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RAPID COMMUNICATION

Effect of vitamin E on oxidative stress status in small intestine of diabetic rat

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Abstract

AIM: To investigate the effect of vitamin E on oxidative stress status in the small intestine of diabetic rats.

METHODS: Twenty-four male Wistar rats were randomly divided into three groups: Control (C), non-treated diabetic (NTD) and vitamin E-treated diabetic (VETD) groups. The increases in lipid peroxidation, protein oxidation and superoxide dismutase (SOD) in these three groups was compared after 6 wk.

RESULTS: There was no significant difference in catalase activity between NTD and control rats. Compared to NTD rats, the treatment with vitamin E significantly decreased lipid peroxidation and protein oxidation, and also increased catalase activity and SOD.

CONCLUSION: The results revealed the occurrence of oxidative stress in the small intestine of diabetic rats. Vitamin E, as an antioxidant, attenuates lipid peroxidation and protein oxidation, and increases antioxidant defense mechanism.

Key words: Diabetes mellitus; Small intestine; Rat; Vitamin E; Oxidative stress

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INTRODUCTION

Gastrointestinal (GI) disorders are common among human beings[1,2]. The entire GI tract from the esophagus to the anorectal region can be affected by diabetes mellitus[3]. Common complaints include hyperplasia and hypertrophy of the epithelial cells[4], elevated levels of digestive enzymes[5], increased absorption of sugars and amino acids[6], enhanced endogenous synthesis of cholesterol and triglycerides[6,7] and decreased fluidity of the brush border membrane[8]. The intestinal mucosa is also vulnerable to oxidative stress and reactive oxygen species (ROS) generated by several conditions, such as ischemia/reperfusion, inflammatory bowel disease[9], surgical stress[10] and diabetes[11]. Recently, several studies have examined the role of oxidative stress on developmental diabetic-mediated disorders, possibly *via* the formation of free radicals[11-13]. Free radicals or ROS generated during oxidative metabolism can inflict damage on all classes of cellular macromolecular components (e.g., mitochondria, endoplasmic reticulum, protein, *etc.*), eventually leading to cell death[14]. The driving force behind the destructive nature of ROS is the unpaired electron residing within their structures, making them unstable and highly reactive. It is well known that metabolic changes brought about by diabetes increase production of ROS (e.g., nitrosonium cation [$OH^{\cdot}$], lipid peroxides [$ROO^{\cdot}$] and hydrogen peroxides [H_2O_2]) as well as reactive nitrogen species (e.g., nitrosonium cation [NO^+], nitroxyl anion [NO^-] and peroxinitrite [$ONOO^-$])[15,16]. These free radicals and non-radical species react with several amino acid residues altering their structures and, by extension, the tertiary structures of the parent protein. These free radicals also degrade the phospholipids of cellular membranes through the process of lipid peroxidation. One promising aspect of understanding the role of oxidative stress in diabetes-mediated disorders is the ability of antioxidant supplementation to attenuate diabetes's adverse effects. Antioxidants, such as ascorbic acid, α-tocopherol (vitamin E), endogenous glutathione peroxidase and the pineal hormone melatonin, have all been tested for efficacy in defending against free-radical-mediated tissue injuries. Melatonin, for example, has been shown to be an effective scavenger of the hydroxyl radical, as well as other radicals such as superoxide, nitric oxide and peroxynitrite, that protects against lipid peroxidation in the brain[17-19]. Vitamin E comprises eight naturally occurring fat-soluble vitamins of which the most predominant, essential and with the highest biological activity is α-tocopherol[20]. Vitamin E is a major antioxidant in biological systems acting as a powerful chain-breaking agent through the scavenging of peroxyl radicals[21]. Vitamin E terminates the chain reaction of lipid peroxidation in membranes and lipoproteins. Thus, a number of studies have been carried out to determine the

protective effects of vitamin E in different biological models of injury[22]. Currently, there is considerable interest in the roles of vitamin E in the protection of membranes lipids against oxidative stress[23]. The present study was, therefore, undertaken to determine whether the small intestine is subjected to oxidative damage during diabetes as well as to examine the accompanying changes in antioxidant status, lipid peroxidation and protein oxidation in order to understand its role in the pathogenesis of the disease. Also, in this study, a possible protective effect of vitamin E against diabetes-induced alterations of enzymatic and oxidative components of antioxidant defense systems in mucosal layer of rat small intestine was investigated.

