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ORIGINAL ARTICLE

Decreased blood pressure with a corresponding decrease in adhesive molecules in diabetic rats caused by vitamin E administration

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Abstract

Background: Hypertension is one of the important clinical problems of diabetic cardiovascular disease. The aim of this study was to determine the effect of vitamin E on blood pressure parameters and adhesive molecule amounts in diabetic rats.

Methods: Twenty-four male Wistar rats were divided into three groups (each of $n = 8$): the controls (C), non-treated diabetic (NTD), and vitamin E treated diabetic (VETD) groups. A single intraperitoneal injection of buffered streptozotocin (60 mg/kg) in cold sodium citrate (pH 4.5) was used to induce diabetes. The VETD group received 300 mg of vitamin E daily intragastrically for 6 weeks. Systolic and diastolic blood pressure, mean arterial pressure, as well as the dicrotic pressure, crest time, systolic and diastolic periods, and plasma levels of intercellular adhesion molecule-1 and E-selectin were measured after 6 weeks.

Results: The results revealed that there was a significant increase in systolic and diastolic blood pressures, mean arterial pressure, crest time, systolic duration, and the amount of sICAM-1 and E-selectin in diabetic rats. There was no significant difference in the heart rate or cardiac cyclic duration among the different groups. Significant improvement of blood pressure parameters as well as attenuation of the elevated ICAM-1 and E-selectin amounts was found in the vitamin E treated group.

Conclusions: These findings indicate that vitamin E significantly improved blood pressure elevation in diabetic rats and that these effects could be associated with reducing adhesive molecule and antioxidant properties of vitamin E.

Keywords: adhesive molecule, blood pressure, diabetes mellitus, rat, vitamin E.

The significant findings of the study: In diabetic rats, crest time and crest time ratio were significantly elevated, but the interval between the first and reflected peaks (T_{DVP}) were significantly lower than controls. A significant elevation of ICAM and E-selectin was also found in diabetic rats. Administration of vitamin E in diabetic animals not only relieved diabetes-induced waveform changes, but also significantly alleviated the elevation of the adhesive molecule.

This study adds: Strong evidence of the usability of pressure waveforms and their alterations in diabetic rats, possibly related to the progressive process of atherogenesis and increasing blood vessel stiffness due to the significant elevation of adhesion molecules. Further support for the alleviating effect of vitamin E in diabetic animals, which strongly suggests the role of oxidative stress-related processes in the complications of diabetes.

ASIA TRACK

Introduction

Despite the controversial underlying pathophysiology of type 1 and type 2 diabetes mellitus, co-existence of hypertension and atherosclerosis in diabetic patients occurs very frequently.[1,2] Our understandings on the mechanisms underlying diabetes-accelerated atherosclerosis are still insufficient to sort out all the pathogenic enigmas. Hyperglycemia and the consequent production of advanced glycation end-products, increased levels of reactive oxygen species, and lipid abnormalities, such as increases in the levels of very low-density lipoproteins and their remnants, all may play a role in the progression of diabetes-accelerated lesions.[3,4]

Monocyte adhesion to the endothelium is one of the initiating steps in the formation of atherosclerotic lesions and it will be followed by entry into the vessel wall and eventual differentiation into macrophages. Characteristic foam cells are the immigrated macrophages that take up the excess modified low-density lipoproteins (LDL) by the scavenger receptors, and it has been recently reported that the proliferation of macrophage-derived foam cells is a critical event in the development of atherosclerotic lesions.[5,6] Adhesion molecules are a heterogeneous family of immunoglobulins, selectins, cadherins, and mucin-like molecules which play crucial roles in cell-to-cell interactions.[7] The presence of several adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and endothelial-leukocyte adhesion molecule-1 (E-selectin), have been identified on endothelial cells and on the cells in human atherosclerotic lesions.[8,9] Enhancement of the monocyte adhesion to the endothelium in the presence of hyperglycemia has been proven in experimental and *in vitro* studies.[10] The reported coexistence of increased expression of adhesive molecules such as ICAM-1 and increased intimal accumulation of leukocytes in both humans and experimental animals implicates the abundance of adhesion molecules on the arterial sites prone to the development of atherosclerotic lesions.[11,12]

