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Vitamin E Modulates the Oxidant –Antioxidant Imbalance during Cigarette Smoke Induced Oxidative Stress in Rats

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Abstract: **Cigarette smoking is one of the major causes of mortality involving respiratory and cardiovascular illness in developing countries. Cigarette smoking is known to contain abundant of free radicals, which is able to cause tissue oxidative damage at various levels. The body has the ability to produce antioxidants. When there are excessive free radicals generated due to smoking, the available tissue antioxidants may become depleted, leading to oxidative stress. Male albino rats were induced with cigarette smoke for 70 days to assess the induced oxidative damage due to lung muscle atrophy. An enhanced lipid per-oxidation was recorded with elevated activity levels of conjucated diens, malondialdehyde and other thiobarbituric acid reactive substances (TABARS) in denervated muscle. The activity levels of antioxidant defense enzymes,viz. superoxide dismutase (SOD), catalase (CAT), glutathioneperoxidase (selenium, non-selenium) (GPx), glutathione reductase (GR), glutathione- s transferase (GST) were depleted in the cigarette smoke induced lung muscle. Vitamin E is a major antioxidant.When the experimental control animal (cigarette smoke induced muscle) was supplemented with vitamin E, revealed a depleted lipid peroxidation and increased activity levels of antioxidant defense enzymes. . In this study we investigate the efficacy of antioxidant, the supplementation of vitamin E could prevent the oxidative damage in the lung muscle despite smoking.**

Keywords: **Oxidative stress, cigarette smoke, Vitamin E, antioxidants.**

I. INTRODUCTION

Vitamin E (a-tocopherol) is considered one of the most important dietary antioxidant in biological systems Vitamin E has many biological functions. The antioxidant function is considered to be the most important function of vitamin E and is the one it is best known for. [1], As it is fat-soluble, it is incorporated into cell membranes, which protects them from oxidative damage. However, there are other functions that have also been recognized to be of importance. α-Tocopherol has a regulatory effect on enzymatic activities. α-Tocopherol is an important lipid-soluble antioxidant. It performs its functions as antioxidant in what is known by the glutathione peroxidase pathway[2], and it protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. [3, 4]. This would remove the free radical intermediates and prevent the oxidative damage from continuing.

In the present study is carried out to evaluate the impact of vitamin E supplementation on the oxidative stress during induced cigarette smoking in lung muscle atrophy [5, 6,7]. The cigarette smoke induced animals kept for 70 days were fed with vitamin E (a-tocopherol) of 200mg/kg body wt. [8,9,10,11] for 30 days [10,12,13].

II. EXPERIMENTAL PROTOCOL

The rats were divided into 4 groups comprising of 10 animals in each group.

Group I Control-sham operated animals (C)

Group II cigarette smoke induced kept for 70 days animals-Control (CSC70)

Group III cigarette smoke induced animals after 70 days given vitamin E supplementation for 30 days (CS vit E given after 70days)

Group IV cigarette smoke given and simultaneous treatment ie immediate vitamin E supplementation from 3rd day for 30 days

A. Cigarette smoke induced

Daily two cigarettes were given to male albino rats in the morning and evening for 70 days. [14].

B. Vitamin E supplementation

Rats were fed with vitamin E (.-tocopherol) of 200mg/Kg body weight, as reported earlier [8, 9], for 30 days. [13, 14].

C. Sample Preparation

The experimental animals were sacrified by cervical dislocation at the end of the appropriate experimental period. The various experimental groups viz. C, (CSC70) (CS vit E30) (CS vit Ei) were dissected and the lung muscles were removed and washed with deionized water, weighed, stored at -20ºC until further analysis.

D. Oxidative damage assay

Oxidative damage assay in the lung muscle tissue was assessed by measuring the levels of TABARS and conjugated dienes in all the lung muscle groups viz. C, (CSC70) (CS vit E30) (CS vit Ei) by the method of ohkawa and klein respectively.[15,16].

E. Antioxidant enzyme assay

Enzymes involved in antioxidant enzyme defense system such as super-oxide-dismutase by the method of Beauchamp and Fridovich [17], catalase by the method of Chance and Machly [18], glutathione peroxidase (selenium dependent and Non selenium dependant) by the method of Rotruck,[19], glutathione reductase by the method of Racker,[20], glutathione-s-transferase by the method of Habig et al.[21] were measured to assess the antioxidant defense mechanism in lung muscle of all 4 groups of animals.

III. RESULTS

The thiobarbituric acid reactive substances and conjugated dienes were significantly elevated to 253.55%, 225.22%. in group II (Table 1) respectively indicating higher lipid peroxidation in the cigarette smoke induced muscle when compared to that of control. The decreased activity levels of the antioxidant defense enzyme viz. super-oxide-dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (both selenium and non selenium dependant), glutathione reductase (GR), and glutathione-s-transferase (GST) in the experimental control animals were recorded a significant augmented activity levels after inducing cigarette smoke, i.e. 75.29%, 73.68%, 86.73%, 97.06%, 90.11% and 87.32%, in Group II respectively (Table).

