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Protective Effect of Selenium and Vitamin E on Lipid Peroxidation in Smoke-Exposed Male Mice

[Sigara Dumanına Maruz Bırakılmış Erkek Farelerde Selenyum ve E-Vitamininin Lipid Peroksidasyonu Üzerine Koruyucu Etkisi]

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ABSTRACT

Exposure to environmental tobacco smoke (passive smoking) has been suggested to be a cause of cancer, and antioxidants have protective effects against reactive oxygen species produced by passive smoking. Recent study was conducted to assess the relationship between the exposure to environmental tobacco smoke (ETS) and membrane damage, and also to show the protective effects of selenium with vitamin E against ETS in mice. Thirty male Balb/c mice were randomly divided into three groups, as follows; (i) 10 mice exposed to ETS; (ii) 10 mice exposed to ETS and treated daily with selenium (3.5 microgram/kg body weight) and vitamin E (2.5 mg/kg body weight); and (iii) 10 control mice neither exposed to ETS nor treated with vitamin E and selenium. Malondialdehyde (MDA) levels in serum of male mice were measured spectrofluorometrically. Lipid peroxidation in serum was remarkably increased in ETS-exposed mice compared with the control group and decreased in ETS-exposed and selenium and vitamin E-treated group relative to the ETS-exposed group. There was no significant differences between the control and ETS-exposed and selenium and vitamin E-treated group. The present study highlights the occurrence of lipid peroxidation and possible protective effect of vitamin E and selenium against environmental tobacco smoke exposure.

Key Words: selenium; vitamin E; lipid peroxidation; smoke-exposed mice.

ÖZET

Sigara dumanına maruz kalmanın kansere neden olduğu ve antioksidanların pasif içicilerde üretilen reaktif oksijen türevlerine karşı koruyucu etkisinin bulunduğu öne sürülmektedir. Çalışmamızda pasif sigara içicilerinde çevresel sigara dumanına maruz bırakılma ile hücre zarı hasarı arasındaki ilişkiyi ortaya koymaya çalıştık ve Selenyum ile E vitamininin birlikte sigara dumanına maruz bırakılmış farelerde koruyucu etkisini gösterdik

Çalışmamızda 30 tane erkek Balb/c fare rastgele üç gruba ayrıldı, bunlar; (i) sigara dumanına maruz bırakılan, (ii) sigara dumanı ve selenyum (3.5 mikrogram/kg vücut ağırlığı) ile E vitaminine (2.5 mg/kg vücut ağırlığı) maruz bırakılan, (iii)sigara dumanı, selenyum ile E vitaminine maruz bırakılmayan kontrol grubu farelerdir. Farelerin serumlarındaki malondialdehit (MDA) düzeyleri spektroflorometric olarak ölçüldü. Kontrol grubu ile karşılaştırıldığında, sigara dumanına maruz bırakılan farelerin serumlarındaki MDA miktarının önemli derecede artmış olduğu, sigara dumanı ve selenyum ile E vitaminine birlikte maruz bırakılan farelerdeki MDA düzeyinin ise sadece sigara dumanına maruz bırakılan farelerin MDA düzeyine göre düşük olduğu bulunmuştur. Kontrol grubu ile sigara dumanı ve selenyum ile E vitaminine birlikte maruz kalan farelerin MDA düzeylerinde istatistiksel olarak bir fark görülmedi. Bu çalışma, çevresel sigara dumanına maruz kalındığında hücre zarında lipid peroksidasyonunun meydana geldiğini ve E vitamini ile selenyumun birlikte çevresel sigara dumanına karşı koruyucu etkisi olduğunu göstermektedir.

Anahtar Kelimeler: selenyum; E vitamini ; lipid peroksidasyonu; Sigara dumanına maruz kalan fareler.

