·Basic Research·

Effect of sodium tungstate on visual evoked potentials in diabetic rats

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Abstract

• **AIM: To evaluate the effect of sodium tungstate on visual evoked potentials (VEPs) in diabetic rats.**

• **METHODS: Wistar rats were randomly divided into three groups as normal control, diabetic control and diabetic rats treated with sodium tungstate. Diabetes was induced by single intraperitoneal injection of streptozotocin (50 mg/kg). Sodium tungstate [40 mg/(kg·d)] was administered for 12wk and then VEPs were recorded. Additionally, thiobarbituric acid reactive substance (TBARS) levels were measured in brain tissues.**

• **RESULTS: The latencies of P1, N1, P2, N2 and P3 waves were significantly prolonged in diabetic rats compared with control group. Diabetes mellitus caused an increase in the lipid peroxidation process that was accompanied by changes in VEPs. However, prolonged latencies of VEPs for all components returned to control levels in sodium tungstate-treated group. The treatment of sodium tungstate significantly decreased brain TBARS levels and depleted the prolonged latencies of VEP components compared with diabetic control group.**

• **CONCLUSION: Sodium tungstate shows protective effects on visual pathway in diabetic rats, and it can be worthy of further study for potential use.**

• **KEYWORDS:** diabetes; retinopathy; sodium tungstate; visual evoked potentials; lipid peroxidation
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INTRODUCTION

Diabetes mellitus (DM) is considered a major health concern worldwide. Approximately 285 million people have diabetes around the world and, an estimated 438 million people will have diabetes by the year 2030 [1]. DM, a chronic metabolic disorder characterized by high blood glucose (hyperglycemia), is divided into two classes: type 1 and type 2 [2]. While progressive failure of β-cells in pancreas is observed in both types, type 1 DM is caused by an autoimmune attack against the β-cells [3]. DM leads to several acute and chronic complications including neuropathy, nephropathy, cardiomyopathy, microangiopathy, atherosclerosis, foot ulcers and retinopathy[4].

Visual anomalies stemming from vascular damage and metabolic imbalances are frequently seen in DM. Furthermore, ganglionic and preganglionic elements of the entire retina and visual pathway may be involved in the development of visual deformity. Therefore, neural conductance might slow down along the postretinal central visual pathways [5]. Besides, considering vasculopathy and neuropathy associated with DM, it is reasonable to expect dysfunction along visual pathway upstream from the retina[6]. Visual evoked potentials (VEPs) are known to be a highly reliable electrophysiological method of detecting the earliest changes in retina, optic pathway, subcortical and visual cortex [7-8]. VEPs elicited by flash stimuli have provided evidence of subclinical visual alterations in diabetic rats. Additionally, delay of VEP components in diabetic rat models have been shown in previous studies[9-10].

Oxidative stress has been suggested to play a crucial role in the pathogenesis and progression of diabetes and its complications by various groups in the literature [11-13]. Hyperglycemia gives rise to oxidative stress which is the consequence of imbalance between production and removal processes of reactive oxygen species (ROS) [11,14]. Overproduction of ROS often leads to damage in cellular macromolecules (DNA, lipids and proteins), contributing to the progress of diabetic complications and various organ injuries [15]. Oxidative stress is indirectly shown by assaying products of oxidative damage such as thiobarbituric acid reactive substance (TBARS) levels indicating membrane lipid peroxidation and cellular injury [16]. Accumulating evidence suggests that radicals derived from reactive oxygen

play a pivotal role in the development of diabetic retinopathy[15]. On the other hand, brain and retina are particularly sensitive to the oxidative stress due to high rate of oxygen consumption. Both in diabetic humans and experimentally diabetic rats, oxidative stress has been shown to mediate brain and retina damage[17-18].

