Int J Clin Exp Med 2019;12(10):12370-12376
www.ijcem.com /ISSN:1940-5901/IJCEM0098994

Original Article

SCN2A expression in the cerebral cortexes of patients with temporal lobe epilepsy and correlation levels with oxidative stress

Yinxu Wang[1*], Qingrong Ouyang[2*], Shunxian Wang[3], Qing Wu[1], Jiameng Jia[1]

[1]Department of Rehabilitation Medicine, Affiliated Hospital of North Sichuan Medical College, Nanchong City, Sichuan Province, China; [2]Department of Neurology, Suining Central Hospital, Suining City, Sichuan Province, China; [3]Department of Neurology, Affiliated Hospital of North Sichuan Medical College, Nanchong City, Sichuan Province, China. [*]Equal contributors.

Received June 27, 2019; Accepted September 3, 2019; Epub October 15, 2019; Published October 30, 2019

Abstract: Epilepsy is a chronic neurological disease caused by excessive synchronized discharging of brain neurons. Epilepsy has been associated with a variety of voltage-gated sodium channel gene mutations, in which type II sodium channel (SCN2A) gene expression plays an important role in maintaining central nervous system function. Cell discharges increase free radicals, leading to induce epilepsy. The current study investigated the relationship between SCN2A expression and oxidative stress in the cortexes of patients with temporal lobe epilepsy. A total of 30 cases of temporal lobe epilepsy were selected, including 15 cases of primary and 15 cases of secondary epilepsy. Moreover, 30 cases of normal brain tissues were used as controls. SCN2A expression was tested via RT-PCR and Western blotting. Neuron-specific enolase (NSE) and 8-hydroxydeoxyguanosine monophosphate (8-OHdG) were determined by ELISA. Malondialdehyde (MDA) and Copper-zinc superoxide dismutase (Cu/Zn SOD) levels were assessed with spectrophotometry. SCN2A protein and mRNA expression levels were significantly reduced, while Cu/Zn SOD activity was obviously decreased. Additionally, NSE, 8-OHdG, and MDA levels were markedly enhanced in the epilepsy group, compared with controls ($P < 0.05$). SCN2A protein expression was positively correlated with Cu/Zn SOD activity ($r = 0.76$, $P < 0.05$) and negatively correlated with 8-OHdG and MDA ($r = -0.68, -0.62$, $P < 0.05$). SCN2A expression in cortical tissues of patients with temporal lobe epilepsy showed a potential relationship with oxidative stress, mediating the development of temporal lobe epilepsy.

Keywords: Temporal lobe epilepsy, SCN2A, oxidative stress, correlation

Introduction

Epilepsy is a common chronic nervous system disease caused by excessive synchronized discharging of brain neurons [1, 2]. This disease can be seen at all ages. Temporal lobe epilepsy is the most common type. Epilepticus is the most serious complication. Most patients experiencing epileptic seizures can be controlled after antiepileptic treatment. However, some cases may develop into intractable epilepsy [3]. Seizure-induced brain injuries are the major cause of frequent and refractory epilepsy. The specific index of the degree of brain injuries caused by seizures plays an important role in assessing the extent of epilepsy [4]. At present, the mechanisms of seizures have not been fully elucidated. Studies have found that occurrence of epilepsy is related to a variety of voltage-gated sodium channel encoding gene mutations [5, 6]. The phenotypes of epileptic syndrome caused by different gene mutations are variant. Voltage-dependent sodium channel plays a crucial role in the production and propagation of action potentials in neurons. Expression of type I and type II sodium channels (SCN1A, SCN2A) plays a key role in maintaining central nervous system function. Mutant SCN2A is related to epilepsy. Previous reports have shown that inhibitory interneuron sodium currents are significantly decreased in SCN2A knockout mice, while there is no statistical change in excitatory vertebral neuron sodium currents [7, 8]. Cell discharge leads to an increase in superoxide radicals and hydroxyl radicals, inhibition of glutamate synthase (GOGAT), and an increase of

