ORIGINAL RESEARCH

Protective effects of vitamin E on central nervous system in streptozotocin-induced diabetic rats

Sibel Canbaz Kabay MD¹

Hilmi Ozden MD²

Gul Guven MD²

M. Cengiz Ustuner, PhD³

Irfan Degirmenci PhD³

Esra Gurlek Olgun MD⁴

Nedim Unal PhD²

¹ Department of Neurology, Department of Pathology,

⁴ Department of Pathology, Faculty of Medicine, Dumlupinar University, 43100 Kutahya, Turkey

² Department of Anatomy, ³ Department of Medical Biology, Faculty of Medicine, Osmangazi University, Eskisehir, Turkey

Manuscript submitted 27th February, 2009

Manuscript accepted 12th July, 2009

Clin Invest Med 2009; 32 (5): E314-E321.

Abstract

Objective: To evaluate the histopathological and antioxidant effects of vitamin E (VE) treatment on brain tissue in streptozotocin (STZ)-induced diabetic rats.

Methods: Thirty two male Wistar albino rats were used. The study comprised four groups of 8 rats: Group A - untreated group, group B - diabetic group, group C - VE and group D - diabetic plus VE. In the diabetic groups, diabetes was induced by a single intraperitoneal injection of 65 mg/kg STZ. Vitamin E was given 50 mg/kg/day i.p. for three weeks. Concentrations of glucose, malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were detected in the haemolysate.

Results: Glucose concentrations were increased in the blood of the STZ-treated rats compared with those in the diabetic groups (group B and D). The MDA concentrations in the brain from diabetic rats increased, whereas the GPx, SOD, CAT concentrations decreased. Treatment with VE returned concentrations of MDA, GPx, SOD and CAT toward control values. The MDA concentration in the diabetic group (20.65 ± 2.24 nmol/mg Hb) was decreased compared with the VE treated group (15.54 ± 1.32 nmol/mg Hb). There were no pathological differences between untreated and VE treated rats' brains. Neuronal ischemic damages were determined in STZ-induced diabetic rats. Ischemic

neuronal alterations in group B (diabetic) had more damage than group D (diabetic + VE).

Conclusion: The study revealed neuroprotective effects of VE on ischemic damage in diabetic central neuronal cells, caused by diabetic oxidative stress.

Diabetes is the most common serious metabolic disorder that cannot be completely treated and may result in complications if with inadequate medication.^{1, 2} Diabetes causes a variety of functional and structural disorders in the central and peripheral nervous systems.³⁻⁵ Diabetes is characterized by hyperglycemia and is associated with long-term vascular complications such as retinopathy, nephropathy, cardiopathy, and neuropathy.⁶ Regulation of blood glucose can prevent the beginning and progression of diabetic complications.⁷ Oxidative stress can lead to damage to the endothelium tissue in the blood vessels, increased blood cholesterol, advanced lipid peroxidation and blood platelet dysfunction.² Diabetes brings about ischemic damages in various brain regions because of increased oxidative stress caused by hyperglycemia.⁸ Increased reactive oxygen species (ROS; e.g. superox-

ide anion, hydroxyl radicals, peroxyxynitrite, hydrogen peroxide) can alter neuronal function because of neuronal death through protein oxidation, DNA damage, elevated nonenzymatic glycosylation, peroxidation of membrane lipids.^{9, 10} Previous studies have shown that treatment with antioxidants prevented neuronal cell damage caused by diabetes-induced oxidative stress. In some experimental studies the administration of free radical scavengers such as superoxide dismutase (SOD), catalase, glutathione, vitamin C, vitamin E (alpha-tocopherol) (VE), α -lipoic acid, beta-carotene, analog trolox C, decreased the concentration of free radicals. Lipid peroxidation in diabetes could be prevented by affecting some biochemical measurements.¹⁰⁻¹² Therefore, increased oxidative stress is the consequence of either enhanced ROS production or attenuated ROS scavenging capacity.¹³ Vitamin E is a lipid-soluble chain-breaking antioxidant which protects, especially, biological membranes from lipid peroxidation.² Few studies have been performed on oxidative stress and antioxidants in relation to the brain.² The mechanism of action of antioxidants are not clear in the diabetes.¹⁴ The aim of this study was to examine histopathological and antioxidant effects of VE on brain tissue in the streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Animals and experimental protocols

