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Selenium and Topiramate Attenuates Blood Oxidative Toxicity in Patients with Epilepsy: A Clinical Pilot Study

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Abstract It is well known that oxidative stress plays an important role in the etiology of epilepsy. We investigated effects of selenium (Se) and topiramate (TPM) combination supplementation on antioxidant and oxidant values in control and patients with epilepsy and refractory epilepsy. For the aim, we used control (n=19), epilepsy + TPM (n=19), epilepsy + TPM + Se (n=15) groups. We also used control (n=15), refractory epilepsy (n=15), and refractory epilepsy + Se (n=8) groups. TPM (0.2 mg/daily) and Se, as sodium selenite (twice daily with 0.1 mg doses), were orally supplemented to the patients for 45 days. Erythrocyte lipid peroxidation levels were higher in refractory epilepsy groups than in control although its level and seizure numbers were decreased in TPM and TPM + Se supplemented groups of the patients. The erythrocyte reduced glutathione (GSH), glutathione peroxidase (GSH-Px), plasma total antioxidant status (TAS), and vitamin E concentration in refractory epilepsy group were lower than in control. However, the erythrocyte and plasma TAS, erythrocyte GSH and GSH-Px, and plasma vitamins A and C values were increased either by Se or Se + TPM in epilepsy and refractory epilepsy groups. There were no effects of TPM and Se on plasma β-carotene values in the groups. In conclusion, TPM and selenium caused protective effects on the epilepsy and refractory epilepsy-induced oxidative injury by inhibiting free radical production and supporting antioxidant redox system.

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Abbreviations

GSH	Glutathione
GSH-Px	Glutathione peroxidase
LP	Lipid peroxidation
NADH	Nicotinamid Adenine Dinucleotide Dehydrogenase
PUFA	Polyunsaturated fatty acid
ROS	Reactive oxygen species
TPM	Topiramate
TAS	Total antioxidant status
VGCC	Voltage-gated calcium channels

Introduction

Epilepsy is a common neurological disorder affecting approximately 1 % of the population worldwide and can cause acute transient complex neurobehavioral disorders resulting from increased excitability of neurons in various brain regions [1]. Although considerable research is being conducted on long-term changes associated with epileptogenesis, the exact cellular and molecular mechanisms involved in epileptogenesis are still unclear. Oxidative stress induces generation of reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, and hydrogen peroxide. The ROS are strongly implicated in a number of neuronal and neuromuscular disorders, including epilepsy [2, 3]. The relationship between free radical and antioxidants has been found in the epilepsy and ROS have been implicated in seizure-induced neurodegeneration [4, 5]. For example, there is a temporal correlation between ROS formation and seizure development [6, 7]. Current researches have suggested that antioxidant compounds may afford

some level of neuroprotection against the neurotoxicity of seizures in cellular level [4, 5].

Certain trace element balance is crucial for a health nervous system and neuronal susceptibility to excitability. The selenium is an essential dietary trace element which plays an important role in a number of biological processes [2]. GSH-Px, a selenium containing enzyme, is responsible for the reduction of hydro- and organic peroxides in the presence of reduced glutathione (GSH) [8]. For example, hydrogen peroxide is converted to water by GSH-Px enzyme. GSH is also a hydroxyl radical and singlet oxygen scavenger and it participates in a wide range of cellular functions [9]. Several reports suggested that body selenium levels play a vital role in seizure condition to develop [9, 10] and selenium deficiency increased susceptibility to glutamate-induced excitotoxicity and seizure activity in HT22 cells [9, 10]. The functions of selenium as an antioxidant trace element are believed to be carried out by selenoproteins that possess antioxidant activities and the ability to promote neuronal cell survival [4, 5].

