J Membrane Biol (2009) 229:131–140
DOI 10.1007/s00232-009-9177-1

Topiramate and Vitamin E Modulate the Electroencephalographic Records, Brain Microsomal and Blood Antioxidant Redox System in Pentylentetrazol-Induced Seizure of Rats

Mustafa Nazıroğlu · Süleyman Kutluhan · Abdulhadi Cihangir Uğuz · Ömer Çelik · Ramazan Bal · Peter J. Butterworth

Received: 19 March 2009 / Accepted: 20 May 2009 / Published online: 10 June 2009

Abstract We investigated the effects of vitamin E and topiramate (TPM) administrations on pentylentetrazol (PTZ)–induced blood and brain toxicity in rats. Forty rats were randomly divided into five equal groups. The first and second groups were used for the control and PTZ groups, respectively. Fifty or 100 mg TPM were administered to rats constituting the third and fourth groups for 7 days, respectively. The TPM and vitamin E combination was given to animals in the fifth group. At the end of 7 days, all groups except the first received a single dose of PTZ. Blood and brain samples were taken at 3 hrs after PTZ administration. Lipid peroxidation levels of plasma, erythrocyte, brain cortex and brain microsomal fraction; nitric oxide levels of serum; and the number of spikes and epileptiform discharges of the EEG were increased by PTZ administration. Plasma and brain vitamin E concentration, erythrocyte glutathione peroxidase (GSH-Px) activity and latency to first spike of the EEG were decreased by PTZ. Plasma lipid peroxidation levels in the third group and plasma and erythrocyte lipid peroxidation levels in the fifth group were decreased compared to the second group, whereas brain vitamin C, vitamin E, erythrocyte GSH-Px and reduced glutathione (GSH) values increased in the fifth group. Brain microsomal GSH levels and EEG records in the third, fourth and fifth groups were restored by the TPM and vitamin E treatment. In conclusion, TPM and vitamin E seems to have protective effects on PTZ-induced blood and brain toxicity by inhibiting free radicals and supporting the antioxidant redox system.

Keywords Topiramate · Antioxidant · Oxidative stress · Brain · Erythrocyte · EEG record

M. Nazıroğlu (✉) · A. C. Uğuz · Ö. Çelik
Department of Biophysics, Medical Faculty, Suleyman Demirel University, Morfoloji Binasi, Cunur, Isparta 32260, Turkey
e-mail: mnaziroglu@med.sdu.edu.tr

S. Kutluhan
Department of Neurology, Medical Faculty, Suleyman Demirel University, Isparta, Turkey

R. Bal
Department of Biophysics, Medical Faculty, Fırat University, Elazığ, Turkey

P. J. Butterworth
Biochemistry Section, Division of Life Sciences, King's College, London, UK

Introduction

Erythrocytes and the brain may be vulnerable to oxidative stress induced by epilepsy and become exposed to reactive oxygen species (ROS) such as superoxide radical, hydrogen peroxide and hydroxyl radical continuously generated via the auto-oxidation of hemoglobin and polyunsaturated fatty acids (PUFAs) (Halliwell 2006; Nazıroğlu 2007a). The brain is extremely susceptible to oxidative damage induced by these ROS because it generates very high levels of ROS due to its very high aerobic metabolism and blood perfusion and has relatively poor enzymatic antioxidant defense (Yatin et al. 2000). Erythrocytes are also extremely susceptible to oxidative damage induced by these ROS because they contain hemoglobin and PUFAs, which can readily be peroxidized (Chung and Wood 1971). Lipid peroxidation (LP) causes injury to cells and intracellular membranes and may lead to cell destruction and subsequently cell death (Yatin et al. 2000; Akyol et al. 2002; Kovacic and Somanathan 2008). The brain and erythrocytes are protected by antioxidants against peroxidative damage (Halliwell 2006;

Nazıroğlu 2007b). Glutathione peroxidase (GSH-Px) catalyzes the reduction of hydrogen peroxide to water. GSH-Px can also remove organic hydroperoxides (Schweizer et al. 2004; Kovacic and Somanathan 2008). Reduced glutathione (GSH) is a hydroxyl radical and singlet oxygen scavenger and participates in a wide range of cellular functions (Rayman 2000). Vitamin E, α-tocopherol, is the most important antioxidant in the lipid phase of cells. Vitamin E acts to protect cells against the effects of free radicals, which are potentially damaging by-products of the body's metabolism (Yatin et al. 2000; Nazıroğlu 2007a). Vitamin C (ascorbic acid), as well as being a free radical scavenger, transforms vitamin E to its active form (Frei et al. 1989). Vitamin A (retinol), serves as a prohormone for retinoids and is involved with signal transduction at cytoplasmic and membrane sites (Czernichow and Hersberg 2001). The brain ascorbic acid concentration is extremely low according to body tissues such as liver and kidney (Frei et al. 1989; Czernichow and Hersberg 2001).

Epilepsy is one of the most common neurological disorders. Oxidative stress and generation of mitochondrial ROS are strongly implicated in seizures of epileptic disorders. The oxidative stress products and nitric oxide (NO) are known both as a cause and as a consequence of epileptic seizures (Patel 2004). Oxidative stress has been shown in several rodent models of experimental epilepsy such as the amygdala kindling model (Frantseva et al. 2000) and the pentylentetrazol (PTZ) model (Uma Devi et al. 2006). Administration of the chemical convulsant leads to a decrease in GABAergic function and to the stimulation and modification of density or sensitivity of different glutamate receptor subtypes (White et al. 2007). Increased activity of glutamatergic systems induced by status epilepticus causes energy impairment and enhanced formation of ROS as described for kainate-evoked seizures.

Topiramate (TPM) is a new antiepileptic drug, which inhibits voltage-gated sodium and calcium channels, blocks glutamate AMPA/kainate receptors and enhances the $GABA_A$ receptor–mediated chloride enhancement (White et al. 2007). TPM is also widely known as a carbonic anhydrase inhibitor (Weiergräber et al. 2006). An increasing body of evidence indicates that TPM possesses not only antiepileptic but also neuroprotective properties due to its multiple mechanisms of action (Cardile et al. 2001). However, it has been reported that it may cause adverse effects such as blood toxicity (White et al. 2007), although there is no information on the effects of oxidative stress and roles of vitamin E or TPM in oxidative stress–induced blood toxicity. Reports on the effects of antiepileptic drugs, except TPM, related to oxidative stress and NO have been presented for some tissues and cells apart from plasma and erythrocytes, but they are also controversial. For example, Cardile et al. (2001) and Pavone and Cardile (2003) reported that TPM increased oxidative stress revealed by LP levels in astrocytes, although Kubera et al. (2004) reported that TPM has an antioxidant role in the piriform cortex of rats.

