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RESEARCH ARTICLE

Effects of Coenzyme Q10 on the Levels of some Trace Elements in Serum of Rats with Bleomycin Induced Pulmonary Fibrosis

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Summary

Coenzyme Q10 (CoQ10) is an antioxidant and an electron carrier in the mitochondrial matrix. The aim of this study was to study the protective effect of CoQ10 on the levels of Fe, Cu, Zn and Mn in serum with bleomycin induced pulmonary fibrosis in rats. Thirty Wistar albino rats were randomly divided into three groups: bleomycin alone, bleomycin + CoQ10, and physiological saline alone (control group). The bleomycin group was given 7.5 mg/kg body weight (single dose) bleomycin hydrochloride intratracheally. The bleomycin + CoQ10 group was also instilled with bleomycin hydrochloride but received administrations of CoQ10 twice a week. The control group was treated with physiological saline alone. Animals were euthanized 14 d after intratracheal instillation of bleomycin. Fe, Cu, Zn and Mn were measured in the serum of rats. Atomic absorption spectroscopy was used to measure the Fe, Cu, Zn and Mn levels. Our data may suggest that bleomycin impairs the equilibrium of Fe, Cu, Zn and Mn which are important in the antioxidant system and CoQ10 tends to rechange the imbalance of some trace elements in pulmonary fibrosis.

Keywords: *Coenzyme Q10, Bleomycin, Pulmonary fibrosis, Trace element, Rat*

Koenzim Q10' nin Bleomisinin Teşvik Ettiği Akciğer Fibrozisli Ratların Serumlarındaki Bazı İz Elementlerin Seviyelerine Etkisi

Özet

Koenzim Q10 (CoQ10) mitokondrial matrikste bulunan antioksidan bir elektron taşıyıcısıdır.Bu çalışmanın amacı bleomisin uygulanarak oluşturulmuş akciğer fibrozisinde CoQ10' nin Fe, Cu, Zn ve Mn düzeyleri üzerine koruyucu etkisini araştırmaktı. Otuz Wistar albino ırkı rat yalnız bleomisin uygulanan grup, bleomisin + CoQ10 uygulanan grup, ve yalnız serum fizyolojik uygulanan grup (kontrol grubu) olmak üzere 3 gruba ayrıldı. Bleomisin grubuna bleomisin hidroklorid 7.5 mg/kg canlı ağırlık oranında intratrakeal olarak tek doz şeklinde uygulandı. Bleomisin + CoQ10 grubuna da bleomisine ilave olarak haftada iki kez CoQ10 uygulandı. Kontrol grubuna ise yalnız serum fizyolojik uygulandı. Bleomisin uygulamasından 14 gün sonra ratlara ötenazi uygulandı. Serumlarında Fe, Cu, Zn ve Mn düzeyleri saptandı. Fe, Cu, Zn ve Mn düzeylerinin belirlenmesinde atomik absorbsiyon spektrofotometresi kullanıldı. Elde ettiğimiz veriler, bleomisinin antioksidan sistemde önemi olan Fe, Cu, Zn ve Mn dengesini bozduğunu ve CoQ10' in akciğer fibrozisinde ağır metal ve iz element dengesizliğini yeniden değiştirdiğini düşündürmektedir.

Anahtar sözcükler: *Koenzim Q10, Bleomisin, Akciğer fibrozisi, İz element, Rat*

INTRODUCTION

Pulmonary fibrosis is a chronic interstitial lung disease. The lung parenchyma is damaged by varying patterns of inflammation and fibrosis with a high mortality rate and poor response to available medical therapy [1,2]. The etiology of this disease is unknown. However, lung inflammation is a major underlying component of a wide variety of pulmonary fibroproliferative disorders. Reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, peroxynitrite, and hydroxyl radical are the major mediators of the lung inflammatory processes [3,4].

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Bleomycin generates reactive oxygen metabolites, including superoxide and hydroxyl radicals. Generation of the reactive species in the lung tissue results in DNA injury, lipid peroxidation, alteration in lung prostaglandin synthesis and degradation, and an increase in lung collagen synthesis [5]. Bleomycin-induced fibrosis is the most commonly used animal model and appears as a significant drug-induced lung disease in the clinical setting [3].

The complex bleomycin-Fe has been the most studied because bleomycin joins the DNA and Fe at the same time, and release of free radicals happens in the presence of molecular oxygen [6]. Fe is typically in the Fe (III) form and will need to be reduced prior to any Fenton activity. Lung lining fluid contains antioxidants, such as glutathione (GSH) and ascorbic acid, which can reduce Fe(III) to Fe(II) [7].

