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Effects of X-Ray Radiation on Oxidation Products of Nitric Oxide in Rabbits Treated with Antioxidant Compounds

[Antioksidan Madde Uygulanan Tavşanlarda Nitrik Oksit Oksidasyon Ürünleri Üzerine X-Işını Radyasyonunun Etkileri]

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ABSTRACT

Objectives: The aim of this study was to investigate the effect of supplemental antioxidant vitamins and minerals on the nitric oxide oxidation products nitrite and nitrate in rabbits after exposure to X-rays.

Methods: The animals were divided into two experimental, one control group. The vitamin-supplemented group 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 mineral-supplemented group. Blood samples were taken from all groups before and after 4 weeks of vitamin and mineral administration and after irradiation with 550-rad X-rays. The nitric oxide oxidation products levels were analyzed in blood serum.

Results: After irradiation, the control group showed increased levels of nitric oxide oxidation products ($p<0.05$). In the vitamin group, the concentration of nitric oxide oxidation products was lower ($p<0.05$) when compared to controls. In the MG, the mineral treatment was not affected nitric oxide oxidation products, but, irradiation was increased nitrate levels.

Conclusion: It is concluded that the elevated levels of nitrate and nitrite concentrations in serum is the result of damage caused by the x-ray radiation. The results suggest that supplementation with antioxidant vitamins and minerals may serve to reinforce the antioxidant systems, thus having a protective effect against cell damage by X-rays.

Key Words: X-ray radiation; erythrocyte; nitric oxide oxidation products; antioxidant compounds

ÖZET

Amaç: Bu çalışma, tavşanlarda antioksidan vitamin ve mineral takviyesinin, X-ışını radyasyonu uygulanmasını takiben serum nitrik oksit oksidasyon ürünlerinin nitrit ve nitrat düzeylerine olan etkisini araştırmak amacıyla planlandı.

Yöntem: Tavşanlar iki deneme ve bir kontrol grubu olarak ayrıldı. Vitamin-takviyeli gruba vitamin E ve C, mineral-takviyeli gruba ise manganez, çinko ve bakır ağız yolu ile günlük olarak uygulandı. Dört hafta boyunca vitamin ve mineral uygulamasından sonra deneme ve kontrol gruplarından kan alındı. Daha sonra toplam 550 rad olmak üzere X-ışını radyasyonu uygulandı. Tekrar kan alındı. Tüm serum örneklerinde nitrik oksit oksidasyon ürünleri düzeyleri ölçüldü.

Bulgular: Radyasyon uygulamasından sonra, nitrik oksit oksidasyon ürünlerinin kontrol grubunda önemli oranda arttığı görüldü ($p<0.05$). Vitamin uygulanan grupta nitrik oksit oksidasyon ürünlerinin kontrole göre önemli oranda düşük olduğu görüldü ($p<0.05$). Mineral grubunda, mineral uygulanmasının nitrik oksit oksidasyon ürünlerini önemli oranda etkilemediği, ancak radyasyon uygulanmasını takiben nitrat düzeylerinde önemli artış olduğu görüldü.

Sonuç: Nitrik oksit oksidasyon ürünlerinin (nitrat ve nitrit) düzeylerinin artmasının X-ışını radyasyonundan kaynaklı bir yıkımın sonucu olduğu kanısına varıldı. Sonuç olarak, özellikle vitamin takviyesinin antioksidan sistemi güçlendirdiği, böylece, X-ışınlarının neden olduğu hücre hasarına karşı koruyucu olarak etkili olduğu görüldü.

Anahtar Kelimeler: X-ışını radyasyon; eritrosit; nitrik oksit oksidasyon ürünleri; antioksidan madde

Introduction

X-rays have been extensively used for both diagnostic and therapeutic purposes. Although generally considered safe, when a water molecule interacts with this type of radiation, it absorbs energy, liberating one electron. These entities are not stable and undergo rapid decomposition, producing highly reactive radical and ionic species capable of irreversible cell membrane damage. Even at relatively low doses or periods of exposure, 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 (1-5).

The formation of reactive oxygen species and free radicals with exposure to radiation is considered one of the most important causes of radiation-induced carcinogenesis (6). They react with body tissues and generate lipid peroxidation, DNA lesions and enzyme inactivation, all of which are mediators of radiation damage. Nitric oxide (NO) is another free radical induced by irradiation (7, 8). NO is a biologic mediator in biochemical reactions, and physiologically, it is synthesized from L-arginine by NO synthase employing cofactor NADPH. The vasodilatory effects of NO on tumor vasculature, suggest that such agents open a new avenue of research in radiation oncology. In the body, the NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) within a very short period of time. This short duration in the conversion of NO to NO_2^- and NO_3^- makes it difficult to accurately measure the concentration of NO. Therefore, by determining the amounts of NO_2^- and NO_3^-, the levels of NO can be assessed. Blood nitrate and nitrite are produced from nitrogen monoxide and its fluctuation may reflect on the fluctuation of nitric oxides. It has been widely reported that the concentration of NO synthesized physiologically increase in some pathologic circumstances, and it acts as a free radical (9-12). In vivo NO formation, resulting in delayed NO synthase expression and NO formation was quantified in mice after exposure to high-dose whole-body X-ray irradiation (13).

Antioxidant systems are known to protect cells against damage by free radicals, a function that may be strengthened by the administration of some antioxidant compounds (14). It has been shown that following exposure to X-rays; natural antioxidants such as vitamin C and E exert a protective effect against chromosomal damage by reactive species generated by the irradiation (2), and show a protective effect against radiation damage (15-18). Trace elements Cu, Zn and Mn have also been show to play a protective effect against X-ray injury as cofactors in enzymatic systems or as free-radical scavengers, that cofactors of several antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), all of which are an important part of the antioxidant system (14,19,20).