Topiramate (TPM) is new antiepileptic drug which inhibits voltage-gated sodium and calcium channels, blocks glutamate AMPA/kainite receptors, and enhances the $GABA_A$ receptor mediated chloride enhances [11]. An increasing body of evidence indicated that TPM possesses not only antiepileptic but also neuroprotective properties due to its multiple mechanisms of action [12]. For example, TPM reduced diabetes-induced oxidative stress through inhibition of mitochondrial carbonic anhydrases activity in the mouse brain and rescues cerebral pericytes dropout [13]. Over Ca^{2+} influx induces over production of ROS through mitochondrial depolarization in cells [14]. To our knowledge, there is no report on oxidative stress and antioxidant values in selenium-treated epileptic and refractory epileptic patients. However, we have recently observed protective effects of selenium and TPM on Ca^{2+} influx, Ca^{2+}-ATPase activity and oxidative stress values in PC12 neuronal cells and rat brain [4, 6]. Hence, selenium and TPM combination may induce protective effects on antioxidant system in patients with epilepsy and refractory epilepsy.

It has not been studied whether TPM and selenium with modify the alterations in lipid peroxidation, total antioxidant states (TAS), GSH, GSH-Px and antioxidant vitamin values in the patients with epilepsy. Hence, we aimed to evaluate whether there would be protective effect of TPM and selenium as sodium selenite on the oxidative stress and antioxidants values in patients with epilepsy and refractory epilepsy.

Materials and Methods

Chemicals

All chemicals were obtained from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA) and all organic solvents from Merck Chemical Inc. (Darmstadt, Germany). All reagents were analytical grade. All reagents except the phosphate buffers were prepared daily and stored at +4 °C. The reagents were equilibrated at room temperature for 0.5 h before an analysis was initiated or reagents containers were refilled. Phosphate buffers were stable at +4 °C for 1 month. Selenium, as sodium selenite (Solgar Seleno-6), was bought from Solgar Inc., İstanbul, Turkey. TPM (Topamax) was obtained from Johnson & Johnson Inc, İstanbul, Turkey.

Patients and Treatments

The study was approved by the local ethical committee of Suleyman Demirel University (SDU) Medical Faculty. The study group was selected from patients admitted to SDU Research Hospital Neurology clinic with epilepsy. Initially, 19 epileptic patients using TPM (0.2 mg) therapy (6 male, 13 female, age 28.6±9.7) and 15 refractory epilepsy group (8 male, 7 female, age 30.8±11.6), 19 sex- and age-matched healthy volunteers were included in the study but in the TPM group, 5 of these patients and in the refractory epilepsy group, 7 of these patients did not come to control. The control group comprised 7 men (36.5 %) and 12 women (64.5 %) and the controls were selected from volunteer hospital persons such as nurses and technicians. There was no statistically significant difference between the ages and gender of the groups in the study. In summary, epilepsy groups in the currents manuscript were control (n=19), TPM (n=19), and TPM + selenium (n=15) and refractory epilepsy groups were control (n=15), refractory epilepsy (n=15), and refractory epilepsy + selenium (n=8).

Blood oxidative stress and antioxidant analyses were evaluated in control and the patients before and selenium treatment. Of the 14 patients with epilepsy, 11 had tonic–clonic seizures, 2 had tonic–clonic and absence seizures, 1 had frontal lobe epilepsy. In the refractory epilepsy group, five had tonic-clonic seizures, two had complex partial seizures, and one had myoclonic seizures. The patients were not taking any other antioxidant agent prior to selenium medication. There were no acute medical conditions such as infection, physical exertion and trauma at the time of blood drawn. All of the patients on topiramate treatment were receiving selenium addition for 45 days. The drug (selenium) was taken twice daily with 0.1 mg doses. Blood samples were obtained from the patients at initial, before selenium medication and 45 days later, after the use of selenium.

Blood Collection and Preparation of Blood Samples

Twelve hours fasting venous blood (5 ml) was taken from the antecubital vein, using a monovette system of blood collection, into anticoagulated tubes containing sodium

EDTA and non-anticoagulated tubes, protected against light. One milliliter of anticoagulated blood was used for hematological analysis. The remaining anticoagulated blood was separated into plasma and erythrocytes by centrifugation at 1,500×*g* for 10 min at +4 °C. The erythrocytes samples were washed three times in cold isotonic saline (0.9 %, *v*/*w*), then hemolysed with a nine-fold volume of phosphate buffer (pH 7.4).