MATERIALS AND METHODS

All procedures on rats were followed according to "Principles of Laboratory Animal Care" (NIH publication no. 85-23, revised 1985), as well as specific rules of "Animal Care and Use Committee", National Medical and Health Service. Sixteen male Wistar rats, weighing 220-240 g, were made diabetic by intraperitoneal injection of streptozotocin (STZ, 60 mg/kg body weight in 0.05 mol/L citrate buffer, pH 4.5). Age- and weight-matched normal control group was injected intraperitoneally with an equivalent amount of buffer. Glucose was determined by glucose oxidase using Biosystem kit (Barcelon, Spain) on blood samples obtained from tail veins 48 h after injection of STZ. Rats with blood glucose higher than 3 g/L were included into the study as diabetic. Three groups (n = 8) of rats were studied for 6 wk after the entry: (1) Control (non-diabetic); (2) untreated diabetic; and (3) vitamin E-treated diabetic (VETD) groups. Rats in VETD group received 300 mg vitamin E (Merck-Germany) in tap water beside regular diet daily. Food was supplied *ad libitum* in all groups throughout the experiment. After 6 wk, all rats were anesthetized by 10% chloral hydrate (5 mL/kg body weight). The body weight was measured at the end of the experiment. Blood samples were directly obtained from the heart of the rats by syringe. Then the abdominal cavity was opened and the whole small intestine was harvested. The small intestine was segmented and each segment was flushed with chilled 115 g/L KCL solution and the mucosa was scraped. A 100 g/L homogenate was prepared in 50 nmol/L phosphate buffer (pH 7.4) and centrifuged at 10000 *g* for 10 min at 4℃ in a refrigerated centrifuge (Hermel Germany). The obtained supernatant was used for all the assays[11]. The protein content was determined by the method of Bradford[24] using bovine serum albumin as the standard. HbA1C or glycosylated hemoglobin was analyzed by HPLC using automated D-10 BioRad hemoglobin analyzers. Protein carbonyl contents were measured in the supernatant using Cayman (Cayman Co. USA) kit. Briefly, 2, 4-dinitropheylhyrayine (DNPH) reacts with protein carbonyls forming a Schiff base to produce the corresponding hydrazone, which can be analyzed spectrophotometrically. Catalase activity was determined in the supernatant using Cayman (Cayman, Co, USA) kit. The method was based on the reaction enzyme with methanol in the presence of optimal concentration of H_2O_2. The formaldehyde produced was measured at 540 nm by spectrophotometry with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole as the chromogen. Tissue SOD activity was determined using a Ransod kit (Randox Laboratories, Crumlin, UK). Briefly, the method uses Xanthine and Xanthine oxidase to generate superoxide radicals, which react with 2-(4-iodophenyl)-3-(4-nitrophenonal)-5-phenyltetrazolium chloride to form a formazan dye. Using spectrophotometer (Perkin-Elmer, Germany), the SOD activity was measured by the degree of inhibition of the reaction. The results were expressed in units per miligram of protein. The 8-isoprotane assay was based on the competition between 8-isoprostane and 8-isoprostane-acetylcholinesterase (AchE) conjugate with specific rabbit antiserum, and then Ellman's reagent was added. The final product gave distinct yellow color which was measured at 412 nm. One way analysis of variance (ANOVA) was used to compare the values among groups. In each test, the data were expressed as mean ± SE and $P < 0.05$ was considered statistically significant.

