IJVAR International Journal of Veterinary and Animal Research
Uluslararası Veteriner ve Hayvan Araştırmaları Dergisi
E-ISSN:2651-3609 1(2): 30-33, 2018

The Investigation of The Effects of Topiramate Treatment on The Role of Zinc Ions in Experimental Epilepsy

Fusun Ak Sonat[1]* Behzat Noyan[2]
[1]Uludag University, Veterinary Faculty, Department of Physiology, 16059, Bursa, TURKEY
[2]Uludag University, Faculty of Medicine, Department of Physiology, 16059, Bursa, TURKEY (Retired)

*Corresponding Author
E-mail:fusunak@uludag.edu.tr

Abstract

The acute effect of topiramate (TPM) treatment on zinc ion translocation in hippocampus and antiepileptic effect on behaviour in SE has been researched in this study. It has also been tried to explain, whether TPM has a role on antiepileptic effect by affecting the zinc ions on the hippocampus.Fortynine male Sprague-Dawley rats, 3 months old, were randomly divided into seven equal groups (n=7 per group). The first group was the naive, the second group was the seizure-control, the third group was seizure+TPM (60 mg/kg, i.p.) treatment, the fourth group was seizure+TPM60+CaEDTA (100 mM,i.c.v.), the fifth group was seizure+TPM60+ZnCl2 (35 mg/kg, i.p.), the sixth group was seizure+CaEDTA, the seventh group was seizure+ZnCl2.Behavioral changes of rats were observed throughout the experiment.End of the study, rats were decapitated under anesthesia to rapidly remove their brains. Hippocampal staining was performed to investigate zinc translocation.Our results show that, there was not significant differences in the SE incidence,SE latency, 24-hour survival, and seizure scoreamong the groups. Also it was observed that TPM is not effective for the zinc hystochemistry in the brain when administered at the dose of 60 mg/kg.On the other hand,TPM can be effective for zinc translocation when it is administered in high doses. For this reason we need to work more.

Key Words: Zinc, Topiramate, Hippocampus, Epilepsy, TSQ

1.INTRODUCTION

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures, is a major health problem that affects ~1-2% of the population worldwide[1]. SE in a neurologic condition with higher morbidity and mortality characterized by seizures that recur in short periods and continue for longer durations [2, 3]. At the same time SE causes serious neuron damage. Using different types of animals and models, acut and chronic SE can be induced experimentally. The degree of excytotoic neuron damage that occur during SE is related to seizure activity and duration [3].

Drugs used for controlling seizure activity (i.e. anticonvulsants or antiepileptics) have been available for many years. Anticonvulsants are used to suppress epileptic seizures without damaging the central nervous system and without causing respiratory depression. TPM [2,3:4,5,-bis-0-(1-methylethylidene) b-D-fuructopyranose sulfamate], ($C_{12}H_{21}NO_8S$) one of the newer antiepileptic drugs, has shown in experiments [4,5] and clinical studies [6] to have broad spectrum antiepileptic activity and neuroprotective effect. Topiramate contains D–fructose sulfamate, natural monosaccharide meaning it is a carbohydrate derivative and because of this it has a different structure than the other antiepileptic drugs and shows wide pharmacodynamic effects and wide spectrum receptor affinity compared to other anticonvulsants drugs [7,8]. It has been said that topiramate shows its effects through; blocking voltage sensitive Na+ channels [7,9] activating GABA receptors, increasing chlor entrance [9] and blocking AMPA receptors. By blocking L-type Ca++ channels which are activated by high voltage, it reduces Ca++ entrance and has been shown to have an activating effect on K+ channels [9]. On the other hand by inhibiting the production glutamate and aspartate it is been said to be neuroprotective [7].