Although various hypoglycemic drugs have been proposed for DM treatment, diabetes related complications continue to be major medical problems. Thus, it is of great interest to develop new pharmacological agents. In the last decade, several inorganic compounds either mimicking the effects of insulin or increasing its action such as chromium, molybdate, cobalt, vanadate, selenate and sodium tungstate (ST) have been suggested for DM treatment [19]. ST has low toxicity profile dependent with the dose and way of administration. Sachdeva *et al* [20] demonstrated that ST increases ROS, catalase and glutathione peroxidase in erythrocytes in a dose dependent manner especially in intraperitoneal administration compared to oral administration. In another study McCain *et al* [21] showed that 200 mg/(kg•d) oral administration of ST significantly decreased food consumption and body weight gain in only male rats but 75 mg/(kg•d) oral administration of ST did not show any observable side effects in both sexes of animals. Together with these limited side effects ST has great benefits reported in experimental animal models. It has been used in diabetic animal models as an antioxidant and antidiabetic agent[22]. In streptozotocin (STZ) induced diabetic rats, oral administration of ST decreases in blood glucose concentration, normalizes diabetes induced alterations in glucose and glycogen metabolism [23-24], either increase antioxidant defense mechanisms or reduce the oxidative stress [20]. Therefore, ST might be protective against the defects in visual system caused by DM.

Despite these encouraging benefits showed in experimental animal studies only one human trial has been performed to date [25]. In this prospective, randomized placebo controlled, double blind study, no evidence of therapeutic effect was found in grade I and II obesity patients[26].

In this study, VEPs were recorded and the latencies of VEP components were analyzed in order to evaluate the effects of ST on alterations of neural transmission in visual pathway induced by DM in diabetic rats. Additionally TBARS levels of brain tissues were measured as an indicator of lipid peroxidation.

MATERIALS AND METHODS

Experimental Design This study protocol was approved by the Institutional Animal Care and Use Committee at Akdeniz University. We confirm adherence to the Association for Research in Vision and Ophthalmology (ARVO) statement for the Use of Animals in Ophthalmic and Vision Research. Twenty seven male Wistar rats, weighing 190-210 g, were housed at 23℃-28℃ on a 12h day-night cycle with a standard diet and water *ad libitum*. Rats were randomly divided into control (C), DM and diabetes mellitus treated with sodium tungstate (DM+ST) groups. Diabetes was constituted by single intraperitoneal injection of 50 mg/kg STZ. One week following injection, blood glucose levels were monitored by Accu-check glucometer (Roche Diagnostic, Turkey). Blood glucose level of rats is higher than 300 mg/dL were considered diabetic. DM group rats were fed with saline and ST [(40 mg/(kg•d)] was administered to DM+ST group both *via* gastric gavage for 12wk. At the end of 12wk, VEPs of rats were recorded under anesthesia.

Visual Evoked Potential Recordings VEPs were recorded subdermally *via* needle electrodes (Medelec 017K024, Medelec Manor Way, Old Working Surrey, UK). Rats were under ether anesthesia throughout recordings. The reference and active electrodes were placed at 0.5 cm anterior and posterior to the bregma, respectively. Ground electrode was located on the tail of each animal. Following a 5min dark adaptation period, a photic stimulator (Nova-Strobe AB; Biopac System, Inc., Santa Barbara, CA, USA) at the lowest intensity level was used to provide the flash stimulus at a distance of 15 cm, which allowed illumination of the entire pupilla from the temporal visual field. Repetition frequency of flash stimulus was adjusted to 1 Hz, and flash energy was 0.1 J. VEPs were obtained from both right and left eyes. During recordings, unstimulated eye was veiled by an appropriate black carbon paper and cotton. Body temperature was maintained between 37.5℃ and 38℃ by a heating pad. The averaging of 100 responses was performed by the average of Biopac MP100 data acquisition equipment (Biopac System, Inc.). Response duration was determined as 300ms. The frequency bandwidth of the amplifier was 1-100 Hz. The gain was selected as 20 mV/div. The microprocessor was programmed to reject any epoch containing large artifacts, and at least 2 averages were obtained to guarantee response reproducibility. Peak latencies of the VEP components were estimated as the duration between the stimulus artifact and the peak in milliseconds. Amplitudes were quantified as the voltage between successive peaks.

Chemical Analysis Under deep urethane anesthesia, brains of rats were perfused transcardially with heparinized saline, removed immediately and stored at -80℃. Brain TBARS levels were quantified by a fluorimetric method described by Wasowicz *et al* [27], using 1.1.3.3-tetraethoxypropane as the standard. Brain tissues were weighed and homogenized (Bio-Gen Pro-200) in ice-cold 50 mmol/L potassium phosphate buffer at pH 7. Homogenates were centrifuged at 10.000 g for 15min at 4℃ (Sigma 3-18 K centrifuge) and supernatants were used for the analysis. Supernatants (50 μL) were transferred into a tube containing 29 mmol/L thiobarbituric acid in acetic acid (8.75 mol/L), samples were

placed in a water bath and heated for 1h at 95℃ -100℃ . Following samples were cooled down, 25 μL of 5 mol/L HCl was added and the reaction mixture was extracted by agitation for 5min with 3.5 mL n-butanol. After centrifugation, the butanol phase was separated and the fluorescence of the butanol extract was assessed in a fluorescence plate reader (Biotek-synergy Mx) using excitation and emission wavelengths of 525 nm and 547 nm respectively. The results are presented as nmol/g protein.

