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THE EFFECTS OF VITAMIN E TREATMENT ON TRACHEAL REACTIVITY AND SOME BIOCHEMICAL PARAMETERS IN DIABETIC RATS

DİYABETİK SIÇANLARDA, VİTAMİN E TEDAVİSİNİN TRAKEAL REAKTİVİTE VE BAZI BİYOKİMSAL PARAMETRELER ÜZERİNE ETKİSİ

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ABSTRACT

The present study investigated the effect of vitamin E treatment on the alterations in reactivity of trachea and some biochemical parameters in diabetic rat. Rats were randomly divided into two groups: Control and diabetic. Diabetes was induced by a single dose injection of streptozotocin (STZ; 60 mglkg ;i.p.). Diabetic rats were divided into two groups as untreated and treated with vitamin E. Vitamin E was given as a high-vitamin E diet in the standard laboratory chow diet (DL-_ tocophenyl acetate 0.5 % w/w) for 8 weeks started 8- week after induction of diabetes. In Vitamin E treated diabetic rats, the increased plasma triglyceride and thiobarbituric reactive substance levels due to diabetes were reduced without any effect on hyperglycemia. Vitamin E significantly restored the increased contractile responses to acetylcholine (ACh) and potassium chloride (KCl) in diabetic trachea. It is concluded that vitamin E treatment restored increased lipid peroxidation and tracheal reactivity in diabetic rat thus Vitamin E treatment may have beneficial effect on diabetic complications.

Key Words: *Diabetes, trachea, Vitamin E, rat*

ÖZET

Bu çalışmada, vitamin E tedavisinin, diyabetik siçanlarda trakea reaktivitesindeki değişiklikler ve bazı biyokimyasal parametreler iizerindeki etkileri araştırılmıştır. Erkek siçanlar kontrol ve diyabetik olmak üzere rastgele iki gruba ayrılmıştır. Diyabet, tek doz streptozotosin enjeksiyonu (STZ; 60 mglkg i.p.) ile yapılmıştır. Sonrasında diyabetik sıçanların bir kısmına, vitamin E tedavisi uygulanmıştır. Vitamin E, standart laboratuvar yemine (DL-_ tocophenyl asetat 0.5 % w/w) katılarak, diyabet

oluştuktan 8 hafta sonra 8 hafta süre ile verilmiştir. Diyabetik sıçanların Vitamin E ile tedavisi, diyabette artan plazma trigliserid ve tiyobarbitürik asit reaktif substans düzeylerini, hiperglisemi üzerine bir etkisi olmaksızın azaltmıştır. Vitamin E, diyabetik trakeada ise artmış asetilkolin (Ask) ve potasyum klorür(KCl) yanıtlarını düzeltmiştir. Bu çalışmanın sonuçları, vitamin E tedavisinin diyabetik sıçanlarda artan lipid peroksidasyonu ve trakea reaktivitesini düzelterek, diyabetik komplikasyonların tedavisinde yararlı etkileri olduğunu göstermektedir.

Anahtar kelimeler: *Diyabet, trakea, vitamin E, sıçan*

INTRODUCTION

Diabetes is a systemic disease that produces some alterations in the structure and function of several tissues with complications such as cardiomyopathy, neuropathy and retinopathy (1,2, 3). The underlying mechanisms of diabetic complications are multifactorial; the various pathogenic factors are interrelated and together contribute to the development and progression of the syndrome. Among these factors, oxidative stress may play a majör role in the pathogenesis of complications and due to excessive production and/or inadequate elimination of free radicals by antioxidant systems in diabetes (4, 5, 6). The deleterious effects of oxidative stress and lipid peroxidation are prevented by non-enzymatic (Vitamin E, Vitamin C, and Vitamin A) and enzymatic (catalase, superoxide dismutase glutathione peroxidase) antioxidant defense systems in diabetes (7, 8, 9). it has been shown that the levels of both antioxidant systems are altered in diabetes. in recent years much attention has been focused on the beneficial role of antioxidant treatment on preventing the various diabetic complications (10,11,12).

Vitamin E, is lipophilic, nonenzymatic antioxidant, acts in membranes or lipoprotein particles by scavenging lipid peroxyl radicals and inhibiting of lipid peroxidation (13,14). it has been reported that vitamin E treatment reduces the severity of diabetic complications and protects tissues in animal and human against oxidative damage in diabetes (15,16,17).

The present study investigated the alterations on the contractile responses of diabetic trachea to some agonists and whether dietary vitamin E treatment affects altered responses. Changes in some biochemical parameters were also measured.