Arterial stiffness is an additional indicator of the severity and progression of atherosclerosis, and it could be assessed using contour analysis of pulse waves as a simple and economic method.[13] Pulse waveforms and their abnormalities carry information about the morphology and functionality of the cardiovascular system that could be used in clinical practice and laboratory animal experiments to assess endothelial dysfunction.[14,15] The pulse wave is composed of the systolic and diastolic components, and an increased boost in the velocity of the aortic blood flow in the stiffened arterial alters both components of the wave form.[16] Pulse wave, crest time, reflecting index, and dicrotic pressure are reported as reliable indices for determining the stiffness of large arteries.[17,18]

In line with our earlier efforts on the elucidation of the role of oxidative stress and ameliorative effect of the antioxidants in the diabetic organopathy,[19–21] the current study was designed to scrutinize the effect of vitamin E on blood pressure parameters and adhesion molecule amounts in diabetic rats.

Materials and methods

"Principles of Laboratory Animal Care"[22] and the specific rules provided by the local animal care and use committee were followed in all procedures utilizing rats. Twenty-four 6-month-old male Wistar rats were included in this study. Their initial body mass was approximately 210 ± 20 g. The rats were assigned to three groups of eight animals each: control (C), non-treated diabetic (NTD), and vitamin E treated (VETD) groups. A single intraperitoneal (IP) injection of buffered streptozotocin (STZ) (60 mg/kg) in cold sodium citrate (pH 4.5) was used to induce diabetes in 16 rats. Control rats received an equivalent amount of the buffer. Hyperglycemia was proved after 48 h of STZ injection by measuring the venous blood glucose level in the tail of each animal using the glucose oxidase-based biosystem kit (Biosystems, Barcelona, Spain). Rats were assigned as diabetic if the blood glucose was >300 mg/dL.[23] Rats in the VETD group received a nontoxic dose equal to 300 mg of vitamin E (Merck, Darmstadt, Germany) intragastrically by gavage. The control and NTD groups were treated with vehicle only (tap water). Foods were supplied *ad libitum* to all groups throughout the experiments. After 6 weeks, an IP injection of ethyl carbamate (urethane) (1 mg/kg) was selected as an anesthetic agent without possible confounding effect on the measured parameters, and pinching a hind paw was used to assess the depth of anesthesia.[24]

Arterial blood pressure monitoring

Arterial blood pressure was directly recorded from the carotid artery. Briefly, the rats were anesthetized with urethane and were placed on a temperature-regulated table in a supine position. A polyethylene catheter was inserted into the left carotid artery for blood pressure measurement. The catheter was filled with normal saline and heparin (75 IU/mL). The catheter was connected to a pressure transducer and a physiograph (Narco Bio-Systems, Austin, TX, USA) was used to measure blood pressure after a 15-min stabilization

period. The pressure catheter was calibrated prior to each experiment. All data were recorded and stored digitally. Data were analyzed using POWERLAB software (ADInstruments, Bella Vista, NSW, Australia).

Blood samples

Blood samples were collected directly from the carotid artery, mixed with ethylene diamine tetra acetic acid (EDTA) as an anticoagulant, and centrifuged at 3000 *g* for 10 min within 30 min of collection. The plasma samples were aliquotted and stored at −80°C without repeated freeze–thaw cycles.