excitatory neurotransmitter glutamate levels, inducing epilepsy. Oxidative stress is closely related to neuronal apoptosis and necrosis in an epileptic state. Neuronal cells activate reactive oxygen species (ROS) and nitric oxide (NO) during ischemia and hypoxia, inducing mitochondrial apoptosis pathways. Mitochondrial dysfunction, oxidative stress damage, and increased free radicals are associated with each other in the pathological process of epilepsy. Brain tissue is susceptible to ROS damage, due to various factors, including high oxidative metabolic rates and low antioxidant levels [9, 10]. Oxidative stress may play an important role in epilepsy. Under oxidative stress, ROS activates apoptosis-related gene c-myc to bind to Max to form dimer. It then binds DNA core sequence through NF-κB, inducing neuronal apoptosis [11, 12]. The degree of oxidation in the body exceeding antioxidant function may aggravate brain damage. MDA levels can reflect the degree of lipid peroxidation and cell damage. SOD is the primary substance that scavenges free radicals and blocks damage of oxygen free radicals in cells [12]. The present study investigated the relationship between SCN2A expression and oxidative stress in the cortexes of patients with temporal lobe epilepsy.

Materials and methods

Materials

Objects: Thirty specimens from patients with temporal lobe epilepsy were selected from November 2016 to 2018. The specimens were obtained from patients that underwent surgical resections, with well preservation and complete follow-up data. All cases were confirmed by pathology. Thirty cases of temporal lobe epilepsy were selected, including 15 cases of primary epilepsy and 15 cases of simple hippocampal sclerosis and secondary epilepsy caused by cavernous hemangiomas, temporal lobe tumors, and arteriovenous malformation. Another 30 cases of normal brain tissues (cranial traumatic anterior sacral resections and decompression) were enrolled as controls. This study was approved by the Ethics Committee and informed consent was obtained from all participants prior to the study.

Main reagents and instruments: MDA, SOD, NSE, and 8-OHdG kits (Nanjing Institute of Bioengineering); TRIzol RNA extraction reagent (Invitrogen, USA); RNase inhibitor and reverse transcription kit (Fermentas, Canada); Horseradish peroxidase-labeled goat anti-rabbit secondary antibody (CST, USA); Primer premier 5.0 designed the SCN2A primer (Invitrogen): Forward: 5'-TTCATTGGATGGGAATGGTACT-3'; Reverse: 5'-CTGTTGCCACAAAGCAGAGC-3'; Real-time PCR amplifier and analysis system (PE, USA); Inverted microscope (Zeiss, Germany); UV spectrophotometer (BioRad, USA).

Methods

SCN2A mRNA and protein expression: (1) Real-time PCR detection of mRNA expression: a) RNA primer design and synthesis; b) RNA extraction; c) Reverse transcription (RT); and d) qPCR reaction to draw a standard curve; (2) Western blot analysis of protein expression: The sample was added with RIPA lysate to extract total protein. The protein was quantified with the Bradford method. A total of 50 μg protein was separated by electrophoresis and transferred to PVDF membranes at 400 mA for 60 minutes. After blocking, the membranes were added with the corresponding monoclonal primary antibody overnight, as well as the corresponding secondary antibody. Next, the membranes were added with ECL chemiluminescence indicator and analyzed with Quantity One image analysis software (BIO-RAD). Relative intensity of target protein expression = the gray value of the target band/gray value of the β-actin band.

ELISA detection of NSE and 8-OHdG and spectrophotometry detection of MDA and Cu/Zn SOD levels: Cortical tissues were added with separation reagent A (1 mL/100 mg), according to kit instructions, preparing the homogenate and centrifuging at 1,000 g and 4°C for 5 minutes. The supernatant was centrifuged at 4°C and 3,500 g for 10 minutes. MDA and Cu/Zn SOD was measured with a spectrophotometer, according to kit instructions. NSE and 8-OHdG levels were determined according to kit instructions.

