ORIGINAL ARTICLE

Investigating the Role of Zinc in a Rat Model of Epilepsy

A. M. Baraka,¹ W. Hassab El Nabi² & S. El Ghotni²¹ Clinical Pharmacology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt² Physiology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt

Keywords

Behavioural neurology; Epilepsy; Movement disorders; Neuropsychopharmacology; Parkinsons disease.

Correspondence

Azza M. Baraka, Clinical Pharmacology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt.

Tel.: (0203) 420-9010;

Fax: (0203) 420-3853;

E-mail: mnhbaraka@yahoo.com

Received 12 April 2010; revision 5 January 2011;

accepted 15 March 2011

doi: 10.1111/j.1755-5949.2011.00252.x

SUMMARY

Aims: The aim of the present study was to investigate the role of zinc (Zn) in pilocarpine-induced seizures and its interrelation with an antiepileptic drug, namely, valproic acid.

Methodology: The study was carried out on 110 male Wistar albino rats that were divided into the following groups: Group I, control rats that received intraperitoneal (i.p.) saline vehicle; Groups II–V received Zn in a medium dose, Zn in a high dose, valproic acid in a therapeutic dose, as well as a combination of valproic acid with medium dose Zn, respectively, for 3 weeks before saline injection, Group VI received i.p. pilocarpine to induce seizures; Groups VII–XI received Zn in a medium dose, Zn in a high dose, valproic acid in a therapeutic dose, a combination of therapeutic dose of valproic acid with medium dose Zn, as well as a combination of subeffective dose of valproic acid with medium dose of Zn, respectively, for 3 weeks before pilocarpine injection. The seizure's latency and severity for each rat was recorded. Blood and brain hippocampal samples were collected for determination of serum neuron specific enolase (NSE), hippocampal Zn, interleukin-1 beta concentrations as well as hippocampal superoxide dismutase and caspase-3 activities. **Results:** The results of the current study demonstrated that pretreatment with high dose of Zn exacerbated pilocarpine-induced seizures. Whereas, a medium dose of Zn and valproic acid either alone or in combination reduced the severity of pilocarpine-induced limbic seizures and increased the latency to attain the forelimb clonus. Also both drugs, either alone or in combination, ameliorated all studied biochemical parameters with the exception of hippocampal Zn concentration, which was only significantly increased by pretreatment with Zn, either alone or in combination with valproic acid. **Conclusions:** The present study highlights the antiepileptic role that could be played by Zn, when given in appropriate doses.

Introduction

Epileptogenesis is a big challenge that refers to a process in which an initial brain-damaging insult triggers a cascade of molecular and cellular changes that eventually lead to increase in brain excitability and the occurrence of spontaneous seizures [1].

Various studies suggested that the homeostasis of trace elements, electrolytes, and antioxidants are crucial for brain function and they are directly or indirectly implicated in the pathophysiology of neuronal excitability and seizure recurrence and its resistance to treatment with antiepileptic drugs (AEDs). In addition, it has been reported that AEDs can alter the homeostasis of trace elements and seriously increase membrane lipid peroxidation at the expense of protective antioxidants, leading to an increase in seizure recurrence [2].

Zinc (Zn) is an essential micronutrient for human health and has numerous structural and biochemical roles. It is a crucial cofactor for many proteins involved in cellular processes like differentiation, proliferation, and apoptosis [3]. Zn is important for main-

taining a healthy immune system, metabolic homeostasis (energy utilization), and antioxidant activity (superoxide dismutase [SOD] enzyme) [4].

Zn is present in high concentration in synaptic vesicles of glutamatergic terminals including hippocampal mossy fibers. This vesicular Zn can be synaptically released during neuronal activity [5]. Releasable, vesicular Zn is most abundant in brain regions that are prone to seizures, namely the limbic regions [6]. Excessive release of Zn has been observed in an animal model of epilepsy [7]. Overall levels of Zn in the hippocampus appear to increase markedly as a result of kindling in rats [8].

Zn is required for normal mammalian brain development and physiology, such that deficiency or excess of zinc has been shown to contribute to alterations in behavior, abnormal central nervous system development, and neurological disease. In this light, it is not surprising that zinc ions have now been shown to play a role in the neuromodulation of synaptic transmission as well as in cortical plasticity [9]. It has been demonstrated that Zn modulates synaptic transmission in the hippocampus [10].

