

Original Article

Oxidative Stress and Antioxidant Defense Status in CSF and Blood Content of the WAG/Rij Rat Models Suffering from Absence Epilepsy

Seyed Mahdi Vatandoust^a, Rafiqeh Ghiasi^b, Ghazaleh Ghamkhari Nejad^c, Parviz Shahabi^{a*}

^aNeurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

^bDepartment of Physiology, Tabriz University of Medical Sciences, Tabriz, Iran

^cStudent Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran

Abstract

Objective: Absence seizure as one of several kinds of seizures has been characterized by short-term episodes of either conscious or unconscious “abnormal excessive or synchronous neuronal activity in the brain. WAG/Rij rats, a genetic model of absence epilepsy, were originally developed as experimental tools to resemble human absence epilepsy. Factors like blood variation and CSF (Cerebrospinal fluid) oxidative stress and antioxidant balance, play a key role in the induction and development of absence epilepsy and its complications. In this study we tried to evaluate the effects of changes in oxidative and antioxidant levels in CSF and blood on absence seizures.

Materials and Methods: 20 male WAG/Rij rats and 20 male Wistar rats (250-350g, 6-7 months old) were used in the study. At the end of the experiment animals were anesthetized, then blood and CSF samples were collected and used for the determination of Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Malondialdehyde (MDA) levels and total antioxidant capacity (TAC).

Results: This study showed that the level of MDA, as an index of oxidative stress, significantly increased in WAG/Rij rats when compared to control group ($p=0.001$ for WD and $p=0.003$ for CSF). It was also found that the levels of antioxidants such as GPx, SOD, and TAC in WAG/Rij rats decreased significantly: $p=0.03$, $p=0.013$, $p=0.001$, $p=0.003$, $p=0.009$ and $p=0.003$, respectively, for GPx WB, GPx CSF, SOD WB, SOD CSF, TAC serum and TAC CSF.

Conclusion: In absence seizure, measurement of oxidative stress markers and antioxidant levels such as MDA, SOD, GPx, in serum and CSF cases can lead to a better diagnosis and treatment of this condition.

Keywords: Absence seizure, Oxidative stress, Antioxidant, Reactive oxygen species, WAG/Rij rats

Correspondence

Parviz Shahabi,
Neurosciences Research Center,
Tabriz University of Medical Sciences,
Tabriz-Iran
Tel: +98-41-33364664
Email: parvizshahabi@gmail.com

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Introduction

Epilepsy is one of the most widespread neurological disorders that affects 1% of the people in the world [1,2]. Several studies have been conducted to investigate the interaction between epilepsy and the antioxidants content of blood and cerebrospinal fluid (CSF). They imply changes on the antioxidant levels in patients. But there is a lack of research concerning the variations of antioxidant levels in patients suffering from absence epilepsy. Therefore, it can be said that the variations in antioxidant levels are common in people with epilepsy and by considering this fact advancing the treatment or prevention of epilepsy may be

significantly enhanced. Absence seizures is a kind of seizure., it has been clinically characterized by a transitory change of consciousness, either with or without other clinical signs, related to electroencephalogram (EEG) with diffuse spike-and-wave discharges (SWD) of variable duration – usually between 3 and 30 seconds [3]. The basic mechanism involves production of unusual impulse from that special neuronal network in thalamocortical pathway. In this pathway it seems that neural impulses are created by interaction between inhibition of GABA and excitation of glutamate and the tension between excitation and inhibition seems to involve the T-type calcium current. Local