It has not been studied whether TPM dose-dependently modifies alterations in the antioxidant enzyme system, LP and NO levels of plasma, erythrocytes, brain cortex and microsomal and electroencephalographic (EEG) records of the brain. We evaluated the effects of TPM and vitamin E on oxidative stress, enzymatic antioxidants, NO status and EEG records in PTZ-induced epilepsy in rats.

Materials and Methods

Animals

Forty male Wistar albino rats weighing 200 ± 20 g were used for the experimental procedures. Rats were allowed 1 week to acclimatize to the surroundings before beginning any experimentation. Animals were housed in individual plastic cages with bedding. Standard rat food and tap water were available ad libitum for the duration of the experiments. The temperature was maintained at 22 ± 2°C. A 12/12 h light/dark cycle was maintained, with lights on at 6:00 a.m., unless otherwise noted. The experimental protocol of the study was approved by the ethical committee of the Medical Faculty of Suleyman Demirel University. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Suleyman Demirel University.

Experimental Design

PTZ and TPM were dissolved in physiological saline (0.9% w/v). Forty animals were randomly divided into five groups as follows:

Group I: Control group ($n = 8$) with placebo (physiological saline) supplementation.
Group II: PTZ (60 mg/kg BW) administered i.p. to rats ($n = 8$) for induction of epilepsy.
Group III: 50 mg TPM + PTZ group. TPM (50 mg/kg/day) was orally (via gastric gavage) given to these animals for 7 consecutive days before PTZ administration.
Group IV: 100 mg TPM + PTZ group. TPM (100 mg/kg/day) was given orally (via gastric gavage) to these animals for 7 consecutive days before PTZ administration.
Group V: Vitamin E + TPM + PTZ group ($n = 8$). TPM (50 mg/kg/day) was orally (via gastric gavage) administered to animals for 7 consecutive days before PTZ administration. The group received also 150 mg/kg

BW i.p. vitamin E (DL-α-tocopherol acetate) every other day, i.e., three times in all before PTZ administration.

Epilepsy was induced in groups II, III, IV and V by administration of PTZ (60 mg/kg). After 3 hrs of PTZ administration, all rats were scarified and blood samples were taken.

Seizure intensity was evaluated using the following modified scale (Ilhan et al. 2005): 0, no response; 1, ear and facial twitching; 2, convulsive waves axially through the body; 3, myoclonic body jerks; 4, generalized clonic convulsion, turn over into side position; 5, generalized convulsions, tonic extension episode and status epilepticus.

Anesthesia, Blood Collection and Preparation of Blood Samples

Rats were anesthetized with a cocktail of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) administered i.p. before being killed and blood samples taken. Blood (4–6 ml) was taken from the heart, using a sterile injector, and put into tubes protected against light.

Serum was obtained from 1 ml of blood without anticoagulation. The remaining blood samples were separated into plasma and red blood cells (RBCs) by centrifugation at 1,500 × *g* for 10 min at +4°C. RBC samples were washed three times in cold isotonic saline (0.9%, v/w), then hemolyzed with a ninefold volume of phosphate buffer (50 mM, pH 7.4). After addition of butylhydroxytoluol (4 μl/ml), hemolyzed RBC and plasma samples were stored at –30°C for <3 months pending measurement of enzymatic activity. NO in serum was immediately determined by a commercial kit (see below). The remaining hemolyzed RBC and plasma samples were used immediately for LP and vitamin assays.

Preparation of Brain Samples

The brain was also taken as follows. The cortex was dissected out after the brain was split in the mid-sagittal plane. Following removal of the cortex, brain tissue was dissected from the total brain as described in our previous study (Nazıroğlu et al. 2008).

Cortical brain tissues were washed twice with cold saline solution, placed into glass bottles, labeled and stored in a deep freeze (–30°C) until processing (maximum 10 h). After weighing, half of the cortex was placed on ice, cut into small pieces using scissors and homogenized (2 min at 5,000 rpm) in 5 volumes (1:5, w/v) of ice-cold Tris-HCl buffer (50 mM, pH 7.4), using a glass Teflon homogenizer (Caliskan Cam Teknik, Ankara, Turkey). All preparation procedures were performed on ice. The homogenate was used for determination of LP and antioxidant levels. Remaining cortical samples were used for Ca^{2+}-ATPase assay and isolation of microsomes by ultracentrifugation.

After addition of butylhydroxytoluol (4 μl/ml), brain homogenate and microsomes samples were used immediately to determine LP levels and enzyme activities. Antioxidant vitamin analyses were performed within 3 months.

Isolation of Brain Microsomes

Tissues were cleaned, minced and then homogenized in 6 volumes of freshly prepared buffer A containing 0.3 mol/l sucrose, 10 mmol/l HEPES-HCl (pH 7.4) and 2 mmol/l dithiothreitol. The material was homogenized with a glass Teflon homogenizer. The homogenate was centrifuged (MS 80; Sanyo) at 85,000 × *g* in an appropriate rotor (Sorvall; Teknolab, Ankara, Turkey) for 75 min. The supernatant was discarded and the pellet was resuspended in the original volume of buffer A containing 0.6 mol/l KCl using four strokes of the pestle and centrifuged again at 85,000 × *g* for 75 min. The pellet was resuspended in the original volume of buffer A. After centrifugation at 85,000 × *g* for 75 min, the pellet was suspended in buffer A using four strokes of the pestle at a protein concentration of 2–7 mg/ml. The procedures took 10–12 h for eight samples. The samples were frozen and stored at –30°C until assayed. The isolation procedure was carried out at +4°C (Nazıroğlu et al. 2008).

LP (as MDA) Determinations

LP levels in the hemolyzed RBC, plasma, brain homogenate and microsomal samples were measured with the thiobarbituric acid reaction by the method of Placer et al. (1966). The quantification of thiobarbituric acid–reactive substances was determined by comparing the absorption to the standard curve of malondialdehyde (MDA) equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetramethoxypropane. The values of lipid peroxidation in the plasma, erythrocytes, brain cortex and brain microsomal were expressed as nanomoles per milliliter and micromoles per gram protein, respectively. Although the method is not specific for LP, measurement of the thiobarbituric acid reaction is an easy and reliable method (Akyol et al. 2002; Akbas et al. 2005; Armağan et al. 2008), which is used as an indicator of LP and ROS activity in biological samples.