Protein Level Protein concentrations in all samples were determined spectrophotometrically (Shimadzu RF-5500, Kyoto, Japan) by a protein assay reagent kit (Pierce, Rockford, IL, USA) by a modified Bradford method [28]. Bovine serum albumin was used as internal standard.

Statistical Analysis The results were expressed as mean ± standard error of the mean. Multiple comparisons among brain TBARS levels of groups were achieved by Kruskal Wallis test and all pairwise comparisons were performed by posthoc Mann-Whitney *U* test. Differences in VEP latencies were evaluated by one-way ANOVA and posthoc Tukey tests. *P* values less than 0.05 were considered statistically significant.

RESULTS

Blood glucose level of C group was lower than both DM and DM+ST groups throughout the experiments. No significant difference was observed between glucose values of DM group with DM+ST group (Table 1).

Latencies of VEP components are presented in Table 2. In all groups, three positive (P1, P2, P3) and two negative (N1, N2) components were analyzed. The mean latencies of each VEP component recorded from all experimental groups were shown in Table 2. The latencies of P1, N1, P2, N2 and P3 components were significantly prolonged in diabetic rats compared with control group. However, prolonged latencies of VEP components in diabetic rats returned to control levels after ST administration. Representative VEP traces of each group are shown in Figure 1.

Brain TBARS level (1.15±0.04 nmol/g protein) was higher in the DM group, compared with C group (0.40±0.01 nmol/g protein). ST administration resulted in lower brain TBARS level in DM+ST group (0.89±0.09 nmol/g protein) compared to DM group. Brain TBARS levels of each group are presented in Figure 2.

DISCUSSION

Being a worldwide major health concern, DM has been studied *via* various experimental animal models. STZ injection is a validated experimental rat DM model by generating insulinopenic type 1 DM and involving impairment of the immune system [15]. As expected, type 1 DM was induced by STZ administration also in our study. That is, high blood glucose levels of STZ injected rats confirmed the accomplishment of the model.

Table 1 Blood glucose levels of experimental groups n=9 ($\bar{x} \pm s$)

Groups	Blood glucose levels (mg/dL)
C	117.2±2.1
DM	464.3±16.7[b]
DM+ST	435.2±19.6[b]

[b]P<0.01 *vs* C group.

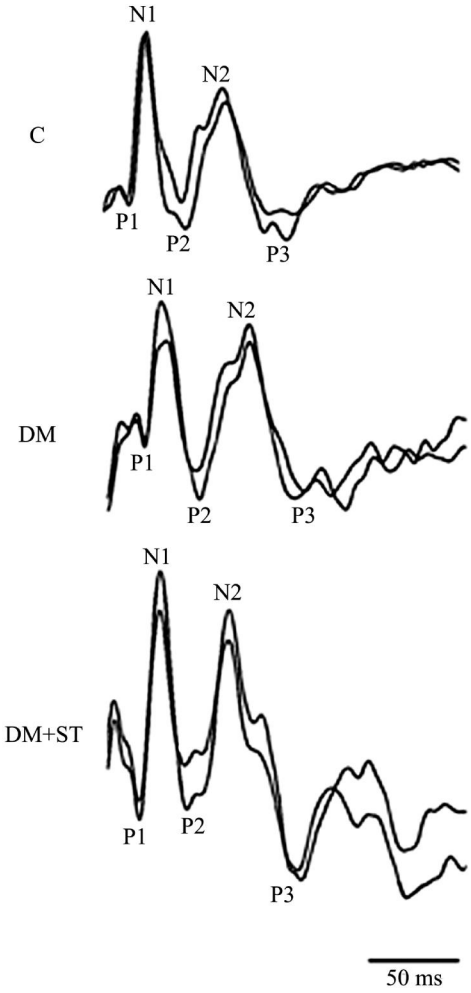

Figure 1 Representative VEPs traces of all experimental groups.

