Available on line at : www.eijppr.com

International Journal of Pharmaceutical and Phytopharmacological Research

(ICV-5.09)

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2013, 2(4): 272-276

(Research Article)

Beneficial Role of Selenium Supplementation Against Experimental Seizures in Rats

Dhammdeep C. Dabhade*, Waseem Rizvi, Anil Kumar

Department of Pharmacology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh - 202002, Uttar Pradesh, India

Received on: 02/02/2013 ***Accepted on:*** 21/02/2013

ABSTRACT

This study was designed to evaluate the antiseizure effect of Sodium selenite in the animal models of maximal electroshock seizures (MES) and pentylenetetrazole (PTZ) induced seizures. Healthy, male albino wistar rats were given sodium selenite supplementation intraperitonially in various doses (100, 200, 300 and 400 µg/Kg/day) for 1 week (day 1 to day 7). On day 0 and day 8, response to MES (150 mA for 0.2 s) was tested. Similarly, in the other groups, the response to PTZ 60 mg/kg i.p. was tested. Sodium selenite has shown protective effect at 200, 300 and 400 µg/Kg doses against MES and at 200 µg/Kg and 300 µg/Kg doses against PTZ induced seizures in rats. Present study reveals that sodium selenite has antiseizure effect when tested against animal models of MES and PTZ-induced seizures.

Key Words: Selenium, Epilepsy, Maximal electroshock seizures (MES), Pentylenetetrazole (PTZ), Electroconvulsiometer .

INTRODUCTION

An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain[1]. It is one of the oldest neurological conditions known to mankind and the most frequent neurological condition after stroke [2]. Around 50 million people worldwide have epilepsy. Close to 80% of epilepsy cases worldwide are found in developing regions [3]. Epilepsy is usually controlled, but not cured, with medication. However, over 30 - 40% of people with epilepsy do not have seizure control even with the best available medications[4, 5]. This limitation invites us to study a newer agent that would overcome this problem.

Various experimental and human studies suggested that the homeostasis of trace elements, membrane lipid peroxidation and antioxidants are crucial for brain function, and they were directly or indirectly implicated as taking part in the pathophysiology of neuronal excitotoxicity and seizure recurrence and its resistance to treatment with antiepileptic drugs (AEDs)[6]. Some clinical reports have demonstrated a direct link between blood selenium levels and the neurological condition of intractable seizures and resistant epilepsy[7,8, 9,10]. In neuronal cell culture, addition of Selenium in the form of selenite within a physiological range protects against exctiotoxic insults and even attenuates primary damage [11]. Selenium is well established as an essential trace element of fundamental importance to human health, is known primarily for its antioxidant activity and in therapeutic aspects for its chemopreventive, antiinflammatory and antiviral properties [12]. Trace amounts of the element are necessary for cellular functions in animals, having a virtue of forming the active center of the enzymes glutathione peroxidase and thioredoxin reductase (which indirectly reduce certain oxidized molecules in the living cells) [13,14]. Various experimental studies reported that treatment with selenium has antiseizure effect in rodent models of chemically induced seizures and may represent a novel approach to treatment of patients with epilepsy[15,16,11,17,13,18,19]. As per our thorough review of literature probably there is no previous study to report the antiseizure effect of selenium supplementation against maximal electroshock seizures in rats. Present study was planned to evaluate the antiseizure effect of sodium selenite in the animal models of maximal electroshock (MES) and pentylenetetrazole (PTZ) induced seizures.

MATERIALS AND METHODS

Animals

Healthy, male, adult albino wistar rats weighing 100 – 250 g were used for the study. The animals used were procured from the central animal house facility, Jawaharlal Nehru Medical College, AMU Aligarh. They were housed in plastic cages in the Pharmacology section of Central Animal House with free access to food and tap water. The animal room was well ventilated and maintained under standard conditions throughout the experimental period. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), Jawaharlal Nehru Medical College, AMU Aligarh. All the animal experiments were carried out

as per the rules and regulations of IAEC and CPCSEA (committee for the purpose of control and supervision on experiments on animals).

Chemicals and Instruments

Sodium selenite powder (Sigma Aldrich, USA), Pentylenetetrazole powder (Sigma Aldrich, USA), Electroconvulsiometer (Orchid scientifics Ltd. Nasik, India).