NO Assay

The NO content of the serum was measured by ELISA (ELx808 Absorbance Microplate Reader; BioTec Instrument, VT) using commercial kits (nitrate/nitrite colorimetric assay kit, catalog 780001; Cayman Chemical, MI).

GSH, GSH-Px and Protein Assay

The GSH content of the RBC, brain cortex and microsomal preparation was measured at 412 nm using the method of Sedlak and Lindsay (1968). GSH-Px activities of RBCs were measured spectrophotometrically at 37°C and 412 nm according to the Lawrence and Burk method (1976). The protein content in RBCs, brain cortex and microsomes was measured by the method of Lowry et al. (1951) with bovine serum albumin as the standard.

Plasma Vitamin A, Vitamin C and Vitamin E Analyses

Vitamins A (retinol) and E (α-tocopherol) were determined in the plasma and brain cortical samples by a modification of the method described by Desai (1984) and Suzuki and Katoh (1990). About 250 µl of plasma and brain samples were saponified by addition of 0.3 ml of 60% (w/v in water) potassium hydroxide and 2 ml of 1% (w/v in ethanol) ascorbic acid, followed by heating at 70°C for 30 min. After cooling the samples on ice, 2 ml of water and 1 ml of *n*-hexane were added and mixed with the samples, which were then rested for 10 min to allow phase separation. An aliquot of 0.5 ml of *n*-hexane extract was taken, and vitamin A levels were measured at 325 nm. Then, reactants were added and the absorbance value of the hexane extract was measured in a spectrophotometer at 535 nm. Calibration was performed using standard solutions of all-*trans* retinol and α-tocopherol in hexane.

Vitamin C (ascorbic acid) in the plasma and brain cortical samples was quantified according to the method of Jagota and Dani (1982). The absorbance of the samples was measured spectrophotometrically at 760 nm.

EEG Recording

Before killing the animals, EEG records were taken by polygraph (ADI Instruments, Australia). In a stereotaxic frame, two monopolar stainless-steel electrodes were placed bilaterally over the frontal and parietal cortical surfaces. Electrodes were connected to a female connector anchored to the skull with dental acrylate. The neutral electrode was connected to the tail or ear of each animal.

EEG recording and behavioral observations were carried out in a Faraday cage. Animals were connected with a male microconnector to the headbox of the recording apparatus with a polygraph system and XA-400 quad channel differential amplifier (ML 870, ADI Instruments). The EEG biopotentials were recorded by the ADI Instruments X-chart 5 program (time constant 1 s, low-pass filter 50 Hz, high-pass filter 1 Hz, range 200 µ*V*). Baseline EEG records were performed 15 min before two doses of TPM and vitamin E treatment or with PTZ administration. After administration of TPM, with or without vitamin E, the records were again taken before PTZ administration. Finally, records were taken for 10 min from groups II, III, IV and V after PTZ administration. Alpha waves are responsible for 70% of brain biopotentials. For calculation of a particular spike, the records were filtered (high cut-off frequency 12 Hz, low cut-off frequency 8 Hz).

A spike was defined as a high-amplitude (twice the baseline) sharply contoured waveform with a duration of 20–70 ms. Latency to the first spike and the total number of spikes were calculated 1 min after PTZ administration. These parameters were chosen according to Kharatishvili et al. (2007) as described in previous studies (Kharatishvili et al. 2007; Nazıroğlu et al. 2008).

PTZ Test

To detect any enhanced seizure susceptibility, the numbers of spikes after a single PTZ administration were considered and the results compared with controls. Spikes occurred within 15 min of PTZ administration, and the number of spikes in PTZ-administered groups was significantly different from normal EEG records. To determine whether the seizure threshold was reduced after TPM and vitamin E administration, seizures in animals receiving two doses of TPM and vitamin E were compared with the PTZ group.

Statistical Analysis

Statistical analysis was performed using the ANOVA-SPSS statistical package (version 9.0; SPSS, Inc., Chicago, IL) for Windows. Least significant difference tests were used to determine the differences between the groups. $P < 0.05$ was considered significant.

Results

The mean serum NO and plasma LP values in the plasma of the five groups are shown in Figure 1, and erythrocyte, brain cortex and brain microsomal lipid peroxidation values are shown in Tables 1, 2 and 3, respectively. The results show that the serum NO ($P < 0.001$) and plasma ($P < 0.05$), erythrocyte ($P < 0.05$), brain ($P < 0.05$) and brain microsomal ($P < 0.001$) LP levels were significantly higher in the PTZ group than in the control group. The two TPM doses caused a decrease in the LP levels of plasma and erythrocytes, brain microsomal ($P < 0.05$) and brain cortex ($P < 0.01$) and NO levels of serum ($P < 0.05$) relative to the PTZ group. However, serum NO, erythrocyte, plasma, brain microsomal and brain cortex LP levels were more significantly decreased in the 50 mg TPM with or without vitamin E group than in the 100 mg TPM group ($P < 0.05$–$P < 0.01$).

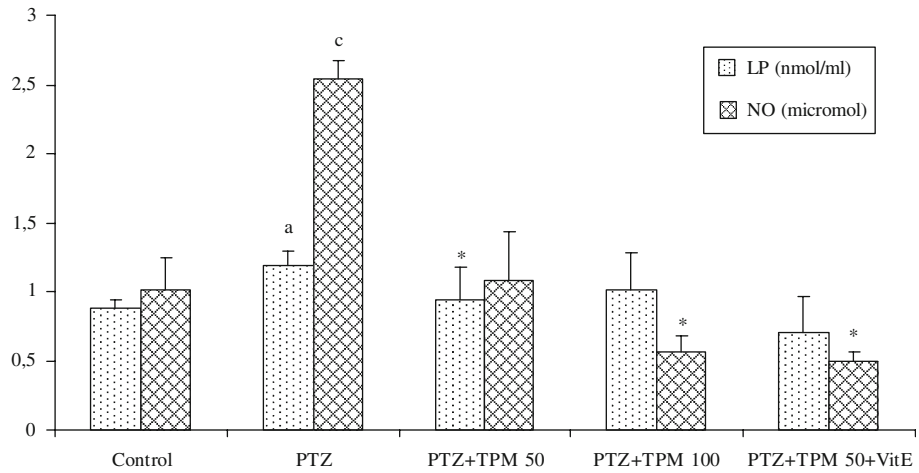

Fig. 1 Effects of TPM and vitamin E on LP and serum NO levels in PTZ-induced toxicity in rats (mean ± SD, $n = 8$). [a] $P < 0.05$ and [c] $P < 0.001$ vs. control group. * $P < 0.05$ vs. PTZ group