Biochemical assays

ICAM-1

Rat ICAM-1 (or CD54), a type I transmembrane glycoprotein, was measured in the plasma samples using a rat soluble-ICAM (sICAM)-1/CD54 immunoassay kit (RIC100; R&D Systems, Minneapolis, MN, USA). This assay employs the quantitative sandwich enzyme immunoassay technique with a specific monoclonal antibody for rat sICAM-1. Briefly, a 50-fold dilution with Calibrator Diluent RD5-26 (1×) was prepared prior to the assay; then, Assay Diluent RD1-21 (50 μL) was mixed with standard, control, or sample (50 μL) in separate wells and covered with the adhesive strip. The plates were incubated for 2 h at room temperature on a horizontal orbital microplate shaker at 500 rpm. Rat sICAM-1 conjugate (100 μL) was added to each well after washing five times with wash buffer, and the plates were covered with the adhesive strip. The aspiration/wash steps were repeated after incubation for 2 h at room temperature on the same horizontal orbital microplate shaker. Substrate solution (100 μL) was added to each well after the aspiration/wash steps and the plate was incubated for 30 min at room temperature. Finally, stop solution (100 μL) was added to each well. The optical density of each well was determined within 30 min, using a microplate reader set to 450 nm.

Soluble endothelium selectin

Rat soluble endothelium-Selectin (sE-Selectin), an early mediator of leukocyte-endothelial adhesion, is a marker of activated endothelium. Soluble E-selectin is present in the supernatant of cytokine-activated endothelial cells, and elevated serum levels are found in a variety of inflammatory conditions.[8,9] An antibody specific to sE-Selectin was measured in plasma samples using a sE-selectin ELISA Kit (E0549r; Uscn Life Science, Wuhan, China).

Briefly, 100 μL of standard, control, or sample was added per well, covered with the adhesive strip, and incubated for 2 h at 37°C. The liquid in each well was removed and 100 μL of Detection Reagent A were added without wash. The aspiration/wash steps were repeated five times after 1 h of incubation at 37°C. Detection Reagent B (100 μL) was added to each well and covered with a new adhesive strip. The aspiration/wash steps were repeated five times after 1 h of incubation at 37°C. Substrate solution (90 μL) was added to each well and was incubated for 30 min at room temperature away from the light. Finally, 50 μL of stop solution was added to each well, and the plate was gently taped to ensure thorough mixing. The optical density of each well was determined within 30 min, using a microplate reader set to 450 nm. The detection range of the kit was 78–5000 pg/mL.

Statistical analysis

Data were expressed as the mean ± SEM. One-way analysis of variance (ANOVA) with pairwise comparisons according to the Tukey method was used in this study. Differences were considered significant if $P < 0.05$.

Results

Figure 1 and Table 1 show the blood pressure parameters in the different experimental groups. As shown in Table 1, the systolic and diastolic blood pressures, mean arterial pressure and dicrotic pressure were significantly higher in the NTD group compared to the control group ($P < 0.05$). There was no significant difference between the VETD and the control rats, or in the heart rate among groups. Pulse pressure in the NTD group was significantly higher than in the control group ($P < 0.05$), but no significant difference was found between the VETD and control groups ($P > 0.05$). There was no significant change in cycle time or duration value among the different groups ($P > 0.05$). The systolic time fraction of the cycle time in the NTD group showed a significant increase compared to the control group ($P < 0.05$), but the diastolic time of the NTD group was significantly lower than that of the control group ($P < 0.05$). There were no significant differences between the systolic and diastolic times of the VETD and control groups. Crest time (CT), which is the time interval from the foot to the peak of a pulse wave, showed significant elevation in the NTD group compared to the control group ($P < 0.05$), but there was no significant differences between the VETD and control groups (Fig. 1). The