Experimental Design

After 7 days of adaptation to the laboratory conditions, the animals were divided into six experimental groups consisting of six rats per group. Group I, II, III and IV were the MES groups in which Sodium selenite 100 µg/Kg i.p, 200 µg/Kg i.p., 300 µg/Kg i.p. and 400 µg/Kg i.p. was administered respectively for a period of 7 days. Group V and VI were PTZ groups in which Sodium selenite 200 µg/Kg i.p. and 300 µg/Kg i.p. was administered respectively for a period of 7 days. Sodium selenite was dissolved in distilled water and injected intraperitonially (i.p.) in all the groups in a volume of 0.2 ml/100 g body weight. In each group seizure induction was done on a day before (day 0) and the day after (day 8) Sodium selenite administration. Each animal was its own control in all the groups. All the tests were performed between 9:00 and 15:00 hr.

Maximal Electroshock Seizures (MES)

This test was done by a modification of the method described by Castel-Branco et al., 2009 [20]. In each animal electroshock stimulation was applied through transauricular electrodes from an electroconvulsiometer at an intensity 150 mA fixed current, a 50-60–Hz pulse frequency for 0.2 sec duration. Parameters noted in each animal were: Onset of seizures, duration of limb flexion, duration of tonic hind limb extension (THLE), duration of clonic phase and total duration of seizures.

Pentylenetetrazole(PTZ) Induced Seizures

PTZ was prepared freshly dissolving in normal saline and administered in a dose of 60 mg/Kg i.p. This dose of PTZ has been standardized as 100% convulsant dose with minimal mortality in rats [21]. Each animal was administered with PTZ in a volume of 0.2 ml/100 g body weight i.p. and was observed for 30 min. Parameters noted in each animal were: Onset of myoclonic jerks, duration of myoclonic jerks, duration of clonic phase, and total duration of seizures.

Statistical Analysis

The data were analyzed with the SPSS 17.0 (Statistical Package for Social Sciences) software package. The paired Students *t*-test was used to compare the data obtained on day 0 and on day 8 in all the groups. Data expressed as mean ± Standard error of mean (SEM). A value of $P<0.05$ was considered to be statistically significant.

RESULTS

Results of Maximal Electroshock Seizures

Onset of seizures was delayed significantly at doses 200 and 300 µg/Kg ($P<0.05$). Duration of THLE and tonic limb flexion was reduced significantly at doses 200, 300 and 400 µg/Kg ($P<0.05$). Duration of clonic phase was reduced significantly at doses 100, 300 and 400 µg/Kg and there was significant reduction in the total duration of seizures at all the doses (Table-1).

Table-1: Effect of Sodium selenite Against Maximal Electroshock Seizures in Rats

Parameter	**Days**	**Group** I (SS 100 µg/Kg, n=6)	**Group** II (SS 200 µg/Kg, n=6)	**Group** III (SS 300 µg/Kg, n=6)	**Group** IV (SS 400 µg/Kg, n=6)
Onset of seizures (sec)	Day 0 Day 8	0.68 ± 0.15 0.62 ± 0.09	0.59 ± 0.03 0.84 ± 0.08*	0.73 ± 0.03 1.20 ± 0.16*	0.68 ± 0.02 1.02 ± 0.13
Duration of TLF (sec)	Day 0 Day 8	4.81 ± 1.14 1.98 ± 0.22	4.12 ± 0.62 1.80 ± 0.29*	6.64 ± 1.10 4.15 ± 1.89*	4.23 ± 1.73 2.69 ± 1.58*
Duration of THLE (sec)	Day 0 Day 8	7.30 ± 1.10 6.13 ± 1.66	10.69 ± 0.32 7.62 ± 0.98*	9.47 ± 1.25 5.56 ± 1.87*	10.04 ± 1.80 5.55 ± 2.05*
Duration of clonic phase (sec)	Day 0 Day 8	30.02 ± 5.26 20.61 ± 1.22*	18.76 ± 1.70 17.12 ± 1.26	19.24 ± 1.31 16.83 ± 1.43**	21.42 ± 2.51 16.12 ±2.27*
Total duration of seizures (sec)	Day 0 Day 8	42.33 ± 4.41 29.26± 2.91**	33.41 ± 1.46 26.57 ± 1.52*	35.14 ± 1.42 28.54 ± 1.09*	35.70 ± 3.27 24.37 ± 2.57**

*Values indicate mean ± SEM, *P<0.05, ** P<0.01, Day 8 vs day 0: Sodium selenite (SS) given from day 1 to 7, THLE→ Tonic hind limb extension, TLF→ Tonic limb flexion.*

Results of PTZ Induced Seizures

There was delayed onset of myoclonic jerks at 200 µg/Kg dose ($P<0.05$) (table 2) and there was significant reduction in the duration of myoclonic jerks, clonic phase and total duration of seizures ($P<0.05$) in doses of 200 and 300 µg/Kg, however with 200 µg/Kg dose this reduction was highly significant ($P<0.01$).