The observation that Zn is present in discrete sites at the nervous system and that it may affect specific physiological functions has led to interesting speculations on its role in neural function and neurological disorders [11].

Neuromodulatory effects of Zn released from sprouted mossy fibers could be proconvulsive or anticonvulsive. One study suggesting proconvulsive effects of Zn showed that the duration of kindling-induced seizures and electrical afterdischarges was decreased by repeated injections of a membrane-permeable zinc chelator before each stimulation [12]. In addition, a large body of evidence shows that excitotoxicity is influenced by Zn released from intracellular stores [13].

Other studies suggest anticonvulsive effects of Zn. Mice lacking vesicular Zn because of the lack of the ZnT-3 Zn transporter [14] or a Zn-deficient diet [15] are much more susceptible to kainic acid-induced seizures than mice with normal vesicular Zn levels, and epilepsy-prone rats have an increased zincergic innervation of their forebrain, perhaps because of an upregulation of Zn to prevent the danger from seizures as the authors speculate [16]. A delay of kindling-induced seizures was seen in cats fed a Zn-enhanced diet, and conversely an acceleration of kindling with a Zn-deficient diet [17]. However, this study did not determine if there was an actual change in hippocampal Zn levels.

Most of the information on effects of Zn is based on studies in brain slices. The implications of slice studies for epilepsy are limited, since only short-term observations are possible and many neuronal connections have been transected. The present study was designed to evaluate potential effects of extracellular Zn in the intact brain.

Thus, the aim of the present study was to investigate the role of medium and high doses of Zn in pilocarpine-induced seizures. The effect of combining medium dose of Zn with therapeutic as well as subeffective doses of a frequently used AED, namely valproic acid, in pilocarpine-induced seizures was also assessed in the current study. Understanding Zn actions will be crucial for determining its potential as preventive and therapeutic agent against excitatory brain damage such as seizures.

Methods

Animal Grouping

The present study was carried out on 110 male Wistar albino rats weighing 150–200 g. The rats were obtained from the Pharmacology Department, Faculty of Medicine, Alexandria University. The rats were housed under the same environmental conditions, natural light/dark cycle with food and water available *ad libitum*. Animals were kept for a minimum period of 7 days prior to use to allow for acclimatization. All experiments were performed in accordance with national animal care guidelines and were preapproved by the Faculty of Medicine, Alexandria University Ethics Committee.

The rats were divided into the following groups of 10 rats each:

Group I: normal control rats that received a single 1 mL intraperitoneal (i.p.) injection of 0.9% saline and served as control for group II.

Group II: normal control rats that received Zn (zinc sulfate, Sigma, St. Louis, MO, USA), in 0.9% saline vehicle, in a medium dose of 3 mg/kg body wt (bwt) daily i.p. [18] for 3 weeks before saline injection.

Group III: normal control rats that received Zn (zinc sulfate, Sigma, USA), in 0.9% saline vehicle, in a high dose of 60 mg/kg bwt [19] daily i.p. for 3 weeks before saline injection.

Group IV: normal control rats that received valproic acid (Depakene, Abbott Pharmaceuticals, North Chicago, IL, USA) in 0.9% saline vehicle, in a therapeutic dose of 100 mg/kg bwt daily i.p. [20] for 3 weeks before saline injection.

Group V: normal control rats that received a combination of valproic acid, in a dose of 100 mg/kg bwt daily i.p. and Zn in a dose of 3 mg/kg bwt for 3 weeks before saline injection.

Group VI: rats in which seizures were induced by i.p. injection of pilocarpine (pilocarpine hydrochloride, Sigma, USA) in 0.9% saline vehicle, in a dose of 400 mg/kg bwt after pretreatment with atropine (1 mg/kg bwt) to avoid undesired peripheral effects of pilocarpine [21]. Pilocarpine-induced seizures in rats provide a widely accepted animal model of temporal lobe epilepsy (TLE) [22].

Group VII: pilocarpine-injected rats that received Zn (zinc sulfate, Sigma, USA), in 0.9% saline vehicle, in a medium dose of 3 mg/kg bwt daily i.p. for 3 weeks before pilocarpine injection which was administered 6 h following the last dose of Zn.

