The Effects of Simultaneous 7 Weeks Oral Supplementation with Antioxidant Vitamin (E) and Aerobic Exercise on Lipid Peroxidation in Erythrocytes After a Bout acute Exhaustive Treadmill Exercise in Rats

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Abstract: The aim of this study was to determine the effects of Simultaneous 7 weeks oral supplementation with antioxidant vitamin (E) and aerobic exercise on lipid peroxidation in erythrocytes after a Bout acute exhaustive treadmill exercise in rats. Thus forty male rats (12 weeks old, Male Wistar) were used in the study. All subjected randomly were divided into trained (N=20) and untrained (N=20) groups. Both groups were further divided equally into two subgroups (n=10). Trained group, 1- Endurance training (ET-C–Exh) 2- Endurance training +100 mg/kg/body weight were administrated a daily dose of E vitamin for 7 weeks (ET-Exh). untrained or control group, 1- sedentary 2- sedentary + 100 mg/kg/body weight were administrated a daily dose of E vitamin for 7 weeks but did not have any exercise training. Endurance training consisted of increase graded time and speed treadmill running was conducted reaching the speed of 1.5 h -1day, 25 m/min, 5 days a week for 7 weeks, reaching the speed of 25 m/min at the end of one week and reaching the1.5 h -1day at the end of three weeks after the start of Endurance training .Results of this study showed that the effect of vitamin E supplementation on the inflammatory response to exercise is unclear. In conclusion, it is reasonable to assume that the administration of vitamins E for 7 weeks can reduce the intensity of oxidant stress by enhancing the antioxidant defense mechanism. Suppression of exertional oxidant stress had greatly minimized the destruction of erythrocytes.

Key words: Antioxidant Vitamin, Lipid Peroxidation, Aerobic Exercise.

INTRODUCTION

Exercise is associated with an increased production of free radicals and reactive oxygen species (ROS), and excess production of ROS has been linked to damage (oxidation) of lipids, proteins, and DNA through a process termed oxidative stress (Jenkins and Goldfarb 1993). Lipid peroxidation of cell membranes changes membrane integrity, leads to increased swelling, and reduces the ability of the cell to maintain ion gradients (Merry et al. 1991). This oxidative damage to cell membranes has been associated with tissue inflammation, muscle fatigue, and impaired recovery following high-intensity exercise (Pyne 1994; Abuja 2001). Erythrocytes are susceptible to oxidative damage because of their continuous exposure to oxygen and their high concentrations of polyunsaturated fatty acids and haem iron (Clemens and Waller 1987). Increased lipid peroxidation in erythrocytes after acute exhaustive treadmill exercise in rats (Senturk et al. 2001) and humans (Miyazaki et al. 2001) has been reported. Antioxidant supplementation can be of value in helping to protect against exercise-induced oxidative stress. Several studies have already examined the effect of antioxidant supplementation on exercise-induced oxidative stress. Vitamin E is a lipid-soluble antioxidant; therefore, it is found mostly in membrane structures within the cell, especially the plasma and mitochondrial membranes. Vitamin E is important in the inhibition of lipid peroxidation because it scavenges superoxide radical and hydroxyl radicals in the lipid phase (Liebler, 1993). Because it is lipid-soluble and works to inhibit lipid peroxidation, vitamin E also acts as a membrane stabilizer. It has also been implicated in the inhibition of oxygen or carbon-centered free radicals (Liebler, 1993). Many studies investigating vitamin E supplementation and exercise-induced oxidative stress have been published (Goldfarb et al., 1994; Reznick et al., 1992; Sen et al., 1997; Sunida et al., 1989). Not all studies have shown beneficial effects in part due to the dose of vitamin E, the form of vitamin E given, and the nature of the exercise. However, it is questionable if vitamin E alone can protect inhibition of lipid peroxidation in erythrocytes. The aim of this study was to investigate the effects of simultaneous 7 weeks oral supplementation with antioxidant vitamin (E) and aerobic exercise on lipid peroxidation in erythrocytes after a Bout acute exhaustive treadmill exercise in rats.
Methods:

Animals and Groups:

Forty male rats (12 weeks old, Male Wistar), fed with standard laboratory chow and water, were used in the study. Animal experimentations were approved by the Ethical Committee of the Borujerd University and carried out in an ethically proper way by following the guidelines provided.