Table 1 Effects of TPM and vitamin E on erythrocyte GSH-Px activity, reduced GSH and LP levels in PTZ-induced toxicity in rats (mean ± SD, $n = 8$)

Parameters	Control ($n = 8$)	PTZ ($n = 8$)	TPM50 ($n = 8$)	TPM100 ($n = 8$)	TPM50 + Vit E ($n = 8$)
GSH-Px (IU/g prot)	32.15 ± 5.20	25.29 ± 5.84*	37.04 ± 9.71[a]	35.45 ± 7.37[a]	34.47 ± 1.28[a]
GSH (μmol/g prot)	3.60 ± 0.89	3.27 ± 0.87	6.00 ± 1.43[b]	5.29 ± 1.52[b]	6.67 ± 1.98[c]
LP (μmol/g prot)	46.49 ± 14.24	56.60 ± 11.74*	46.44 ± 9.33*	48.82 ± 14.00	42.11 ± 9.11[b]

* $P < 0.05$ vs. control group. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. PTZ group

Table 2 Effects of TPM and vitamin E on brain cortex LP, GSH-Px, GSH and antioxidant vitamin values in PTZ-induced rats (mean ± SD, $n = 8$)

Parameters	Control ($n = 8$)	PTZ ($n = 8$)	TPM50 ($n = 8$)	TPM100 ($n = 8$)	TPM50 + Vit E ($n = 8$)
GSH-Px (IU/g prot)	120.09 ± 11.57	119.43 ± 8.11	123.58 ± 13.24	120.81 ± 8.61	119.87 ± 12.79
GSH (μmol/g prot)	7.37 ± 1.26	7.43 ± 0.66	8.58 ± 1.05[a]	8.18 ± 1.28[a]	8.37 ± 0.78[a]
LP (μmol/g prot)	871.9 ± 167.0	999.3 ± 240.6*	763.1 ± 126.0[b]	759.2 ± 246.7[b]	708.3 ± 110.9[b]
Vitamin C (μmol/g brain)	1.31 ± 0.15	1.04 ± 0.27	1.25 ± 0.25[a]	2.00 ± 0.38[b]	1.50 ± 0.23[a]
Vitamin E (μmol/g brain)	12.11 ± 1.51	7.10 ± 1.34**	8.20 ± 1.16	7.60 ± 1.24	10.61 ± 2.55[b]
β-carotene (μmol/g brain)	2.37 ± 0.28	2.27 ± 0.38	2.48 ± 0.12	2.61 ± 0.14	2.42 ± 0.15
Vitamin A (μmol/g brain)	3.22 ± 0.56	2.78 ± 0.46	2.89 ± 0.10	3.24 ± 0.53	2.78 ± 0.46

* $P < 0.05$ and ** $P < 0.01$ vs. control. [a] $P < 0.05$ and [b] $P < 0.01$ vs. PTZ group

Table 3 Effects of TPM and vitamin E on brain microsomal LP, GSH-Px and GSH values in PTZ-induced rats (mean ± SD, $n = 8$)

Parameters	Control ($n = 8$)	PTZ ($n = 8$)	TPM50 ($n = 8$)	TPM100 ($n = 8$)	TPM50 + Vit E ($n = 8$)
GSH-Px (IU/g prot)	40.34 ± 7.10	34.56 ± 12.12	45.23 ± 11.42[a]	44.80 ± 7.66[a]	43.46 ± 7.13[a]
GSH (μmol/g prot)	3.43 ± 0.68	1.95 ± 0.66**	3.63 ± 0.95[c]	3.08 ± 0.39[c]	3.75 ± 0.54[c]
LP (μmol/g prot)	43.32 ± 4.40	180.91 ± 17.71**	133.69 ± 10.90[a]	150.48 ± 35.41[a]	138.07 ± 16.67[a]

* $P < 0.05$ and ** $P < 0.001$ vs. control. [a] $P < 0.05$ and [c] $P < 0.001$ vs. PTZ group

The mean LP and GSH levels and GSH-Px activities in erythrocytes, brain cortex and microsomes of the five groups are shown in Tables 1, 2 and 3, respectively. The results show that the erythrocyte GSH-Px activities ($P < 0.05$) and brain microsomal GSH levels ($P < 0.01$) were significantly lower in the PTZ group than the control group. However, administration of TPM caused an increase in the erythrocyte and brain microsomal enzyme activities of rats receiving PTZ plus 50 mg and 100 mg TPM and vitamin E ($P < 0.05$). TPM 50 mg did not bring about a greater increase in GSH-Px activities compared to 100 mg, and there was no statistical difference in GSH-Px values

Table 4 Effects of TPM and vitamin E on plasma concentrations of vitamins A, C and E in PTZ-induced toxicity in rats (mean ± SD, $n = 8$)

Parameters	Control ($n = 8$)	PTZ ($n = 8$)	TPM50 ($n = 8$)	TPM100 ($n = 8$)	TPM50 + Vit E ($n = 8$)
Vitamin A (nmol/l)	1.21 ± 0.35	1.13 ± 0.35	1.41 ± 0.17	1.18 ± 0.26	1.35 ± 0.25
Vitamin C (μmol/l)	0.97 ± 0.24	0.99 ± 0.33	0.95 ± 0.34	0.87 ± 0.46	1.23 ± 0.12*,[a]
Vitamin E (μmol/l)	4.11 ± 1.01	3.44 ± 0.43*	3.31 ± 0.76*	3.59 ± 0.98*	4.50 ± 0.90[a]

* $P < 0.05$ vs. control group. [a] $P < 0.05$ vs. PTZ group

between the 50 mg and 100 mg TPM-administered groups. The brain cortical GSH-Px activities did not change in the five groups. However, erythrocyte GSH levels were increased to a greater degree in the 50 mg with vitamin E group ($P < 0.001$) compared to the 100 mg TPM group ($P < 0.01$). There was no statistically significant difference in erythrocytes and brain cortical GSH levels between control and PTZ-administered groups, although microsomal GSH levels were significantly different ($P < 0.001$) in the PTZ group than in controls.