Group VIII: pilocarpine-injected rats that received Zn (zinc sulfate, Sigma, USA), in 0.9% saline vehicle, in a high dose of 60 mg/kg bwt daily i.p. for 3 weeks before pilocarpine injection which was administered 6 h following the last dose of Zn.

Group IX: pilocarpine-injected rats that received valproic acid (Depakene, Abbott Pharmaceuticals, USA) in 0.9% saline vehicle, in a therapeutic dose of 100 mg/kg bwt daily i.p. for 3 weeks before pilocarpine injection which was administered 6 h following the last dose of valproic acid.

Group X: pilocarpine-injected rats that received a combination of i.p. valproic acid in a therapeutic dose of 100 mg/kg bwt and Zn in a dose of 3 mg/kg bwt daily for 3 weeks before pilocarpine injection which was administered 6 h following the last doses of Zn and valproic acid.

Group XI: pilocarpine-injected rats that received a combination of a subeffective dose of i.p. valproic acid (54 mg/kg bwt) [23] and i.p. Zn (3 mg/kg bwt) daily for 3 weeks before pilocarpine injection which was administered 6 h following the last doses of Zn and valproic acid.

The period of 3 weeks Zn administration prior to pilocarpine injection was chosen since it has been reported that chronic treatment with Zn induces enhancement of presynaptic/extracellular Zn concentration in the rat hippocampus [24].

Behavioral Assessment

The seizure latency and severity for each rat was recorded [25]. The animals were observed for the progression of limbic seizures every 30 min for 2 h as follows: No response = 0; gustatory movements and/or fictive scratching = 1; tremors = 2; head bobbing = 3; forelimb clonus = 4; rearing, falling, and clonus = 5. Latency to forelimb clonus was recorded for score 4 only.

Two hours following pilocarpine injection rats were treated with diazepam 5 mg/kg bwt i.p. to block seizures activity.

Biochemical Assessment

Twenty-four hours after pilocarpine injection, blood was withdrawn from retroorbital vein plexus, serum was separated and used for the determination of serum neuron specific enolase (NSE) concentration (as a marker of brain injury) by enzyme-linked immunoassay (ELIZA, ALPCO Diagnostics, Windham, New Hampshire, USA) [26].

Then rats were killed by exsanguination and the brains were quickly removed and rinsed in ice cold 0.9% w/v NaCl to remove blood. The hippocampi were immediately frozen on dry ice and stored at -80°C until analysis for determination of:

- Zn concentration measured with an atomic absorption spectrophotometer Perkin-Elmer Model 2280 (Flame) (AA-880 Mark-II, Nippon Jarrel-Ash Co. Ltd., Kyoto, Japan) [27].
- SOD enzyme activity performed according to Misra and Fridovich [28].
- Interleukin-1 beta (IL-1 β) concentration by ELIZA (Biosource, CA, USA) [29].
- Caspase-3 activity as a marker of neuronal apoptosis by fluorometric kit (Assay Design Inc., MI, USA) [30].

Statistical Analysis

Data were fed to the microcomputer program Statistical Package for Social Science (SPSS) version 17.0. Results were expressed as a mean $\pm$ S.E.M. Tabulation and analysis of data was done using analysis of variance (ANOVA) test. Significance of differences between the groups studied was determined with least significant test (LSD) test. Statistically significant differences were assumed at *P* less than or equal 0.05 [31].

Results

Mortality

Three rats died in groups VI and VIII and one rat died in each of groups III and VII, while no rats died in the rest of the groups.

Effects on Behavior

Intraperitoneal injection of pilocarpine to rats resulted in progression to limbic seizures with progressing behavioral score at various time intervals (recorded every 30 min up to 2 h). High-dose Zn resulted in a significant increase in the severity of pilocarpine-induced seizures. Pretreatment with medium dose Zn reduced the severity of pilocarpine-induced limbic seizures and delayed latency to forelimb clonus. Also, pretreatment with valproic acid either alone or in combination reduced the severity of pilocarpine-induced limbic seizures and animals did not attain the forelimb clonus (score 4). A significant difference was observed between rats that received a combination of Zn and therapeutic dose of valproic compared to those that received valproic acid alone or that

received a combination of medium dose Zn and subeffective dose of valproic acid (Table 1).