RESULTS

Table 1 shows body mass gain, blood glucose levels in the normal, 48 h after STZ injection and at the end of study, HbA1C and total protein of rats in control, non-treated diabetic and VETD groups. Body gain was significantly decreased in the non-treated diabetic rats compared to the control (P = 0.002); however, there is no significant difference between VETD and control (P = 0.09) groups. As shown in Table 1, blood glucose level was significantly increased after 48 h of STZ injection compared to control rats (P = 0.005). In the end of study, blood glucose in the VETD rats significantly decreased compared to the non-treated diabetic rats and also after 48 h of STZ injection ($P < 0.05$). HbA1c was significantly elevated in the non-treated diabetic rats compared to the control rats (P = 0.005), but it was normalized in the VETD group (Table 1). The intestinal levels of total protein found in the control and non-treated diabetic rats did not differ significantly (Table 1). Total protein level in the VETD rats elevated significantly compared to the control rats ($P < 0.05$). As shown in Figure 1, 8-isoprostanoide level was significant increased in the non-treated diabetic rats compared to the control rats (P = 0.005), while it was significantly decreased in VETD rats compared to the control and non-treated diabetic rats ($P < 0.05$). VETD group showed a significant increase in the small intestine SOD activities as compared to the control (P = 0.005) and non-treated diabetic rats ($P < 0.01$) (Figure 1B). Catalase activity in the non-treated diabetic rats was significantly decreased compared to the control rats ($P < 0.05$) (Figure 1C), but it was normalized in the VETD rats compared with the control mice ($P < 0.5$). In addition, there was a significant increase in protein carbonyl contents in the non-treated diabetic rats compared to the control rats (P = 0.0005). In the VETD rats, protein carbonyl was significantly reduced below the level of the control rats (P = 0.004).

DISCUSSION

Our study demonstrated that vitamin E treatment can improve oxidative stress status *via* normalization of lipid per-

Table 1 Body gain, blood glucose, HbA1C and protein content in the three groups (mean ± SEM)

	Body gain (g)	Blood glucose (mg/L)			HbA_{1C}%	Total protein
		Initial	48 h after STZ	End of study		
Control	6.2 ± 3	1450 ± 150	1560 ± 130	1510 ± 120	5.7 ± 0.3	7.2 ± 0.22
Non-treated diabetic	-70.2 ± 7[a]	1480 ± 170	4925 ± 210[a]	6044.6 ± 480[a]	28.8 ± 1[a]	7.5 ± 0.18
V_ETD	-17 ± 12	1440 ± 200	4580 ± 280	3803 ± 650[c]	4 ± 0.3[c]	8.02 ± 1[a]

[a]$P < 0.05$ *vs* control; [c]$P < 0.05$ *vs* non-treated diabetic.