After addition of butylhydroxytoluol (4 μl per ml), the hemolysed erythrocytes and plasma were stored at −30 °C for <3 months pending measurements of lipid peroxidation, TOS and GSH levels. The remaining hemolysed erythrocytes and plasma was used for immediate GSH-Px activity and plasma vitamin concentrations.

Lipid Peroxidation and TOS Level Determinations

Lipid peroxidation levels in the plasma and hemolyzate were measured with the thiobarbituric-acid reaction at 532 nm by the method of Placer et al. [15]. The quantification of thiobarbituric acid reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde equivalents generated by acid catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of lipid peroxidation (LP) in the plasma and hemolyzate were expressed as micromoles per gram protein.

The erythrocyte and plasma TAS levels were measured calorimetrically using the TAS kit (Mega Tıp Inc, Gaziantep, Turkey) [16]. The results in the plasma and erythrocytes were expressed in micromole H_2O_2 equivalent per liter (μmol H_2O_2 equiv./l) and micromole H_2O_2 equivalent per gram protein (μmol H_2O_2 equiv/g prot), respectively.

Reduced Glutathione, Glutathione Peroxidase and Protein Assay

The GSH content of the brain homogenate and microsomal was measured at 412 nm using the method of Sedlak and Lindsay [17] as described own studies [6, 7]. GSH-Px activities of erythrocytes were measured spectrophotometrically at 37 °C and 412 nm according to the Lawrence and Burk [18]. The protein content in the erythrocyte was measured by the method of Lowry et al. [19] with bovine serum albumin as the standard.

Plasma β-Carotene, Vitamins A, C, and E Analyses

Concentrations of vitamins A and E in the plasma samples were determined by spectrofluorometrically (Infinitepro200 Plate reader, Tecan Group Ltd. Männedorf, Switzerland) according to methods of Desai [20] as described in previous studies [21, 22]. Samples were saponified with sodium hydroxide in the presence of pyrogallol (saturated form in water) as an antioxidant for 30 min at 70 °C. The vitamin A and E were extracted from the plasma sample with hexane and the levels were monitored spectrofluorometrically (excitation: 330 nm, emission: 470 nm for vitamin A; excitation: 295 nm, emission: 330 nm for vitamin E). Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in hexane and the results are expressed in nanomole per liter and micromole per liter of plasma, respectively.

The levels of β-carotene in plasma samples were determined according to the method of Suzuki and Katoh [23]. Two milliliters of hexane were mixed with 250 μl plasma. The value of β-carotene in hexane was measured at 453 nm in a spectrophotometer.

Plasma vitamin C was determined by the spectrophotometry [24] and is expressed in micromoles per liter.

Statistical Analyses

All results are expressed as means ± SD. *p* values of less than 0.05 were regarded as significant. We firstly tested presence of statistical significance by LSD test. Then, significant values were assessed with student *t* test. Data was analyzed using the SPSS statistical program (version 17.0 software, SPSS Inc. Chicago, Illinois, USA).

Results

Demographic Values

Demographic values of the groups are shown in Tables 1 and 2. There were no statistical significances on the demographic values in the groups.

Plasma and Erythrocytes Lipid Peroxidation

The plasma and erythrocyte lipid peroxidation concentrations in the groups of epilepsy and refractory epilepsy are shown in Tables 3, 4, 5, and 6. The erythrocyte lipid peroxidation levels in patient with refractory epilepsy were significantly ($p<0.01$) higher than in control although its levels

Table 1 Demographic values of control and patients with epilepsy (mean ± SD)

Parameters	Control ($n=19$)	Patients ($n=19$)	*p* values
Age	22.9±9.0	28.6±9.7	>0.05
Gender (F/M)	12/7	13/6	>0.05
Disease duration (year)	–	17.1±7.7	–
Topiramate take time (month)	–	41.7±23.2	–

Table 2 Demographic values of control and patients with refractory epilepsy (mean ± SD)

Parameters	Control (n=15)	Patients (n=15)	p values
Age	26.1±7.3	30.8±11.6	>0.05
Gender (F/M)	10/6	7/8	>0.05
Disease duration and antiepileptic intake time (year)	–	16.7.0±10.2	–

were decreased by selenium (p<0.001). The mean plasma and erythrocytes lipid peroxidation values in patients with epilepsy were decreased by the TPM (p<0.001) and TPM plus selenium (p<0.05 and p<0.001) supplementations.