MATERIAL AND METHODS

Treatment ofanimals

Wistar male rats (200 - 250 g) were used and were randomly divided into two groups: Control and diabetic. Diabetes was induced by a single injection of streptozotocin (STZ; 60

mg/kg ;i.p.). 2 day after the injection, development of diabetes was confirmed by measuring blood glucose levels from tail vein. Rats with blood glucose levels of > 150 mmol/1 were considered to be diabetic. Diabetic rats were divided as untreated diabetic and treated with vitamin E. Vitamin E was given as a high-vitamin E diet added in the standard laboratory chow diet (DL-_ tocophenyl acetate 0.5 % w/w) for 8 weeks, starting 8-week after, induction of diabetes. During the experimental periods (16 weeks), the rats fed with food and water ad libitum.

Biochemical analysis:

Blood samples were taken from cardiac puncture and plasma was used for determination of lipids, thiobarbituric acid reactive substances (TBARS) and stored at -70 °C until assayed. Blood glucose concentrations were measured by an Accutrend Glucometer (Boehringer, Manheim, Germany). Plasma triglycerides were determined with an automatic analyzer by using a commercially available enzyme kit (Wako, Osaka, Japan). TBARS reactivity is the most widely used method for assessing lipid peroxidation (18).

Trachea preparation

The trachea was excised, rapidly placed in physiological solution (PSS) and carefully cleaned excess connective tissue, to avoid possible differences in reactivity due to localization, tracheal spiral strips were prepared from the larynx to 3-4th cartilage. The strips were suspended horizontally between stainless hooks in 10 ml organ baths, filled with PSS of the following composition in (mol/1): NaCl 118.0; KC1 7.4; $CaCl_2$ $2H_20$ 2.5; KH_2P0_4 1.2; $MgS0_4$ $7H_20$ 1.2; $NaHC0_3$ 25 and glucose 10.0. The PSS was aerated with 95% 0_2 - 5% $C0_2$ at 37°C. Each strip was connected to a isotonic transducer (Ugo-Basile, No 7004). Changes in isotonic tension were recorded on a microdynamometer (Ugo Basile No 7050). The trachea was allowed to equilibrate for 60 min under a resting tension of lg. During this period, bath solution was replaced every 10 min. At the end of equilibration period, concentration-response curves to cumulative concentrations of acetylcholine (ACh; 10^{-7} - 10^{-2} M) were performed on each preparations. After maximum contraction reaching plateau, each ring was serially washed to baseline and equilibrated and then concentration-response curve of KC1 (10 - 50 mM) was obtained to evaluate tracheal reactivity.

Drugs

All chemicals except Vitamin E used in the experiments were purchased from Sigma Chemical (ST. Louis, MO, U.SA) and dissolved in saline. Vitamin E was given as a gift from Roche Company Istanbul, Turkey.

Statistical analysis

Results are expressed as the mean ± SEM. The changes in contractility to ACh and KC1 were calculated as a percentage of control values. The sensitivities to the agonists were

evaluated as the pD_2 (-log EC_{50}). Statistical analysis was carried out using one-way analysis of variance followed by Neuman-Keul's test. Results were considered significantly different if $p<0.05$.

RESULTS

Metabolic parameters

Blood glucose and plasma triglyceride levels were significantly increased in diabetic rats. Plasma triglyceride levels of diabetic rats were reduced with the treatment of Vitamin E. Vitamin E-treated diabetic rats were still hyperglycemic. Plasma TBARS levels were increased in diabetic rats than control rats. Vitamin-E treatment partially normalized the increased TBARS levels in diabetic rats (Table **1).**

Table 1: Some of biochemical characteristics of experimental groups.

Characteristics	Control (n=7)	Untreated diabetic (n=9)	Vitamin-E-treated diabetic (n=10)
Final body weight (gr)	395 + 9	272 ± 8#	285 ± 12#
Blood glucose (mmol/L)	96 ±4	482 ± 10'	475 ± 7'
Plasma triglyceride (mmol/L)	42 ±6	165 ± 4*	78 ±8*+
Plasma TBARS (nmol/ml)	0.72 ±0.03	1.58 ±0.05'	0.96 ± 0.02*+

Values are means ± SEM. n = number of animals. *p <0.05, *p<0.001 significantly different from control rats; +p <0.01 significantly different from diabetic rats.