Biochemical Results

The results of the present study demonstrated a significant decrease in hippocampal Zn concentration and SOD activity, a significant increase in hippocampal IL-1 β concentration, caspase-3 activity as well as serum NSE in nontreated pilocarpine-injected rats as well as in high dose Zn-treated rats compared to normal control ones.

Pretreatment with medium dose zinc or valproic acid either alone or in combination resulted in a significant increase in hippocampal SOD activity as well as a significant decrease in hippocampal IL-1 β concentration, hippocampal caspase-3 activity, and serum NSE concentration. A significant difference in all these studied parameters was observed between rats that received a combination of Zn and valproic acid compared to those that received valproic acid alone. A significant increase in hippocampal Zn concentration was only observed in rats that received Zn either alone or in a combination with valproic acid (Table 2).

Discussion

In the present study, pilocarpine-injected rats exhibited a sequence of behavioral alterations including staring spells, olfactory and gustatory automatisms, and motor limbic seizures that developed over 1–2 h. These findings are in accordance with other studies reporting that the stages of pilocarpine-induced seizures progress from stages I–II (mouth and facial movements, head nodding) to stage III (forelimb clonus), and then progress rapidly to stages IV–V (generalized limbic seizures; rearing, and rearing with falling) followed by status epilepticus [32].

The present work showed a significant increase in NSE, which has been established as a reliable marker of neuronal damage in various neurological disorders. The determination of biochemical markers of neuronal damage offers many advantages since they are noninvasive and can be repeated [33]. In agreement with our results, Palmio *et al.* [34] reported an increase in the levels of NSE in patients with TLE and suggested that TLE may be associated with brain damage.

A significant increase in oxidative stress has been also found in pilocarpine-injected rats as reflected by significant decrease in hippocampal SOD activity. This consumption of antioxidants has been reported to be causally involved in some forms of epilepsies [35].

Moreover, the present work demonstrated an increase in hippocampal apoptosis evidenced by the significant increase in hippocampal caspase-3 activity in pilocarpine-injected rats. This observed apoptotic activity is also consistent with previous reports where seizures cause mitochondrial dysfunction and activate intrinsic pathway components including proapoptotic caspases [36]. Recent studies provide strong evidence for the involvement of mitochondrially generated ROS production in the induction of cell death [37]. Evidence has accumulated that apoptotic cell death contributes to brain damage following experimental seizures. Although longer periods of seizures consistently result in brain

Table 1 Behavioral score at various time intervals (in min) and latency to forelimb clonus, (Mean $\pm$ SD), within 2 h following pilocarpine injection in rats

Rat group	Behavioral score				Latency of forelimb clonus (min)
	30 min	60 min	90 min	120 min	
Group I: Normal control, n = 10	ND	ND	ND	ND	ND
Group II: medium-dose Zn (3 mg/kg bwt) pretreated saline-injected rats, n = 10	ND	ND	ND	ND	ND
Group III: high-dose Zn (60 mg/kg bwt) pretreated saline-injected rats, n = 9	ND	ND	ND	ND	ND
Group IV: valproic acid (100 mg/kg bwt) pretreated saline injected rats, n = 10	ND	ND	ND	ND	ND
Group V: medium-dose Zn (3 mg/kg bwt) & valproic acid (100 mg/kg bwt) pretreated saline-injected rats, n = 10	ND	ND	ND	ND	ND
Group VI: pilocarpine-injected rats, n = 7	2.67 $\pm$ 0.29	4.29 $\pm$ 0.89	4.76 $\pm$ 0.79	4.79 $\pm$ 1.43	45.73 $\pm$ 2.27
Group VII: medium-dose Zn pretreated (3 mg/kg bwt) pilocarpine-injected rats, n = 9	1.04 $\pm$ 0.18 ^a	2.96 $\pm$ 0.43 ^a	4.00 $\pm$ 0.78 ^a	4.02 $\pm$ 0.76 ^a	98.15 $\pm$ 2.19 ^a
Group VIII: high-dose Zn (60 mg/kg bwt) pretreated pilocarpine-injected rats, n = 7	2.97 $\pm$ 0.12 ^a	4.99 $\pm$ 0.84 ^a	4.96 $\pm$ 0.93 ^a	5.00 $\pm$ 0.43 ^a	35.73 $\pm$ 2.27 ^a
Group IX: valproic acid (100 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	0.54 $\pm$ 0.08 ^a	1.21 $\pm$ 0.16 ^a	1.34 $\pm$ 0.45 ^a	2.51 $\pm$ 0.39 ^a	NC
Group X: medium-dose Zn (3 mg/kg bwt) & valproic acid (100 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	0.34 $\pm$ 0.04 ^{a,b}	0.91 $\pm$ 0.12 ^{a,b}	1.06 $\pm$ 0.22 ^{a,b}	1.12 $\pm$ 0.32 ^{a,b}	NC
Group XI: medium-dose Zn (3 mg/kg bwt) & subeffective valproic acid (54 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	0.59 $\pm$ 0.06 ^a	1.41 $\pm$ 0.20 ^a	1.40 $\pm$ 0.32 ^a	2.71 $\pm$ 0.37 ^a	NC
F test	47.67	60.34	50.23	39.66	22.90
P value	0.001	0.001	0.001	0.001	0.001