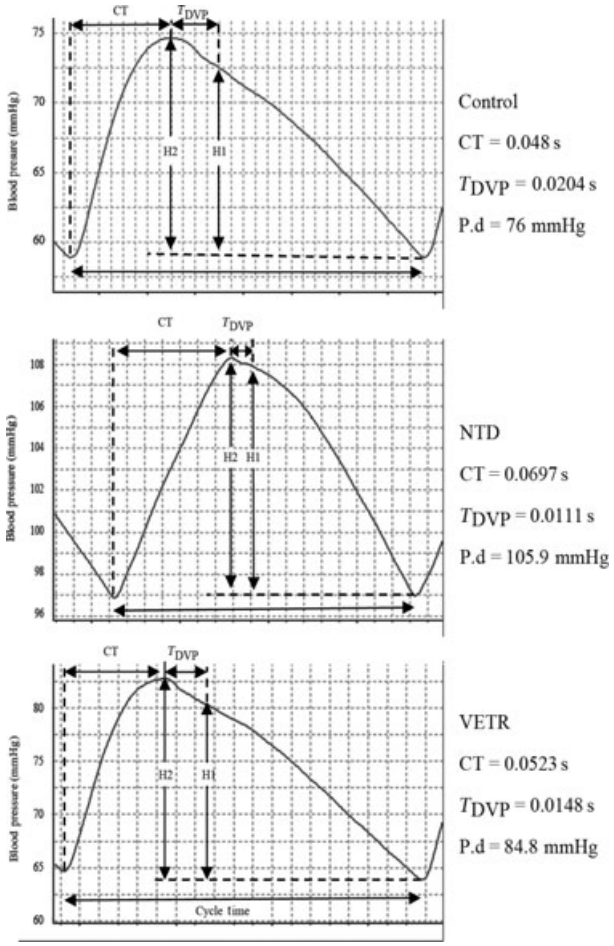

Figure 1 Effect of vitamin E on the diabetes induced waveforms. Crest time (CT), time interval from the foot point of wave to the first peak in milliseconds. DVP, digital volume pulse; H1, amplitude of the second peak; H2, amplitude of the first peak; P.d, dicrotic pressure; T_{DVP}, time difference between first and second peak in milliseconds.

crest time ratio (CT/R) or crest time/cycle time showed a significant increase in the NTD group compared to the control group ($P < 0.05$). The CT/R in the VETD rats significantly decreased in the VETD rats compared to the NTD rats ($P < 0.05$), but it was still significantly higher than in the control rats ($P < 0.05$). The interval between the first and reflected peaks (T_{DVP}) was significantly shorter in the NTD group than in the control group ($P < 0.05$), but it was restored in the VETD rats as compared to the control group (Fig. 1 and Table 1). The amplitude of the first peak (H_2) and second peak (H_1) showed a significant decrease in the NTD and VETD groups compared to the control group ($P < 0.05$) (Table 1).

As shown in Fig. 2a, the plasma levels of sICAM-1 in the control, NTD, and VETD groups were 81.28 ± 6.36, 879.71 ± 26.11, and 377.73 ± 38 pg/mL, respectively. Therefore, the level of sICAM-1 in the NTD group was drastically higher than in the control group ($P < 0.05$). Vitamin E treatment ameliorated sICAM elevation significantly compared to the NTD group, but there are still significant differences between the control and VETD groups ($P < 0.05$).

The plasma levels of E-selectin in the control, NTD, and VETD groups were 75.57 ± 11.81, 168 ± 7.58, and 79.25 ± 3.41 pg/mL, respectively. A significant difference was found between the NTD and control groups ($P < 0.05$), but no significant difference was found between the VETD and control groups ($P > 0.05$) (Fig. 2b).

Discussion

Significant alterations of the pressure waveforms, such as elevated mean arterial pressures, as well as elevated systolic and diastolic blood pressures, are risk factors of atherosclerosis that were observed in the diabetic group. Waveform contour analysis has gained further popularity in recent years as it is relatively inexpensive, easy to operate, and can yield useful biological information in the development of cardiovascular disease.[13,25,26] Besides significantly increased systolic and diastolic blood pressures and mean arterial pressure, we found a significant increase in the dicrotic or reflected pressure. It has been reported that dicrotic notch height elevation is an evidence of diminished nitric oxide (NO) production, and altered NO synthesis initiates subsequent alterations in the physical and geometrical properties of vessels.[18] Recent studies of the pulse wave in the human finger and the rabbit auricular artery have also proved that alteration of the NO pathway correlates with the changes of the relative height of the dicrotic notch.[27,28] NO has an important vasodilatory effect on vessel diameters, tone, resistance, and compliance of vasculature.[27] Crest time and the crest time ratio are accurate markers of arterial stiffness that can be simply determined by subtracting the foot point from the easily discernible duration to reach the systolic peak.[29] Our results showed that crest time and the crest time ratio were significantly elevated in the diabetic group compared to the control rats. Results of previous studies have showed that crest time and the crest time ratio are markedly prolonged in the aged population with atherosclerosis.[29] We found that the interval between the first and reflected peaks (T_{DVP}) in the NTD group were significantly lower than in the controls. Significant decreases of T_{DVP} in middle-aged, hypertensive diabetic subjects compared to young normotensive subjects has reported