circuitry within the thalamus may influence these oscillatory rhythms by GABA-mediated inhibition. Neurotransmitters that are active in this pathways affect the thalamus and/or cortex originate from different sources [4]. WAG/Rij rats (Wistar Albino Glaxo Rijswijk), are genetic animal models for human absence epilepsy. In these rats, in the cortico-thalamo-cortical pathway spike-and-wave discharges (SWDs) are generated then they circulate in this pathway that are identified in the perioral somatosensory cortex (S1po) [5,6]. In the somatosensory cortex the local hyperexcitability phenomenon is consistent with the *theory of Cortical Focus* for the development of absence epilepsy. Some studies support impairments in balance between the inhibitory and excitatory networks in WAG/Rij rats [7]. Seizures were developed at this area and be transferred rapidly to the other zones of the thalamus and cortex [8]. SWD's in WAG/Rij rats have a frequency of 7-11 Hz and the amplitude varies in a range of 300-1000 μ V with a mean duration of 8.0 ± 1.0 s(9). Thus, the oxidative stress, defined by the extensive generation of free radicals, can greatly modify the cell function and an excessive production of these components has been linked to seizure-induced neuronal death [10]. Due to some biochemical, physiological and anatomical reasons, the nervous system is more susceptible to reactive oxygen species (ROS) as compared to other organs of the body [11,12]. Production of reactive oxygen species leads to widespread lipid peroxidation in biological membrane which caused loss of fluidity, decreases in membrane potential and enhancement of permeability to H^+ ions, leading to a tissue injury [13-15]. ROS led to the generation of peroxidation of unsaturated fatty acids, one of the compounds of neuronal membrane, resulting in depolarization. ROS also stimulates generation of compounds including neurotoxicity effects such as guanidino compounds (e.g. methyl guanidine, guanidine) which are recognized to be inductors in the brain. Such responses can be followed by alterations in excitatory and inhibitory neurotransmitter amounts, principally, enhancing the release of excitatory amino acids such as aspartic acid and reducing the release of inhibitory amino acid such as GABA. These alterations in transmitter release can be linked to epileptogenicity by production of ROS. The ROS will dramatically increase the generation of neurotoxic guanidino compounds [16,17], therefore may induce convulsion. The aim of this study was to investigate the changes in oxidative and antioxidant levels in CSF and blood in cases with absence seizures.

Material and Methods

Animals

In this study, we used twenty male Wistar rats (250-350g, 6-7 months old) as the control group and twenty male WAG/Rij rats (250-350g, 6-7 months old) as the test group. WAG/Rij rats were obtained from Shefa Neuroscience Research Center, Tehran, and Wistar rats were purchased from Animal's House of Tabriz University of Medical Sciences. All rats were kept in small groups in separate cages and at a temperature of 20-22°C and in a 12 h light/12 h darkness cycle putting the lights off at 6:00 pm. Rats were given ad libitum access to food and water.

Electrode implantation for EEG recording

The rats were anesthetized with ketamine (85 mg/kg) and xylazine (3 mg/kg) administered intraperitoneally [18]. All animals were implanted with two cortical stainless steel electrodes for EEG recording. The monopolar EEG recording electrode was at the frontal cortical region of the right hemisphere (coordinates: AP = 0.22, L = 0.24, and V = 0.26), while the reference electrode was on the occipital cortex. Electrodes were fixed in the sockets using pins. One week after conducting electroencephalography (EEG) recordings on animals moving freely in all directions, EEG was recorded with the sampling rate of 1 kHz. The observed SWD waves for the rats is provided in Figure 1. SWD mainly occurs during drowsiness and light sleep.

Blood collection and analysis

Rats were initially anesthetized and then their blood was collected by puncturing the orbital sinus by an approximate amount of 5 ml volumes, and immediately frozen at $-80^{\circ}C$ and then used to determine GPx, SOD, MDA and TAC levels.

Surgical procedure for the exposure of Dura and collection of CSF from cisterna magna

All surgical appliances were sterilized with 70% ethyl alcohol before any application. The simplest way for rat CSF sampling involves removal of the musculature overlying the atlanto-occipital membrane to expose the cisterna magna (Figure 2). The pool of CSF is then sampled by needle and syringe aspiration [19]. In this method an anesthetized rat was placed in a stereotaxic device (Stoelting, USA) and the skin of the neck was shaved [20]. The surgical site was swabbed with 10% povidone iodine, and later by 70% ethanol, and a

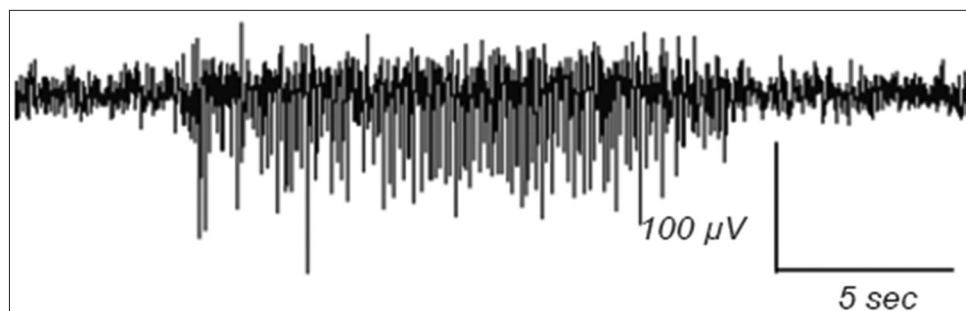


Figure 1. Typical spike and wave discharge recorded from the S1po cortex of a WAG/Rij rat.

sagittal incision of the skin was made inferior to the occiput. The dura mater of the cisterna magna appeared as a glistening and clear reverse triangle through which the medulla oblongata and a major blood vessel (arteria dorsalis spinalis), and the CSF space was visible [21]. After the exposure of translucent dura mater, a 1ml syringe, to which a 29G needle was attached, was carefully inserted at a 45° angle to the Dura, from the caudal end of the incision. The needle was inserted approximately 1mm deep to the Dura. The syringe plunger was pulled back, CSF was aspirated in less than 1min and about 100-250 μ l CSF were collected and transferred to Eppendorf tube and frozen at -80°C and then used for determination of GPx, SOD, MDA and TAC levels.