The mean vitamin A, vitamin C and vitamin E concentrations in plasma and brain cortex of the five groups are shown in Tables 2 and 4, respectively. Vitamin E levels in plasma ($P < 0.05$) and brain cortex ($P < 0.01$) were significantly decreased in PTZ groups compared with controls. Decreased plasma vitamin E concentrations were not improved by 50 and 100 mg TPM administrations. There were no significant differences in plasma vitamin C concentration in the groups except for the 50 mg TPM plus vitamin E group. Vitamin C and E concentrations in plasma and brain cortex were significantly ($P < 0.05$) higher in the 50 mg TPM plus vitamin E–administered groups than in the PTZ group. Vitamin A and β-carotene concentrations in the five groups did not change statistically.

The mean EEG records and alpha waves (filtered records) are shown in Figs. 2 and 3, respectively. The mean spike numbers in the brain of the five groups are shown in Fig. 4. The total number of spikes during 1-min follow-up was higher in the PTZ group compared to the controls (mean 34 vs. 1, $P < 0.001$), whereas the total numbers of spikes in the TPM50 (mean 25.6 and $P < 0.05$), TPM100 (mean 20 and $P < 0.01$) and TPM50 + Vit E (mean 16 and $P < 0.001$) groups were significantly lower than that in the PTZ group. Also, the total number of epileptiform discharges was higher in the PTZ group than the controls (mean 31 vs. 2.8, $P < 0.001$), whereas the total numbers of epileptiform discharges in the TPM50 (mean 24 and $P < 0.05$), TPM100 (mean 18 and $P < 0.01$) and TPM50 + Vit E (mean 15 and $P < 0.001$) groups were significantly lower compared to the PTZ group.

The latency to the first spike in the brain of the five groups is shown in Fig. 5. The latency to the first spike during follow-up was shorter in the PTZ group than in the controls (mean 118 vs. 51, $P < 0.001$). The latency to the

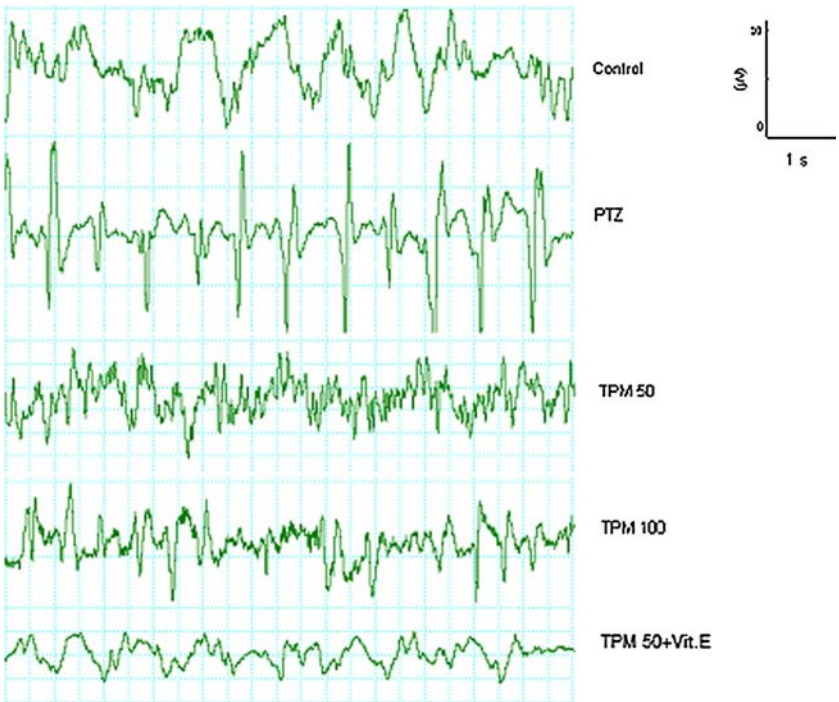

Fig. 2 Examples of EEG records in the five groups. The EEG biopotentials were recorded by ADI Instruments X-chart 5 program (time constant 1 s, low-pass filter 50 Hz, high-pass filter 1 Hz, range 200 μ*V*). Baseline EEG records were performed 15 min before TPM and vitamin E treatment or with the PTZ administration. After administration of TPM, with or without vitamin E, the records were again taken before PTZ administration. The last records were taken for 10 min from the TPM50, TPM100 and TPM50 + Vit E groups after PTZ administration ($n = 8$)

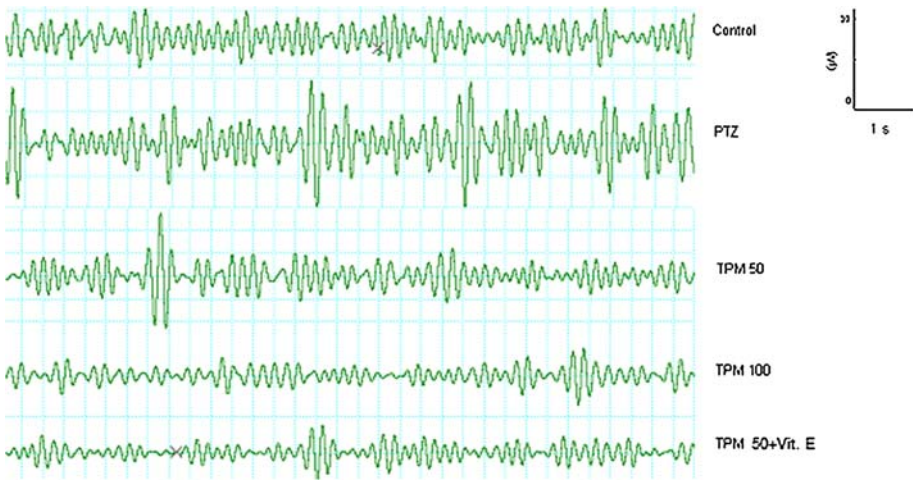

Fig. 3 Alpha wave examples of EEG records in the five groups. The mean records were filtered (high cut-off frequency 12 Hz, low cut-off frequency 8 Hz) for obtaining alpha records ($n = 8$)