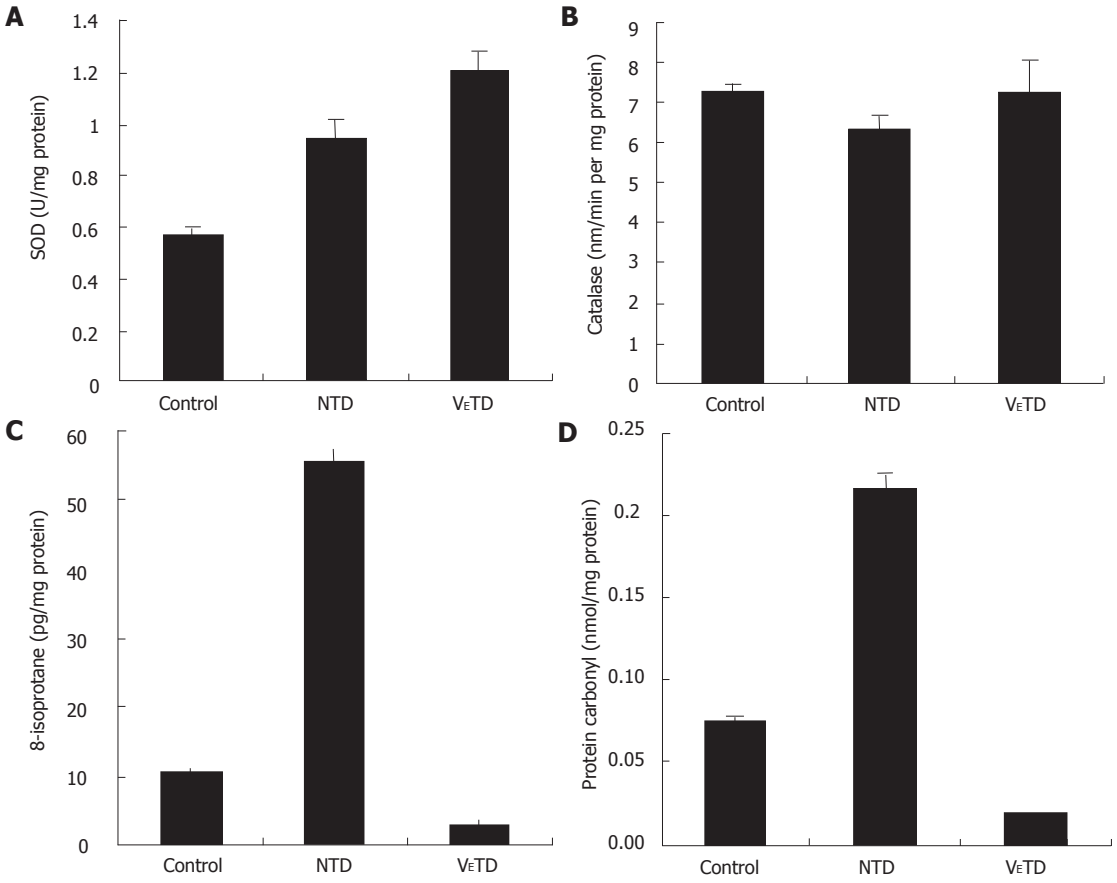

Figure 1 Biochemical markers which indicate status of oxidative stress. Significant high levels of superoxide dismutase activity (**A**) were observed in the non-treated diabetic rats instead of low level of catalase activity (**B**); 8-isoprostan (**C**) and protein carbonyl (**D**) contents of the small intestine of the non-treated diabetic rats were also significantly high. Vitamin E-treated diabetic rats showed elevated levels of superoxide dismutase (**A**) and catalase activities (**B**) accompanying with significant decrease in 8-isoprostan (**C**) and protein carbonyl (**D**) contents.

oxidation, protein oxidation and partially glycemic control in diabetic Wistar rats. Since the oxidative stress status did not alter in non-treated diabetic rats, the vitamin E treatment probably exerts its effects by protecting the small intestine from the toxic effects of ROS produced under hyperglycemic condition. Our previous study and others showed that the diabetes-induced small intestine morphologic changes include increasing of weight, length, crypt depth and villus height in diabetic rats[11,25,26]. Our previous[26] study also showed that vitamin E treatment restored all morphologic changes induced by diabetes in all parts of the small intestine. Vitamin E or α-tocopherol is highly soluble in lipids, so that it is the main antioxidant of lipoproteins and cell membrane. Some studies[27,28] have shown that the control of diabetes improves with the administration of vitamin E to patients, since it protects the fatty acids of cell membrane and thereby preserves their reaction with respect to insulin. Our results showed a significant increment of lipid peroxidation in the small intestine in the non-treated diabetic rats. The observed increases in lipid peroxidation levels in the small intestine are in agreement with similar finding in other tissues[29]. Lipid peroxidation may bring about protein damage and inactivation of membrane-bound enzymes either through direct attachment by free radicals or through chemical modification by its end products, malondialdehyde and 4-hydroxynonenal[9]. It is also known to decrease the fluidity of the intestinal brush border membrane[30]. Therefore, the observed increase in lipid peroxidation could provide an additional explanation for the previously reported decrease in fluidity

of the intestinal brush border membrane during diabetes attributed to changes in lipid composition alone[31]. In this study, significantly higher levels of protein-bound carbonyls were found in the small intestine of the non-treated diabetic rats. Recently, it has been proposed that carbonyl stress, i.e. the increase in reactive carbonyl compounds derived from oxidative and non-oxidative reactions, leads to increased chemical modification of proteins and, at a later stage, to oxidative stress and tissue damage. A deficit in the detoxification of carbonyl compounds by the enzymes of glyoxalase pathway and aldose reductase is believed to be partly responsible for carbonyl stress and consequent oxidative stress[32]. In the present study, VETD rats showed significant decrement in lipid peroxidation and protein oxidation compared to the non-treated diabetic and control rats. Elimination of lipid and protein oxidation as two important free radical generation sources by vitamin E may be resulted in recovery of cell membrane to its normal physiologic state, and thus the insulin binds the cell readily.