Statistical analysis

Statistical analyses were performed using SPSS 20.0 software. Measurement data were tested for normality and are presented as mean ± standard deviation. Data was compared with one-way ANOVA and least significant difference (LSD) tests. Pearson's correlation analysis was

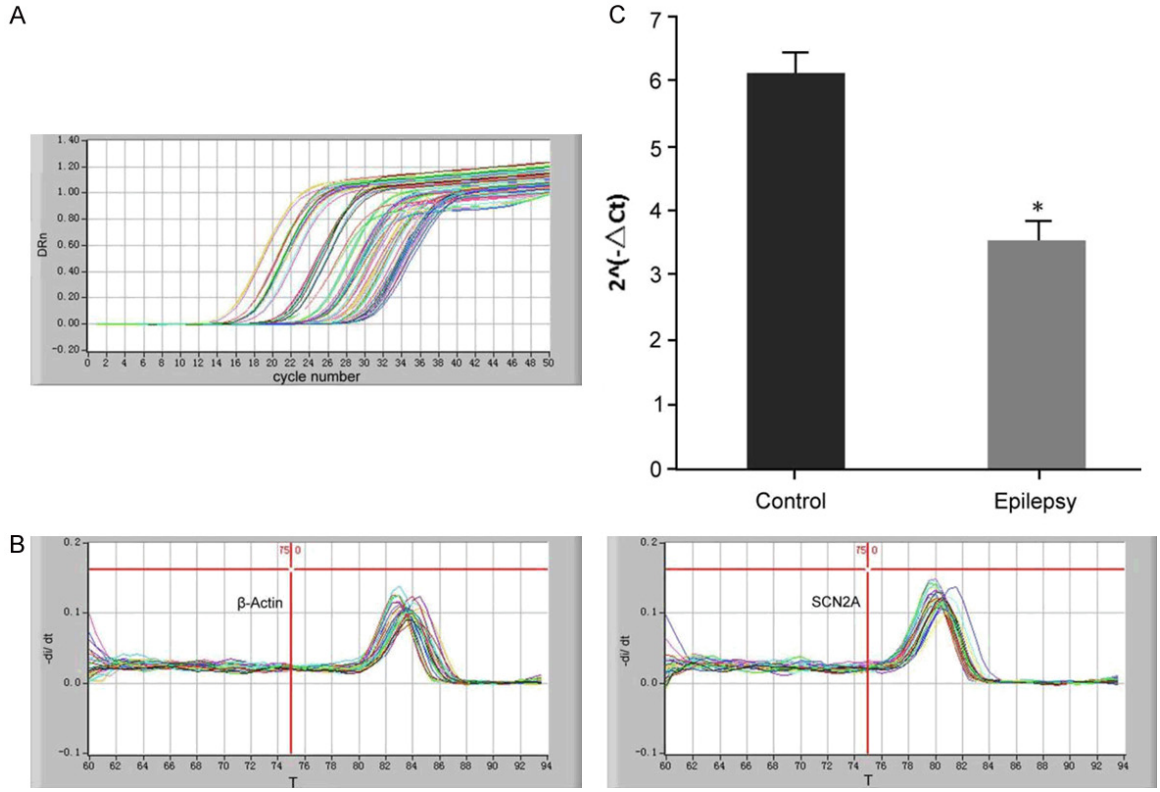

Figure 1. SCN2A mRNA expression in cortical tissues of patients with temporal lobe epilepsy. A. qPCR amplification curve; B. qPCR melting curve; C. Relative SCN2A mRNA expression. *P < 0.05, compared with controls.

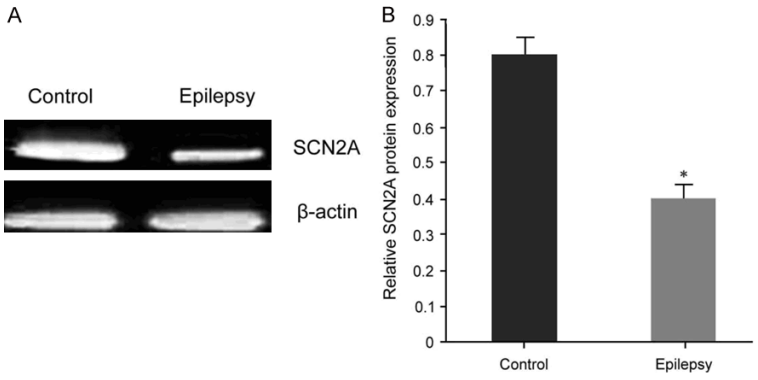

Figure 2. SCN2A protein expression in cortical tissues of patients with temporal lobe epilepsy. A. SCN2A protein expression in the cortical tissue; B. Relative SCN2A protein expression. *P < 0.05, compared with controls.

also performed. P < 0.05 indicates statistical significance.

