Reversal of Defective Nerve Conduction With Vitamin E Supplementation in Type 2 Diabetes

A preliminary study

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OBJECTIVE — The present study has examined the effect of vitamin E, the principal modulator of free radical activity, on electrophysiological parameters in patients with diabetic peripheral sensorimotor polyneuropathy, matched for duration of disease and metabolic control.

RESEARCH DESIGN AND METHODS — A total of 21 subjects with type 2 diabetes were enrolled in this double-blind randomized placebo-controlled study (vitamin E, 11 patients; placebo, 10 patients). Patients were randomly assigned to receive either 900 mg vitamin E or placebo for 6 months. The average dietary vitamin E consumption of the subjects was similar during the study. The main outcome measure was the electrophysiological tests assessing nerve conduction. Fasting plasma glucose, HbA_1, postprandial plasma glucose, and electrophysiological parameters in the basal state and after 6 months of treatment were studied.

RESULTS — Glycemic indexes did not show any significant changes during the study, whereas nerve conduction improved significantly in 2 of the 12 studied electrophysiological parameters after 6 months in patients on vitamin E supplementation. The changes in the electrophysiological parameters were obvious in the median motor nerve fibers and tibial motor nerve fibers. Nerve conduction velocity in the median motor nerve fibers ($P = 0.0019$) and tibial motor nerve distal latency ($P = 0.0284$) improved significantly after 6 months of vitamin E supplementation.

CONCLUSIONS — This study shows that defective nerve conduction in diabetic subjects with mild-to-moderate peripheral neuropathy may be improved by pharmacological doses of vitamin E supplementation. Further studies with a larger number of patients for longer periods of time are needed.

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The pathogenesis of diabetic neuropathy is not yet clearly defined. Several metabolic and microvascular factors are considered to play important roles. Among the metabolic factors, the potential contribution of increased oxidative stress to the development of complications in diabetes was recognized several decades ago (1,2) and is still the focus of increasing attention (3–5).

An increase in oxidative stress may occur because of either an increase in free radical production or a reduction in antioxidant defenses (6). Increased production of free radicals in diabetes may arise in a number of ways, including autooxidation of glucose and glycated proteins, particularly in the presence of transition metals. Diabetic monocytes also have an increased capacity to produce superoxide (7,8). In addition, there are widespread disturbances of antioxidant defense systems, suggesting that reduced resistance to free radical–induced tissue damage may also occur. This is reflected by increased levels of biochemical markers of lipid peroxidation and free radical activity, particularly in the presence of microangiopathic complications (9–11).

Oxidative stress has an important role in the pathogenesis of diabetic neuropathy (3,12,13). The presence of increased oxygen free radical activity in diabetic nerve tissue is supported by the finding of increased conjugated dienes (formed via lipid peroxidation) and sciatic nerve norepinephrine in experimental diabetes. In addition, superoxide dismutase, which has the important role of neutralizing superoxide radicals, may be reduced in diabetic peripheral nerve tissue, thus compounding any enhancement of free radical formation (13,14).

Electrophysiological studies play an important role in detecting, characterizing, and measuring the progress of the different forms of diabetic neuropathies (15). In diffuse neuropathies, slowing of conduction velocity may become more apparent if measurement is obtained over long nerve segments. The slowing of the nerve conduction velocity is among the earliest neuropathic abnormalities that occur in diabetes and often is present even at diagnosis of diabetes. After diagnosis, the slowing of the nerve conduction velocity generally progresses and is correlated directly with duration of diabetes. Sensory fibers usually are affected first, followed by motor fibers (12). Lower-extremity distal nerves, in particular, the peroneal and sural nerves, frequently show the most significant abnormalities (12).

Although major questions about the pathogenesis of diabetic neuropathy remain unanswered and require further intense investigation, significant recent progress is pushing us into future therapy modalities directed against one or more elements of the complex pathogenetic process responsible for diabetic neuropathy. Oxidative stress

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A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Characteristics of the patients enrolled in the study*

	Placebo	Vitamin E
Age (years)	59.3 ± 9.8	57.2 ± 13.0
Sex (M/F)	1/9	2/9
BMI (kg/m^2)	26.7 ± 5.2	28.1 ± 6.1
Type 2 diabetes duration (years)	8.9 ± 2.3	8.8 ± 7.4
Fasting plasma glucose (mmol/l)		
At beginning	7.0 ± 1.9	7.4 ± 1.1
At 6 months	7.4 ± 2.4	7.9 ± 2.4
Postprandial plasma glucose (mmol/l)		
At beginning	9.1 ± 2.5	9.1 ± 2.6
At 6 months	8.6 ± 1.8	9.0 ± 3.9
Mean HbA_1 (%)		
At beginning	7.4 ± 2.8	9.2 ± 2.5
At 6 months	8.5 ± 0.8	7.4 ± 1.5

Data are means ± SD.

plays a role in the development of macrovascular and endoneurial disease in the diabetic neural tissue (9,13). Here in our double-blind, randomized, placebo-controlled study, we investigated the effect of the chain-breaking antioxidant, vitamin E, in patients with electrophysiologically documented peripheral sensorimotor neuropathy, on the electrophysiological parameters.