Coenzyme Q10 (CoQ10), also known as ubiquinone, is a vitamin like substance present in all human cells in the membranes of endoplasmic reticulum, peroxisomes, lysosomes, and the inner membrane of mitochondria. It is an important component of the electron transport chain in mitochondria responsible for generation of energy. Furthermore, its reduced form serves as an important antioxidant in both mitochondria and lipid membranes where it protects the body against the deleterious effects of reactive oxygen species (ROS). Therefore, the enzyme has been therapeutically employed in many disorders for its cytoprotective and antioxidant properties [8].

Huang et al.[9] reported that many trace elements play an important role in a number of biological processes by activating or inhibiting enzymes, by competing with other elements and metalloproteins for binding sites, by affecting the permeability of cell membranes or by other mechanisms. Trace elements function as activators of enzyme systems or as constituents of organic compounds. Trace elements are necessary for humans and animals [10].

The purpose of this study was to evaluate the effects of CoQ10 on the serum levels of some of the trace elements (Fe, Cu, Zn and Mn) in rats with bleomycin induced pulmonary fibrosis.

MATERIAL and METHODS

Thirty male Wistar albino rats, six months old, weighing 180-200 g, were obtained from the animal laboratory of Yuzuncu Yil University. All procedures involving animals were approved by the institutional ethics committee (Document No: 28.02.2007/04). Rats were housed in specific cages. A12-h light/dark cycle was maintained and the rats had free access to water and food *ad libitum*. The animals were divided randomly in the following three groups: Control group (*n*=10): Sterile physiological saline solution was given intraperitoneally. Bleomycin treated group (*n*=10): Bleomycin hydrochloride (Nippon Kayaku, Tokyo, Japan) was dissolved in 250 µL of phosphate- buffered saline (PBS) solution and instilled into the animals at a dose of 7.5 mg/kg body weight (single dose) intratracheally under chloroform anesthesia. The animals were shaken to facilitate distribution of the bleomycin and physiological saline [11]. Bleomycin+CoQ10 treated group (*n*=10): CoQ10 was given as CoQ10 (Sigma, St. Louis, MO, USA) at a dose of 10 mg/kg body weight (twice a week) during the experiment [12]. To evaluate the serum trace elements of bleomycin induced pulmonary fibrosis, animals were euthanized 14 d after its instillation. Blood samples were collected for analysis. They were centrifuged at 1500 rpm for 15 min and serum was obtained. The levels of trace elements (Fe, Cu, Zn and Mn) were analyzed by atomic absorption spectrophotometry (Unicam 929 atomic absorption spectrophometer).

All analyses were made using the SPSS statistical software package. Data are expressed as means±standard deviation. Statistical analyses were made using the independent sample t test.

RESULTS

The levels of Fe, Cu, Zn and Mn in the groups are indicated in *Table 1*. The concentration of Fe was lower in bleomycin + CoQ10 administrated groups as compared to the other groups (P<0.05). The levels of Zn were significantly decreased in all groups as compared to the

Table 1. *Concentrations of Fe, Zn, Mn and Cu in serum of the control, bleomycin administered and bleomycin + CoenzymeQ10-administered groups*

Tablo 1. *Bleomisin, bleomisin + Coenzyme Q10 uygulanmış ve kontrol gruplarının serumlarında Fe, Cu, Zn ve Mn konsantrasyonları*

Parameters	n	Bleomycin M±SD	Bleomycin + CoenzymeQ10 M±SD	Control M±SD
Fe (µg/dl)	10	0.446±0.14	0.211±0.20 [c]	0.597±0.38
Zn (µg/dl)	10	0.188±0.06 [b]	0.101±0.09 [a]	0.250±0.03
Mn (µg/dl)	10	0.006±0.00 [a]	0.005±0.00 [c]	0.003±0.00
Cu (µg/dl)	10	0.225±0.08 [b]	0.143±0.14 [b]	0.315±0.04

[a] P<0.001, [b] P<0.01, [c] P<0.05

control. It was found that the levels of Mn were significantly increased in all groups than that of the control. The level of Mn was lower in CoQ10 + bleomycin administrated group as compared to the only bleomycin administered group. The concentration of Cu was significantly decreased in all groups as compared to the control. The level of Cu was higher in bleomycin administered group than that of the CoQ10 + bleomycin treated groups.