Thirty two male Wistar albino rats (250-300 g) were used in this study which was subject to the Guiding Principles for the Care and Use of Laboratory Animals and the Recommendations of the Declaration of Helsinki. All animal procedures were conducted in agreement with Eskisehir Osmangazi University guidelines for the care and animal ethics committee. Animals were housed in polycarbonate cages in a room with controlled temperature ($22\pm 2^\circ\text{C}$), humidity ($50\pm 5\%$), a 12 h cycle of light and dark, and were fed laboratory pellet chows and water *ad libitum*. The ex-

periment was performed after a stabilization period in the laboratory for several days. Animals were obtained from the experimental research centre of the institute and all experiments were carried out in the same centre. Rats were anesthetized intraperitoneally with ketamine (50 mg/kg) and Xylazine (5 mg/kg). In the diabetic groups, diabetes was induced by a single intraperitoneal injection of 65 mg/kg STZ prepared in a 0.1 M citrate buffer solution, pH 4.5 (Sigma Chemical Co, St Luis, MO, USA). Blood glucose concentrations of all groups were measured at 24 h and 4 wks after STZ administration.

The study comprised four groups of 8 animals: group A - untreated group, group B - diabetic, group C - VE administration and group D - diabetic plus VE treatment. The VE was injected 50 mg/kg/day i.p for three weeks. To determine the antioxidant effect of VE on STZ-induced diabetic rats, 2 ml cardiac blood were taken from each rat into EDTA-tubes for measurement of serum glucose, malondialdehyde (MDA), SOD, catalase (CAT), and glutathione peroxidase (GPx) concentrations.

Determination of MDA

MDA concentrations were determined for products of lipid peroxidation by monitoring thiobarbituric acid reactive substance formation as described previously.¹⁵ Activity was expressed as nmol/mg Hb.

Determination of SOD activity

SOD activity was assayed spectrophotometrically with a commercial kit. The Fluka SOD kit USA contains the reagents and solutions required for determining superoxide dismutase activity in an indirect assay method based on xanthine oxidase and a novel colour reagent. The haemolysate SOD activity was determined by inhibition of formosan dye (450 nm) employing the xanthin-xanthin oxidase enzymatic method to generate superoxide radicals and expressed as U/mg of hemoglobin.

Determination of CAT activity

One unit (1U) of CAT equals the enzyme activity that recognized 1 μ mol of hydrogen peroxide in 60 sec at 37°C. Three blank samples were prepared according to Goth, 1991. CAT activity was measured with determination of absorbance of three blank samples at 405 nm in spectrophotometer. CAT activity (kU/L) was calculated as = [(Abs_{blank1} - Abs_{blanksample}) / Abs_{blank 2} - Abs_{blank 3}] x 271. Results were divided to sample hemoglobin amount ml/mg Hb.¹⁶

Determination of GPx activity

GPx activity was assayed spectrophotometrically with a commercial kit (GPx, Calbiochem kit, USA). Cellular glutathione peroxidase (c-GPx) is a member of a family of GPx enzymes whose function is to detoxify peroxides in the cell. Oxidation of NADPH to NADP⁺ is accompanied by a decrease in absorbance at 340 nm, thus providing a spectrophotometric means for monitoring GPx enzyme activity and expressed as U/mg of hemoglobin. After the cardiac perfusion, brain was isolated for histopathological examinations.

Histopathological examination

For light microscopic investigation, brain tissue specimens were fixed in a 10% formaldehyde, dehydrated in alcohol solution and were embedded in paraffin used for histopathological examination. Five mi-

crometer (μ m) thick sections were cut, deparaffinized, hydrated and stained with hematoxyline and eosin (H&E) under a photomicroscope (Olympus BH 2, Tokyo, Japan). All tissue sections were examined microscopically to characterize the histopathological changes, by an experienced histologist who was unaware of the treatment conditions.

Statistical Analysis

All data are expressed as the mean $\pm$ SD. The comparison between groups was performed by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test to compare the mean values in groups. A probability value of $P < 0.05$, $P < 0.001$ were considered statistically significant.

Results

At the end of the experiment, blood glucose concentrations were increased in STZ-induced rats compared with group A and C (table 1). These high levels ($P < 0.1$) were altered in the animals with VE treatment at end of the 4th week. The MDA concentrations of diabetic rats increased ($P < 0.01$), whereas the GPx ($P < 0.1$), SOD ($P < 0.1$), CAT concentrations ($P < 0.001$) decreased. Treatment with VE returned concentrations of MDA, GPx, SOD and CAT toward their control values (table 2).

TABLE 1. Multiple comparisons of Blood glucose concentrations for all groups at groups at 24 hr and 4 wks.