^aSignificant compared to non-treated pilocarpine-injected group.^bSignificant compared to valproic acid-treated rats.

n, number of rats in each group; ND, not determined; NC, no observed forelimb clonus within 2 h following pilocarpine-injection.

damage, it has previously not been clear whether brief single or intermittent seizures lead to cell death. However, results indicate that also single seizures lead to apoptotic neuronal death. A brief, nonconvulsive seizure evoked by kindling stimulation was reported to produce apoptotic neurons bilaterally in the rat dentate gyrus [38]. In the current study, though apoptosis was observed after 24 h, this does not necessarily mean that this is the most relevant time point for cell death, thus the 24-h time point might be a limitation of our findings and further research is needed on the longer term cell death.

In this study, we reported a significant increase in the level of hippocampal IL-1 β in pilocarpine-injected rats compared to normal animals. This is in accordance with other studies reporting a role for inflammatory processes in the clinical manifestations and neuropathological sequelae of epilepsy [39,40].

It has been suggested that the transformation of normal pattern of neuronal activity to the paroxysmal one is associated with the increased production of brain cytokines. However, the expression of these cytokines is associated with cell injury rather than with seizures *per se*. This finding suggested the presence of another brain damaging factor in epilepsy in addition to apoptosis [41].

Regarding hippocampal Zn concentration, a significant decrease in this trace element has been demonstrated in nontreated

pilocarpine-injected rats. Excessive release of Zn and glutamate from the neuron terminals under pilocarpine-induced seizures might be responsible for the loss of Zn from the brain. The decrease in actively functioning Zn in the brain may be associated with the increase in susceptibility to seizure [42].

Though valproic acid exerted a significant protective effect against pilocarpine-induced seizures but it failed to cause a significant increase in hippocampal Zn concentration. This situation suggests an association between the side effects that develop during valproic acid treatment and zinc deficiency [43]. Indeed, valproic acid given to normal rats resulted in a significant decrease in hippocampal Zn concentration, which might be due to the ability of valproic acid to bind to Zn.

Considering the effect of administered Zn, our data demonstrated the ability of medium doses of Zn to inhibit seizure activity and prolong seizure latency in pilocarpine-injected rats. In addition, combination of a medium dose of Zn with a subeffective dose of valproic acid normalized the decrease in hippocampal Zn concentration and enhanced valproic acid-induced protective effect against pilocarpine-induced seizures.