Results

SCN2A mRNA expression in cortical tissues of patients with temporal lobe epilepsy

SCN2A mRNA expression was detected by Real-time PCR. SCN2A mRNA expression was significantly reduced in the epilepsy group, compared to the control group (P < 0.05) (**Figure 1**).

SCN2A protein expression in cortical tissues of patients with temporal lobe epilepsy

SCN2A protein expression was detected by Western blotting. SCN2A protein expression was significantly reduced in the epilepsy group, compared with the control group (P < 0.05) (**Figure 2**).

NSE, 8-OHdG, MDA, and Cu/Zn SOD levels in cortical tissues of patients with temporal lobe epilepsy

Cu/Zn SOD activity was significantly decreased and NSE, 8-OHdG, and MDA levels were significantly enhanced in the epilepsy group, compared with levels in the control group (P < 0.05) (**Figure 3A-D**). SCN2A protein expression was positively correlated with Cu/Zn SOD activity (r

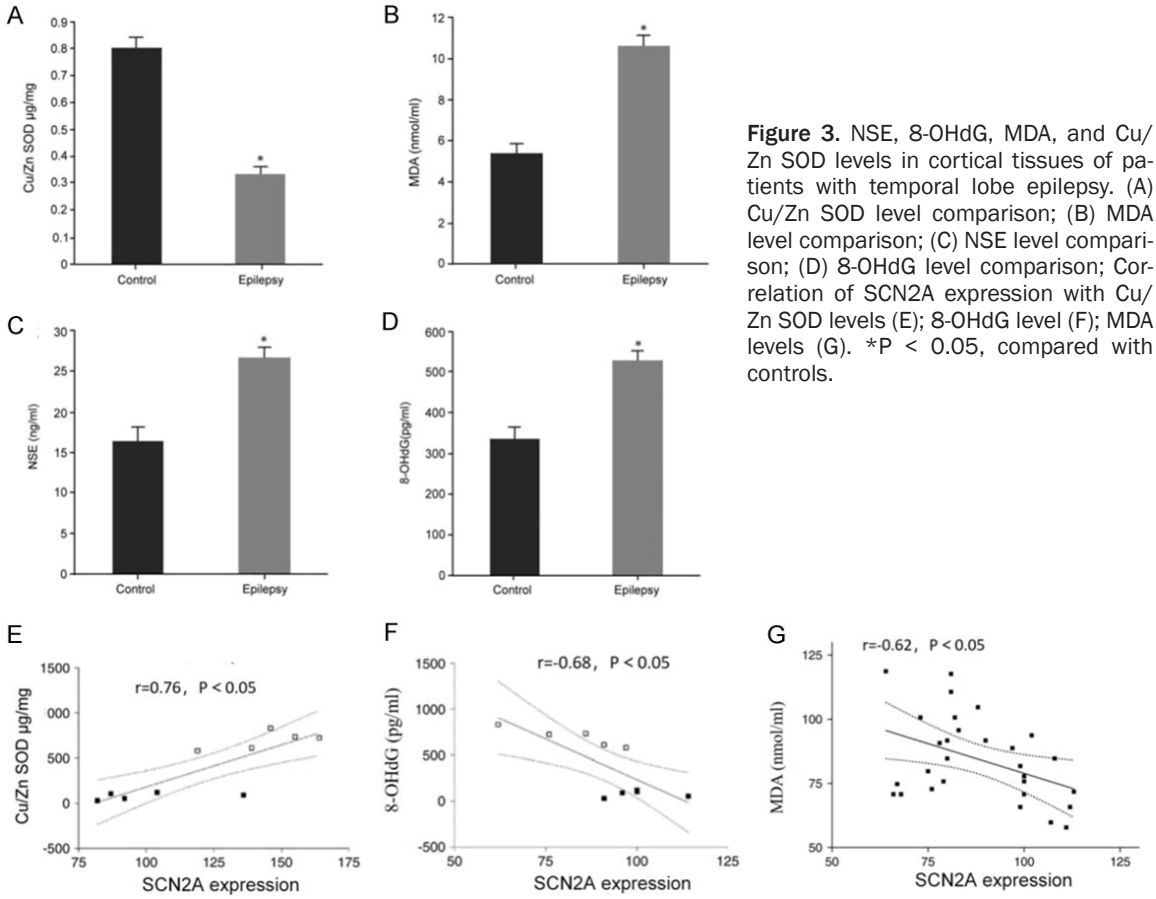

Figure 3. NSE, 8-OHdG, MDA, and Cu/Zn SOD levels in cortical tissues of patients with temporal lobe epilepsy. (A) Cu/Zn SOD level comparison; (B) MDA level comparison; (C) NSE level comparison; (D) 8-OHdG level comparison; Correlation of SCN2A expression with Cu/Zn SOD levels (E); 8-OHdG level (F); MDA levels (G). *P < 0.05, compared with controls.