Blood Glucose (mg/100 ml)			
Group A (Untreated)		Group B (Diabetes Mellitus)	
24 hour	88 $\pm$ 7.37	24 hour	312 $\pm$ 42.28
4th Week	91 $\pm$ 8.23	4th Week	435 $\pm$ 37.37
Group C (Vitamin E)		Group D (Diabetes Mellitus+ Vitamin E)	
24 hour	93 $\pm$ 12.67	24 hour	323 $\pm$ 21.63
4th Week	90 $\pm$ 11.14	4th Week	410 $\pm$ 17.19
		24 hr Group A-B***	4 wk Group A-B***
		24 hr Group B-C***	4 wk Group B-C***
			4 wk Group B-D*

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

TABLE 2. Multiple comparisons of MDA levels and antioxidant enzyme activities for all groups at end of 4th week.

	MDA (nmol/mg Hb)	SOD (U/mg Hb)	CAT (U/mg Hb)	GPx (U/mg Hb)
Group A (Untreated)	14.23±1.52	11.048±1.34	110.5±5.95	12.38±1.89
Group B (Diabetes Mellitus)	20.65±2.24	8.052±1.26	67.32±4.8	9.45±1.67
Group C (Vitamin E)	13.33±1.45	10.217±1.76	108.53±7.8	12.97±1.52
Group D (Diabetes Mellitus+Vitamin E)	15.54±1.32	10.974±1.36	101.76±6.27	12.17±2.06
	GROUP A-B** GROUP A-D** GROUP B-C** GROUP B-D**	GROUP A-B* GROUP B-C* GROUP B-D*	GROUP A-B*** GROUP B-C*** GROUP B-D*** GROUP C-D*	GROUP A-B* GROUP B-C* GROUP B-D*

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Histological Findings

Groups A and C contained euchromatic nuclei, basophilic cytosol, axon and dendrites of normal multipolar neurons, (figure 1A) with normal heterochromatic nuclear structure of neuroglia cells (figure 1B). Group B contained neuronal necrosis areas, hemorrhagic foci, damaged blood vessels (figure 2A, 2B) and distorted cell membrane with degeneration in pyramidal cells (figure 2C). Group D showed decreased neuronal necrotic areas (figure 3A), contain partially blood ves-

sels, neuroglia cells, pyramidal cells degeneration and stained heterochromatic cells with undefined nucleus structure (figure 3B).

Discussion

STZ-induced diabetes is a well-recognised model of experimental diabetes. It provides a relevant example of endogenous chronic oxidative stress due to the resulting hyperglycemia.¹⁷ The roles of oxidative stress in nerve damage have been performed in experimental

FIGURE 1. (A) Untreated group; Euchromatic nucleus, basophilic cytosol, axon and dendrites of normal multipolar neurons (arrows) were observed, H&E X40, (B) Vitamin E; Normal heterochromatic nucleus structure of Neuroglia cells (arrows) observed, H&E X40.

FIGURE 2. (A) Diabetic group; Neuronal necrosis areas (arrows) were observed, H&E X20, (B) Diabetic group; Hemorrhagic foci and damaged blood vessels (arrows) were observed, H&E X20, (C) The distorted cell membrane with degeneration in pyramidal cells (arrows) were observed, H&E X40.

diabetes and diabetic patients.¹⁸ In the present study we showed that MDA concentrations increased in STZ-induced diabetes rats even though SOD, CAT and GPx activities were decreased. VE decreased the MDA concentration. In addition, VE increased SOD, CAT and GPx activities when VE was administered with STZ induced rat's hemolysate. These results con-

firm a previous study that STZ-induced diabetes was accompanied by increased generation reactive species.^{19,20} In the current study, we further examined the histopathological effects of treatment with vitamin E. There was no pathological change between untreated and VE treated rat's brain. Neuronal ischemic damages were observed in STZ-induced diabetic rats.

FIGURE 3. (A) Diabetic + vitamin E decreased neuronal necrotic areas (arrows). H&E X20, (B) Diabetic + vitamin E contain partially blood vessels (thick arrow), neuroglia cells (thin arrow), pyramidal cells degeneration and stained heterochromatic cells (head arrow) with undefined nucleus structure, H&E X20.