Tracheal responses to agonists

Cumulative applications of ACh (10^{-7} - 1(T^2 M) and KC1 (10 - 50 raM) to the isolated organ bath resulted the increased contractions in a dose-dependent manner. The maximum responses to ACh and KC1 were increased in tracheal preparations from untreated diabetic group, compared to their age- matched controls. Vitamin E treatment restored the increased contractile responses to both ACh and KC1 in diabetic group (Figure 1 A, B respectively).

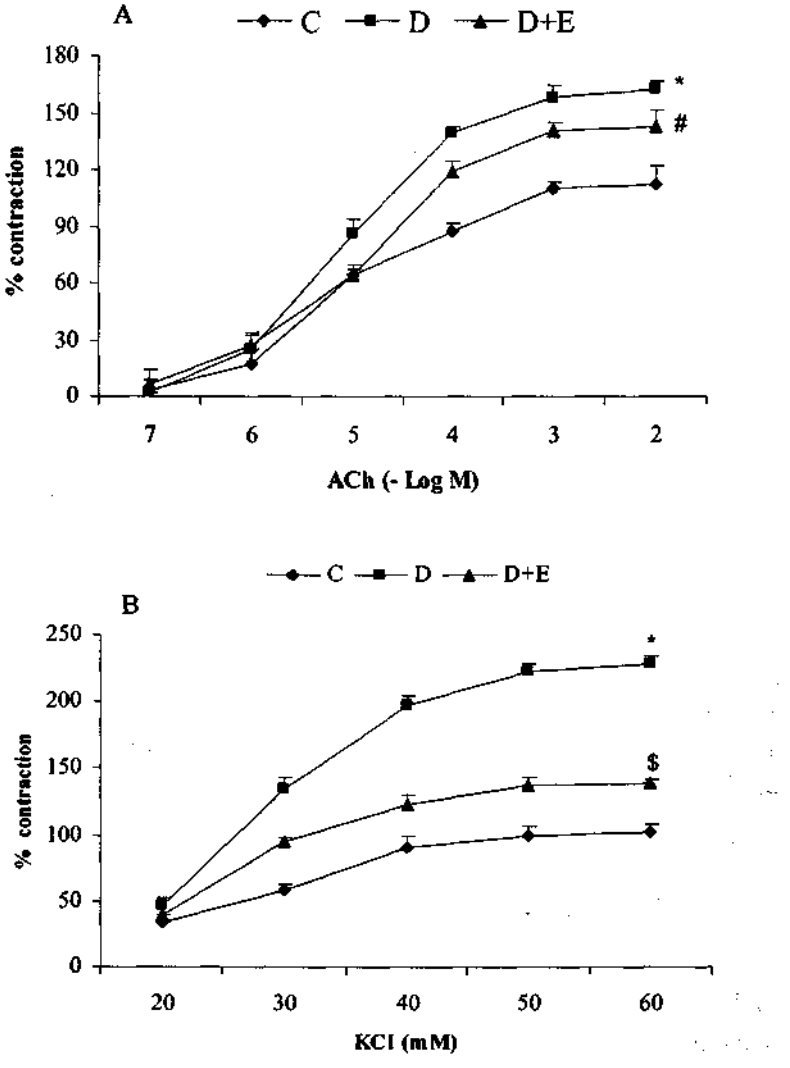

Figure 1: Concentration-response curves for Ach (A) and KC1 (B) induced-contractions of trachea strips obtained from control (C), diabetic (D) and Vitamin E-treated diabetic rats. .*p<0.001, *p<0.05 significantly different from control rats; $p< 0.01 significantly different from diabetic rats.

pD_2 values (agonist affinity) to ACh and KC1 were not significantly different in all groups. The maximal contractile responses and pD_2 values to ACh and KC1 are summarized in Table 2.

Table 2: pD_2 values for ACh and KC1 in tracheas from control, untreated-diabetic, and vitamin E- treated diabetic groups. pD_2 values are expressed as - log M.

Groups	ACh	KC1
Control	4.92 ± 0.003	1.35 ±0.002
Untreated-diabetic	4.90 ±0.002	1.36 ± 0.006
Vitamin E- treated- diabetic	4.89 ±0.003	1.33 ±0.004

DISCUSSION:

The results of present investigation revealed that the alterations of tracheal reactivity were accompanied with some biochemical changes in diabetic rats. The contractile responses to ACh and KC1 are increased in diabetic trachea. Blood glucose, plasma triglyceride levels and TBARS are high in diabetic rats compared to control rats. It can be suggested that oxidative stress and dyslipidemia may play a role in the impaired contractile responses in diabetic trachea. Treatment with vitamin E not only restored the contractile responses of trachea but also improved the increased blood lipids and lipid peroxidation.

