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Studies on the Effects of X-Ray on Erythrocyte Zinc and Copper Concentrations in Rabbits After Treatment with Antioxidants

S. Dede,*[,1] Y. Değer,[1] N. Mert,[1]
T. Kahraman,[3] M. Alkan,[4] and I. Keleş[2]

Departments of [1]Biochemistry and [2]Internal Medicine, Faculty of Veterinary Medicine, University of Yuzuncu Yıl, Van 65080 Turkey; [3]High School of Tatvan, Bitlis, Turkey; and [4]Sap Institute, Ulus/Ankara, Turkey

ABSTRACT

The aim of this study was to investigate the effect of supplemental antioxidant vitamins and minerals on the erythrocyte concentrations of zinc and copper in rabbits after exposure to X-rays. The animals were divided into two experimental and one control group (CG). The first group (VG) was given daily oral doses of vitamins E and C; supplemental amounts of manganese, zinc, and copper were mixed with the feed and given to the second group of experimental animals (MG). Blood samples were taken from all groups before and after 4 wk of vitamin and mineral administration and after irradiation with a total dose of 550-rad X-rays. The administration of minerals caused the most significant increases of Zn and Cu. Even after irradiation, the zinc levels in the irradiated animals were higher than in the nonirradiated vitamin-supplemented animals ($p<0.05$). The results suggest that supplementation with antioxidant vitamins and minerals may have a protective effect against X-ray-induced damage.

Index Entries: X-ray radiation; erythrocyte; trace element; antioxidant compounds.

INTRODUCTION

For decades, X-rays have been in use for the diagnosis and treatment of some diseases. Even at relatively low doses or periods of exposure, X-

*Author to whom all correspondence and reprint requests should be addressed.

rays and other ionizing radiation induce the production of free radicals, known to have adverse effects on cells and tissues that may or may not be a part of the target area *(1,2).* Antioxidant systems such as glutathione, superoxide dismutase, and vitamins E, C, and A, among others, are known to protect cells against damage by free radicals, a function that may be strengthened by the administration of some antioxidant compounds *(3).*

Zinc and copper are known to be an essential part of many enzymes that play an important role in growth, immune system function, cellular respiration, redox processes, and protein synthesis. They also have a remarkable function in the prevention of cancer and other chronic, noncancerous diseases *(1,3,4).* Trace elements are cofactors of several antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSHPx), and catalase (CAT), all of which are an important part of the antioxidant system *(5).* Zinc and copper also play a role in the quenching of free radicals through the reduction of the peroxidation ratio and in breaking the free-radical production chain *(6).* It has been reported that there is an initial rise of the copper concentration after the application of radiation *(7,8),* although Chatterjee et al. indicated that the serum Cu concentration decreased in technicians chronically exposed to X-rays *(9).*

In this study, we report the effects of supplemental antioxidant vitamins E and C and of copper, zinc, and manganese on the erythrocyte levels of copper and zinc in rabbits before and after being exposed to X-ray radiation.

MATERIALS AND METHODS

The healthy native rabbits weighing 2100–2400 g used in this study were divided into three groups of seven animals each. One of these sets was used as control and all were kept under normal conditions for 1 mo. The animals were fed a standard commercially available diet and were allowed to eat and drink water ad libitum. The animals were treated with a mixture of tetramezathin, oxytetracyclin, and sulfadimidin sodium to prevent infectious diseases. Before the start of the experiment, 1-mL blood samples were collected from the 21 animals and placed into heparinized tubes and frozen until needed for analysis.

The animals in the control group (CG) were fed only the commercial food and water throughout the study. The vitamin-supplemented group (VG), in addition to the standard diet, received daily oral doses of vitamin E as α-tocopherol (Sigma, St. Louis, MD, USA) (460 mg/kg body wt) and 100 mg/mL vitamin C (Roche, Basel, Switzerland). The mineral-supplemented group (MG) was fed the standard diet with 60 mg $MnCl_2$, 40 mg $ZnSO_4$, and 5 mg $CuSO_4$ (Merck, Germany). One week after supplementation with vitamins and minerals, blood samples were again taken from the two experimental groups. Then, all of the animals were irradiated using a Shimadzu Co. (Kyoto, Japan) X-ray apparatus. The conditions of the irra-

Table 1
Erythrocyte Zn and Cu Concentrations in Rabbits for the Control, Vitamin, and Mineral Groups Before and After Vitamin and Mineral Administration and Irradiation