GSH and GSH-Px Values

The mean values of erythrocyte GSH and GSH-Px in the groups are shown in Tables 3 and 5, respectively. The GSH and GSH-Px values in the patients with refractory epilepsy was significantly (p<0.05) lower than in control although their values were increased in the patients by selenium treatment (p<0.05 and p<0.001). The erythrocyte GSH and GSH-Px values were also increased in the patients with epilepsy by TPM (p<0.05) and TPM + selenium (p<0.05 and p<0.001) supplementation.

Antioxidant Vitamin Concentrations

The mean values of plasma vitamins A, C, E, and β-carotene in the groups are shown in Tables 4 and 6, respectively. The vitamin A (p<0.05), vitamin C (p<0.05), and vitamin E (p<0.01) concentrations in patients with epilepsy were significantly higher in TPM group and TPM + selenium groups than in control group. However, vitamin E concentrations in patients with refractory epilepsy were lower (p<0.001) than in control although its concentration in the patient was increased by selenium supplementation (p<0.05). However, vitamin A and β-carotene concentrations in patients with refractory epilepsy did not change in patients and treatment groups. The β-carotene concentrations in patients with epilepsy did not also change in TPM and TPM + selenium groups.

Table 3 Effects of topiramate (TPM) and selenium (Se) on erythrocytes lipid peroxidation (LP), total antioxidant status (TAS), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) in patients with epilepsy (mean ± SD)

Parameters	Control (n=19)	TPM (n=19)	TPM + Se (n=14)
LP (μmol/g protein)	17.74±1.60	13.10±1.11[c]	11.86±1.00[c,d]
TAS (μmol H_2O_2 equiv./l)	2.20±1.50	3.42±1.73[c]	3.74±1.11[c,,d]
GSH (μmol/g protein)	5.74±0.71	6.82±1.01[a]	7.55±0.99[b,c]
GSH-Px (IU/g protein)	9.17±0.74	12.20±2.03[a]	14.50±1.95[d,e]

[a] p<0.05 versus control group
[b] p<0.01 versus control group
[c] p<0.001 versus control group
[d] p<0.05 versus TOPM group
[e] p<0.01 versus TOPM group

Table 4 Effects of topiramate (TPM) and selenium (Se) on plasma lipid peroxidation (LP), total antioxidant status (TAS), vitamin A, β-carotene and vitamin E levels in patients with epilepsy (mean ± SD)

Parameters	Control (n=19)	TPM (n=19)	TPM + Se (n=14)
LP (nmol/ml)	1.80±0.21	1.48±0.12[c]	1.38±0.16[c]
TAS (μmol H_2O_2 equiv/ g prot)	5.49±1.49	6.69±2.22[a]	7.77±1.48[a,d]
Vitamin A (nmol/l)	1.92±0.25	2.56±0.36[a]	2.82±0.31[b]
Vitamin C (μmol/l)	140.8±48.8	164.9±32.8[a]	165.7±34.3[a]
β-carotene (μmol/l)	0.85±0.12	0.95±0.13	0.90±0.17
Vitamin E (μmol/l)	12.65±1.49	16.59±1.81[b]	17.19±1.14[b]

[a] p<0.05 versus control group
[b] p<0.01 versus control group
[c] p<0.001 versus control group
[d] p<0.01 versus TPM group

TAS Levels

Erythrocyte and plasma TAS levels in the groups are shown in Tables 4, 5, and 6. The TAS levels in patients with epilepsy were significantly higher in TPM (p<0.05) and TPM + selenium groups (p<0.01) than in control. The TAS values in patients with epilepsy were also significantly (p<0.05) higher in TPM + selenium group than in only TPM group. The erythrocyte (p<0.05) and plasma (p<0.01) TAS