Table 2: Effect of Sodium selenite Against PTZ Induced Seizures in Rats

Parameter (sec)	Days	Group V (SS 200 µg/Kg, n=6)	Group VI (SS 300 µg/Kg, n=6)
Onset of myoclonic jerks	Day 0	40.70 ± 6.45	34.41±.7.52
	Day 8	51.11 ± 4.01*	23.35 ± 7.64
Duration of myoclonic jerks	Day 0	5.97 ± 0.78	5.12 ± 0.60
	Day 8	2.37 ± 0.81**	2.06 ± 0.61*
Duration of clonic phase	Day 0	121.80 ±. 32.71	93.33 ± 17.17
	Day 8	44.83 ± 16.57**	28.39 ± 14.15*
Total duration of seizures	Day 0	127.78 ± 32.37	98.45 ± 17.36
	Day 8	47.21 ± 16.73**	30.46 ± 14.53*

*Values indicate mean ± SEM, *p<0.05, ** P<0.01, Day 8 vs day 0:Sodium selenite(SS) given from day 1 to 7.*

DISCUSSION

In the present study selenium supplementation in the form of sodium selenite appears to exert protective effect in the animal models of maximal electroshock seizures (MES) and pentylenetetrazole (PTZ) induced seizures.

The trace element Selenium (Se) plays a critical role in the maintenance of proper functioning of the nervous system. Se is a potent protective agent for neurons through the expression of selenoproteins, which are mostly involved in regulation of redox status under physiological conditions and in antioxidant defense and Se is involved in protection of astroglial and neuronal cells against oxidative stress [22]. Selenium linked protection has been ascribed to the involvement of selenoenzymes like glutathione peroxidase (GPx) and phospholipid hydroperoxide GPx (GPx4). GPx is a family capable of eliminating peroxides in the neurons by reducing them to H_2O or alcohols, with Glutathione as reducing substrate[23,24,25]. Thus, in the form of GPx selenoenzymes, Se is involved in the protection of neuronal cells in a diverse manner [26]. Selenium can reduce oxidative stress and generation of reactive oxygen species which is an important cause and consequence of epileptic seizures [27,17]. Experimental studies have shown that a single convulsive dose of PTZ resulted insignificant changes in many parameters such as whole brain hydroxyl radicals (except cerebellum) [28] and GPx activity in specific brain areas [29]. PTZ-induced reductions in antioxidant content were observed in rat brain homogenates [30]. Maximal electroshock induced changes in some markers of oxidative stress in mice were reported by Nieoczym et al., 2008 [31]. Reeta et al.,2011[32] reported that PTZ and MES caused an oxidative stress probably due to free radical generation. The observed protective effect in the present study is more possibly due to the antioxidant property of selenium against the oxidative stress associated with the PTZ as well as MES seizure models.

In the previous studies[17,13] (Naziroglu et al.,2008 and Kutluhan et al., 2009), Selenium as Sodium selenite in the dose of 300µg/Kg i.p. for 7 days has shown antiseizure effect in PTZ-induced seizures in rats. Similar effect was observed in the present study at 300 µg/Kg dose of sodium selenite against PTZ as well as MES induced seizures. In addition, in present study selenium has shown antiseizure effect at lower dose i.e. 200 µg/kg. The doses of sodium selenite (200 and 300 µg/Kg) were considered effective doses as there was significant reduction in THLE (Table 1) along with delayed onset of seizures (Table 1 and 2). Observed protective effect of Selenium in PTZ induced seizures is supported by some of the previously reported studies [17,13,18]. Willmore, 1981 [15] has reported anticonvulsant activity of Selenium against iron induced seizures in rats and Ethel et al., 2012 [19] against pilocarpine induced seizures in rats. As per our thorough review of literature antiseizure effect of selenium against MES has been reported first time in our study.