Table 1 Effect of vitamin E on diabetic induced changes in blood pressure parameters

	Control (n = 8)	NTD (n = 8)	VETD (n = 8)
SBP (mmHg)	82.7 ± 7.1	106.8 ± 2.3*	91.2 ± 3.4**
DBP (mmHg)	63.9 ± 7.4	95.0 ± 2.6*	79.4 ± 4.5**
MAP (mmHg)	70.2 ± 7.3	99.3 ± 2.5*	83.3 ± 4.3
PP (mmHg)	18.8 ± 1.3	13.1 ± 1.0*	11.7 ± 1.0
HR (bpm)	357.6 ± 26.4	362.1 ± 9.6	388.0 ± 7.1
Cycle duration (s)	0.177 ± 0.019	0.169 ± 0.004	0.158 ± 0.002
Crest time (s)	0.048 ± 0.005	0.069 ± 0.002*	0.052 ± 0.003**
CTR	0.272 ± 0.011	0.412 ± 0.009*	0.332 ± 0.014**
T_{DVP} (s)	0.0204 ± 0.0030	0.0111 ± 0.0006*	0.0148 ± 0.0003
H_1 (mmHg)	14.9 ± 2.1	11.0 ± 1.2	8.6 ± 1.0
H_2 (mmHg)	18.8 ± 1.3	13.1 ± 1.0*	11.7 ± 1.0
Dicrotic pressure (mmHg)	76.0 ± 6.8	105.9 ± 2.7*	84.8 ± 2.4**
ST (s)	0.0561 ± 0.0010	0.0729 ± 0.0028*	0.0588 ± 0.0036**
DT (s)	0.1192 ± 0.0130	0.0946 ± 0.0019*	0.0994 ± 0.0036

Values are given as mean ± SEM. *P < 0.05 compared with the control group; **P < 0.05 compared with the non-treated diabetic (NTD) group.

CTR, crest time ratio; DBP, diastolic blood pressure; DT, diastolic time; H_1, amplitude of the second peak; H_2, amplitude of the first peak; HR, heart rate; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; ST, systolic time; T_{DVP}, time difference between first and second peak; VETD, vitamin E treated diabetic (group).