Zinc is highly concentrated in the hippocampus, particularly in the mossy fibers. Most zinc ions in the mammalian brain are tightly bound to metalloenzymes, but more loosely bound zinc ions (about 8%) are found in synaptic vesicles in a subset of the glutamatergic neurons in cerebrum[10]. Although the vesicular zinc is a small fraction of total zinc in the brain, it is the only fraction of zinc that can be traced histochemically. The N-(6-methoxy-8-quinolyl)-para-toluenesulfonamide (TSQ) detectable zinc is present as free or loosely bound zinc ions exist in the synaptic vesicules of some glutamatergic neurons. Vesicular zinc ions are believed to be released during normal synaptic transmission [11,12]. It is suggested that the release of zinc ions stored in synaptic vesicles and the translocation of these ions to the postsynaptic compartment may trigger a degeneration of nerve cells following brain insults such as epilepsy [13], ischemia [14] and traumatic brain injury [15]. In addition, prevention of this zinc translocation has been shown to be neuroprotective in both ischemia and seizures [15].

The hypothetical link between mechanisms of TPM and the release of zinc ions after seizures induced by pilocarpine have not been previously investigated. Thus, we planned our study on the basis of assessing the effects of acutely administered, single dose of topiramate on behaviour and zinc translocation as a result of high dose pilocarpine HCl induced SE. Detecting wheather TPM would show antiepileptic effect via new path, especially by influencing the brain's zinc ions makes our study important and original. Therefore, we have examined whether or not TPM treatment would modulate the pilocarpine-induced seizures and/or change the histochemical distribution of zinc ions in hippocampus, basing on the outcome that receptor types of topiramate and the zinc ions are identical.

2.MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley rats (n=70), about three month old, weighting 250 g., (Uludag University, Experimental Animals Breeding and Research Center, Bursa, TURKEY) were used throughout the experiments. The study was approved by the Animal Ethical Committee of Uludag University (permit number:08062005/3). The animals were kept in

plastic cages in a room (12/12 hour light/dark schedule and at a temperature of 20-22° C, 50% humidity). All experimental procedures were conducted accordingly to NIH and EU guidelines for care and use of animals.

2.2.Surgical procedure

The rats were anesthetized with thiopenthal sodium (40 mg/kg; i.p., Pentothal Sodium-Abott). For intracerebroventricular (i.c.v.) administration of CaEDTA, a burr hole was drilled through the skull 1.5 mm lateral to midline and 1.0 mm posterior to bregma and lowered 4.2 mm below. (Figure 1) A 22-gauge stainless steel cannula was directed in the hole towards the lateral ventricleusing stereotaxic equipment (Figure 2). The cannula was fixed to skull with dental cement (ADHESOR®, CARBOFINE, SpofaDental). Local antibiotic (Furacin®, ECZACIBASI) and analgesics (Xylocaine® Pump Spray, AstraZeneca) applied and the skin was closed.

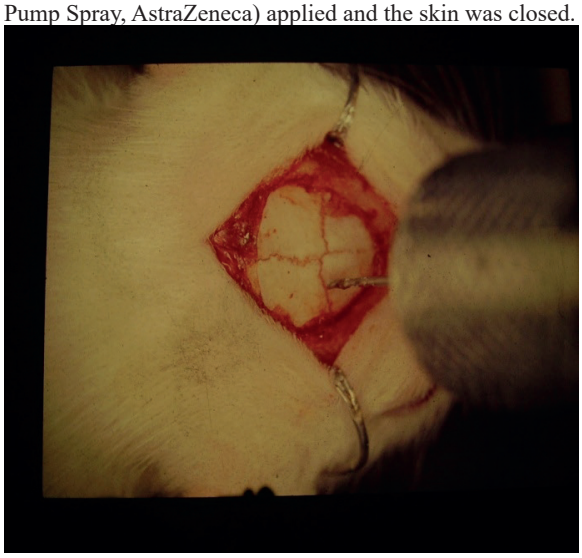

Figure 1: For intracerebroventricular (i.c.v.) administration of CaEDTA, a hole is drilled in the skull