RESEARCH DESIGN AND METHODS

— A total of 21 type 2 diabetic patients with clinically documented peripheral neuropathy were selected for the present study from outpatient diabetic clinic in Hacettepe University Hospital. All patients had symptomatic symmetrical distal neuropathy (i.e., reduced or absent ankle reflexes, reduced tactile, pinprick, and/or position sensation) with at least moderate severity of one or more of the typical symptoms (burning, paresthesia, numbness) in the feet. These patients were divided into two groups randomly. One group (n = 11) was given 900 mg of vitamin E daily, and the other group (n = 10) was given placebo for 6 months.

Vitamin E supplement was provided as Ephynal tablets containing 100 mg of DL-α-tocopheryl acetate (courtesy of Roche Pharmaceuticals, Istanbul, Turkey). Placebo tablets were also provided by the same company, identical in shape and weight, containing the same ingredients and coating except the active substance, DL-α-tocopheryl acetate.

The assignment of a group to vitamin E treatment was randomized and unknown to the physician. Two groups were matched for age, duration of disease, and metabolic control (Table 1).

All patients were given informed consent to participate in the study. The study protocol was approved by the ethical committee of Hacettepe University Hospital.

The patients were seen on a monthly basis and metabolic evaluation was made throughout the study. Clinical neurologic examination of the patients were repeated at each visit.

Patients enrolled in our study all had type 2 diabetes and were treated with diet alone or with oral antidiabetic agents. None of the patients were taking vitamin E supplements routinely before the study. Patients were told to write their daily food consumption on three consecutive days at the beginning of the study, at the third month, and the end of the study. Those lists were analyzed by the same hospital dietitian to determine the α-tocopherol content of the diet. According to the lists, none of the subjects were accustomed to eating large amounts of food containing high levels of vitamin E or food containing vitamin E supplements before or during the study. This analysis by the dietitians revealed that dietary levels of vitamin E were similar in both study groups.

Fasting plasma glucose determinations were maintained from venous sampling after 12 h of overnight fasting, by glucose-oxidase method (Boehringer Mannheim, Mannheim, Germany).

HbA_1 determinations were made from the same venous sample by colorimetric method using a commercially available kit (Glycohemoglobin HbA_1 Test; Stanbio Laboratory, San Antonio, TX) (16). Postprandial plasma glucose determinations were made from postprandial second hour venous plasma samples.

All patients had electrophysiological evaluation of the upper and lower extremities at the beginning and at the end of the study. Nerve conduction studies of the patients were carried by the same clinical neurophysiologist in the Neurology Department of Hacettepe University at the beginning and at the end of the study.

Electrophysiological study

For the electrophysiological follow-up, median and posterior tibial motor nerve, orthodromic median (2nd finger-wrist), and sural nerve sensory conduction studies were performed by using standard techniques as described by Kimura (17), and the equipment used was Medelec MS 25 Mystro. Stimulating electrodes (Medelec, bipolar stimulating electrode, 10 mm, code: 16894), recording electrodes (Medelec, bar recording electrode, 40 mm, code: 16934), and ground electrodes (Medelec, wraparound ground electrode, 2 mm, code: 54483) were used for all the stimulations and recordings. Lower and upper filter frequencies were 20 Hz to 10 kHz for the motor nerve studies and 500 Hz to 10 kHz for the sensory nerve studies. Negative peak amplitude and peak-to-peak amplitude measurements were done for the motor and sensory responses, respectively. While studying extremities, skin temperature was kept between 32 and 34°C.

Measurement of F response latency was conducted with sweep duration of 100 ms (frequency 5–500 Hz, repetition rate 0.5 Hz). At least 10 consecutive stimuli were given and the minimum latency values observed were considered for evaluation.