The aim of this study was to investigate the effects of supplemental antioxidant vitamin E and C and of copper, zinc, and manganese on the status of nitric oxide oxidation products in rabbits before and after being exposed to X-ray radiation.

Materials and methods

Experimental animals

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 a control and all were kept under normal conditions for 1 month. 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. All animals received humane care according to the criteria outlined in the "*Guide for the Care and Use of Laboratory Animals*" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Experimental design

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 feed 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 irradiation were 100-kV, 60-mA X-ray transmission and a 0.5-mm Cu^{1+} mm Al filter. The irradiation continued for 1 wk in daily sessions of 100 rad/min until a total dose of 550 rad. Twenty-four hours after irradiation, blood samples were again taken from all groups. Serum samples obtained after centrifugation.

NO oxidation products analysis

The concentrations of oxidation products of serum NO were determined by coupling reagent according to Sthar modified method (21).

Statistical analysis

Analysis of variance was used to test whether there is a significant difference between groups. Duncan's multiple comparison tests was used for finding the groups which are significantly different from the others.

Results and Discussion

In first blood samples, nitrite and nitrate levels of the groups were not different. In VG, nitric oxide oxidation products were decreased via vitamin administration, and, were increased after radiation exposure. But, these elevations of nitric oxide oxidation products were lower as compared to CG and MG. In MG, nitrite levels were not affected by mineral administration or radiation exposure. Nitrate levels were not changed after mineral treatment, but, were increased by radiation exposure.

Exposure to ionizing radiation adversely affects several physiological systems. Radiation damage in mammals may cause death from diseases, such as central neuron death, digestive tract damage, malignant tumors and leukemia, and acute death through failure of the bone marrow. Reactive oxygen species or free radicals generated by radiation are well known to induce inflammation and tumorigenesis in target tissues (3-5). The irradiation-induced increase in nitric oxide may be related to lethal injury (12). NO is another free radical induced by irradiation (7-11). NO is produced by an enzyme-catalyzed reaction. The inducible form of NOS is generally considered to be absent under physiological conditions, and induced by radiation (22, 23). The presence of nitric oxidation products NO_2, NO_3 are taken as an indicator of free radical damage through membrane lipid peroxidation (8-12).

The results for all determinations in the three sampling periods are given in Table 1. Our results show that after irradiation, the control group showed increased levels of nitric oxide oxidation products ($p<0.05$).

Ohata et al (12) were investigated the role of nitric oxide in relation to radiation damage, by examining changes in mouse serum nitrate concentrations after irradiation, and were reported that post-irradiation serum nitrate concentrations increased dose-dependently with irradiation dose, and, claim the known physiological functions of nitric oxide imply that it should prevent radiation-induced death.

This is consistent with previous reports about the protective effects of vitamin E and C against lipid peroxidation. In the VG, the concentration of nitric oxide oxidation products was lower ($p<0.05$) when compared to controls by vitamin administration. After irradiation, the nitric oxide oxidation products levels were increased, but, rising of this levels were still lower than control and mineral treated groups, statistically.

Undesirable damage to healthy tissue during radiotherapy could be better managed by the administration of antioxidants (1, 15) to strengthen endogenous antioxidant systems (19, 25). Endogenous and exogenous vitamins protect the organism against the damaging effects of free radicals. The natural antioxidants like vitamin E and C have a protective effect toward chromosomal damage by radiation, in particular when they are used in relatively low doses during a long period of time (2). Baraboi et al. (26) reported that although the administration of antioxidant vitamins after irradiation helped balance the pro-oxidant and antioxidant distributions, it did not represent a complete cure for the deleterious effects of radiation. There are reports of the beneficial effects of vitamin reinforcement in the protection against radiation and in the treatment of deleterious effects of irradiation (15, 16).

Trace elements are a cofactor of antioxidant enzymes, playing an important role in the antioxidant system (19, 27). In the MG, the concentrations of nitrite levels were not affected by the both of mineral and radiation treatment. Nitrate levels were not changed after mineral treatment, but, were increased by radiation exposure. Also, the administration of minerals seems to ineffective help the overall antioxidant status of the organism.

Table 1. Concentration of nitric oxide oxidation products NO2− and nitrate NO3− in the controls and in the vitamin and mineral supplemented groups before and after supplementation and/or irradiation

	Blood Sampling Stage	Controls	Vitamin Supplemented Group	Mineral Supplemented Group
Nitrite (μg/ml)	**1**[a]	1.587±0.059	1.797±0.466	1.977±0.311
	2[b]		0.914±0.0585[e]	2.143±0.525
	3[c]	2.044±0.528[d]	1.187±0.165*[d]	1.790±0.509
Nitrate (μg/ml)	**1**[a]	5.387±0.546	5.853±0.866	5.973±0.235
	2[b]		4.573±0.175[e]	5.783±0.624
	3[c]	8.783±1,274[d]	7.033±1.275*[d]	8.210±0.454[d]

Values are mean ± standard deviation

[a] Before supplementation

[b] Before irradiation

[c] After irradiation

[d] Significantly different from first blood sampling, $p<0.05$

[e] Significantly different from 1 and 3 blood sampling, $p<0.05$

* Significantly different from control group

These results suggest that the administration of antioxidant vitamins does have a positive effect against damage by ionizing radiation. In the present study, it could be summarized obtained results: NO is an important radical for radiation-induced oxidative stress in rabbits. On the other hand, the group receiving vitamins E and C seemed to have the greater resistance against radiation. Vitamin and mineral supplementation may then be considered as coadjuvants in the management of radiotherapy patients. 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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