Selenium has neuroprotective activity against brain insults [33,34,35] which can lead to its antiseizure effect, as a strong co-relation between neuroprotective activity and antiseizure effect of a agent is reported by Stepienket al., 2005 [36]. Observed antiseizure effect with 200 µg/kg dose of sodium selenite can be possibly due its neuroprotective activity at 200 µg/kg given for 7 days as reported by Ansari et al., 2004[33] and Mehta et al., 2012 [37].

Further studies are required to validate the results in the present study and to evaluate the effect of selenium supplementation on the activity of standard antiepileptic drugs.

Our findings in the present study propose a crucial role for the trace element Selenium in epilepsy and mandate further research on the place for selenium supplements in epilepsy therapeutic regimens.

CONCLUSION

Selenium supplementation in the form of Sodium selenite appears to exert protective effect in the animal models of maximal electroshock seizures (MES) and pentylenetetrazole (PTZ) induced seizures. This suggests potential utility of Sodium selenite supplementation in the management of generalized tonic-clonic seizures and absence seizures.

ACKNOWLEDGEMENT

The authors are grateful to Sigma Aldrich, USA for providing Sodium selenite and Pentylenetetrazole for this study.

REFERENCES

1) Fisher RS, van Emde Boas W, et al "Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE)" *Epilepsia*, 2005,46(4):470-2.
2) Strine TW, Kobau R, et al "Psychological distress, comorbidities, and health behaviors among U.S. adults with seizures: results from the 2002 National Health Interview Survey" *Epilepsia*, 2005, 46:1133–1139.
3) World health organization (WHO) epilepsy fact sheet [Internet]. 2012 Oct [cited 2012 Oct 30]. Available