Our result also showed significant decrement in blood glucose and HbA1c levels in the VETD rats as compared with the non-treated diabetic rats. It is well known that hyperglycemia leads to autoxidation of glucose, lipid peroxidation and protein oxidation, that are three major ROS generation sources and consequently oxidative stress in diabetic subjects[33-35]. HbA1c was found to increase in patients with diabetes mellitus and the amount of increase was directly proportional to the fasting glucose level[36]. During diabetes mellitus, the excess glucose present in the blood reacts with hemoglobin to form HbA1c[37]. HbA1c is used as a marker for estimating the degree of protein glycation in diabetes mellitus[38]. Administration of vitamin E to diabetic rats reduced the glycation of hemoglobin, and thus decreased the levels of glycosylated hemoglobin in diabetic rats. This normalization of glycosylated hemoglobin indicates decreased glycation of protein. The activity of SOD was increased in the small intestine of non-treated diabetic and VETD rats. It is known that diabetes induces oxidative stress by production of superoxide anion radicals[35] and it is reasonable to expect an increased activity of SOD[39]. In physiological conditions, SOD is an important intracellular antioxidant which catalyses the conversion of the superoxide anion radical to molecular oxygen and hydrogen peroxide (H_2O_2) and thus protects against superoxide-induced damage[40]. Compared with the control rats, catalase activity significantly decreased in the non-treated diabetic rats. Vitamin E treatment normalized the catalase activity in the control group. In contrast to our results, increases in catalase activity in the small intestine of diabetic rats had been reported[11]. Giron *et al*[41] reported a lack of changes in the activity of intestinal catalase of diabetic rats fed with diets containing different fat supplements. This inconsistency in the reported literature might be due to the difference in the strain of the animals used, the duration of the experiment and/or severity of diabetes. Moreover, vitamin E has a number of effects at the cellular level that are not dependent on its antioxidant activity and may potentially contribute to improved insulin action. For example, vitamin E inhibits protein kinase C by a non-antioxidant mechanism[42]. Vitamin E also accelerates diacylglycerol kinase activity, thereby decreasing levels of diacylglycerol, which is an allosteric activator or protein kinase C[43]. Increased protein kinase C activity apparently impairs insulin action by phosphorylating serine or threonine residues on insulin receptor and insulin receptor-1 proteins[44]. This decreases insulin-stimulated, phosphatidylinositol 3-kinase-catalyzed phosphorylation of tyrosine residues in these proteins, which is required for effective insulin action. Recent evidence suggests that vitamin E may influence the activity of these enzymes by decreasing the curvature of plasma membranes[24].

In conclusion, our results clearly demonstrate that vitamin E administration improves type 1 diabetes-induced oxidative stress *via* decreasing lipid peroxidation and protein oxidation as a free radical generation sources and elevating antioxidant defense system enzymes like SOD and catalase activities.

COMMENTS

Background

The entire GI tract from the esophagus to the anorectal region can be affected by diabetes mellitus. Recently, several studies have examined the role of oxidative stress on developmental diabetic-mediated disorders, possibly *via* the formation of free radicals. Free radicals or reactive oxygen species are generated during oxidative metabolism and can inflict damage on all classes of cellular macromolecular components (e.g. mitochondria, endoplasmic reticulum, protein, *etc.*), eventually leading to cell death. One promising aspect of understanding the role of oxidative stress in diabetes-mediated disorders is the ability of antioxidant supplementation to attenuate adverse effect of diabetes. Antioxidants, such as ascorbic acid, α-tocopherol (vitamin E), endogenous glutathione peroxidase and the pineal hormone melatonin, have all been tested for efficacy in defending against free-radical-mediated tissue injuries.

Research frontiers

Currently, there is considerable interest in the roles of vitamin E in the protection of membranes lipids, proteins or other biomolecules against oxidative stress as well as precise molecular mechanism by which they may occur.

Related publications

For more information about each section, please refer to articles which included in reference list.

Innovations and breakthrough

This study tried to open new horizons on molecular base of protective effects of vitamin E on diabetes-induced injuries in the small intestine.

Applications

The results of current study launch a new view on pathogenesis of diabetes mellitus; hence, further studies may be designed to elucidate more informative details.

Terminology

Free radicals or reactive oxygen species are the molecules which generated during oxidative metabolism and can inflict damage on all classes of cellular macromolecular components called oxidative damage.

Peer review

The authors in this manuscript use a streptozotocin model of type I diabetes to examine the effects of vitamin E on the gut. Improved glucose control, less weight loss, and reduced oxidative injury in the gut were observed. The authors conclude that vitamin E may play a beneficial role in preventing oxidative injury associated with diabetes.

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