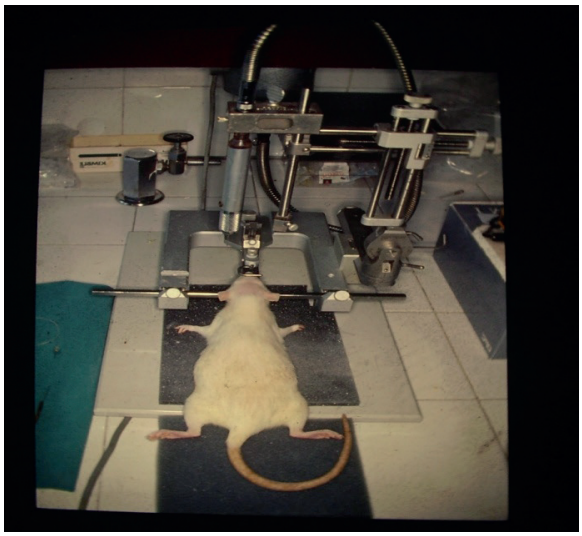

Figure 2: The placement of rat to stereotaxic equipment

2.3. Seizure induction and treatment groups

Naive group of rats (n=7) that were recevied vehicle (2 ml NaCl, 0.9% i.p.), control group of rats (n=7) that were recevied vehicle (2 ml NaCl, 0.9% i.p.) and then pilocarpine HCl (380 mg/kg i.p.) was injected 30 minutes later in order to induce SE. TPM groups (n=21) that were recevied (60 mg/kg i.p.) TPM. In these groups, TPM was given 30 minutes before the injection of pilocarpine HCl. In the CaEDTA groups (100 mM i.c.v.), CaEDTA (with or without TPM) was given 30 minutes before the injection of pilocarpine HCl. In the $ZnCl_2$ gropus (35 mg/kg, i.p.), $ZnCl_2$ (with or without TPM) was given 30 minutes before the injection of pilocarpine HCl. Peripheral cholinergic side effects were reduced by a methylscopolamine (1 mg/kg, s.c.) injected forty minutes before the injection of pilocarpine HCl. Topiramate, pilocarpine HCl and methyl-scopolamine were purchased from Sigma Chemical Co. (St. Louis, MO).

2.4. Behavioral evaluation of SE activity

The effects of TPM on pilocarpine-induced SE were assessed on the basis of SE incidence, latency to SE and 24-hour survival. The behavior of the animals was observed for 24 hours and the intensity of their seizures was scored. The Racine scale [16] was used to evaluate the intensity of the seizures. Scoring was made according to the most intense seizure that was witnessed during 24 hours observation time. The scores given to the behavioral signs of limbic motor seizures were as follow:

0 point: Immobility, no seizures
1 point: Facial automatisms
2 point: Head nodding
3 point: Unilateral or bilateral forelimb clonus
4 point: Bilateral forelimb clonus and rearing
5 point: Rearing, falling and generalized convulsions, SE

2.5. Tissue preparation and zinc-spesific fluorescence staining

Rats were anaesthetized with thiopental sodium (40 mg/kg, i.p.,Pentothal sodium, Abott) and decapitated 24h after the pilocarpine HCl injection. The brains were removed rapidly, frozen in CO_2 snow then stored at – 80 °C. Coronal sections (10 µm thick) of hippocampus were cut using a cryostat and mounted on poly-L-lysine coated glass slides. For the fluorescence visualization of loosely bound synaptic (vesicular) and free zinc ions, the sections were thawed and stained with TSQ (Invitrogen Co., USA), by immersing the sections in a solution of TSQ (4,5 µM) and 140 mM sodium acetate and 140 mM sodium barbital buffer for 60 s, rinsed for 1 min, in normal saline (NaCl, 0.9 %), then viewed and imaged using a compound (Nikon Eclipses E600) fluorescence microscope (UV filter: excitation, 330-385 nm; barrier, 420 nm) [17]. The photos of hippocampus taken by a camera connected the microscope have been saved to the computer.