Ischemic neuronal alterations in group B (diabetic) had more damage than group D (diabetic + VE). One previous study showed that brain damage caused by hypoglycemia increased the vulnerability of specific brain areas to neuronal damage like cortex and the hippocampus. In this study, untreated and VE groups had normal histological structure. Despite this, STZ caused neuronal necrosis area, hemorrhagic focuses, damaged blood vessels and distorted cell membrane with degeneration in pyramidal cells in diabetic group.²¹

Piotrowski et al. demonstrated that oxidative stress in diabetic rat's brain led to neuronal cell death inashion a necrotic or apoptotic way.²² Dheen et al. applied morphometric analysis and showed hypertrophy in supraoptic neurons of diabetic rats with semithin sections. However, they observed a lot of histopathological change under the electron microscope.²³

Neuronal damages after 30 min-middle cerebral artery occlusion were seen earlier and massive than in nondiabetic rats. At the 7th day of recovery, the mean damage area was larger in diabetic than in nondiabetic

rats. These results suggest that activation of apoptotic cell death pathway may play an important role in aggravating brain damage in diabetic subjects.¹⁴ Antioxidative vitamins used not only decreased the processes of lipid peroxides but also reduced structural damage and the impairment of tissue microcirculation. Dietary VE has been shown to be excellent for strengthening the antioxidative defense system by increasing the activity of SOD and GPx and increasing membrane fluidity in the brain of STZ-diabetic rats.² When neuroaxonal dystrophy was developed in gracile/cuneate nuclei, in chronic VE deficient rats, the distribution of axonopathy did not match the pattern of experimental diabetes and aging.²⁴ It has been suggested that antioxidant vitamin C might prevent leukocyte adhesion to the cerebral endothelium in diabetes mellitus and, thus, reduce the occlusion of capillaries by leukocytes in diabetic rats.²⁵

Diabetes has teratogenic effects in embryos. The addition of VE normalizes the embryonic antioxidant defense mechanism, reducing diabetes-induced embryonic damage.²⁶ Some studies have shown that VE

deficiency changes the dynamics of the development of the antigen-specific CD8⁺ T cell response both in the periphery and in the central nervous system (CNS) via a combination of dendritic cell activation in the periphery and development of T regulatory cells.²⁷ Most of the studies have shown a protective or therapeutic effect of VE against the free radical injury and oxidative stress in the aging brain or alcohol-induced oxidative damage in brain.^{28, 29} Administration of vitamin E exerts a protective effect on the locus coeruleus (LC) neurons in an early model of Parkinson's disease (PD).³⁰ The activity of chaperone-mediated autophagy, a selective pathway for the degradation of cytosolic proteins in lysosomes, is enhanced during oxidative stress. Also Cao et al. showed that vitamin E may partially inhibit activated chaperone-mediated autophagy during oxidative stress.³¹

Diabetes leads to long-term complications in the brain, such as increased risk of stroke and small vessel disease.^{8,32} On the basis of above studies and our study we suggest the use of VE in diabetic patients in order to protect from the cerebrovascular complications associated with diabetes.

In conclusion, oxidative stress develops in streptozotocin-induced diabetes in rats. This study revealed the neuroprotective effects of VE treatment on ischemic damages in diabetic central neuronal cells, caused by diabetic oxidative stress.

References

1. Tuzcu M, Baydas G. Effect of melatonin and vitamin E on diabetes-induced learning and memory impairment in rats. *Eur J Pharmacol.* 2006; 537:106-10.
2. Hong JH, Kim MJ, Park MR, et al. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. *Clin Chim Acta.* 2004; 340:107-15.
3. Gispen WH, Biessels GJ. Cognition and synaptic plasticity in diabetes mellitus. *Trends Neurosci.* 2000; 23:542-9.
4. McCall AL. The impact of diabetes on the CNS. *Diabetes.* 1992; 41:557-70.
5. Ellenberg M. Development of urinary bladder dysfunction in diabetes mellitus. *Ann Intern Med.* 1980; 92:321-3.
6. Auslander W, Haire-Joshu D, Houston C, et al. A controlled evaluation of staging dietary patterns to reduce the risk of diabetes in African-American women. *Diabetes Care.* 2002; 25:809-14.
7. Diabetes Control and Complications Trial Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin dependent diabetes mellitus. *N Engl J Med.* 1993; 329:977-86.
8. Dalal PM, Parab PV. Cerebrovascular disease in type 2 diabetes mellitus. *Neurol India.* 2002; 50:380-5.
9. Hawkins CL, Davies MJ. Generation and propagation of radical reactions on proteins. *Biochim Biophys Acta.* 2001; 1504:196-219.
10. Ozkan Y, Yilmaz O, Oztürk AI, Ersan Y. Effects of triple antioxidant combination (vitamin E, vitamin C and alpha-lipoic acid) with insulin on lipid and cholesterol levels and fatty acid composition of brain tissue in experimental diabetic and non-diabetic rats. *Cell Biol Int.* 2005; 29:754-60.
11. Barros DO, Xavier SM, Barbosa CO, et al. Effects of the vitamin E in catalase activities in hippocampus after status epilepticus induced by pilocarpine in Wistar rats. *Neurosci Lett.* 2007; 416:227-30.
12. Kowluru RA, Engerman RL, Kern TS. Diabetes-induced metabolic abnormalities in myocardium: effect of antioxidant therapy. *Free Radic Res.* 2000; 32:67-74.
13. Dickinson PJ, Carrington AL, Frost GS, Boulton AJ. Neurovascular disease, antioxidants and glycation in diabetes. *Diabetes Metab Res Rev* 2002; 18:260-72.
14. Muranyi M, Fujioka M, He Q, et al. Diabetes activates cell death pathway after transient focal cerebral ischemia. *Diabetes.* 2003; 52:481-6.
15. Mihara M, Uchiyama M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. *Anal Biochem.* 1978; 86:271-8.
16. Goth LA. Simple method for determination of serum CAT activity and revision of reference range. *Clin Chim Acta.* 1991; 196:143-52.
17. Low PA, Nickander KK, Tritschler H.J. The role of oxidative stress and antioxidant treatment in experimental diabetic neuropathy. *Diabetes.* 1997;46:38-42.
18. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes.* 1991;40:405-12.
19. Celik S, Baydas G, Yilmaz O. Influence of vitamin E on the levels of fatty acids and MDA in some tissues of diabetic rats. *Cell Biochem Funct.* 2002;20:67-71.