= 0.76, P < 0.05) and negatively correlated with 8-OHdG and MDA (r = -0.68, -0.62, P < 0.05) (**Figure 3E-G**).

Discussion

At present, the pathological mechanisms of seizures have not been fully elucidated. In epileptic seizures, brain tissue is hypoxic and damaged due to high-intensity discharging. Under physiological conditions, oxidation and anti-oxidation functions are maintained in a dynamic balance state. This balance is disrupted during high-intensity discharges, leading to brain tissue hypoxia, increased oxidative stress levels, and aggravated brain damage. Increased free radicals may change neuronal membrane and synaptic function, affect the cell membrane structure and permeability, and induce epilepsy [13, 14]. One of the oxygen peroxide and cell membrane unsaturated fatty acid lipid peroxidation end products, MDA can reflect the degree of lipid peroxidation and cell damage. SOD is the primary substance that scavenges free radicals and blocks damage of oxygen free radicals in cells. Moreover, 8-OHdG reflects the degree of DNA oxidative damage. When DNA is attacked by oxygen free radicals, guanine is oxidized [15, 16]. SOD, MDA, and 8-OHdG are common indicators reflecting oxidation stress [14-16]. Cu/Zn SOD is a key dismutase for superoxide anion radicals. After epileptic seizures, multiple free radicals may interact with each other. However, there remains a lack of information concerning the roles of ion channel regulation on oxidative stress. The current study analyzed the relationship between SCN2A expression and oxidative stress in brain bulging tissues of patients with temporal lobe epilepsy from gene and protein levels, providing reference for the study and prevention of epilepsy.

Under physiological condition, the antioxidant system maintains a dynamic balance with ROS production. Under pathological conditions, br-

ain tissue hypoxia and other conditions attenuate antioxidant capacity and produce excessive free radicals, leading to oxidative stress damage. Hypoxia-induced oxidative stress has been associated with a variety of factors, including decreased function of the antioxidant system and elevated inflammatory factor levels, resulting in brain cell damage [17, 18]. NF-κB signaling pathways are involved in cell ischemia and hypoxia. Both *in vitro* and *in vivo* tests have indicated that NF-κB is involved in the pathological processes of cerebral ischemia and hypoxia. Secondary damage around the hematoma after cerebral ischemia and hypoxia has been associated with NF-κB activation [17]. NSE can reflect neuronal damage. It is closely related to synaptic plasticity, gliosis, memory deposition, and formation of nerve cells. Furthermore, it plays an important role in the involvement of nerve cells in cognitive function [18]. Present results showed that Cu/Zn SOD activity was obviously decreased and NSE, 8-OHdG, and MDA levels were markedly enhanced in the epilepsy group, compared with controls. Results suggest that oxidative stress may mediate occurrence and development of temporal lobe epilepsy. SOD can increase antioxidant enzyme activity and reduce brain damage via inhibiting oxidative stress levels. This may be related to mitochondrial ATP-sensitive potassium channels and mitochondrial membrane stability [19].