Table 5 Effects of selenium (Se) on erythrocytes lipid peroxidation (LP), total antioxidant status (TAS), reduced glutathione (GSH) and glutathione peroxidase (GSH-Px) in patients with refractory epilepsy (mean ± SD)

Parameters	Control (n=15)	Patient (n=15)	Patient + Se (n=8)
LP (μmol/g protein)	12.90±1.16	17.47±1.28[b]	11.40±0.49[d]
TAS (μmol H_2O_2 equiv/g prot)	3.10±0.87	2.04±0.61[a]	3.27±0.89[d]
GSH (μmol/g protein)	5.86±0.67	5.40±0.80[a]	8.05±0.82[d]
GSH-Px(IU/g protein)	10.10±0.81	8.46±1.29[a]	10.80±1.84[c]

[a] p<0.05 versus control group
[b] p<0.01 versus control group
[c] p<0.05 versus patient group
[d] p<0.001 versus patient group

Table 6 Effects of selenium (Se) on plasma lipid peroxidation (LP), total antioxidant status (TAS), vitamin A, β-carotene and vitamin E levels in patients with refractory epilepsy (mean ± SD)

Parameters	Control (n=15)	Patient (n=15)	Patient + Se (n=8)
LP (nmol/ml)	1.77±0.18	1.77±0.10	1.64±0.16
TAS (μmol H_2O_2 equiv./l)	7.51±1.56	5.52±2.10[b]	7.78±2.15[e]
Vitamin A (nmol/l)	1.93±0.24	2.12±0.27	1.98±0.38
Vitamin C (μmol/l)	131.1±39.5	144.7±39.7	161.8±39.2[a,d]
β-carotene (μmol/l)	0.86±0.12	0.75±0.12	0.87±0.17
Vitamin E (μmol/l)	18.98±2.23	12.26±0.89[c]	15.54±2.60[d]

[a] $p<0.05$ versus control group
[b] $p<0.01$ versus control group
[c] $p<0.001$ versus control group
[d] $p<0.05$ versus patient group
[e] $p<0.001$ versus patient group

levels in patients with refractory epilepsy were significantly lower in patient group than in control. However, the TAS values in the patients with refractory epilepsy were significantly ($p<0.001$) higher in selenium group than in the patient group.

Seizure Rate

Some epileptic patients (n=3) were exposure to epileptic seizure in every day before the treatment. During the 45-day selenium treatment, the seizure rates in the two patients of three were decreased from every day to every week.

Discussion

We found that the erythrocyte lipid peroxidation levels were increased by refractory epilepsy although erythrocyte GSH, GSH-Px, plasma TAS, and vitamin E concentrations decreased. Hence, epilepsy and refractory epilepsy in the patients are characterized by increased oxidative stress and decreased GSH, GSH-Px and vitamin E antioxidant values. Administration of TPM and Se caused decrease in lipid peroxidation levels although GSH, GSH-Px, TAS, vitamins A, C, and E values increased. A limited number of cell culture [4] and experimental animal [6, 7] studies except human study have been reported regarding the effects of TPM on antioxidant vitamin system and lipid peroxidation values. To the best of our knowledge, the current study is the first to compare the medicine and Se with particular reference to its effects on oxidative stress and antioxidant redox system in epilepsy and refractory epilepsy-induced oxidative toxicity in the patients.

Neuronal hyperexcitability and excessive production of free radicals have been implicated in the pathogenesis of a considerable range of neurological disorders, including epilepsy. The high rate of oxidative metabolism, coupled with the low antioxidant defenses and the richness in polyunsaturated fatty acids, makes the brain highly vulnerable to free radical damage [25]. The increased susceptibility of the brain to oxidative damage highlights the importance of understanding the role of oxidative stress in the pathophysiology of seizures [26]. In the current study, erythrocyte lipid peroxidation levels were higher in the refractory epilepsy groups than in the control group and, therefore demonstrated and confirmed the possible role of oxidative toxicity after seizure of refractory epilepsy.