20. Baydas G, Sonkaya E, Tuzcu M, et al. Novel role for gabapentin in neuroprotection of central nervous system in streptozotocine-induced diabetic rats. *Acta Pharmacol Sin.* 2005;26:417-22.
21. Bree AJ, Puente EC, Daphna-Iken D, et al. Diabetes increases brain damage caused by severe hypoglycemia. *Am J Physiol Endocrinol Metab.* 2009;297:194-201.
22. Piotrowski P. Morphology of experimental diabetes and cerebral ischemia in the rat brain. *Folia Neuropathol.* 1999;37:252-5.
23. Dheen ST, Tay SSW, Wong WC. Ultrastructural changes in the hypothalamic supraoptic nucleus of the streptozotocin-induced diabetic rat. *J Anat.* 1994;184:615-23.
24. Schmidt RE, Coleman BD, Nelson JS. Differential effect of chronic vitamin E deficiency on the development of neuroaxonal dystrophy in rat gracile/cuneate nuclei and prevertebral sympathetic ganglia. *Neurosci Lett.* 1991;123:102-6.
25. Jariyapongskul A, Patumraj S, Yamaguchi S, Niimi H. The effect of long-term supplementation of vitamin C on leukocyte adhesion to the cerebral endothelium in STZ-induced diabetic rats. *Clin Hemorheol Microcirc.* 2002;27:67-76.
26. Zaken V, Kohen R, Ornoy A. Vitamin C and E improve rat embryonic antioxidant defense mechanism in diabetic culture medium. *Teratology.* 2001;64:33-44.
27. Sheridan PA, Beck MA. The dendritic and T cell responses to herpes simplex virus-1 are modulated by dietary vitamin E. *Free Radic Biol Med.* 2009;15:1581-8.
28. Asha Devi S. Aging brain: prevention of oxidative stress by vitamin E and exercise. *ScientificWorldJournal.* 2009;22:366-72.
29. Kaur J, Bansal MP. Effect of vitamin E on alcohol-induced changes in oxidative stress and expression of transcription factors NF kappa B and AP-1 in mice brain cerebral hemispheres. *Indian J Exp Biol.* 2008;46:562-7.
30. Pasbakhsh P, Omid N, Mehrannia K, et al. The protective effect of vitamin E on locus coeruleus in early model of Parkinson's disease in rat: immunoreactivity evidence. *Iran Biomed J.* 2008; 12:217-22.
31. Cao L, Chen R, Xu J, Lin Y, Wang R, Chi Z. Vitamin E inhibits activated chaperone-mediated autophagy in rats with status epilepticus. *Neuroscience.* 2009;16:73-7.
32. Makar TK, Rimpel-Lamhaouar K, Abraham DG, et al. Antioxidant defense systems in the brains of type II diabetic mice. *J Neurochem.* 1995;65:287-91.

Correspondence to:

Sibel Canbaz Kabay MD
 Dumlupinar University Faculty of Medicine
 Department of Neurology, 43100 Kutahya, Turkey
 E-mail:skabay@yahoo.com