Voltage-dependent sodium channels play an important role in the generation and propagation of neuronal action potentials. There are 11 genes encoding the sodium channel α subunit in humans, while only SCN8A, SCN1A, SCN2A, and SCN3A are expressed in the central nervous system. The current produced by type I and type II sodium channels in the central nervous system accounts for 70%. It has been observed that downregulation of SCN2A gene expression is associated with the development of epilepsy. There are no significant changes in sodium current of excitatory vertebral neurons in SCN2A knockout mice. Inhibitory inter-neuronal sodium currents were significantly reduced and SCN2A mutant mice showed persistent seizures [20-22]. In the current study, expression levels of SCN2A mRNA and proteins in the cortexes of patients with temporal lobe epilepsy were determined by RT-PCR and Western blot. It was revealed that expression levels of SCN2A mRNA and proteins in the cortexes of patients with temporal lobe epilepsy were significantly lower than those of normal tissues. Cu/Zn SOD activity was obviously decreased and NSE, 8-OHdG, and MDA levels were markedly enhanced in the epilepsy group, compared with controls. SCN2A protein expression was positively correlated with Cu/Zn SOD activity and negatively correlated with 8-OHdG and MDA, indicating that SCN2A expression in cortical tissues of patients with temporal lobe epilepsy may have a potential relationship with oxidative stress.

With a deepening of epilepsy and neuronal damage caused by free radicals, anti-free radical treatments, which protect nerve cells, have increased. In the epilepsy artery model, free radical scavengers can exert neuroprotective effects through various pathways. However, the effects require further investigation. The present study only explored the possible relationship between SCN2A expression in cortical tissues and oxidative stress in patients with temporal lobe epilepsy. Reducing ROS levels may help to alleviate epileptic mediated neuronal damage. The specific pathogenesis of temporal lobe epilepsy should be further explored.

Conclusion

SCN2A expression in cortical tissues of patients with temporal lobe epilepsy showed a potential relationship with oxidative stress, mediating the development of temporal lobe epilepsy.

Acknowledgements

This work was supported by 13A0031 repetitive transcranial magnetic stimulation with different frequency- and intensity-inhibited seizures in epileptic rats (2013 Nanchong Applied Technology Research and Development Fund Project).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shunxian Wang, Department of Neurology, Affiliated Hospital of North Sichuan Medical College, No. 1 Maoyuan South Road, Shunqing District, Nanchong City, Sichuan

Province, China. Tel: +86-0817-2162011; Fax: +86-0817-2162011; E-mail: shentuo414xt@126.com

References

[1] Xiang J and Jiang YG. Regulation of Cu-Zn superoxide dismutase on SCN2A in SH-SY5Y cells as a potential therapy for temporal lobe epilepsy. Mol Med Rep 2014; 9: 16-22.

[2] Manno I, Macchi F, Caleo M and Bozzi Y. Environmental enrichment reduces spontaneous seizures in the Q54 transgenic mouse model of temporal lobe epilepsy. Epilepsia 2011; 52: E113-E117.

[3] Wang WZ, Takashima S, Segawa Y, Itoh M, Shi XY, Hwang SK, Nabeshima K, Takeshita M and Hirose S. The developmental changes of Na(v)1.1 and Na(v)1.2 expression in the human hippocampus and temporal lobe. Brain Res 2011; 1389: 61-70.

[4] Petkova Z, Tchekalarova J, Pechlivanova D, Moyanova S, Kortenska L, Mitreva R, Popov D, Markova P, Lozanov V, Atanasova D, Lazarov N and Stoynev A. Treatment with melatonin after status epilepticus attenuates seizure activity and neuronal damage but does not prevent the disturbance in diurnal rhythms and behavioral alterations in spontaneously hypertensive rats in kainate model of temporal lobe epilepsy. Epilepsy Behav 2014; 31: 198-208.

[5] Brown EC, Muzik O, Rothermel R, Juhasz C, Shah AK, Fuerst D, Mittal S, Sood S and Asano E. Evaluating signal-correlated noise as a control task with language-related gamma activity on electrocorticography. Clin Neurophysiol 2014; 125: 1312-1323.

[6] Cho CH. Molecular mechanism of circadian rhythmicity of seizures in temporal lobe epilepsy. Front Cell Neurosci 2012; 6: 55.

[7] Persike DS, Marques-Carneiro JE, Stein MLL, Yacubian EMT, Centeno R, Canzian M and Fernandes MJDS. Altered proteins in the hippocampus of patients with mesial temporal lobe epilepsy. Pharmaceuticals (Basel) 2018; 11.

[8] Kwon JY, Jung UJ, Kim DW, Kim S, Moon GJ, Hong J, Jeon MT, Shin M, Chang JH and Kim SR. Beneficial effects of hesperetin in a mouse model of temporal lobe epilepsy. J Med Food 2018; [Epub ahead of print].