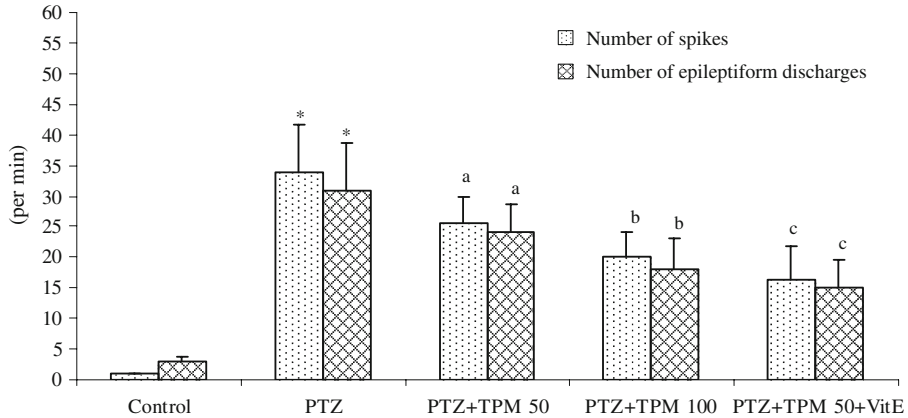

Fig. 4 PTZ test results of number of spikes and number of epileptiform discharges in alpha waves in the control, PTZ, PTZ + TPM50, PTZ + TPM100 and PTZ + TPM50 + Vit E groups. Rats with PTZ had increased numbers of spikes and of epileptiform discharges, indicating hyperexcitability. In animals with TPM50, TPM100 and TPM50 + Vit E before PTZ administration, the seizure duration, number of spikes and number of epileptiform discharges decreased, indicating both doses of TPM with and without vitamin E exerted protective effects on hyperexcitability (mean ± SD, $n = 8$). * $P < 0.001$ vs. control group. [a] $P < 0.05$, [b] $P < 0.01$ and [c] $P < 0.001$ vs. PTZ group

first spike was recovered in the TPM, vitamin E and TPM + Vit E groups ($P < 0.001$).

According to the results, rats treated with PTZ had shorter latency to the first spike, an increased number of spikes and an increased number of epileptiform discharges indicating hyperexcitability. In animals represented by TPM50, TPM100 and TPM50 + Vit E before PTZ administration, the seizure duration was shorter. Thus, both doses of TPM and vitamin E had protective effects on hyperexcitability.

Discussion

We found that serum NO; plasma, brain cortex and brain microsomal and erythrocytic LP values; and spike numbers of EEG were increased by PTZ administration, whereas erythrocyte GSH-Px activity, plasma and brain vitamin E levels and latency to the first EEG spike were decreased. Hence, PTZ administration to the animals is characterized by increased LP and NO levels and decreased GSH-Px, GSH and vitamin E antioxidant values. Administration of TPM and vitamin E caused a decrease in serum NO, erythrocyte and plasma LP levels and brain spike numbers, whereas GSH-Px, GSH, vitamin C and vitamin E levels and latency to the first spike of EEG increased.

The current study indicates that PTZ administration, at a convulsive dose of 60 mg/kg, produced significant increases in LP levels of plasma, erythrocytes, brain cortex and brain microsomal preparations as well as in serum NO levels. Our results are in accordance with previous reports of LP and NO increments in brain, erythrocytes and liver during epileptic seizures (Kubera et al. 2004; Akbas et al. 2005; Nazıroğlu et al. 2008; Deniz Onay et al. 2008). On the other hand, the present study is the first report regarding the increased plasma, erythrocyte LP, brain cortex, brain

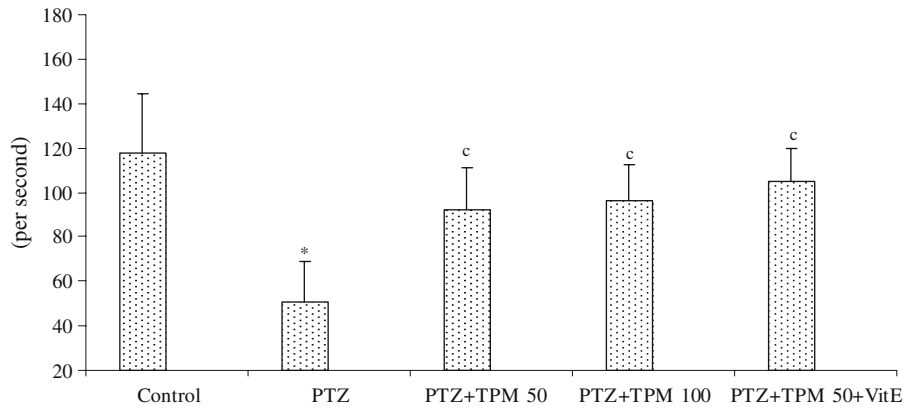

Fig. 5 PTZ test results as latency to the first spike in the control, PTZ, PTZ + TPM50, PTZ + TPM100 and PTZ + TPM50 + Vit E groups. Rats with PTZ had shorter latency to the first spike, indicating hyperexcitability. In animals with TPM50, TPM100 and TPM50 + Vit E before PTZ administration, the latency to the first spike increased. Thus, vitamin E and TPM had protective effects on hyperexcitability (mean ± SD, $n = 8$). * $P < 0.001$ vs. control group. [c] $P < 0.001$ vs. PTZ group

microsomal and serum NO levels in TPM and vitamin E–administered epileptic rats. PTZ may trigger a variety of biochemical process including the activation of membrane phospholipases, proteases and nucleases (Wu et al. 2004). Marked alteration in membrane phospholipid metabolism results in LP and formation of ROS. Hence, involvement of LP as MDA in PTZ-induced epilepsy can be attributed to the activation of membrane phospholipases. In the current study, GSH-Px activities in erythrocytes were decreased by PTZ-induced epilepsy. If GSH-Px activity decreases, superoxide radical production may increase and finally leads to oxidative stress and LP (Schweizer et al. 2004; Halliwell 2006).

Inactivation of ROS can be carried out by antioxidative enzymes (Nazıroğlu 2007a, 2007b). Hydrogen peroxide formed by the catalytic reaction of superoxide dismutase is both a reactive form of oxygen and a normal cellular metabolite, and it is further detoxified by GSH-Px and catalase (Rayman 2000). Activity of the enzymes in erythrocytes and brain is relatively low. Therefore, a combination of the high iron contents in hemoglobin, low antioxidant enzyme activities and high content of PUFA result in limited antioxidant defense in erythrocytes as well as brain. GSH-Px activity in erythrocytes was decreased in the PTZ group, whereas the activity in erythrocytes was increased in the TPM and vitamin E–treated groups. The increased activity of GSH-Px could be due to its depletion on production of free radicals. The increase in erythrocyte GSH-Px in animals during TPM treatments has been attributed to the inhibition of free radicals and LP (Kubera et al. 2004).

