CHANGES IN LEVELS OF ZINC, COPPER, COBALT, AND MANGANESE IN SOFT TISSUES OF FLUORIDE-EXPOSED RABBITS

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SUMMARY: Changes in the concentrations of zinc (Zn) copper (Cu), cobalt (Co), and manganese (Mn) in wet soft tissues of 4–6 week old male and female New Zealand white rabbits administered increasing amounts of fluoride (F) were investigated. Four groups of six rabbits consisting of an equal number of individually housed males and females were given drinking water containing 0 (Group I, control), 50 (Group II), 100 (Group III), and 200 (Group IV) μg NaF/mL. After 90 days the animals were sacrificed by anaesthesia with chloroform, and samples of liver, kidney, muscle, heart, and lung tissue were collected. Zn concentrations were significantly lower in liver, kidney, heart, and lung tissues in Group II than in the control Group I, but the lower level in muscle tissue was not significantly different from that of the control level. Cu concentrations, although lower in all five tissues of the Group II rabbits, differed significantly only in the muscle, heart, and lung tissues between the control Group I and the treatment Groups II, III, and IV. Co concentrations in all tissues in Group II were also significantly lower than in Group I, but in the other two Groups III and IV no definite pattern of change was evident. Mn concentrations in all the tissues of Group II were lower than in Group I and were significantly lower only in the liver, heart, and lung tissues of Group II but not in Groups III and IV. Although the levels of Zn, Cu, Co, and Mn declined with increasing F intake, the maximum decrease occurred in the lowest F-exposure Group II rabbits, suggesting a paradoxical F dose response relationship. A likely mechanism for these toxic effects is the interaction of these electropositive trace elements with F inside the gut tract and other body tissues. However, further studies are needed to elucidate exactly how these interactions occur.

Keywords: Cobalt; Copper; Fluoride interactions; Manganese; Rabbit soft tissues; Trace elements; Zinc.

INTRODUCTION

Excess fluoride (F) intake in mammals exerts toxic effects in many ways, including inhibition of enzymes, generation of free radicals, and deposition in various tissues, especially bones and teeth.[1-3] Being highly electronegative, the F anion can interact with many elements forming soluble or insoluble complexes.[4] Therefore, changes in blood or tissue levels of macro, and trace minerals during chronic F toxicity are expected. Alteration in blood, and bone levels of calcium, phosphorus, and magnesium have already been reported in humans, and animals afflicted with chronic F intoxication.[5,6] Various investigations of tissue levels of trace minerals have been reported without reaching definite conclusions.[3,7,8] Biologically important trace minerals include zinc (Zn), copper (Cu), cobalt (Co), and manganese (Mn) that are essential for certain body functions, and serve as

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vital cofactors of many enzymes.[9] They may also serve to mitigate ill effects arising from oxidative stress induced by fluorosis.[10] Co, for example, is required for the synthesis of vitamin B_{12} in ruminants. Mn is essential for normal development of bones and tendons and proper functioning of reproductive processes in both males, and females.[11] Recently, a decrease in Mn and an increase in Co levels in blood of animals suffering from fluorosis have been reported.[3,8] However, the status and role of trace minerals in soft tissues of animals suffering from fluorosis still require further investigation. The present study was designed to determine the levels of Zn, Cu, Co, and Mn in soft tissues at different levels of F intoxication in rabbits.

MATERIALS AND METHODS

Animals: Twenty-four New Zealand white rabbits, 12 males and 12 females, 4 to 6 weeks old, weighing 600 to 800 g, were procured from the Laboratory Animal Resource Section of the Indian Veterinary Research Institute, Izatnagar, India. They were housed individually and maintained on a standard rabbit diet and tap water (0.17 ppm F) *ad libitum* with a 12-hr photoperiod at room temperature (17–25ºC) and were acclimatized for 15 days before starting the experiment. The study was conducted according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Government of India, after approval from the Animal Ethics Committee of the Institute.

Fluoride exposure, and sample collection: The rabbits were divided equally by sex into four equal groups of six animals each: Group I (control), and Groups II, III, and IV (experimental animals). Drinking water containing 0, 50, 100, and 200 µg of sodium fluoride (NaF)/mL, was provided *ad libitum* to Groups I, II, III, and IV, respectively, for a period of 90 days. The rabbits were then sacrificed under chloroform anaesthesia, and about 5 g of liver, kidney, muscle, heart, and lung tissues were collected in clean polyethylene bags.