Alterations of functional effects on pulmonary system particularly the in trachea have been reported a few in diabetes. Results of these studies were controversial with regard to sensivity and the maximal contractile responses to cholinergic agonists. Cros et al showed that the supersensitivity and hyperresponsivity to the cholinergic agonist were developed after 4 months diabetes induction and the parameters were not changed after 5 weeks in diabetic trachea (19). Previously we have been shown that the contractile responses to ACh were decreased in 5-6 week-diabetic rat trachea but the contractions were increased in 11-12 week-diabetic rats (20). The mechanisms by which alter of the contractile responses to agonists is not known clearly. The discrepancies between the results of the studies may be related to duration and severity of diabetes. This view was further supported by Cros et al who proposed that there is a major time-dependence in tracheal responsiveness during diabetes (19). They suggested that these alterations may be related to diabetes-induced vagal autonomic neuropathy, but based on the binding studies of cholinergic receptors using the antagonist ligand, they did not detect any change in diabetic tracheas compared to control ones. (19). Although we did not investigate the effect of diabetes on the binding characteristics of muscarinic receptors in rat trachea, the increased contractilities to ACh and KC1 in diabetic tracheas without any change in the sensitivity (pD_2) indicate that the hyper-responsiveness may be dependent to the alterations in

the post- receptor regulatory mechanisms other than impairment in receptor activation. In diabetes the increased contractile responses of trachea to ACh and KC1 are related with an increase of intracellular Ca^{+2} concentrations. It is well known that contractions of KC1 are mediated by a Ca^{+2} influx through voltage-dependent Ca^{+2} channels, contractions of ACh are mediated through both voltage-dependent Ca^{+2} channels and released of stored Ca^{+2} from intracellular stores by inositol triphosphate (IP_3) pathway through protein kinase C (22, 23, 24). It has been shown that oxidative stress is responsible for the increased contractile responsiveness of diabetic vasculature by increasing diacylglycerol -protein kinase C (DAC-PKC) activity which stimulates IP_3 induced Ca^{+2} release from intracellular organelles leading to intracellular Ca^{+2} accumulation in diabetes mellitus (25, 26). In addition, similarly it has been reported that oxidative stress-induces alterations in both voltage-dependent and receptor operated calcium channel activities in aorta, in smooth muscle cells and platelets of diabetic rat (26, 27). During diabetes, persistent hyperglycemia causes an excessive endogenous formation of free radicals apparently overcomes cellular antioxidant defense mechanisms, resulting in free radicals-initiated modification of lipids, proteins, carbohydrates and DNA (28). One of the commonly findings of the oxidative stress is the increased production of malondialdehyde as TBARS levels which has been found in the plasma and various tissues of diabetic patients and animals (29, 30, 31, 32). In agreement with previous studies, we also found that TBARS levels of plasma were increased in diabetic group compared with control group. This indicates that oxidative stress was increased in diabetic rats .

In the present study, blood glucose level in the diabetic group were found unaffected by vitamin E which is in agreement with the other findings (33, 34). In addition, Vitamin E treatment significantly decreased the enhanced contractility in diabetic trachea accompanied with decreased lipid peroxidation. In addition, similar results in previous studies have been reported in vasculature in diabetes (16, 35). On the other hand, it was reported that Vitamin E treatment reduces the contractile responses of trachea to various agonists in experimentally oxidative stress induced rats (36, 37, 38). Vitamin E is a membrane-bound, lipid soluble, non-enzymatic antioxidant in the body (37, 38). It seems to have a particularly important role in protecting cell membranes from the detrimental effects of oxidative stress. In number of free radical-initiated processes in membranes such as lipid peroxidation are inhibited by vitamin E and its deficiency exacerbates to prevent biological membranes against oxidative injury (39). A great number of studies have been supported that vitamin E treatment can improve the imbalance between oxidative and antioxidant status in various tissue types in diabetes (39, 40, 41, 42). It was also shown that Vitamin E restored the accumulation of Ca^{+2} and inhibition of Ca^{+2} - ATP-ase activity via inhibition of the DAC-PKC pathway in heart and kidney in diabetes (26,43,44).

The results of this study indicate that the restoring effect of Vitamin E on diabetic trachea might due to it's antioxidant effect. In conclusion, Vitamin E may have a beneficial effect in treatment of various complications of diabetes.

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