2.6. Statistical analysis

All observations are given as means ± SEM. Statistical differences of the results were performed using analysis of variance (ANOVA) to compare the latency to SE.The post-hoc test didn't made since there is no significance in analysis of variance. Kruskal-Wallis variance analysis was performed toseizure scores.The SPSS program (SPSS 15.0 for windows) was used to perform these tests.Statistical differences of the results were performed using Fischer's Exact Test with Instat (GraphPad Instat software V2.02) for comparing the percentage of SE and the 24-hour survival because there are five animals in some groups.

3. RESULT AND DISCUSSION

3.1.Behavioral evaluation of seizure activity

In all groups, approximately 5 minutes after pilocarpine was administered, cholinergic stimulation signs; salivation, piloerection, diuresis, tremor, automatic limbic movements like chewing was formed. After 15-20 minutes

head shaking, rearing and forelimb clonus was seen and then SE was formed some animals 39-41 minutes (average) after pilocarpine injection. There was not significant differences in incidence of SE,SE latency, 24-hour survival, seizure score among the groups (table 1).

Table 1. Mean values (mean ± SEM) and comparison of percentage of status epilepticus (SE), latency to SE, 24-hour survival and seizure scores between at the control group (380 mg/kg pilocarpine HCl injected group) and experimental groups.

	SE	Latency to	24-hour	Seizure Scores
	(%)	SE (min)	Survival(%)	(point)
Naive Group (n=7)	-	-	100	-
Seizure-control (n=7)	90	40.7±1.8	80	4.5±0.2
S+TPM 60 mg/kg (n=7)	50	45.0±1.6	90	3.8±0.4
S+TPM60+CaEDTA (n=7)	60	44.5±2.1	90	3.9±0.3
S+TPM60+ZnCl2 (n=7)	90	42.1±2.6	80	4.3±0.3
S+CaEDTA (n=7)	70	44.7±3.3	90	4.2±0.2
S+ZnCl2 (n=7)	90	44.5±2.1	70	4.6±0.2

ANOVA, p>0.05, not significant differences among the groups compare to seizure-control group

3.2. Evaluation of zinc-spesific fluorescence staining

In order to evaluate a possible seizure induced postsynaptic accumulation of free zinc ions in the hippocampal pyramidal cells, we used TSQ staining, furthermore, microscopic pictures of the TSQ fluorescence intensities in area CA3 was compared between groups.In our study a meaning result hadn't been achieved in the means of distribution and amount of zinc ions. As expected, it is observed that in the naive group, the zinc ions that are in the CA3 area, hanging on the presynaptic ends of the mossy fibers kept being fluorescent in the normal levels. (Figure 3).When TPM administered at the dose of 60 mg/kg and control group it is observed that zinc could pass to the postsynaptic neuron from the presynaptic neuron (Figure5and4) When 60 mg/kg was applied, TPM was not effective on the zinc translocation. Only the images of the three groups were used because of the images of the other groups are similar.

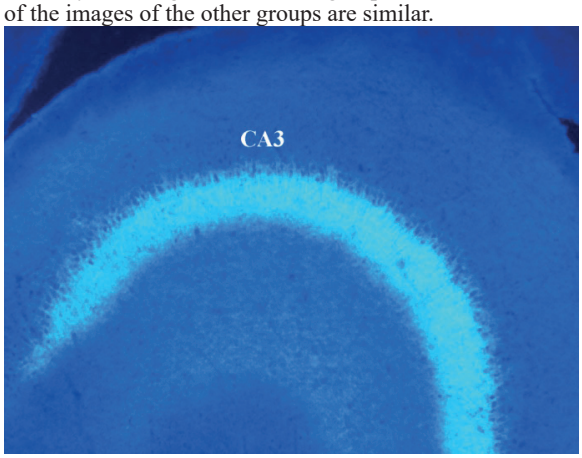

Figure 3: TSQ staining. Samples of TSQ staining from mossy fibers and Cornu Ammonis (CA3) area of naive group. It is observed that in the naive group, the zinc ions that are in the CA3 area, hanging on the presynaptic ends of the mossy fibers kept being fluorescent in the normal levels.