Mineral concentrations: Tissue samples were blotted to remove extra water, weighed, and wet digested with a 3:1 mixture of 70% nitric acid and 70% perchloric acid by heating below 80ºC.[12] The digested samples were cooled, and diluted with triple glass-distilled water to a final volume 5.0 mL. The concentrations of Zn, Cu, Co, and Mn were measured with an atomic absorption spectrophotometer (AAS 4141, ECIL, Hyderabad, India) at appropriate wavelengths with acetylene and air as the fuel-oxidant mixture. The concentrations of the various minerals in wet tissues were estimated by multiplying by the corresponding dilution factor and are expressed in µg/g.

Statistical analysis: The values were recorded as means±SE. The data were statistically analyzed using analysis of variance.[13]

RESULTS

The concentrations of Zn, Cu, Co, and Mn in the liver, kidney, muscle, heart, and lung tissues of the rabbits after the 90-day experimental period are shown in the table.

Table. Concentrations (µg/g) of Zn, Cu, Co, and Mn in soft (wet) tissues of rabbits (n = 6 in each group) exposed to 0, 50, 100, and 200 µg NaF/mL in their drinking water for 90 days. Values (means±SEM) bearing different superscripts in a row differ significantly (p<0.05)

Mineral	Organ/ tissue	Group			
		I (Control, no NaF)	II (50 µg/mLNaF)	III (100 µg/mLNaF)	IV (200 µg/mL NaF)
Zinc	Liver	14.58±0.66[b]	7.06±0.26[a]	12.97±0.91[b]	12.65±0.58[b]
	Kidney	11.32±0.38[b]	7.43±0.36[a]	10.51±0.66[b]	11.04±0.62[b]
	Muscle	5.23±0.32[a]	3.88±0.28[a]	4.01±0.61[a]	4.68±0.37[a]
	Heart	10.36±0.51[b]	6.27±0.17[a]	9.03±0.43[b]	9.00±0.19[b]
	Lung	9.06±0.53[b]	6.32±0.48[a]	10.29±0.47[b]	9.53±0.27[b]
Copper	Liver	2.70±0.12[a]	2.29±0.16[a]	2.86±0.33[a]	2.51±0.26[a]
	Kidney	2.30±0.09[a]	1.96±0.07[a]	1.56±0.07[a]	2.47±0.45[a]
	Muscle	0.44±0.03[b]	0.33±0.05[a]	0.23±0.08[a]	0.21±0.07[a]
	Heart	3.46±0.10[b]	2.46±0.40[a]	2.65±0.20[a]	2.58±0.18[a]
	Lung	1.37±0.04[c]	1.16±0.13[b]	0.79±0.10[a]	1.06±0.08[ab]
Cobalt	Liver	1.34±0.11[b]	0.68±0.07[a]	0.73±0.20[a]	1.33±0.15[b]
	Kidney	1.38±0.09[bc]	0.83±0.06[a]	1.12±0.13[ab]	1.60±0.16[c]
	Muscle	1.40±0.16[b]	0.71±0.07[a]	1.13±0.08[b]	1.26±0.17[b]
	Heart	1.80±0.19[b]	0.72±0.07[a]	1.18±0.29[ab]	1.76±0.18[b]
	Lung	1.52±0.14[b]	0.88±0.08[a]	1.45±0.27[b]	1.54±0.22[b]
Manganese	Liver	3.66±0.43[b]	1.77±0.13[a]	2.65±0.55[ab]	2.78±0.63[ab]
	Kidney	2.23±0.29[a]	1.60±0.25[a]	2.08±0.32[a]	1.87±0.15[a]
	Muscle	2.10±0.12[a]	1.40±0.41[a]	2.14±0.54[a]	1.63±0.14[a]
	Heart	2.17±0.58[b]	0.38±0.05[a]	2.04±0.38[b]	2.17±0.15[b]
	Lung	2.34±0.55[b]	0.42±0.01[a]	1.76±0.22[b]	2.01±0.48[b]

In the Group II rabbits (lowest F), the Zn levels were significantly lower than in the control Group I in the tissues of all five organs except the muscle tissue, in which they were not significantly lower. In the control Group I, and in the F Groups III and IV, they were significantly higher in the liver, kidney, heart, and lung tissues than in Group II.