Statistical analysis

All the results are given as means ± SD. Statistical analysis was made by GraphPad Instat, V2.02 software. Our data were tested and found suitable for evaluation with parametric tests. We used Student's t test for the comparisons among the groups, the paired t test for comparing the beginning and 6-month values, and the unpaired t test for the comparisons between placebo and vitamin E groups. In all the comparisons, two-sided P values were used. Descriptive statistics of the data and results of comparisons are given in Table 2.

RESULTS

— Characteristics of the patients are shown in Table 1. Of the 21 patients enrolled in the study, 3 were men and 18 were women. The mean age of the study subjects was 58 years (range 35–75 years). Comparison of the groups revealed no statistical difference between age, BMI, and duration of disease ($P > 0.05$).

The respective changes of the HbA_1 values during the 6-month treatment period within the placebo and the vitamin E groups were compared. The changes showed no statistical significance in each group ($P > 0.05$). The groups were also compared with each other at the beginning and at the end of the study. No statistically significant difference was found between the groups for HbA_1 levels both at the beginning and at the end of the study ($P >$

Table 2—*Descriptive statistics and results of comparisons of placebo and vitamin E groups*

	Placebo			Vitamin E		
	0 months	6 months	*P* value	0 months	6 months	*P* value
Median motor nerve						
DL	4.2 ± 0.87	4.2 ± 1.19	1.0	3.9 ± 0.79	3.8 ± 1.0	0.808
NCV	54.4 ± 5.38	54.3 ± 7.31	0.985	49.4 ± 4.23	55.1 ± 2.51	0.002*
A	5.1 ± 3.01	6.0 ± 3.24	0.062	3.6 ± 1.98	5.2 ± 3.57	0.103
F	27.8 ± 2.26	27.8 ± 1.52	0.065	28.1 ± 2.66	27.5 ± 2.83	0.277
Δ in NCV	—	−0.03	—	—	5.36	0.019†
Median sensory nerve						
NCV	45.0 ± 7.85	44.6 ± 7.55	0.673	42.6 ± 8.24	46.2 ± 5.81	0.062
A	4.2 ± 2.09	4.5 ± 1.76	0.588	4.9 ± 2.93	6.2 ± 4.85	0.314
Tibial motor nerve						
DL	4.7 ± 0.76	4.6 ± 0.91	0.527	4.9 ± 0.79	4.2 ± 0.56	0.028*
NCV	42.1 ± 5.15	44.5 ± 4.98	0.192	39.5 ± 6.02	42.1 ± 10.59	0.236
A	5.8 ± 2.51	6.3 ± 2.37	0.634	4.4 ± 3.04	5.3 ± 2.95	0.319
F	50.3 ± 6.06	43.1 ± 4.96	0.343	54.4 ± 7.78	53.2 ± 10.27	0.680
Sural sensory nerve						
NCV	42.2 ± 4.79	42.4 ± 6.69	0.918	39.4 ± 7.32	39.5 ± 8.27	0.979
A	7.6 ± 6.45	8.6 ± 7.07	0.208	3.8 ± 2.57	6.2 ± 6.67	0.116

Data are means ± SD. Δ in NCV, change in NCV during 6-month period. *Statistically significant improvement in the electrophysiological parameter after 6 months of vitamin E supplementation, when compared with beginning values; †statistically significant change in the electrophysiological parameter during 6-month period when the placebo and vitamin E groups were compared.

0.05). When the change in HbA_1 levels of both groups during a 6-month period was compared, it was found to be statistically significant ($P = 0.033$).

Descriptive statistics of electrophysiological parameters of the patients at the beginning and end of the study are given in Table 2. After 6 months of treatment, the diabetic group having vitamin E supplementation had significant increases in median motor nerve conduction velocity ($P = 0.0019$), and the tibial motor nerve distal latency decreased significantly ($P = 0.0284$). *F* latencies and the amplitudes of the motor nerves did not show statistically significant changes ($P > 0.05$). The electrophysiological parameters of the sensory nerves also demonstrated no statistically significant changes ($P > 0.05$).

CONCLUSIONS — In the present study, supplementation of vitamin E for 6 months resulted in improvement in 2 of 12 electrophysiological parameters of nerve conduction in diabetic patients with mild sensorimotor neuropathy. Among the chain-breaking antioxidants, which prevent the propagation of free radical–induced chain reactions, ascorbate and tocopherol are particularly important. Tocopherol is a major lipid-phase antioxidant. The importance of tocopherol as an antioxidant has been increasingly recognized in recent years (18). We are not aware of any reports describing the effects of tocopherol supplementation to patients with diabetic peripheral sensorimotor neuropathy. But there are few clinical reports about the supplementation of vitamin E to diabetic patients. In the study by Paolisso et al. (19), they showed the improvement of insulin action in healthy and NIDDM patients by supplementation of pharmacological doses of vitamin E. Again in the study by Ceriello et al. (20), it was shown that vitamin E could decrease protein glycation in type 2 diabetes and was argued that vitamin E could be a new prospect for the prevention of diabetic complications.