Duration And Parameters		Control Group (X±Sx)	Vitamin Group (X±Sx)	Mineral Group (X±Sx)
Before vitamin and mineral administration	Zn(μg/dl)	4.94±0.27	4.56±0.42	4.29±0.46
	Cu(μg/dl)	2.59 ±0.08	2.64 ±0.32	2.67±0.11
After vitamin and mineral administration	Zn(μg/dl)		5.87 ±0.45	7.08±0.60*
	Cu(μg/dl)		2.75 ±0.18	3.52±0.76*
After irradiation	Zn(μg/dl)	3.91±0.22	4.79±0.37*	5.55±0.42**,a
	Cu(μg/dl)	3.77 ±0.07	2.73±0.19*	2.66±0.18*
n		7	7	7

* $p<0.05$.
** $p<0.01$.
[a] Higher than vitamin-treated group ($p<0.05$).

diation were 100-kV, 60-mA X-ray transmission and a 0.5-mm Cu^{1+} + 1 mm Al filter. The irradiation continued for 1 wk in daily sessions of 100 rad/min until a total dose of 550 rad *(10)*. Twenty-four hours after irradiation, blood samples were again taken from all groups. The erythrocytes were separated and diluted 1 : 10 with double-distilled water. The samples were then analyzed for zinc and copper by means of a Unicam 929 (United Kingdom) atomic absorption spectrophotometer following established procedures *(11,12)*. The results were analyzed statistically according to Duncan's test.

RESULTS

The zinc and copper concentrations for the two study groups and controls are given in Table 1. The Cu and Zn concentrations in the blood collected before vitamin and mineral administration are statistically the same.

The Cu levels of the MG significantly increased after mineral supplementation ($p<0.05$), but they remained unchanged by irradiation. Copper levels in the VG group are the same before and after vitamin administration. After irradiation, the Cu concentration in all groups decreased significantly ($p<0.01$) compared to the original control values.

The Zn levels of the animals in MG increased significantly ($p<0.05$) after supplementation, but they were not affected after irradiation. The Zn values remained unchanged after irradiation and were lower in the CG than in the postirradiation values in the MG ($p<0.01$) and VG ($p<0.05$) after irradiation. Furthermore, Zn levels in the MG animals were significantly

higher when compared to the values obtained from the other groups after irradiation ($p<0.05$).

DISCUSSION

At certain doses, ionizing radiation results in cleavage of water in cells forming hydroxyl *(2)* and superoxide *(13)* radicals that then result in cell membrane damage by lipid peroxidation *(6).*

Trace elements are a cofactor of antioxidant enzymes, playing an important role in the antioxidant system *(5).* Zhou et al. determined that supplemental Cu and Zn increased serum SOD activity *(14).* Similarly, several investigators have reported that use of irradiation for both therapy and diagnosis causes an initial increase of serum Cu concentration, followed by an increase of ceruloplasmin values. This acute-phase protein is the most important Cu carrier protein and is considered as an indicator of damage and disease caused by irradiation. Our result showed that the Cu concentration of CG increased ($p<0.05$) after X-ray irradiation, in agreement with the literature reports *(7,8).*

Endogenous and exogenous vitamins protect the organism against the damaging effects of free radicals *(15).* There are reports of the beneficial effects of vitamin reinforcement in the protection against radiation and in the treatment of deleterious effects of irradiation *(16–18).* Fomenko et al. compared the protective effects against radiation of Zn and selenium (Se) together with a mixture of β-carotene, α-tocopherol, and sodium ascorbate and concluded that daily vitamin administration better prevented chromosomal damage than the Zn–Se mixture alone *(19).* In the present study, erythrocyte Zn and Cu levels of the animals in the control group were considerable affected by irradiation, but not so in the vitamin-supplemented group.

Russano et al. reported that the Cu cofactor of SOD and CAT has a protective effect against X-ray-originated lipid peroxidation and that the activity of these enzymes increased after irradiation *(20).* In our study, the erythrocyte Cu values increased in MG ($p<0.05$) but were not significantly changed by irradiation. Irradiation caused a significant increase in erythrocyte Cu level of the controls, but not in the vitamin group. These findings thus support the premise that vitamin and mineral administration have a positive effect on the erythrocyte Cu status.

Chatterjee et al. reported that serum and tissue Zn concentrations in humans decrease after a long irradiation time *(9).* Ogata et al. reported that before 24 h irradiation, Zn administration induces production of methallothionein (MT), a free-radical scavenger, thus being a factor in the protection against radiation *(21).* We find that, after irradiation, the Zn concentration of the animals in CG were lower than in MG ($p<0.01$) and VG ($p<0.05$). Lower Zn concentrations observed in the control group indi-

cate that the vitamin and mineral supplements used in this study indeed have protective effects against the damaging effects of X-ray irradiation.

In conclusion, the administration of vitamins and minerals appear to be useful in the reinforcement of antioxidant systems that protect the organism against cell damage by free radicals produced by X-ray irradiation. Particularly, the results obtained from the vitamin group support the claim that administration of vitamins is necessary in the protection against X-ray damage.

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