Determination of antioxidant enzymes

After adding EDTA (Vacuette, Greiner Bio-One, Kremmunster, Austria) to micropipette containing whole blood and CSF samples to prevent from coagulation they were used for the determination of GPx and SOD. SOD activity was determined using a commercial kit (RANSOD, Randox co., Antrim, United Kingdom) [22]. According to DelmasBeauvieux et al. SOD activity was measured at 505 nm by a spectrophotometer (Pharmacia Biotech; England). In this method, xanthine and xanthine oxidase were used to generate superoxide radicals that reacted with 2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium chloride (ITN) to form a red formazan dye. Concentrations of substrates were 0.05 mmol/L for xanthine and 0.025 mmol/L for ITN. SOD activity was measured based on its reaction inhibition rate. After calculating the percent of inhibition by using related formula, SOD activity value was calculated by comparing with the standard curve and was expressed in blood. GPx activity was determined using commercial kit (RANSEL, Randox co., Antrim, United Kingdom) according to the method by Paglia and Valentine [23]. GPx catalyses the oxidation of glutathione (at a concentration of 4 mmol/L) using Cumenehydroperoxide. In the presence of glutathione reductase (at a concentration ≥ 0.5 units/L) and 0.28 mmol/L of NADPH, oxidized glutathione was immediately converted to the reduced form as a result of concomitant oxidation of NADPH to NADP^+ . The decrease in absorbance at 340 nm (37°C) was measured using a spectrophotometer (Pharmacia Biotech; England), and then the GPx concentration was calculated by the related formula and expressed in blood [24].

MDA assessment

MDA, as the end-product of lipid peroxidation, was measured in the blood and CSF samples according to the Esterbauer and Cheeseman method. MDA reacts with thiobarbituric acid and produces a pink pigment that has a maximum absorption at 532 nm [25, 26].

Statistical analysis

Data were statistically analyzed using independent t- test. The significant level was set at $P < 0.05$. Results are expressed as means \pm SD.

Results

MDA level in serum and CSF samples

In this study the serum levels of MDA, as an index of oxidative stress, was 4.56 nmol/ml (nanomole/milliliter) for WAG/Rij rats and 2.74 nmol/ml for Wistar rats which significantly ($P < 0.05$) increased in epileptic WAG/Rij rats compared to the control group. Also, CSF levels of MDA, in WAG/Rij rats, 4.03 nmol/ml, and in Wistar rats, 2.56 nmol/ml, showed a significant ($P < 0.05$) increase in epileptic WAG/Rij rats as compared to the control group (Figure 3).

SOD level in whole blood and CSF samples

The SOD levels in whole blood samples for WAG/Rij rats was 7.36 ng/ml (nanogram per milliliter) and for Wistar rats it was 27.56 ng/ml which significantly ($P < 0.05$) decreased in WAG/Rij rats as compared to the control group. Also the CSF levels of SOD in WAG/Rij and Wistar rats were 0.25 and 1.52 ng/ml, respectively, which decreased significantly ($P < 0.05$) in WAG/Rij as compared to that of the control group (Figure 4).

GPx level in whole blood and CSF samples

Based on our findings, the GPx levels in the whole blood of epileptic rats was 58.23 nmol/min per ml and in control rats it was 212.50 nmol/min per ml which decreased significantly ($P < 0.05$) in WAG/Rij as compared to the control group. And the CSF level of GPx decreased significantly ($P < 0.05$) in WAG/Rij

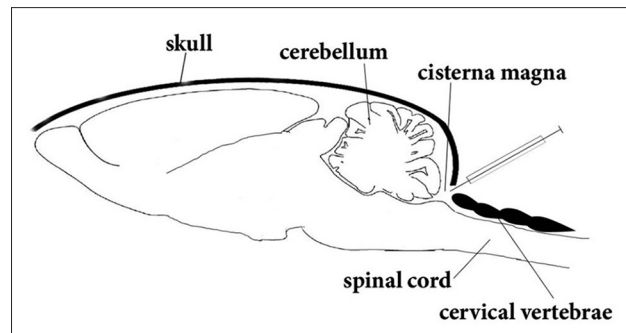


Figure 2. Schematic diagram of cisterna magna and syringe plunger.