INTRODUCTION

Cigarette smoke is a heterogenous aerosol, which contains many thousands of chemicals (1). These include various compounds, which are capable of causing an increase in the generation of various reactive oxygen species, such as $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$, and $ROO^{\cdot}$. These reactive oxygen species in turn are capable of initiating and promoting oxidative damage in the form of lipid peroxidation (2). Cigarette smoking may thus be associated with an increase in the incidence and severity of various diseases such as cancer. For non-smoking women living with a spouse who smoked, there is 50% increase in risk for development of lung adenocarcinomas. In addition there is a significant increase in risk for non-smoking women exposed to environmental tobacco smoke (ETS) at work or during social activities (3).

Various organs may control or prevent the damaging effects of the oxidant species by antioxidant defence systems. Although there is not enough evidence that indicates increased intake of antioxidant nutrients is beneficial, the observation that smokers have lower circulating levels of certain nutrients raises concern (4).

A variety of studies points to dietary selenium as a potential chemopreventive nutrient. Men in the Health Professionals Follow-Up Study in the highest quintile for toenail selenium content were at a significantly lower risk of advanced prostate cancer (OR=0.35) than those in the lowest quintile. Considerable evidence points to vitamin E as a protector against free radical generation (5, 6). Based on the encouraging research data linking selenium and vitamin E with reduced prostate cancer risk, the Selenium and Vitamin E cancer Prevention Trial (SELECT) has been designed by the American National Cancer Institute to test vitamin E and selenium (7).

Daily selenium and vitamin E uptake in humans changes according to geographical region and feeding habits. The balanced uptake of selenium rich foods such as crops especially wheat, barley, oats and soybeans and vitamin E rich foods such as nuts and fish oil may reduce the risk of ETS related lipid peroxidation and selenium and vitamin E enriched foods may protect against oxidative damage by ETS.

MATERIALS AND METHOD

Animals

During the study, a total of 30 male Balb/c mice (Akdeniz University, Experimental Animals Unit), aged eight weeks and weighing 20-25 gr, were used. The animals were housed in plastic cages (ten mice per cage) on sawdust bedding, and provided with standard pellet mice diet and tap water ad libitum. The animal room temperature was a 23 $\pm$ 2 ºC, with relative humidity of 45%, and a 12 h day-night-light cycle. The housing and treatment of mice were in accordance with national and institutional guidelines.

Treatment groups

After 5 days acclimatisation, the mice were randomly divided into three groups. A total of 20 mice were exposed to cigarette smoke generated by one cigarette during 20 minutes; Namely, 3 cigarettes and 60 minutes per day for up to 12 weeks. Ten smoke-exposed mice were treated daily with selenium (3.5 microgram/kg body weight) and vitamin E (2.5 mg/kg body weight) starting as same day as exposure to cigarette smoke, and continuing for up to 12 weeks. The other 10 mice will be kept for the same time interval in a similar environment, but in the absence of cigarette smoke.

Whole-body exposure to main stream cigarette smoke was obtained by using filter-tipped commercial cigarettes (Maltepe, Turkishtabac). Briefly, each one of the groups of mice undergoing this treatment was placed in a 110 x 43 x 20 cm closed glass chamber. The mainstream cigarette smoke in the chamber was obtained by fan (0.4 Watt, 6 cm radius) during treatment.

Blood samples were collected from the hearts of ten mice of each group which are anesthesized with diethyl ether. The serum was obtained from blood by centrifugation.

Biochemical parameters

Malondialdehyde (MDA) contents of the samples were determined in 24 hours by a fluorimetric method previously described by Wasowics et al (8) by measuring thiobarbituric acid-reactive substances (TBARS) in serum which is based on the reaction between malondialdeyhde and thiobarbituric acid. The product of this reaction was extracted into butanol and measured at 525 nm of excitation and 547 nm of emission wavelengts.

Protein was determined by the method of Lowry et al. (9) with bovine serum albumin as a standard.

Statistical analyses

Comparisons between groups were evaluated by Student's t-test for unpaired data, and by means of ANOVA for repeated measurements (10, 11).