Inactivation of ROS can be carried out by antioxidant vitamins. Vitamin E, alpha-tocopherol, is the most important antioxidant in the lipid phase of cells. The vitamin E has revealed many important molecular properties such as the scavenging of ROS and nitrogen species and the modulation of signal transduction in antioxidant and non-antioxidant molecular pathways [26]. Vitamin C, as well as being a free radical scavenger, also transforms vitamin E to its active form [26] and it is also a potent anticonvulsant and neuroprotector [27]. Levels of the antioxidants in brain are considerable low. Therefore, low antioxidant levels and high content of PUFA, results in limited antioxidant defense in brain. The plasma vitamin E concentrations in the patients with refractory epilepsy were although vitamin A, C, and E concentrations in the serum were increased in the TPM and selenium treatment groups. The increased concentrations of the antioxidant vitamins could be due to its depletion or inhibition as a result of the increased production of free radicals. The increase in the brain cortex vitamins A, C, and vitamin E values in animals during TPM and selenium treatments has been attributed to the inhibition of free radicals and LP [6–9]. Similarly, we recently observed increased brain cortex vitamin A, E and C concentrations in PTZ-induced epileptic rats by selenium and TPM administrations. Gupta et al. reported also that pretreatment of rats with an vitamin E (100 mg/kg, i.p./day for 3 days) attenuated depletion of high-energy phosphates and oxidative stresses caused by kainic acid-induced status epilepticus of rats [28]. Hence, the human epilepsy results were confirmed by the experimental animal epilepsy study [6–9, 28].

Equilibrium of trace elements is essential for physiology of nervous system because most of them contribute to the activation of specific enzymes that play important roles in many pathways of the central nervous system [29]. GSH-Px which is a selenium-dependent enzyme that is involved in antioxidative defense mechanisms controls the intracellular levels of hydrogen peroxide [2]. GSH-Px and GSH values were lower in refractory epilepsy groups than in controls although their values were increased in the selenium and selenium plus TPM groups. Increased ROS production in epilepsy can also lead further enhancement of production of

superoxide radical in a redox cycle. As a consequence, synthesis of NADH will be decreased and, which may lead to depletion of ATP pool and in addition to exhaustion of GSH and GSH-Px pools although antioxidants modulated the cycle through inhibition of over production of ROS [29]. Similarly, decreased erythrocytes GSH-Px activity and serum selenium levels were reported in epileptic children [10, 30] and rats [31]. Hence, the GSH-Px results of the current study were supported by results of the studies [10, 30, 31].

Occurrence of epilepsy in humans also increases with mitochondrial oxidative stress. Mitochondria have critical cellular function that influences neuronal excitability including production of ATP, fatty acid oxidation and regulation of Ca^{2+} homeostasis [32, 33]. Mitochondria are the primary site of ROS production and are uniquely vulnerable to oxidative damage that may play a critical role in controlling neuronal excitability [34]. Mitochondrial depolarization induces over production of ROS and Ca^{2+} influx through the cation channels induces mitochondrial depolarization [6]. In a previous study, we observed modulator role of TPM and selenium on plasma membrane calcium ATPase activity in brain cortex microsomes of rats [6]. In a recent study, we observed also modulator role of TPM and selenium on Ca^{2+} influx through VGCC in PC12 neuronal cells [4]. The lipid peroxidation levels decreased in TPM with/without Se decreased although TAS, GSH, and antioxidant vitamin values were increased by TPM and selenium treatments. These finding suggested that TPM may interfere production of ROS due to modulator role of Ca^{2+} channels on Ca^{2+} influx and mitochondrial function in the patients.

In conclusion, we firstly observed oxidative stress abnormalities in patients with refractory epilepsy. However, selenium and topiramate supplementation induced protective effect on the oxidative stress and antioxidant redox system in the blood of the patients with epilepsy and refractory epilepsy. The beneficial effect of topiramate and selenium on the antioxidant systems was regulation of GSH, antioxidant vitamins, TAS, and LP levels in the blood samples. Results of the current study demonstrate that selenium plus antiepileptic treatment especially with topiramate have protective effects on oxidative stress in epileptic patients. Hence, use of the selenium with topiramate could be a potential approach in arresting or inhibiting the seizure genesis caused by oxidative stress agents.

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