from:http://www.who.int/mediacentre/ factsheets/fs999/en/

4) Cascino GD, "Epilepsy: contemporary perspectives on evaluation and treatment" *Mayo Clin Proc*, 1994, 69(12):1199-211.
5) Bialer M, White HS, "Key factors in the discovery and development of new antiepileptic drugs" *Nat Rev Drug Discov*, 2010, 9:68–82.
6) Hamed SA, AbdellahMM, "Trace elements and electrolytes homeostasis and their relation to antioxidant enzyme activity in brain hyperexcitability of epileptic patients" *J Pharmacol Sci*, 2004, 96(4):349-59.
7) Muth OH, Weswig PH, et al "Effect of feeding selenium-deficient ration to the subhuman primate" *Am J Vet Res*, 1971, 32:1603–1605.
8) Weber GF, Maertens P, et al "Glutathione peroxidase deficiency and childhood seizures" *Lancet*, 1991, 337:1443–4.15.
9) Ramaekers VT, Calomme M, et al "Selenium deficiency triggering intractable seizures" *Neuropediatrics*, 1994, 25(4):217-223.
10) Seven M, Basaran SY, et al "Deficiency of selenium and zinc as a causative factor for idiopathic intractable epilepsy" *Epilepsy Res*, 2012 Oct 25, [Epub ahead of print].
11) Savaskan NE, Bräuer AU, et al "Selenium deficiency increases susceptibility to glutamate-induced excitotoxicity" *FASEB J*, 2003, 17(1):112-4.
12) Rayman MP. The importance of selenium to human health.Lancet, 2000, 356: 233–241.
13) Kutluhan S, Naziroglu M, et al "Effects of selenium and topiramate on lipid peroxidation and antioxidant vitamin levels in blood of pentylentetrazol-induced epileptic rats" *Biol Trace Elem Res*, 2009, 129(1-3):181-189.
14) Ozdemir E, Cetinkaya S, et al "Serum selenium and plasma malondialdehyde levels and antioxidant enzyme activities in patients with obsessive-compulsive disorders" *Prog Neuropsycho pharmacol Biol Psychiatry*, 2009, 33:62–5.
15) Willmore LJ, Rubin JJ, "Antiperoxidant pretreatment and iron-induced epileptiform discharges in the rat: EEG and histopathologic studies" *Neurology*,1981,31(1):63-9.
16) Oztas B, Kilic S, et al "Influence of antioxidants on the blood–brain barrier permeability during epileptic seizures" *J Neurosci Res*, 2001,66:674–678.
17) Naziroglu M, Kutluhan S, et al "Selenium and topiramate modulates brain microsomal oxidative stress values, Ca2+-ATPase activity, and EEG records in pentylentetrazol-induced seizures in rats" *J Membr Biol*, 2008, 225(1-3):39-49.
18) Rehni AK, Singh TG, "Selenium induced anticonvulsant effect: A potential role of prostaglandin E1 receptor activation linked mechanism" *J Trace Elem Med Biol*, 2013, 27(1):31-9
19) Ethel AW, Bibiana MG, et al "Involvement of GABAergic and glutamatergic systems in the anticonvulsant activity of 3-alkynyl selenophene in 21 day-old rats" *Mol Cell Biochem*, 2012, 365:175–180.
20) Castel-Branco MM, Alves GL, et al "The maximal electroshock seizure (MES) model in the preclinical assessment of potential new antiepileptic drugs" *Methods Find Exp Clin Pharmacol*, 2009,31(2):101-6.
21) Malhotra J, Gupta YK, "Effect of adenosine receptors modulation on pentylenetetrazole induced seizures in rats" *Br J Pharmacol*,1997,120:282–8.
22) Behne D, Hilmert H, et al "Evidence for specific selenocysteine target tissues and new biologically important selenoproteins" *Biochem Biophys Acta*,1988,966:12-21.
23) Al Deeb S, Al Moutaery K, et al "Neuroprotective effect of selenium on iminodipropionitrile-induced toxicity" *J Psychiatry Neurosci*, 1995, 20:189–92.
24) Imam SZ, Newport GD, et al "Selenium, an antioxidant, protects against methamphetamine-induced dopaminergic neurotoxicity" *Brain Res*, 1999,818:575–8.
25) Atif F, Yousuf S, et al "Restraint stress-induced oxidative damage and its amelioration with selenium" *Eur J Pharmacol*, 2008, 600:59–63.
26) Rhee SG, Chae HZ, Kim K, "Peroxiredoxns: a historical overview and speculative preview in cell signalling" *Free Radic Biol Med*, 2005, 38:1543–52.
27) Lores AS, Travacio M, et al "Regional vulnerability to oxidative stress in a model of experimental epilepsy" *Neurochem Res*, 1998, 23(12):1477-83.
28) Rauca C, Zerbe R, Jantze H, "Formation of free hydroxyl radicals after pentylenetetrazol-induced seizure and kindling" *Brain Res*, 1999, 847:347–351.
29) Erakovic V, Zupan G, et al "Pentylenetetrazol-induced seizures and kindling: changes in free fatty acids, superoxide dismutase, and glutathione peroxidase activity" *Neurochem Int*, 2003, 42:173–178.
30) Rauca C, Wiswedel I, et al "The role of superoxide dismutase and alpha-tocopherol in the development of seizures and kindling induced by pentylenetetrazol - influence of the radical scavenger alpha-phenyl-N-tert-butyl nitrone" *Brain Res*,2004,1009(1-2):203-12.
31) Nieoczym D, Albera E, et al "Maximal electroshock induces changes in some markers of oxidative stress in mice" *J Neural Transm*, 2008, 115(1):19-25.
32) Reeta KH, Mehla J, et al "Pharmacokinetic and pharmacodynamic interactions of valproate, phenytoin, phenobarbitone and carbamazepine with curcumin in experimental models of epilepsy in rats" *Pharmacol Biochem Behav*, 2011, 99(3):399-407.
33) Ansari MA, Ahmad AS, et al "Selenium protects cerebral ischemia in rat brain mitochondria" *Biol Trace Elem Res*, 2004, 101(1):73-86.
34) Ishrat T, Parveen K, et al "Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type" *Brain Res*, 2009, 1281:117-127.
35) Ahmad A, Khan MM, et al "Synergistic effect of selenium and melatonin on neuroprotection in cerebral ischemia in rats" *Biol Trace Elem Res*, 2011, 139(1):81-96.
36) Stepień K, Tomaszewski M, Czuczwar SJ, "Profile of anticonvulsant activity and neuroprotective effects of novel and potential antiepileptic drugs--an update" *Pharmacol Rep*, 2005,57(6):719-33.
37) Mehta SL, Kumari S, et al "Selenium preserves mitochondrial function, stimulates mitochondrial

biogenesis and reduces infarct volume after focal cerebral ischemia" *BMC Neurosci*, 2012, 13:79.

Corresponding Author:
Dr. Dhammdeep C. Dabhade,
Department of Pharmacology
Jawaharlal Nehru Medical College,
Aligarh Muslim University,
Aligarh - 202002, Uttar Pradesh, India
Email : drdhammadeep@gmail.com
Mobile No.: +91-7895226395