Training and Acute Exhaustive Exercise:

All Male Wistar rats followed a familiarization treadmill protocol for one week then were randomly divided equally into two subgroups (n=10). Trained group (n=20), 1- Endurance training (ET-C–Exh) 2- Endurance training +100 mg/kg/body weight were administrated a daily dose of E vitamin for 7 weeks (ET-Exh) untrained (Control n=20) 1- sedentary 2- sedentary + 100 mg/kg/body weight were administrated a daily dose of E vitamin for 7 weeks but did not have any exercise training. Endurance training consisted of increase graded time and speed treadmill running was conducted reaching the speed of 1.5 h⁻¹day, 25 m/min, 5 days a week for 7 weeks, reaching the speed of 25 m/min at the end of one week and reaching the 1.5 h⁻¹day at the end of three weeks after the start of Endurance training. At the end of the training period, half of the rats were randomly selected into the acute exhaustive treadmill exercise group. In acute exhaustive exercise, running speed was 10 m/min (5% uphill gradient) for the first 10 min; after that the speed was increased gradually to 25 m/min (5% uphill gradient) and kept constant until the rats were exhausted. The loss of the righting reflex when the rats were turned on their backs was the criterion of exhaustion.

Determination of Erythrocyte Malondialdehyde (MDA):

At the end of the experiment, all the animals of untrained (Control n=20) group and half the animals of trained groups (n=10, each subgroup 5 rats ET-C–Exh n=5, ET-Exh n=5) before the acute exhaustive treadmill exercise and other half the animals of trained groups (n=10, each subgroup 5 rats ET-C–Exh n=5, ET-Exh n=5) after the exhaustion by acute exhaustive treadmill exercise were anaesthetized with ketamine-HCl (Ketalar, 20 mg/kg, i.p.), and the blood was collected by cardiac puncture after thoracotomy. Blood samples were collected in vacutainer tubes with K3-EDTA as anticoagulant (1/10, v/v). They were centrifuged at 3000 g for 15 min and plasma was removed using a Pasteur pipette. Then, erythrocytes were washed with 0.9% NaCl solution three times, and washed erythrocytes were haemolysed by dilution with deionized water (50-fold). Haemoglobin (Hb) values of the samples were measured using a GEN-S counter hematology analyser. The haemolysate was kept at –80° C until biochemical determinations. The erythrocyte MDA level was estimated by the method described by Jain et al. (1989) based on thiobarbituric acid reactivity. MDA, an end-product of fatty acid peroxidation, reacts with TBA to form a coloured complex that has maximum absorbency at 532 nm. For this purpose, 0.2 ml hemolysate was suspended in 0.8 ml phosphate-buffered saline (pH 7.4) and 0.025 ml butylated hydroxytoluene. Thirty percent trichloroacetic acid (0.5 ml) was added. Tubes were vortexed and allowed to stand in ice for at least 2 h. They were then centrifuged at 2000 rpm for 15 min. One millilitre of each supernatant was transferred to another tube, and 0.075 ml of 0.1 mol.l⁻¹ EDTA and 0.25 ml of 1% TBA were added. Tubes were mixed and kept in a boiling water bath for 15 min. Absorbency was read at 532 and 600 nm (600 nm reading is for preventing haemoglobin interference), after tubes were cooled to room temperature. Butylated hydroxytoluene, an antioxidant, was added to prevent MDA formation during the assay, which could result in falsely elevated TBA activity. Absorbency at 600 nm was subtracted from absorbency at 532 nm. The concentration of MDA was calculated using 1.56×10⁵ cm⁻¹ mol⁻¹, the absorbency coefficient of the MDA–thiobarbituric acid complex at 532 nm.