TABARS and conjugated dienes were recorded a significant decrement of 39.35% and 48.66% (Group III),69.53% and 68.18% (Group IV) (Table)respectively indicating lowered lipid peroxidation in the cigarette smoke induced muscle supplemented with vitaminE. The study on the antioxidant defense enzyme reveled a significant elevation of the activity levels of enzymes viz.SOD, CAT, GPx (selenium and non selenium dependant),GR and GST in the lung muscles of vitamin E fed cigarette smoke induced animals the elevation of activity levels of enzymes ranges 42.85%, 160%, 73.33%, 30%,144.44%, 53.52% in Group III, 235.71% , 246.67%, 253.33%, 200%, 288.88%, 271.83% in Group IV respectively (Table).

IV. DISCUSSION

The increased lipid peroxidation in the cigarette smoke dis-use lung muscle of rat might be due to increased generation of reactive oxygen species (ROS) in the muscle thereby disturbing both enzymatic and non enzymatic antioxidant defense system in the muscle. Vitamin E (α-tocopherol) serves as potent peroxyl radical scavenger. Excess generation of ROS

may overhelm natural antioxidant defenses such as muscle membrane vitamin E leading to lipid peroxidation in further contributing to muscle damage [22, 23, 24, 25].

Therefore in the present study has been carried out to evaluate the antioxidant effect of vitamin E dietary supplementation on oxidative stress during induced cigarette smoke-disuse lung muscle atrophy [26]. The decrease levels of TBARS and conjugated dienes in the present study indicate the reduced lipid peroxidation which might be due to the non enzymatic antioxidant vitamin E impact on the disuse lung muscle. Similar studies where vitamin E reduced lipid peroxidation.were recorded [22, 27]. The deranged antioxidant enzymatic defense system with the depleted activity levels of SOD, CAT, GPx (selenium and non selenium dependant), GR and GST were significantly restored indicating the elevated activity levels of SOD, CAT, GPx (selenium and non selenium dependant), GR and GST in the lung muscle of cigarette smoke induced (experimental control) animals supplemented with vitamin E. This might be due to the free radicals scavenging act of vitamin E, thus reducing the free radical concentration and the probable regain of the antioxidant enzymatic defense system.

The above results envisage that the supplementation of vitamin E is an important lipid soluble antioxidant *in vivo*, and it is presumed that its principle role is to protect membrane lipids from lipid peroxidation *invivo*, by scavenging lipid alkoxy or peroxy radicals which are capable of abstracting hydrogen from adjacent polyunsaturated lipid molecules to propagate a lipid peroxidation reaction and thus prevent the muscle damage due to oxidative stress [28].

Table – Levels of Thiobarbituric acid reactive substances (TABARS) and conjucated dienes (CD), antioxidant enzymes [Super-oxide-dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) (both selenium and non selenium dependant), Glutathione reductase (GR) and gluthaione s-transferase (GST) in control (C), Experiment control (cigarette smoke induced) (CSC70), Cigaratte smoke induced treated with vitamin E (CS vit E30) and cigarette smoke induced immediately treated with vitamin E (CS vitEi30).

PARAMETERS	Control C	Experimental control (cigarette smoke induced CSC70)	Cigarette smoke induced, treated with Vitamin E (CS VitE30)	Cigarette smoke induced, immediately treated with Vitamin E (CS VitEi 30)
1. SOD	0.85 ± 0.007	0.21 ± 0.07 -75.29	0.30 ± 0.08 +42.85	0.705 ± 0.04 +235.71
2. CAT	0.114 ± 0.001	0.03 ± 0.004 -73.68	0.078 ± 0.006 +160	0.104 ± 0.005 +246.67
3. GPX (selenium)	0.113 ± 0.002	0.015 ± 0.002 -86.73	0.026 ± 0.006 +73.33	0.053 ± 0.007 +253.33
4. GPX (Non-selenium)	0.034 ± 0.002	0.002 ± 0.0002 -97.06	0.0026 ± 0.006 +30.00	0.006 ± 0.0006 +200.00
5. Glutathione Reductase	0.91 ± 0.07	0.09 ± 0.008 -90.11	0.22 ± 0.06 +144.44	0.35 ± 0.004 +288.88
6. GST	0.56 ± 0.032	0.071 ± 0.008 -87.32	0.109 ± 0.02 +53.52	0.264 ± 0.02 +271.83
7.TABARS	0.956 ± 0.03	3.38 ± 0.004 +253.55	2.05 ± 0.01 -39.35	1.03 ± 0.03 -69.53
8. Conjugated Diene	1.15 ± 0.05	3.74 ± 0.15 +225.22	1.92 ± 0.2 -48.66	1.19 ± 0.09 -68.18

Unit: *µ moles/mg protein; µ units/mg protein/ min

P values : <0.001

Values are mean + SD from 10 animals from each group. Figures in parenthesis are % increase (+) or % decrease (-) 0ver control (C) in Experimental control (cigarette smoke induced) (CSC), and over Experimental control (cigarette smoke induced) (CSC30) in cigarette smoke induced treated with vitamin E (CS vitE30) and cigarette smoke induced immediately treated with vitamin E (CS vitEi30).

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