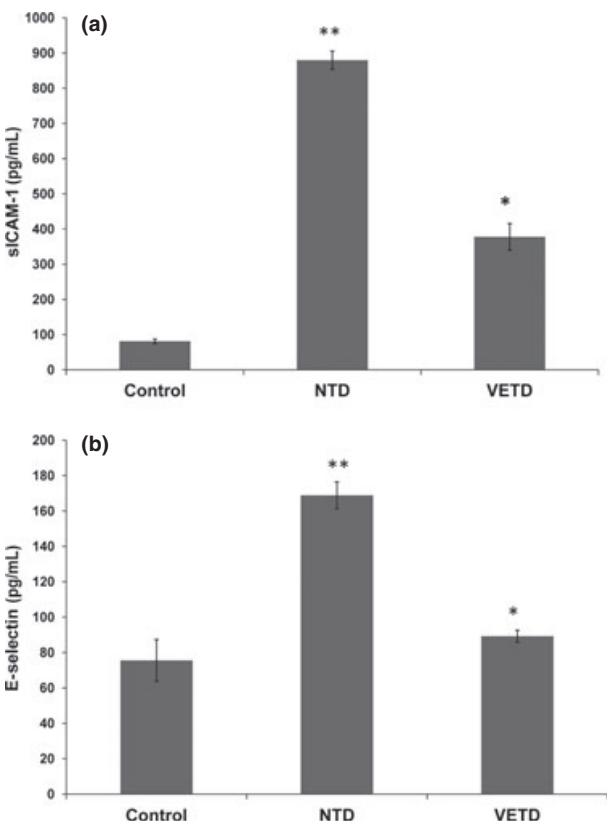

Figure 2 Effect of vitamin E on the plasma level of (a) soluble intercellular adhesion molecule-1 and (b) E-selectin. Values are given as mean ± SEM. *P < 0.05 compared with the control group; **P < 0.05 compared with the non-treated diabetic (NTD) group. VETD, vitamin E treated diabetic (group).

by Woodman and Watts,[30] which is additional evidence for diabetes-induced artery stiffness and atherosclerosis. Although we didn't find a significant change in the heart rate duration in the different groups, the systolic time showed a significant increase in the diabetic group, as well as a decrease in the diastolic time. It is an indicative of an abnormal injection of blood from the heart to the vessels due to increasing blood vessel stiffness.

Adhesion molecules such as ICAM and E-selectin are indicators of endothelial dysfunction and their expression on the surface of the vascular endothelium in response to lesions plays an important role in atherosclerosis.[31] ICAM-1 enables the adhesion and transmigration of inflammatory cells to the vascular wall.[32] The soluble forms of these molecules in plasma can be considered as a marker of atherogenesis and endothelial dysfunction.[33] In particular, E-selectin, an adhesion molecule exclusively expressed on endothelial cells, is an independent predictor of cardiovascular disease incidence.[34] In the current study, a significant elevation of ICAM and E-selectin was also found in the diabetic group. In addition, the impact of hyperglycemia-related glycation and increased oxidative stress in atherogenesis and endothelial dysfunction has been reported.[34] It is believed that the oxidative modification of LDL-induced by diabetes is a key initiating mechanism of atherosclerosis, in which the accumulation of oxidized LDL on the vascular wall, the activation of macrophages and endothelial cells, and the impairment of the physiologic action of nitric oxide

have been identified.[35–39] The preventive effect of vitamin E on oxidative stress-induced disorders in diabetic animal complications have been discussed in several publications by ourselves and others.[19–21,40] Results of the current study have also shown that administration of vitamin E to diabetic animals not only improved diabetes-induced waveform changes, but also significantly alleviated the elevation of the adhesive molecule. While no direct correlation was found between vitamin E treatment and blood pressure, the observed effects of vitamin E provide further support for the role of oxidative stress in the pathophysiological processes of diabetes and the potent antioxidant property of vitamin E, which consequently holds back the accumulation of free radicals or toxic materials and the subsequent induction of arterial wall dysfunction.[41]

Regulation of blood pressure in diabetic models and the mechanism of diabetic hypertension has not been fully understood and, to the best of our knowledge, this is the first study of the underling factors that play a vital role in the pathogenesis of diabetes. In addition, the alleviating effect of vitamin E is a new finding that helps to explain the oxidative background of the observed complications of diabetes in blood vessels. In the current study, the blood pressure was measured by a direct approach using catheterization instead of routine cuff-based indirect measurements, hence the recorded values are more accurate than the reported ones, which could be considered a main advantage. On the other hand, the aggressive nature of catheterization could also be considered as a disadvantage.

In conclusion, we mainly found that the alteration of the pressure waveforms in diabetic rats are significantly higher than in the controls, which is possibly related to the progressive process of atherogenesis and increasing blood vessel stiffness due to the significant elevation of adhesion molecules. The alleviating effect of vitamin E in diabetic animals strongly suggests the role that oxidative stress-related processes have in the complications of diabetes.

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Disclosure

None declared.

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