Statistics:

The results are expressed as means ± SE, and statistical analyses were done by one-way ANOVA with post-hoc LSD test was used for multiple comparison among means was used to compare intergroup differences. P<0.05 was accepted as significant.

Results:

The erythrocyte MDA level results are presented in Fig. 1. Erythrocyte MDA level was significantly increased after exhaustion in the all two subgroups TR animals compared with Erythrocyte MDA level before the acute exhaustive treadmill exercise in the all two subgroups TR animals and two UTR subgroup. Antioxidant treatment caused a significant decrease in TBARS levels of ET-Exh compared with ET-C–Exh animals. TBARS level was significantly higher in ET-C–Exh than in ET-Exh. The lower TBARS levels in ET-Exh subgroup indicated that erythrocytes became more resistant after the administration of vitamins E for 7 weeks.
Discussion:

The rise in oxygen utilization during physical exercise may lead to an increase in metabolic leakage of damaging free radicals of oxygen from the mitochondria into the cytosol, resulting in the formation of lipid peroxide (Davies KJA, 1982, Lowlin R, 1987, Pincemail J, 1990). The MDA production has been considered as a basic reaction in the membrane alterations due to free radical interaction with polyunsaturated fatty acids (Alessio H. Goldfarb, 1988, Kanter MM, 1988). We hereby show that erythrocyte TBARS values significantly increased after exhaustion in the all two subgroups TR animals compared with Erythrocyte MDA level before the acute exhaustive treadmill exercise in the all two subgroups TR animals as well as two Control subgroup. Smith et al. 1995, showed that a single episode of submaximal exercise enhanced the exposures of erythrocytes to oxidative and osmotic stresses, resulting in erythrocyte deterioration. We show that fortifying antioxidant defense mechanisms with 7 weeks of treatment with vitamin E results in reduced erythrocyte deterioration in relation to decreased oxidant stress after exhaustive running episodes in ET-Exh compared with ET- C-Exh animals subgroup Fig. 1. To examine the effect of exhaustive exercise on erythrocytes with enhanced antioxidant defense, rats were given vitamins E for 7 weeks before exhaustive exercise. Vitamin therapy resulted in significant decrements in TBARS levels measured after exhaustion (ET-Exh). Additionally, after antioxidant vitamin therapy, the resistance of erythrocytes from these rats against free radicals induces of exhaustive exercise, thereby reducing the free radicals and TBARS levels. Since vitamin E has been found to protect cellular membranes from lipid peroxidation, studies have focused on the ability of vitamin E supplementation to reduce the increase in oxidative stress caused by exercise. A beneficial effect of vitamin E supplementation on exercise induced oxidative stress seems to be supported by the current literature (Goldfarb, 1999). Exercise induced oxidative stress has been shown to be reduced in both humans and animals as a result of vitamin E supplementation (Sumida et al., 1989; Reznick et al., 1992; Goldfarb et al., 1994; Sen et al., 1997). Conversely, this beneficial effect has not been found in other studies (Boyer et al., 1996; Siciliano et al., 1997). In 1989, Sumida et al. reported that subjects who ingested 300 mg vitamin E/day for 4 weeks was shown to reduce the increase in MDA in response to strenuous exercise (Sumida et al., 1989). Niess et al. (2000) examined the effect of 28 days of 500 IU vitamin E/day in a double blind, placebo controlled, cross-over study on exercise induced cytoplasmic expression of inducible nitric oxide synthase (iNOS) and antioxidant stress protein heme oxygenase-1 (HO-1) in leukocytes. Induction of these proteins may depend on increases in free radicals and cytokines, which are both altered by exercise. The results showed that a 30 min exhaustive treadmill exercise induced expression of iNOS and HO-1 but this change was not altered by vitamin E supplementation. At present, the effect of vitamin E supplementation on the inflammatory response to exercise is unclear. In conclusion, it is reasonable to assume that the administration of vitamins E for 7 weeks can reduce the intensity of oxidant stress by enhancing the antioxidant defense mechanism. Suppression of exertional oxidant stress had greatly minimized the destruction of erythrocytes.

REFERENCES