DISCUSSION

Bleomycin has been used as cytostatic treatment of many malignant tumors, such as germ cell tumors, lymphomas, head and neck tumors, and Kaposi's sarcomas [13,14]. Its mechanism of action is breaking the DNA double helix by the production of free radicals, which is oxygen and iron dependent [14,15]. Pulmonary fibrosis has more common side effect of bleomycin in cancer treatment. In a previous study, Mert et al.[16] showed that α-tocopherol and vitamin D_3 levels in the lung tissue of the bleomycin treated rats decreased appreciably more than that of the control group. Hence, the histological changes observed in the bleomycin administered rats. Large fibrous areas in the interalveolar septa tissue, peribronchial and peri-vascular regions were observed with apparent infiltration of lymphocytes. In another study, Ertekin et al.[3] have investigated the preventive effect of vitamin E on bleomycin induced rats. In their study, the amounts of Cu, Fe, and carbonic anhydrase found in the lung were increased in both bleomycin and bleomycin+vitamin E administered rats. Zn and Mn levels were decreased, except for the Mn level in the bleomycin group. The levels of Zn, Mn, and Cu were decreased in both experimental groups compared to the control group, whereas Fe and carbonic anhydrase activity increased in comparison to the control group. Dede et al. [17] had also showed that the lungs of rats induced with bleomycin were damaged and α-tocopherol prevented it from certain damage as indicated by the difference of the concentrations in major elements of serum. The levels of Fe and Mg in the group of bleomycin were lower than that of the control group and the levels of Cu, Ca and K were not changed among the groups.

In this study, we have investigated the protective effect of CoQ10 on some trace elements (Fe, Cu, Zn, Mn) in the serum of rats with lung fibrosis induced by bleomycin. Cu was decreased significantly in bleomycin and bleomycin + CoQ10 administered groups ($P<0.01$). The level of Zn was significantly decreased bleomycin treated group ($P<0.01$). Zn is a part of superoxide dismutase which is an important antioxidant enzyme in the cell. The change of the level of Zn means an imbalance of the antioxidant system. The Mn concentrations were higher in all of the groups than that of the control. Mn is also part of superoxide dismutase enzyme. The less increase of the level of Mn in bleomycin + CoQ10 treated group than that of the bleomycin administered group may imply an amelioration in the antioxidant system. Because, CoQ10 is an antioxidant and found in the mitochondria. Manganese superoxide dismutase (MnSOD) is the major ROS detoxifying enzyme of cells because of its localization to mitochondria. Altered function or expression of MnSOD can have remarkable consequences on mitochondrial function and the overall health of cells due to oxidative damage to various mitochondria-localized metabolic processes, leading to the development of different diseases [18]. CoQ10 may show an effect in the mitochondrial matrix tending to regulate Mn balance. However it is difficult to say that the protective effect of CoQ10 barely by measuring trace elements. It could be more helpful that to measure the antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase. In another study, the effects of CoQ10 on the levels of serum biochemistry and malondialdehyde (MDA) in rats were investigated. The decreased levels of alkaline phosphatase (ALP) and increased levels of phosphorus (P) in CoQ10 treated rats were found compared to the control rats [19].

In a research, serum CoQ10 levels were measured at rest and during incremental exercise in 21 patients with chronic obstructive pulmonary disease (COPD) and 9 patients with idiopathic pulmonary fibrosis (IPF). The mean of serum CoQ10 levels were found lower in both experimental groups than that of the healthy subjects. Hence, they investigated the effects of the oral administration of CoQ10 on pulmonary function and exercise performance in patients with COPD. Serum CoQ10 levels were found significantly higher in association with an improvement in hypoxemia at rest, whereas pulmonary function was unchanged [20].

Reju et al.[21] indicated that in the cancerous tissue of testis, the concentrations of K, Cr and Cu were higher while the concentrations of Fe, Co and Zn were lower when compared to those in normal tissue of testis. The concentrations of Cl, Ca, Ti and Mn were in agreement in both cancerous and normal tissues of testis. Cunzhi et al.[22] investigated the relationship between trace elements and the incidence of cervical cancer. They measured tissue and serum levels of six elements Cu, Zn, Fe, Mn, Ca and Se and Cu/Zn ratio in patients belonging to cervical cancer and uterine myoma. Their results show that the tissue contents of Zn, Se and Ca were significantly lower and the Cu and Fe levels and Cu/Zn ratio were significantly higher in cervical cancer tissue. The same trend was also observed in serum levels also.

Free radical species affect all important components of cells such as lipids, proteins, carbohydrates and nucleic acids. It is known that CoQ10 is an important free radical scavenger. In the present study, we may conclude that bleomycin impairs the equilibrium of Fe, Cu, Zn and Mn which are important in the antioxidant system. We

can not suggest that CoQ10 has protective effect on bleomycin induced pulmonary fibrosis as measuring trace elements. However, we can say that CoQ10 rechanges to imbalance of trace elements in pulmonary fibrosis. Further investigations are needed to find how the mechanism works related the trace elements and CoQ10 in pulmonary fibrosis.

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