Another interesting finding of the current study is the comparable antiepileptic activity of a combination of medium dose Zn and subeffective dose of valproic acid to that exerted by a

Table 2 Hippocampal zinc, superoxide dismutase (SOD), interleukin-1 beta (IL-1 β), caspase-3, and serum neurone specific enolase (NSE), (Mean $\pm$ SD), 24 h following pilocarpine-injection (400 mg/kg) in rats

Rat group	Hippocampal Zinc (μ g/g wet tissue)	Hippocampal SOD (U/mg protein)	Hippocampal IL-1 β (ng/g wet tissue)	Hippocampal caspase-3 (μ g/g protein/min)	Serum NSE (ng/mL)
Group I: Normal Control, n = 10	16.37 $\pm$ 1.45	6.51 $\pm$ 0.94	0.20 $\pm$ 0.01	2.42 $\pm$ 0.06	6.31 $\pm$ 0.54
Group II: medium-dose Zn (3 mg/kg bwt) pretreated saline injected rats, n = 10	20.27 $\pm$ 2.14 ^a	7.01 $\pm$ 1.05	0.24 $\pm$ 0.01	2.18 $\pm$ 0.05	5.89 $\pm$ 0.84
Group III: high-dose Zn (60 mg/kg bwt) pretreated saline-injected rats, n = 9	29.84 $\pm$ 2.65 ^a	4.48 $\pm$ 0.46 ^a	1.15 $\pm$ 0.13 ^a	4.16 $\pm$ 0.03 ^a	10.58 $\pm$ 1.87 ^a
Group IV: valproic acid (100 mg/kg bwt) pretreated saline-injected rats, n = 10	10.55 $\pm$ 1.27 ^a	5.98 $\pm$ 0.16	0.30 $\pm$ 0.02	2.42 $\pm$ 0.12	6.09 $\pm$ 0.41
Group V: medium-dose Zn (3 mg/kg bwt) & valproic acid (100 mg/kg bwt) pretreated saline-injected rats, n = 10	15.81 $\pm$ 2.31	6.13 $\pm$ 0.56	0.23 $\pm$ 0.01	2.89 $\pm$ 0.24	5.91 $\pm$ 0.61
Group VI: pilocarpine-injected rats, n = 7	11.12 $\pm$ 1.56 ^a	3.97 $\pm$ 0.54 ^a	2.45 $\pm$ 0.08 ^a	17.65 $\pm$ 1.09 ^a	24.32 $\pm$ 2.56 ^a
Group VII: medium-dose Zn pretreated (3 mg/kg bwt) pilocarpine-injected rats, n = 9	17.27 $\pm$ 2.14 ^b	4.43 $\pm$ 0.42 ^b	1.63 $\pm$ 0.04 ^b	11.65 $\pm$ 1.76 ^b	18.14 $\pm$ 1.89 ^b
Group VIII: high-dose Zn (60 mg/kg bwt) pretreated pilocarpine-injected rats, n = 7	27.12 $\pm$ 2.32 ^{a,b}	2.38 $\pm$ 0.12 ^{a,b}	4.16 $\pm$ 0.03 ^{a,b}	23.65 $\pm$ 2.32 ^{a,b}	28.84 $\pm$ 4.29 ^{a,b}
Group IX: valproic acid (100 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	12.42 $\pm$ 2.54	5.98 $\pm$ 0.14 ^b	1.01 $\pm$ 0.03 ^b	9.12 $\pm$ 0.89 ^b	14.21 $\pm$ 1.01 ^b
Group X: medium-dose Zn (3 mg/kg bwt) & valproic acid (100 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	15.15 $\pm$ 3.67 ^{b,c}	6.26 $\pm$ 0.18 ^{b,c}	0.87 $\pm$ 0.02 ^{b,c}	7.02 $\pm$ 0.53 ^{b,c}	11.31 $\pm$ 2.01 ^{b,c}
Group XI: medium-dose Zn (3 mg/kg bwt) & subeffective valproic acid (54 mg/kg bwt) pretreated pilocarpine-injected rats, n = 10	16.09 $\pm$ 2.54 ^{b,c}	5.03 $\pm$ 0.14 ^b	1.65 $\pm$ 0.04 ^b	10.32 $\pm$ 1.29 ^b	16.27 $\pm$ 1.65 ^b
F test	64.98	27.16	52.16	40.15	57.18
P value	0.001	0.001	0.001	0.001	0.001

^aSignificant compared to control group (I)^bSignificant compared to non-treated pilocarpine-injected group.^cSignificant compared to valproic acid-treated rats.

n, number of rats in each group.

therapeutic dose of valproic acid which denotes a synergistic antiepileptic effect between Zn and valproic acid.