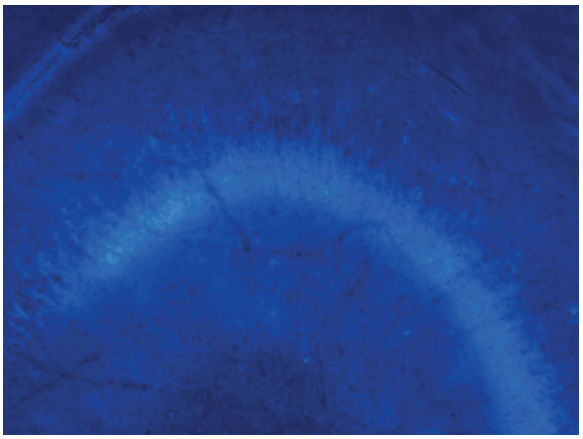

Figure 4: TSQ staining. Samples of TSQ staining from mossy fibers and Cornu Ammonis (CA3) area of seizure-control group. The zinc ions could pass to the postsynaptic neuron from the presynaptic neuron in the this group.

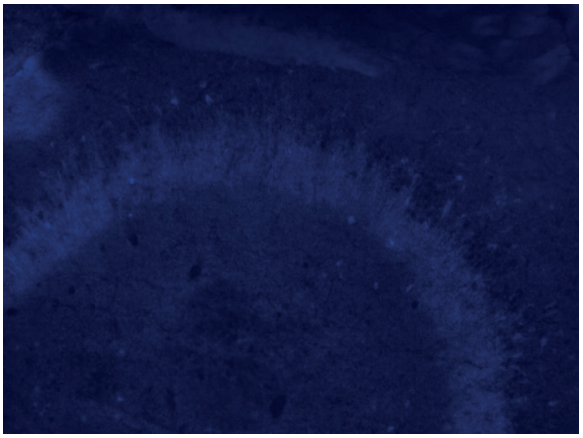

Figure 5: *TSQ staining. Samples of TSQ staining from mossy fibers and Cornu Ammonis (CA3) area of TPM60 group. The zinc ions could pass to the postsynaptic neuron from the presynaptic neuron when TPM administered at the dose of 60 mg/kg in this group.*

The fact that Niebauer and Gruenthal [18] do not find a difference between the animals that given and not given TPM in terms of behavior supports our findings.Fisher et al. [19] found that any dose of TPM (20-320 mg / kg) did not terminate the SE.Rigoulot et al. [20] found that TPM was not effective on behavior.The findings of these researchers support us.In a study of post-ischemic epilepsy in rats, TPM prevented tonic and clonic seizures [21].In another study of mice with seizures with pentylenetetrazole, TPM increased to seizure threshold [22,23].The tonic phase of generalized seizures was found to be suppressed by topiramate in young and adult rats [24].Sills et al.[25] have suggested that TPM is not effective on GABA levels in a study with mice.Rigoulot et al. [20] claimed that GABA and glutamate levels could be altered by TPM injection in GAERS rats.In a study with humans, it has been reported that TPM has an antiepileptic effect by increasing GABA level in healthy subjects [26] and epileptic patients [27].The fact that these findings do not support us may be due to differences between species and the difference in use of the seizure methods.Placidi et al. [28] found that TPM significantly reduced both partial and generalized tonic-clonic seizures (≥50%) in humans.But the treatment in this study is a chronic treatment in humans.The difference in our findings may be due to our study is acute.A study by Kudin et al. [29] found that TPM did not terminate SE at doses of 100 mg / kg or less. In recent years studies, SE

was controlled in 68.6% of patients receiving TPM in human [30].In Acon-Chen et al work,results suggest that treatment with TPM is not effective at reducing soman-induced seizure activity and neuropathology in rats [31].In another study in human,TPM was not a predictor of successful SE termination in neither the overall cohort, nor in the subgroup of complex-partial RSE [32].

