs. 0.13 mg/Hb in the healthy sheep. Nitrite and nitrate levels from oxidation of nitric oxide were 2.31 and 3.32 vs. 0.51 and 0.84 ppm in the fluorotic and healthy sheep, respectively. These results have important health implications for the widespread occurrence of fluorosis among people and animals living in these areas of Turkey.

Keywords: Carbonic anhydrase; Catalase; Fluorosis in sheep; Fluorosis in Turkey; Morkaraman sheep; Nitric oxide oxidation products.

INTRODUCTION

The element fluorine plays an important role in biological functions that alter teeth and bones. Excess intake of fluoride (F), apart from causing dental and skeletal abnormalities, can inhibit the activities of many enzymes.¹ Depending on how it is bound, F can cross cell membranes and enter soft tissues causing impairment of their function. F is also very noxious in the environment, affecting the health of humans and animals as well as plant life. Its toxicity occurs mainly in the form of industrial air pollution or from F in drinking water² and/or food, especially from domestic use of high-F coal.³ Chronic F intoxication or fluorosis is a worldwide health problem and is endemic in areas where the F content of drinking waters is high. Its primary manifestations in humans and mammals are mottling of teeth and osteosclerosis of the skeleton.⁴

On the other hand, the kinetics and pathogenesis of F toxicity in the whole body require further understanding and study. Generation of free radicals causing lipid peroxidation and promotion of altered antioxidant defense system are recognized as toxic effects of F.^{5–8} They play a role in fluorosis as a metabolic hard tissue disease caused by ingestion of excessive amounts of F from water or food in endemic areas.⁹ In the eastern part of Turkey, fluorosis is also a serious health problem of humans and animals. Near the volcanic areas of Mt Ararat and Mt Tendürek, humans consume considerable amounts of F from drinking water, thus accounting for the extensive amount of human tooth and bone deformities in these areas. In region of Doğubeyazıt in Ağrı, levels of 12.5 ppm F in the water have been reported, and, in the region of Muradiye in Van, levels of 5.7–15.2 ppm F are

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on record. Moreover, F concentrations of 8.1 ppm in sheep urine, 4.3 ppm in human urine, 3374–5149 ppm in sheep bone ash, and concentrations of 3787–5299 ppm have been found in teeth of sheep in Eastern Anatolia.¹⁰

The aim of this study was to investigate the levels of nitrite and nitrate, and the activities of the catalase and carbonic anhydrase in the blood of sheep with endemic fluorosis.

MATERIALS AND METHODS

In this investigation, blood samples from twenty 3–4 year-old fluorotic and ten healthy Morkaraman sheep in the region of Çaldıran, in Van, Turkey, were examined. The samples were collected by leg venipuncture into EDTA solution (1 mL per 4 mL of blood). Plasma and buffy coat (consisting of leukocytes and platelets) were removed by centrifugation at 3000 rpm for 15 min. Red blood cells were washed three times with 0.9% saline in 0.01 M pH 7.4 phosphate buffer. The packed cells were then suspended in an equal volume of the buffered saline. Blood samples without anti-coagulant were also collected. Nitrite and nitrate in the serum was analyzed spectrophotometrically according to the method of Stahr.¹¹ In the red cell lysates, carbonic anhydrase activity was determined by the Maren method,¹² and catalase (CAT) by the method of Aebi.¹³ The hemoglobin (Hb) content in the cell lysates was estimated by the Daring method.¹⁴ Statistical significance of the results was assessed by the Student t test.

RESULTS

The results of the analyses of nitrite, nitrate, and the activities of carbonic anhydrase and catalase levels are shown in the Table. The serum nitrite and nitrate levels of the fluorotic sheep were significantly higher than those of the control sheep ($p < 0.05$). In the cell lysates, the carbonic anhydrase activity of the fluorotic sheep was higher compared to that of the control sheep ($p < 0.001$), whereas the CAT activity of the fluorotic sheep was lower than in the control sheep ($p < 0.001$).

Table. Serum nitrite and nitrate levels and blood lysate activities of carbonic anhydrase and catalase of fluorotic and control sheep (values: mean \pm SE)

Parameters	Control group (n=10)	Exposed group (n=20)	p
Nitrite (ppm)	0.51 \pm 0.042	0.84 \pm 0.066	0.003
Nitrate (ppm)	2.31 \pm 0.11	3.32 \pm 0.29	0.026
Carbonic anhydrase (mg/Hb)	0.39 \pm 0.78	2.13 \pm 0.22	0.000
Catalase (mg/Hb)	11.23 \pm 1.33	1.72 \pm 0.69	0.000

DISCUSSION

At biochemical and molecular levels, extensive studies have been made of the effects of F on the composition of bone, urine, plasma, and the levels of hormones.^{9,15,16} F has been shown to inhibit many enzymes involved in the

pentose phosphate pathway, antioxidant defense systems, and in myosin-ATPase activity.^{17,18} Since F impairs the production free radical scavengers such as GSH (glutathione), CAT, GSH-Px (GSH peroxidase), SOD (superoxide dismutase) and GST (glutathione-S-transferase), oxidative stress produced by free radicals and hydrogen peroxide is greater.⁵ Decreases in the activities of CAT, GPx, SOD, and GST have been found in tissues animal subjected to F intoxication.¹ Metabolic imbalances, oxidative stress, and lipid peroxidation are evidently involved in the pathogenesis of chronic fluorosis, but the results of studies are often conflicting and contradictory to one another.

For example, decreases in the activity of SOD and GPx have been found in people living in endemic fluorosis areas.² On the other hand, Reddy et al.¹⁹ found no significant changes in any of the antioxidant parameters tested, including ascorbic acid, GSH, CAT, SOD, GPx, and GST in fluorotic humans and rabbits. In another study,¹ F injected into rats for 14 days at 20 mg/kg bw/day/ip caused decreases in GST, SOD, and CAT activity in the brain. Similar results in rats from F in their drinking water have also been reported.²⁰

It is also worth noting that the increases in serum NO might be due to the generation of peroxynitrite by F.²¹ With an increase in nitric oxide synthase (NOS), release of NO occurs, and NO combines with superoxide radicals to form highly toxic peroxynitrites responsible for neuronal injury.²² Bhatnagar et al.²³ reported that excessive F intake caused morphological changes in NADPH-d/NOS positive neurons in the brain, thus increasing NO synthesis implicated in F-induced neuron cell death. It has also been shown that NaF significantly increases NOS activity.²⁴ An increased extracellular influx of Ca^{++} reported during formation of varicosities and intersections may activate NOS and thus release NO, which eventually can cause injury to neurons.²⁵

As seen in our study, a highly significant increase in carbonic anhydrase activity was found in the fluorosed sheep compared to that in the control sheep, whereas a similarly significant decrease was observed in the activity of CAT. We also found that nitrite and nitrate levels were higher in the fluorosed sheep compared to those of the control sheep. Clearly, these findings have important implications for the high levels of fluorosis in humans and animals in these regions of Turkey.

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