GSH is an important free radical scavenger in the mammalian nervous system (Wu et al. 2004) and an endogenous anticonvulsant (Abe et al. 2000). If free radicals and ROS are generated by the increased neuronal activity associated with seizure initiation and GSH interacts with these reactive species, then one might predict an acute decrease in the available GSH early in the seizure induced by PTZ. Ono et al. (2000) demonstrated that some anticonvulsants caused a significant decrease in plasma total GSH levels, suggesting that oxidative stress could occur during anticonvulsant treatment. Therefore, GSH was measured in erythrocytes of animals given anticonvulsants and PTZ, the model in which some of the antioxidant was effective. In the current study, the GSH concentration of erythrocyte and microsomal preparations decreased in PTZ-injected rats without any epileptic drug therapy, whereas it increased with TPM administration. Hence, the study supports the conclusion that oxidative stress does not occur with anticonvulsant drugs such as TPM but seizures themselves might directly induce oxidative stress.

Exposure of mitochondria to high cytosolic free Ca^{+2} was shown to increase formation of ROS (Weiergräber et al. 2006). It has been reported that in PTZ-induced epileptic cells of mice and vitamin E in hippocampal slice cultures (Kovács et al. 2002), TPM treatment (Weiergräber et al. 2006) modulated cytosolic Ca^{+2} levels by regulation of voltage-gated calcium channels. It has also been suggested that antiepileptic drugs are associated with detrimental effects on blood antioxidant defense systems (Maertens et al. 1995; Kutluhan et al. 2009). TPM is a new drug and LP and NO levels decreased following treatment with TPM with or without a vitamin E decrease. These findings suggest that TPM may interfere with the production of ROS by inhibition of voltage-gated calcium channels and of carbonic anhydrase activity in erythrocytes. In the current study LP levels of erythrocytes, plasma, brain cortex and brain microsomes and serum NO levels were lower in the TPM groups than in the PTZ group. Modulation of voltage-gated calcium channels in blood cells by treatment with TPM with or without vitamin E may have caused a decrease in mitochondrial ROS and NO production. This observation agrees with the finding of other

studies that TPM was effective against kainate-induced LP production in the piriform cortex of brain (Kubera et al. 2004), ischemia-induced neuronal damage (Lee et al. 2000) and PTZ-induced kidney damage (Armağan et al. 2008).

It is well known that carbonic acid is converted to water and bicarbonate by carbonic anhydrase, which is highly expressed in erythrocytes. During the process, electron transport occurs in the blood cells and causes an increase in ROS production. Batcioglu et al. (2005) reported that vitamin E had a modulatory effect on carbonic anhydrase activity against 7,12-DMBA-induced oxidative stress in mouse brain. Wang et al. (1996) reported that α-tocopherol helps protect against thiol group modification of carbonic anhydrase III. TPM is also widely known as a carbonic anhydrase inhibitor (Weiergräber et al. 2006). Hence, the decrease may be caused in erythrocytes via regulatory roles of TPM and vitamin E on the enzyme.

In the EEG records, we evaluated protective effects of TPM and vitamin E on oxidative stress. Some recent studies observed increases in oxidative status in different tissues of animals, which were probably caused by recurrent seizures (Ilhan et al. 2005). On the other hand, a deficiency of antioxidant redox systems could exacerbate the etiology of epilepsy (Eraković et al. 2003; Verrotti et al. 2008). There is evidence for the formation of superoxide anion and hydroxyl radicals after ferric chloride injection into the rat cerebral cortex (Uma Devi et al. 2006). Increased LP of brain tissues has been reported in PTZ-induced epilepsy in rats (Nazıroğlu et al. 2008; Deniz Onay et al. 2008). Kubera et al. (2004) reported that TPM has an antioxidant role and that it attenuated LP levels in the piriform cortex of rats. To our knowledge, there is no report on the effect of TPM and vitamin E on EEG records in rats. In the current study, the number of spikes and epileptiform discharges of EEG increased in the PTZ group, whereas latency to the first spike of EEG was decreased by PTZ. The changes were reversed by TPM and vitamin E administrations.

In conclusion, our blood and brain results in the PTZ group are consistent with a generalized antioxidant abnormality in different tissues of epileptic animals. However, TPM and vitamin E supplementations have a protective effect on EEG records via regulation of oxidative stress and antioxidant redox systems in erythrocytes, plasma, brain cortex and brain microsomal. The results in blood and brain may be of help to physicians in the treatment with vitamin E of oxidative stress–dependent epileptic toxicity.

Acknowledgments S. K. and M. N. formulated the present hypothesis and were responsible for writing the report. A. C. U. and O. C. were responsible for analysis of the data. P. J. B. and R. B. made critical revisions to the manuscript.

References

Abe K, Nakanishi K, Saito H (2000) The possible role of endogenous glutathione as an anticonvulsant in mice. Brain Res 854:235–238

Akbas SH, Yegin A, Ozben T (2005) Effect of pentylenetetrazol-induced epileptic seizure on the antioxidant enzyme activities, glutathione and lipid peroxidation levels in rat erythrocytes and liver tissues. Clin Biochem 38:1009–1014

Akyol O, Herken H, Uz E, Fadillioglu E, Unal S, Sogut S, Ozyurt H, Savas HA (2002) The indices of endogenous oxidative and antioxidative processes in plasma from schizophrenic patients. The possible role of oxidant/antioxidant imbalance. Prog Neuropsychopharmacol Biol Psychiatry 26:995–1005

Armağan M, Kutluhan S, Yılmaz M, Yılmaz N, Bülbül M, Vural H, Soyupek S, Nazıroğlu M (2008) Topiramate and vitamin E modulates antioxidant enzymes, nitric oxide and lipid peroxidation in pentylentetrazol-induced nephrotoxicity in rats. Basic Clin Pharm Toxicol 103:166–170

Batcioglu K, Karagözler AA, Ozturk IC, Genc M, Bay A, Ozturk F, Aydogdu N (2005) Comparison of chemopreventive effects of vitamin E plus selenium versus melatonin in 7,12-dimethylbenz(a)anthracene-induced mouse brain damage. Cancer Detect Prev 29:54–58

Cardile V, Pavone A, Renis M, Maci T, Perciavalle V (2001) Effects of gabapentin and topiramate in primary rat astrocyte cultures. NeuroReport 12:1705–1708

Chung J, Wood JL (1971) Oxidation of thiocyanate to cyanide catalysed by hemoglobin. J Biol Chem 246:555–560

Czernichow S, Hersberg S (2001) Interventional studies concerning the role of antioxidant vitamins in cardiovascular disease: a review. J Nutr Health Aging 5:188–195