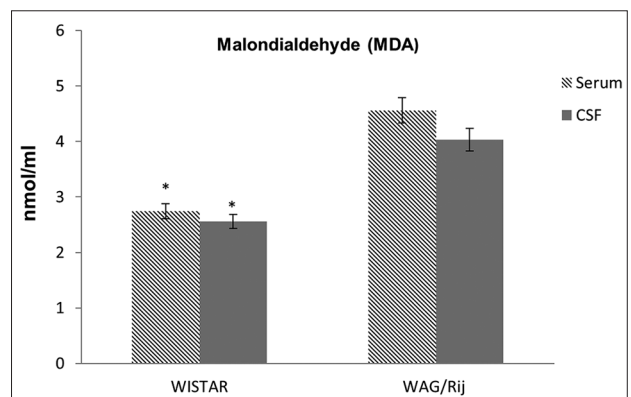


Figure 3. Malondialdehyde (MDA) levels in serum and CSF samples of WAG/Rij and control group. Data are expressed as Mean \pm SD, for (n=20) each group. * $p < 0.05$ when compared to the control group.

rats (4.71nmol/min per ml) in regards with the control group (11.55nmol/min per ml) (Figure 5).

TAC level in serum and CSF samples

Also, as shown in Figure 6, the serum TAC levels in WAG/Rij group was 1.47 mmol/L and the serum TAC levels in control group was 2.25 mmol/L which was significantly ($P<0.05$) lower in WAG/Rij rats in comparison with the control group. And the CSF level of TAC in WAG/Rij rats, 1.25 mmol/L, and in Wistar rats, 2.03 mmol/L, showed a significant decrease ($P<0.05$) in WAG/Rij group unlike the control group.

Discussion

The development of epilepsy is probably related to the homeostatic impairment of oxidant and antioxidants levels. Many experimental models of seizure have been expanded to study the role of endogenous antioxidants markers in response to oxidative stress. Disorders in the endogenous antioxidant markers that act against oxidative stress can develop the seizure. Current study was conducted to investigate the antioxidant and oxidative levels in CSF and blood on absence seizure cases. WAG/Rij rats, as genetics animal models of human absence seizure, show many behavioral symptoms and electroencephalogram waves similar to humans absence seizure, including the action of various antiepileptic drugs [9]. Today there have been many studies conducted on these rats including studies on absence epilepsy, depression, memory and etc. Since they are a reliable source of research there is an increasing number of researches being conducted on them every day (Drinkenburg et al., 2003), (Cryan and Holmes, 2005) and (McGlinchey et al., 2006; Overstreet, 1993, 2002; Overstreet et al. 2005; Willner and Mitchell, 2002). In the present study, lipid peroxidation product such as MDA measured in serum and CSF was found to be increased significantly compared to the control group. This result is in consistent with the results found by Ramaekes et al [27]. Also it is comparable with other studies [28,29]. Increased CSF MDA levels suggest the lipid peroxidation in epileptic condition. Nikushkin Braslavskii et al. has reported that lipid peroxidation in the neuronal membranes participate in the development of epileptic activity [30]. Membrane lipids which have unsaturated fatty acids contents are mostly sensitive to oxidative stress and peroxidation of the membrane lipids destroys the integrity of the membrane [31,32]. Since the nervous system relatively has high levels of polyunsaturated fatty acids that are susceptible to lipid peroxidation, it is more susceptible to the injurious effect of oxidative stress [33,34]. Because of high metabolic activity, it receives a high level of oxygen and is comparatively a defect in the antioxidant enzymes. It is believed that an increase in the free radicals may cause neuronal degeneration and acute or chronic diseases of the brain, like epilepsy, cerebrovascular disease, Alzheimer’s disease, and etc. [35]. An important factor in epileptogenesis is oxidative stress and oxidative damage that achieved from cell death and consequences of epileptic seizures. Other research has shown that, antioxidant and oxidative stress levels regulation with the chemical or natural treatments reduce the incidence of epilepsy [36] in this study. GPx and SOD are the

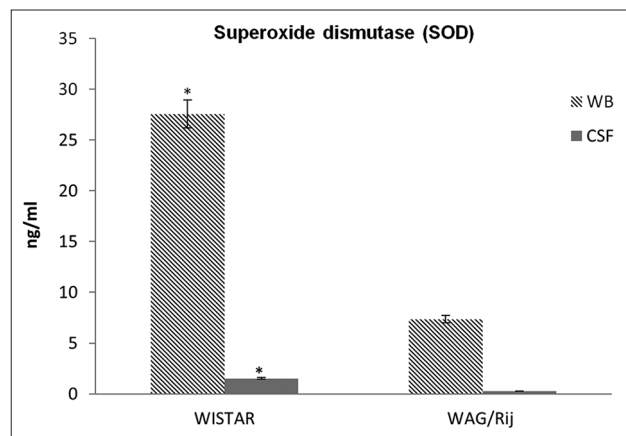


Figure 4. Superoxide dismutase (SOD) levels in Whole blood and CSF samples of WAG/Rij and the control group. Data are expressed as Mean±SD, for (n=20) each group. * $p < 0.05$ when compared to the control group.