Also, there was no literature which supports us either pozitively or negatively about TPM and zinc histochemistry. The hypothetical link between mechanisms of TPM and the release of zinc ions after seizures induced by pilocarpine have not been previously investigated.On the other hand when TPM is administered in higher doses, it can be seen, in addition to its known antiepileptic mechanisms, has an other antiepileptic effect via a possible pathway by blocking brain's zinc translocation. also higher doses of topiramate may be effective on SE behaviours.As a result of insufficient studies investigating TPM and zinc ions in the brain and not knowing much about their relation, we considered this issue worth to research.

REFERENCES

[1]. Löscher W. 2002. Current status and future directions in the pharmacotherapy of epilepsy. Trends Pharmacol. Sci., 23: 113-118.

[2]. Hussain N, Appleton R, Thorburn K. 2007. Aetiology, course and outcome of children admitted to paediatric intensive care with convulsive status epilepticus: A retrospective 5-year review. Seizure, 16: 305-312.

[3]. Sloviter RS. 1983. Epileptic brain demage in rats induced by sustained electrical stimulation of the perforant path, I: acute electrophysiological and light microscopic studies. Brain Res Bull, 10: 675-697.

[4]. Shank RP, Gardocki JF, Vaught JL. 1994. Topiramate: preclinical evaluation of structurally novel anticonvulsant. Epilepsia, 35: 450-460.

[5]. Wauquier A, Zhou S. 1996. Topiramate: a potent anticonvulsant in the amygdala-kindled rat. Epilepsy Res, 24: 73-77.

[6]. Bourgeois BF. 1996. New antiepileptic drugs. Curr Opin Pediatr, 8: 543-548.

[7]. Ben-Menachem E, Sander JW, Stefan H, Schwalen S, Schauble B. 2008. Topiramate monotherapy in the treatment of newly or recently diagnosed epilepsy. Clin. Ther., 30: 1180-1195.

[8]. Latini G, Verrotti A, Manco R, Scardapane A, Del Vecchio A, Chiarelli F. 2008. Topiramate: its pharmacological properties and therapeutic efficacy in epilepsy. Mini Rev. Med. Chem., 8: 10-23.

[9]. Ashtari, F., Shaygannejad, V., Akbari, M., 2008. A double-blind, randomized trial of low-dose topiramate vs propranolol in migraine prophylaxis. Acta. Neurol. Scand. 118: 301-5.

[10]. Green AL, Weaver DF. 2008. Potential proconvulsant effects of oral zinc supplementation-A case report. NeuroToxicology, 29: 476-477.

[11]. Frederickson CJ, Suh SW, Silva D, Frederickson JC, Thompson RB. 2000. Importance of zinc in the central nervous system: the zinc-containing neuron. J. Nutr., 130: 1471–1483.

[12]. Takeda A. 2000. Movement of zinc and its functional significance in the brain. Brain Res. Brain Res. Rev. 34: 137–148.

[13]. Frederickson CJ, Hernandez MD, McGinty JF. 1989. Translocation of zinc may contribute to seizure-induced death of neurons. Brain Res., 480: 317–321.

[14]. Lee JM, Zipfel GJ, Park KH, He YY, Hsu CY, Choi DW. 2002. Zinc translocation accelerates infarction after mild transient focal ischemia. Neuroscience,115: 871-878.

[15]. Suh SW, Chen JW, Motamedi M, Bell B, Listiak K, Pons, NF. 2000. Evidence that synaptically-released zinc contributes to neuronal injury after traumatic brain injury. Brain Res., 852: 268-273.