RESULTS

In this study we observed the protective effects of vitamin E and selenium against oxidative stress of ETS-exposed male mice. The amount of MDA in serum was accepted as a biomarker of lipid peroxidation which indicates the membrane damage. MDA level in serum

was significantly increased in ETS-exposed mice (13.81 nmol MDA/mg protein) when compared with the ones of control group ($p<0.001$) and ETS-exposed with selenium and vitamin E treated group ($p<0.01$). MDA levels in ETS-exposed receiving selenium and vitamin E treatment is in less than ETS-exposed mice but higher than that of the control group (table 1). These results showed the protective effect of selenium and vitamin E on lipid peroxidation in smoke-exposed male mice.

DISCUSSION

There is sound evidence that cigarette smoke represents a major risk factor in the pathogenesis of cancers and of many other chronic degenerative disease (12). The induction, persistence and modulation of cytogenic alterations in cells of smoke-exposed mice were shown previously (13). If cigarette-smoke induced damage is oxidant mediated, then the antioxidant defence mechanism may play a protective role against to this deffect. Thereby, it was announced that antioxidants such as SOD, glutathione and catalase decreased the free radical generation in Hep G2 cells, MCF7 and H1299 cells treated with anticancer drugs (14, 15, 16). It has been known that free radicals generated in biological systems by cigarette smoke inhalation can cause oxidative stress in tissues, resulting in lipid peroxidation. Alpha-tocopherol exhibited a role either directly by scavenging the oxidative species or indirectly by modulating the GSH levels (17). Lipid peroxidase levels as well as antioxidant enzyme activities in liver, lung, and kidney were reported to be enhanced in rats exposed to cigarette smoke (18).

Accumulating evidance indicates that vitamin E, the most patent lipid peroxyl radical scavenger, may reduce free radical induced chromosomal damages (19). Based on the encouraging research data linking selenium and vitamin E with reduced prostate cancer risk, the Selenium and Vitamin E cancer Prevention Trial (SELECT) has been designed to test vitamin E and selenium (7).

Table 1 shows that lipid peroxidation was significantly increased in ETS-exposed mice (13.81 nmol MDA/mg protein) when compared with that of the control group ($p<0.001$) and the one of ETS-exposed with selenium and vitamin E treated group ($p<0.01$ level). MDA level in ETS-exposed receiving selenium and vitamin E treatment is in less than ETS-exposed mice but higher than the control group. These results showed the protective effect of selenium and vitamin E on lipid peroxidation in smoke-exposed male mice. Another study showed that lipid peroxidation was markedly increased and enzymic antioxidants were decreased in erythrocytes of cigarette smokers (20). Selenium, vitamin C and vitamin E showed protective effects against oxidative stress caused by cigarette smoke in rats (21). Also, selenium in combination with other minerals and vitamins, such as vitamin E, is promising approach toward inhibiting genetic damage and cancer development (22).

In summary, evidence has been obtained to suggest that nutritional status may influence MDA level in passive-smoker. Increasing MDA level as a result of production of free radicals may be delayed by the presence of antioxidants. Our results indicate that taking vitamin E and selenium is associated with lower risk of lipid peroxidation in passive smoker.

Table 1: Levels of malondialdehyde (MDA) in serum of ETS-exposed mice

Parameters	ETS Mean ± SE	ETS+Vitamin E+Selenium Mean ± SE	Control Mean ± SE
MDA levels in serum (nanomol MDA / mg protein)	13.8 ± 0.4*	10.8 ± 0.8	8.9 ± 0.9

*, Amount of MDA in ETS group is statistically different from control group at $P<0.001$ level and ETS+Vitamin E+Selenium group at $P<0.01$ level. In ETS group; 10 mice were exposed to environmental tobacco smoke (ETS), in ETS+vitaminE+selenium group; 10 mice were exposed to ETS and treated daily selenium (3.5 microgram/kg body weight) and vitamin E (2.5 mg/kg body weight); and in control group, 10 mice were neither exposed ETS nor treated Vitamin E and Selenium.

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