Cu levels, although lower in all five organ tissues of Group II than in the control Group I, were significantly lower only in the muscle, heart, and lung tissues of Group II, and in these tissues of Groups III and IV.

Co levels in all five tissues of Group II were significantly lower than in Group I, but in Groups III and IV no clear pattern of differences was evident.

Finally, Mn levels in all five tissues in Group II were also lower than in Group I, but were significantly lower only in the liver, heart, and lung tissues of Group II, but not in these tissues in Groups III and IV.

DISCUSSION

As seen in the table, depletion of Zn in the rabbits occurred mostly in liver and kidneys, in agreement with an earlier report on F-intoxicated mice.[7] Zn is required by more than 100 enzymes including carbonic anhydrase, alkaline phosphatase, and Cu-Zn superoxide dismutase. The activity of some Zn-dependent enzymes, such as alkaline phosphatase, increases during F toxicity.[14,15] Oxidative stress and increased superoxide dismutase (SOD) activity with Zn involvement have also been observed in experimental F intoxication.[10,16] In response to increased free radical production, the antioxidant defense system of the body is up-regulated,[9.17] and an increase in SOD activity occurs in blood and soft tissues during heavy metal intoxication.[18] A decrease in Zn and Cu concentrations may be due to increased need for synthesis of Zn-dependent enzymes. Zn is poorly stored in body tissues, and therefore a decrease in its level occurs when increased demand is not accompanied with increased availability in the diet. Lower blood levels of Cu, and Zn in various diseases in which oxidative stress oxidative stress is present,[17,19] plus beneficial effects of Zn supplementation, further substantiate this hypothesis.[20]

Liver is known to be as a storehouse for copper, and the kidneys and heart also maintain elevated Cu concentrations.[21] Hepatic storage, and biliary Cu secretion are predominant pathways for adjustment to fluctuations in Cu intake.[11] In the present study, only the liver in Group III and the kidney in Group IV had higher Cu concentrations than in the tissues of the control and Group II rabbits, thus reflecting a negative Cu balance in the lowest level of F exposure. Interference in absorption and utilization of Cu owing to F binding may be responsible. Cu deficiency in animals can result from inadequate absorption, and improper utilization despite adequate dietary intake.[22] Beneficial effects of extra Cu supplementation in fluorosis also support this hypothesis.[3]

Because of its extremely high chemical reactivity, elemental fluorine does not exist in nature.[4] It reacts with almost all elements to form fluorides.[23] This chemical interaction is perhaps responsible for enhanced total body excretion of F in animals co-treated with Cu, magnesium, and selenium.[24] Hypocalcemia observed in cases of chronic F intoxication is also attributed to chemical interaction between calcium, and F, lowering the bioavailability of ingested Ca.[25] Likewise, beneficial effects of aluminium salts in F toxicity may be due to formation of insoluble complexes in gastrointestinal tract.[26]

Co concentrations in tissues of F-exposed rabbits did not differ significantly from the control, except for the Group II rabbits receiving the lowest dose of F. No definitive dose-response relationship could be observed, although a trend toward a lower Co concentration in the F-exposed animals was evident. Low normal levels of Co in different tissues may be a possible reason for these observations.

Mn is one of the least abundant essential trace elements in animal tissues, and, except in poultry, its deficiency is rarely observed under natural conditions.[11] Its level in tissues is extremely variable, and only a slight decline is noticed in animals deprived of Mn.[11] In the present study, Mn levels in the tissues varied widely. The only significant difference from control values was observed in Group II, in which it was the lowest. In humans suffering from fluorosis, decreases in zinc, manganese, and molybdenum concentrations, along with high F deposition, are reported in ossified ligamenta flava.[27]

From the present study, it can be concluded that a downward trend of Zn, Cu, Co, and Mn is observed in soft tissue levels of animals like rabbits during F intoxication. Interaction of different trace minerals with highly electronegative F ions within the gut, and other body tissues may be primarily responsible for these changes. However, in view of their diverse biological roles and variable chemical nature, further research is required for elucidating the various pathways of their interaction with F. The present study results also indicated paradoxical dose response relationship in F toxicity. The dose response relationship showed a non-linear pattern, and changes were more prominent in most of the tissues of the rabbits exposed to the lowest level F intake. This result is therefore in agreement with evidence of a paradoxical dose-response relationship for lower rather than higher exposure to F in various biological systems.[28]

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