Once induced and accompanied by high blood glucose concentration, DM ultimately gives rise to numerous complications, one of which is retinopathy[4]. VEP alterations have been considered as indexes of optic neuropathy in diabetic patients by several investigators [29-30]. Paralleling to previous researches[7,31-32], STZ-induced diabetic rats displayed elongation of all positive (P1, P2, P3) and negative (N1, N2) VEP component latencies in the present study. DM related such VEP changes might be in relation with the alterations in the electro conductive properties of myelin sheath caused by various factors such as metabolic disturbances, impaired incorporation of acetate and glucose into nerve lipid and increased lipid peroxidation[16].

ST is a newly described agent that mimics the effects of insulin or increases its action when given orally to diabetic rats [23-24,33-34]. As oral administration of ST has recently emerged as an efficient therapeutic for DM, we aimed to

Table 2 The latencies of VEP components in the control and experimental groups n=9 ($\bar{x} \pm s$)

Groups	P1 (ms)	N1 (ms)	P2 (ms)	N2 (ms)	P3 (ms)
C	17.8±0.37	30.8±0.42	48.0±0.82	70.0±1.33	93.6±0.95
DM	21.3±0.61[b]	35.2±0.64[b]	54.4±1.17[b]	78.1±0.97[b]	107.7±0.70[b]
DM+ST	17.55±0.55[d]	30.22±0.49[d]	49.0±0.64[d]	71.1±1.20[d]	98.1±1.01[d]

[b]P<0.01 *vs* C group; [d]P<0.01 *vs* DM group.

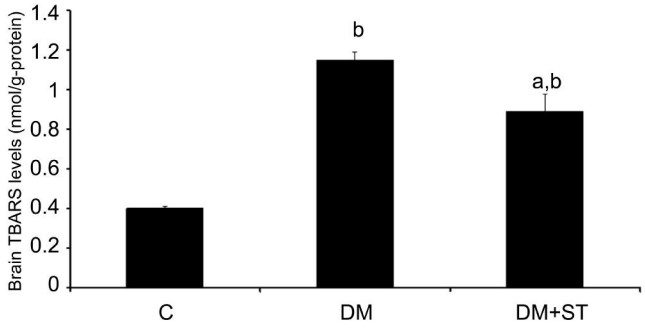

Figure 2 TBARS levels of all groups Bars represent the group means±SEM. [a]P<0.05 *vs* DM group, [b]P<0.01 *vs* C group (n=6 for all groups).

investigate possible effects of ST on VEP alterations arising from DM. ST treatment was observed to return prolonged VEP component latencies back to control levels in DM+ST rats. Due to the fact that VEPs have been shown to be a sensitive and reliable method to evaluate the earliest changes in the visual system [7], our results indicate that ST can ameliorate DM mediated visual system defects.

Several studies have clearly demonstrated that DM is associated with increased oxidative stress in retina and brain [9,22,35]. Monitoring of oxidative stress in experimental research can be done indirectly by assaying products of oxidative damage as TBARS levels and malondialdehyde (MDA) that indicate membrane lipid peroxidation and cellular injury [16,36]. Lipid peroxidation of cellular structures, a consequence of increased free radicals is thought to play an important role in long-term complications of DM [37-38]. Oxidative damage to lipids in the brain and retina of experimental diabetic rats has been reported [39-41]. In agreement with previous studies [9,20], an increase of brain TBARS level due to DM was observed in the present study. ST administration reduced the effect of DM and resulted in a lower brain TBARS level of ST treated diabetic rats. Such action of ST might be attributed to its hypoglycemic property as well as its antioxidant effect by eliminating free oxygen radicals.

The present study showed the occurrence of oxidative stress in diabetic rats as elongation of VEP component latencies. Additionally, the fact that decrease of TBARS levels are accompanied with the shortening of elongated VEP latencies points out that oxidative stress might play a pivotal role in DM induced VEP alterations. Although ST was suggested to reduce brain TBARS levels previously, it is the first time that ST treatment is correlated with VEP alterations. Therefore, ST may be proposed for DM induced visual system injury treatment and introduced as an antioxidant agent besides being antidiabetic provided that supporting data are obtained in further studies.

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