However, various studies considering the role of Zn in epilepsy, demonstrated too many contradictions. Some studies have shown that Zn is neuroprotective whereas others demonstrated its neurotoxic action. It has been suggested that Zn dyshomeostasis is detrimental to neurons and that Zn might be neurotoxic or neuroprotective depending on its concentration [16,35,44].

This reported dose-dependent effect of Zn has been confirmed in the current study where a medium dose of Zn resulted in a significant protective effect against pilocarpine-induced seizures, whereas, high dose augmented pilocarpine-induced effects.

These observed protective effects of medium dose of Zn had been proved by other studies which suggested various neuroprotective mechanisms for Zn [45,46]. In the brain, the highest amount of Zn is in the mossy fiber system in the hippocampus where it has been suggested to modulate synaptic transmission [47]. Zn released from these fibers may be a negative-feedback factor against presynaptic activity during tetanic stimulation [48]. Moreover, by activating the presynaptic ATP-sensitive potassium

channels, Zn can protect neurons from hyperexcitation, excessive transmitter release, and excitotoxicity. Thus, Zn may act as an endogenous neuroprotective in conditions such as epilepsy [49]. Indeed, Ni *et al.* found that the long-term adverse effects of recurrent neonatal seizures on cognition might be associated with downregulation of Zn transporter 1 in the hippocampus [27].

As Zn functions as a signaling molecule in the nervous system and modulates many ionic channels, it has been reported to exhibit differential modulatory effects on T-type calcium channels. This may partly explain the complex features of Zn modulation of the neuronal excitability in normal and disease states [50]. It is likely that abnormal calcium mobilization in neurons is involved in seizure susceptibility in Zn-deficient animals [51].

The demonstrated attenuation of apoptosis, oxidative stress as well as IL-1 β concentration in medium dose Zn-treated pilocarpine-injected rats, could account, in part, for the Zn neuroprotective effect observed in the current study. These findings are in line with previous studies that reported a similar decrease in caspases-3 with Zn supplementation [3]. Indeed, it has been reported that increased neuronal apoptosis and lowered seizure

threshold especially in hippocampus, the epileptic focus in human TLE, occurs more easily in Zn deficiency [44].

Other studies demonstrated that Zn reduces the activities of IL-1 receptor-associated kinase and reported that the potent immunomodulatory effects of zinc are via the modulation of cytokine signaling [3,52]. Zn, in a medium dose, might also decrease IL-1 β through its reported ability to block glutamate receptors since it has been suggested that the induction of IL-1 β in epilepsy is mainly indirect through the release of endogenous glutamate [53].

Studies indicate that Zn acts as a potent antioxidant by scavenging ROS, possibly elevating tissue antioxidant enzyme activities [54,55], which is consistent with our findings, in which hippocampal SOD was significantly higher after medium dose Zn administration compared to nontreated pilocarpine injected rats.

On the other hand, high dose Zn-induced oxidative stress and apoptosis might explain, in part, the proconvulsive effect of this dose of Zn observed in the current study. These results indicate that high-dose Zn is neurotoxic and indeed exacerbates pilocarpine-induced seizures. Our results are emphasized in the context of conflicting results for Zn in the literature where some studies demonstrate anticonvulsive effect of Zn [17,46], whereas others demonstrate proconvulsive effect [56].

Several additional mechanisms have been suggested that could account for high dose Zn-induced proconvulsive potential, including inhibition of glutamic acid decarboxylase [56]. Another speculation that might be put to clarify the dose dependent effect of Zn in epilepsy is that medium Zn concentration might inhibit whereas, high dose might stimulate NMDA glutaminergic receptors.

In conclusion, the present study supports the notion that the trace element Zn, in appropriate doses, plays an important role in mitigating seizures by suppression of oxidative stress, apoptotic activity, and IL-1 β levels, resulting in neuroprotective effects. Furthermore, since medium-dose Zn exerted a synergistic effect with subeffective dose of valproic acid and normalized the decrease in hippocampal Zn concentration associated with valproic acid, thus it would be interesting to further investigate the add-on effect of zinc supplementation in valproic acid as well as other AED treatment of epilepsy especially in the light of the reported potential zinc-depleting effects of these drugs.

Conflict of Interest

The authors have no conflict of interest.

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