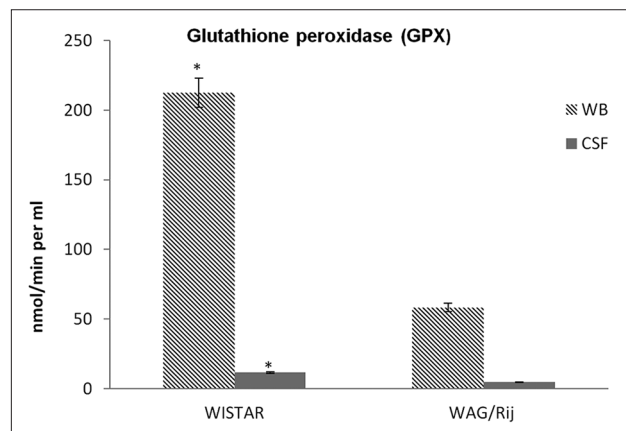


Figure 5. Glutathione peroxidase (GPx) levels in whole blood and CSF samples of WAG/Rij and the control group. Data are expressed as Mean±SD, for (n=20) each group. * $p < 0.05$ when compared to the control group.

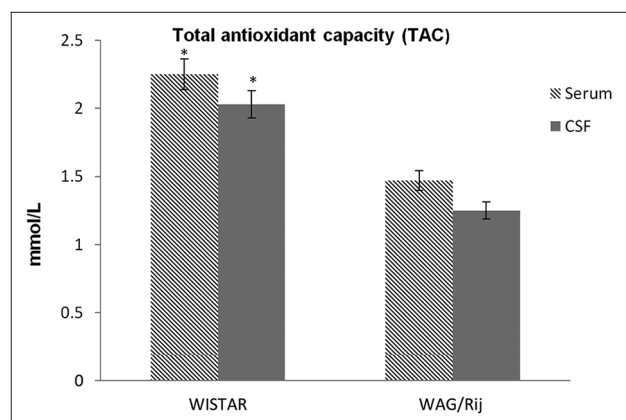


Figure 6. Total antioxidant capacity (TAC) levels in serum and CSF samples of WAG/Rij and the control group. Data are expressed as Mean±SD, for (n=20) each group. * $p < 0.05$ when compared to the control group.

most important members of antioxidant defense mechanisms, protecting cells against oxidative injury by catalyzing hydro

peroxide reduction. In the study a significant reduction in the serum and CSF levels of GPx and SOD was observed in epileptic rats as compared to the control. In this study, the serum and CSF levels of SOD among epileptic rats was reduced with regard to the control group. This was in consistent with the findings of other researchers [30]. There is a controversial report about the glutathione peroxidase activity and concentrations in epileptic conditions. Some studies indicated that the glutathione peroxidase activity and level was similar in epileptic group as compared to the control group [37]. Other studies showed an increase in the activity and the level of these antioxidant enzymes in epileptic subjects compared to the control group [38]. Parallel to our results, some studies reported lower levels of GPx in epileptic group as compared to the control [39]. The relatively low levels of Gpx in brain tissue appeared to prevent from peroxide resulting damages. In addition, the membranes of nerve cells have a high level of unsaturated fatty acids that are highly susceptible to peroxidation harms [40]; therefore, reflecting the important role of homeostasis with respect to peroxidative vs. antioxidant stress in the epilepsy [24, 41]. Also, there is contradiction in some other studies regarding the relation between the changes in antioxidant level and the prevalence of epilepsy. For example, in studies by Tejada and Bellissimo the amount of GPX in patients suffering from epilepsy was reported to be higher compared to healthy people [42, 43]. One of the outputs of the present study that can be considered as an innovation was the investigation of antioxidant level in patients with absence epilepsy which was considered previously.

Conclusion

Our results indicate that the oxidant – anti oxidant disturbances in epileptic rats, can play an important role in the pathophysiology of the epilepsy. We suggest that further studies should be done on the role of oxidative stress in cases with epilepsy.

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