In our study at the end of 6 months, median motor nerve conduction velocity increased significantly ($P = 0.0019$) and the tibial motor nerve distal latency decreased significantly ($P = 0.0284$) in the group having vitamin E supplementation. Reversal of the defective nerve conduction velocity is more readily appreciated in the upper-extremity motor nerves. This may be due to the fact that the lower-extremity nerves, being longer in length, are insulted by the diabetic metabolic complications earlier than are the upper-extremity nerves. Having been exposed to the biochemical insults for a longer time may cause more irreversible damage to the nerves (12).

The slowing of the nerve conduction is among the earliest neuropathic abnormalities that occur in type 2 diabetes. Sensory fibers usually are affected first, followed by motor fibers (12). For this reason, the diabetic microangiopathic damage of the sensory fibers may be more pronounced and thus may be irreversible (12). In our case also, sensory fiber defects both in the lower and the upper extremity showed no improvement at the end of 6 months.

Sensory action potentials and somatosensory-evoked responses have been shown to be more sensitive methods than motor studies in detecting early nerve involvement (12). Lower-extremity distal nerves, in particular, the peroneal and the sural nerves, frequently show the most significant abnormalities. In diffuse peripheric neuropathy cases, having both the motor and sensory nerve fiber involvement, the slowing of the nerve conduction velocity is more obvious when the longer nerves are studied (12,15). At the beginning of the study, lower-extremity electrophysiological study revealed more pronounced abnormalities than the upper-extremity electrophysiological parameters. There was no significant variation among the electrophysiological parameters of the patients at the beginning ($P > 0.05$). Latency measurements are highly specific for the motor nerve fiber conduction evaluation. Although nerve conduction velocity measurement is also highly sensitive, it is not that specific (12,15). These two parameters are important not for the diagnosis but for the follow-up of the patient.

Endoneural microangiopathy with resulting decreased endoneural oxygen tension and endoneural blood flow have been demonstrated in patients with diabetic neuropathy (5,14,21). In diabetes, the increased levels of the free oxygen radicals neutralizes the endoneural nitric oxide and thus inhibits the vasorelaxation. At the same time, these radicals form the very reactive hydroxyl radicals, which are responsible for the endothelial cell toxicity. The free radicals thus cause microangiopathy characterized by endoneural vascular damage and endoneural hypoxia (22,23). Vitamin E can inhibit the free radical–induced endoneural damage (24). Vitamin E can also improve the antioxidant tone in the diabetic individual in whom the antioxidant capacity is defective because of the active polyol pathway (13).

It is shown by Ceriello et al. (20) that protein glycosylation is reduced in diabetes by vitamin E supplementation. In our study, although the mean HbA_1 level of the vitamin E group is higher than that of the placebo group at the beginning of the study, statistical analysis of the groups at the beginning revealed that the difference is not significant. On the other hand, the mean HbA_1 level of the vitamin E group at the end of the study decreased, while the mean HbA_1 level of the placebo group increased. But again, the changes were without statistical significance. Still, we cannot exclude the beneficial effects of improved metabolic status, possibly induced by vitamin E supplementation, on amelioration of diabetic neuropathy because the change in HbA_1 levels during the 6-month supplementation period was found to be statistically significant.

In the in vivo studies carried on the erythrocytes and the platelets, it has been demonstrated that there is deficiency of vitamin E in diabetic patients. It is also shown that vitamin E deficiency is correlated with the microvascular complications of diabetes (3,25,26). However, there is still controversy about the place of vitamin E in the treatment of chronic degenerative complications of diabetes. It is usually advised that vitamin E supplementation should aim for the optimal tissue concentration but this optimal level is not known yet. Although it was initially intended to measure serum vitamin E levels and determine the possible therapeutic levels in the design of this study, determination of serum vitamin E levels of the subjects before and after the study could not be achieved because of technical details, which constitutes a major limitation to the study.

This short-term study, being a preliminary one, provides us data about the possible effects of pharmacological treatment with vitamin E supplementation on the electrophysiological parameters of diabetic patients with mild to moderate peripheral neuropathy and provides the basis for future studies with larger groups of patients with longer duration of treatment.

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