[16]. Racine RJ. 1972. Modification of seizure activity by electrical stimulation II: motor seizure. Electroencephalogr. Clin Neurophysiol, 32: 281-294.

[17]. Noyan B, Jensen MS, Danscher G. 2007. The lack of effects of zinc and nitric oxide in initial state of pilocarpine-induced seizures. Seizure, 16: 410-416.

[18]. Niebauer M, Gruenthal M. 1999. Topiramate reduces neuronal injury after experimental stetus epilepticus. Brain Research, 837: 263-269.

[19]. Fisher A, Wang X, Cock HR, Thom M, Patsalos PN, Walker MC. 2004. Synergism between topiramate and budipine in refractory status epilepticus in the rat. Epilepsia, 45: 1300-1307.

[20]. Rigoulot MA, Boehrer A, Nehlig A. 2003. Effects of topiramate in two models of genetically determined generalized epilepsy, the GAERS and the audiogenic Wistar AS. Epilepsia, 44(1): 14-19.

[21].Edmonds HL Jr, Jiang YD, Zhang PY.1996. Anticonvulsant activity of topiramate and phenytoin in a rat model of ischemia induced epilepsy. Life Science, 59: 127-131.

[22]. White HS, Brown SD, Woodhead JH, Skeen GA, Wolf HH. 1997. Topiramate enhances GABA mediated chloride flux and GABA evoked chloride currents in murine brain neurons and increases seizure threshold. Epilepsy Research, 28:167-179.

[23]. White HS, Brown SD, Woodhead JH, Skeen GA, Wolf HH. 2000. Topiramate modulates GABA evoked current in murine cortical neurons by a nonbenzodiazepine mechanism. Epilepsia, 41: 17-20.

[24]. Haugvicova R, Kubova H, Skutova M. 2000. Anticonvulsant action of topiramate against motor seizures in developing rats. Epilepsia, 41: 1235-1240.

[25].Sills GJ, Leach JP, Kilpatrick WS. 2000. Concentration effect studies with topiramate on selected enzymes and intermediates of the GABA shunt. Epilepsia, 41: 30-34.

[26]. Kohling R, Vreugdenhil M, Bracci E. 2000. Ictal epileptiform activity is facilitated by hippocampal GABAA receptor mediated oscillations. Journal of Neuroscience, 20: 6820-6829.

[27]. Petrof OAC, Hyder F, Mattson RH. 1999. Topiramate increases brain GABA, homocarnosine and pyrrolidinone in patients with epilepsy. Neurology, 52: 473-478.

[28]. Placidi F, Tombini M, Romigi A, Bianchi L, Izzi F, Sperli F, Mattia D, Cervellino A, Marciani MG. 2004. Topiramate: effect on EEG interictal abnormalities and background activity in patients affected by focal epilepsy. Epilepsy Research, 58: 43-52.

[29]. Kudin AP, Debska-Vielhaber G, Vielhaber S, Elger CE, Kunz WS. 2004. The mechanism of neuroprotection by topiramate in an animal model of epilepsy. Epilepsia, 45: 1478-1487.

[30]. Brigo F, Bragazzi NL, Igwe SC. Nardone R, Trinka E. 2017.Topiramate in the Treatment of Generalized Convulsive StatusEpilepticus in Adults: A Systematic Review with IndividualPatient Data Analysis,Drugs 77:67–74.

[31]. Acon-Chen C, Koenig JA, Smith GR, Truitt AR, Thomas TP, Shih TM. 2016. Evaluation of acetylcholine, seizure activity and neuropathology following high-dose nerve agent exposure and delayed neuroprotective treatment drugs in freely moving rats, Toxicol Mech Methods. 26:378-88.

[32]. Dominik Madzar D, Kuramatsu JB, Gerner ST, Huttner HB. 2016.Assessing the value of topiramate in refractory status epilepticus, Seizure, 38: 7–10.