Deniz Onay B, Tasdemir E, Tümer C, Bilgin HM, Atmaca M (2008) Dose dependent effects of ghrelin on pentylenetetrazole-induced oxidative stress in a rat seizure model. Peptides 29:448–455

Desai ID (1984) Vitamin E analysis methods for animal tissues. Methods Enzymol 105:138–147

Eraković V, Zupan G, Varljen J, Simonić A (2003) Pentylenetetrazol-induced seizures and kindling: changes in free fatty acids, superoxide dismutase, and glutathione peroxidase activity. Neurochem Int 42:173–178

Frantseva MV, Perez Velazquez JL, Tsoraklidis G, Mendonca AJ, Adamchik Y, Mills LR, Carlen PL, Burnham MW (2000) Oxidative stress is involved in seizure-induced neurodegeneration in the kindling model of epilepsy. Neuroscience 97:431–435

Frei B, England L, Ames BN (1989) Ascorbate is an outstanding antioxidant in human blood plasma. Proc Natl Acad Sci USA 86:6377–6381

Halliwell B (2006) Oxidative stress and neurodegeneration: where are we now? J Neurochem 97:1634–1658

Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M (2005) Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology 49:456–464

Jagota SK, Dani HM (1982) A new colorimetric technique for the estimation of vitamin C using Folin phenol reagent. Anal Biochem 127:178–182

Kharatishvili I, Immonen R, Gröhn O, Pitkänen A (2007) Quantitative diffusion MRI of hippocampus as a surrogate marker for post-traumatic epileptogenesis. Brain 130(Pt 12):3155–3168

Kovacic P, Somanathan R (2008) Unifying mechanism for eye toxicity: electron transfer, reactive oxygen species, antioxidant benefits, cell signaling and cell membranes. Cell Membr Free Radic Res 2:56–69

Kovács R, Schuchmann S, Gabriel S, Kann O, Kardos J, Heinemann U (2002) Free radical-mediated cell damage after experimental

status epilepticus in hippocampal slice cultures. J Neurophysiol 88:2909–2918

Kubera M, Budziszewska B, Jaworska-Feil L, Basta-Kaim A, Leśkiewicz M, Tetich M, Maes M, Kenis G, Marciniak A, Czuczwar SJ, Jagła G, Nowak W, Lasoń W (2004) Effect of topiramate on the kainate-induced status epilepticus, lipid peroxidation and immunoreactivity of rats. Pol J Pharmacol 56:553–561

Kutluhan S, Nazıroğlu M, Çelik Ö, Yılmaz M (2009) Protective effects of selenium and topiramate on lipid peroxidation and antioxidant vitamin levels in blood of pentylentetrazol-induced epileptic rats. Biol Trace Elem Res 129:181–189

Lawrence RA, Burk RF (1976) Glutathione peroxidase activity in selenium-deficient rat liver. Biochem Biophys Res Commun 71:952–958

Lee SR, Kim SP, Kim JE (2000) Protective effect of topiramate against hippocampal neuronal damage after global ischemia in the gerbils. Neurosci Lett 281:183–186

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin-phenol reagent. J Biol Chem 193:265–275

Maertens P, Dyken P, Graf W, Pippenger C, Chronister R, Shah A (1995) Free radical, anticonvulsants, and the neuronal ceroid-lipofuscinoses. Am J Med Genet 57:225–228

Nazıroğlu M (2007a) Molecular mechanisms of vitamin E on intracellular signaling pathways in brain. In: Goth L (ed) Reactive oxygen species and diseases. Research Signpost Press, Kerala, India, pp 239–256

Nazıroğlu M (2007b) New molecular mechanisms on the activation of TRPM2 channels by oxidative stress and ADP-ribose. Neurochem Res 32:1990–2001

Nazıroğlu M, Kutluhan S, Yılmaz M (2008) Selenium and topiramate modulates oxidative stress and Ca^{+2}-ATPase, EEG records in pentylentetrazol-induced brain seizures in rats. J Membr Biol 225:39–49

Ono H, Sakamato A, Sakura N (2000) Plasma total glutathione concentrations in epileptic patients taking anticonvulsants. Clin Chim Acta 298:135–143

Patel M (2004) Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med 37:1951–1962

Pavone A, Cardile V (2003) An in vitro study of new antiepileptic drugs and astrocytes. Epilepsia 44(Suppl 10):34–39

Placer ZA, Cushman L, Johnson BC (1966) Estimation of products of lipid peroxidation (malonyl dialdehyde) in biological fluids. Anal Biochem 16:359–364

Rayman MP (2000) The importance of selenium to human health. Lancet 356:233–241

Schweizer U, Bräuer AU, Köhrle J, Nitsch R, Savaskan NE (2004) Selenium and brain function: a poorly recognized liaison. Brain Res Brain Res Rev 45:164–178

Sedlak J, Lindsay RHC (1968) Estimation of total, protein bound and non-protein sulfhydryl groups in tissue with Ellmann's reagent. Anal Biochem 25:192–205

Suzuki J, Katoh N (1990) A simple and cheap method for measuring vitamin A in cattle using only a spectrophotometer. Jpn J Vet Sci 52:1282–1284

Uma Devi P, Pillai KK, Vohora D (2006) Modulation of pentylenetetrazole-induced seizures and oxidative stress parameters by sodium valproate in the absence and presence of *N*-acetylcysteine. Fundam Clin Pharmacol 20:247–253

Verrotti A, Scardapane A, Franzoni E, Manco R, Chiarelli F (2008) Increased oxidative stress in epileptic children treated with propoic acid. Epilepsy Res 78:171–177

Wang ST, Kuo JH, Chou RG, Lii CK (1996) Vitamin E protection of cell morphology and protein thiols in rat hepatocytes treated with tert-butyl hydroperoxide. Toxicol Lett 89:91–98

Weiergräber M, Henry M, Krieger A, Kamp M, Radhakrishnan K, Hescheler J, Schneider T (2006) Altered seizure susceptibility in mice lacking the Ca(v)2.3 E-type Ca^{2+} channel. Epilepsia 47:839–850

White HS, Smith MD, Wilcox KS (2007) Mechanisms of action of antiepileptic drugs. Int Rev Neurobiol 81:85–110

Wu G, Fang YZ, Yang S, Lupton JR, Turner ND (2004) Glutathione metabolism and its implications for health. J Nutr 134:489–492

Yatin SM, Varadarajan S, Butterfield DA (2000) Vitamin E prevents Alzheimer's amyloid beta-peptide (1–42)–induced neuronal protein oxidation and reactive oxygen species production. J Alzheimers Dis 2:123–131