[9] Drion CM, van Scheppingen J, Arena A, Geijtenbeek KW, Kooijman L, van Vliet EA, Aronica E and Gorter JA. Effects of rapamycin and curcumin on inflammation and oxidative stress in vitro and in vivo-in search of potential anti-epileptogenic strategies for temporal lobe epilepsy. J Neuroinflammation 2018; 15: 212.

[10] Pottoo FH, Tabassum N, Javed MN, Nigar S, Rasheed R, Khan A, Barkat MA, Alam MS, Maqbool A, Ansari MA, Barreto GE and Ashraf GM. The synergistic effect of raloxifene, fluoxetine, and bromocriptine protects against pilocarpine-induced status epilepticus and temporal lobe epilepsy. Mol Neurobiol 2019; 56: 1233-1247.

[11] Pardo-Pena K, Sanchez-Lira A, Salazar-Sanchez JC and Morales-Villagran A. A novel online fluorescence method for in-vivo measurement of hydrogen peroxide during oxidative stress produced in a temporal lobe epilepsy model. Neuroreport 2018; 29: 621-630.

[12] de Araújo Filho GM, Martins DP, Lopes AM, de Jesus Brait B, Furlan AER, Oliveira CIF, Marques LHN, Souza DRS and de Almeida EA. Oxidative stress in patients with refractory temporal lobe epilepsy and mesial temporal sclerosis: possible association with major depressive disorder? Epilepsy Behav 2018; 80: 191-196.

[13] Sharma S, Puttachary S and Thippeswamy T. Glial source of nitric oxide in epileptogenesis: a target for disease modification in epilepsy. J Neurosci Res 2019; 97: 1363-1377.

[14] Sharma S, Carlson S, Puttachary S, Sarkar S, Showman L, Putra M, Kanthasamy AG and Thippeswamy T. Role of the Fyn-PKCdelta signaling in SE-induced neuroinflammation and epileptogenesis in experimental models of temporal lobe epilepsy. Neurobiol Dis 2018; 110: 102-121.

[15] Huang LG, Zou J and Lu QC. Silencing rno-miR-155-5p in rat temporal lobe epilepsy model reduces pathophysiological features and cell apoptosis by activating Sestrin-3. Brain Research 2018; 1689: 109-122.

[16] Castro OW, Upadhya D, Kodali M and Shetty AK. Resveratrol for easing status epilepticus induced brain injury, inflammation, epileptogenesis, and cognitive and memory dysfunction-are we there yet? Front Neurol 2017; 8: 603.

[17] Busquets O, Ettcheto M, Verdaguer E, Castro-Torres RD, Auladell C, Beas-Zarate C, Folch J and Camins A. JNK1 inhibition by Licochalcone A leads to neuronal protection against excitotoxic insults derived of kainic acid. Neuropharmacology 2018; 131: 440-452.

[18] Jeong JH, Choi BY, Kho AR, Lee SH, Hong DK, Lee SY, Song HK, Choi HC and Suh SW. Diverse effects of an acetylcholinesterase inhibitor, donepezil, on hippocampal neuronal death after pilocarpine-induced seizure. Int J Mol Sci 2017; 18.

[19] Juarez-Rebollar D, Alonso-Vanegas M, Nava-Ruiz C, Buentello-Garcia M, Yescas-Gomez P, Diaz-Ruiz A, Rios C and Mendez-Armenta M. Immunohistochemical study of Metallothionein in patients with temporal lobe epilepsy. J Clin Neurosci 2017; 39: 87-90.

[20] Liang LP and Patel M. Plasma cysteine/cystine redox couple disruption in animal models of temporal lobe epilepsy. Redox Biol 2016; 9: 45-49.

[21] Pearson JN, Warren E, Liang LP, Roberts LJ 2nd and Patel M. Scavenging of highly reactive gamma-ketoaldehydes attenuates cognitive dysfunction associated with epileptogenesis. Neurobiol Dis 2017; 98: 88-99.

[22] Shakeel S, Rehman MU, Tabassum N, Amin U and Mir MUR. Effect of naringenin (a naturally occurring flavanone) against pilocarpine-induced status epilepticus and oxidative stress in mice. Pharmacogn Mag 2017; 13 Suppl 1: S154-S160.