Chapter 6

Free Radicals and Antioxidants: Opportunities for Enhancing Treatment of Epilepsy with Personalized Medicine

Jerzy Majkowski, Tuomas Westermarck and Faik Atroshi

Additional information is available at the end of the chapter

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Abstract

Introduction: Epileptic seizures and antiepileptic drugs (AEDs) are a source of oxygen stress. Oxygen stress can have negative effects. These effects which can be prevented are largely unknown in clinical epileptology. Objective: The objective of the study is to discuss (a) homeostatic oxidant, antioxidant imbalance due to epileptic seizures and AEDs, (b) the protective factors that help prevent oxygen stress (OS), and personalized medicine based on pharmacogenomics and diet as therapeutic challenges in epilepsy. Discussion: Experimental models of epileptic seizures evoked by various means suggest that seizures can cause neuronal destruction. This is accompanied by an increased activity of free radicals and a reduction of total antioxidant capacity (in red blood cells, blood serum, and cerebrospinal fluid). A number of antioxidants have been found to attenuate the negative effects of OS and act neuroprotectively if they are administered prior to seizure occurrence: vitamins (C, E), trace elements (Se, Zn), melatonin, erdosteine, or natural herbal extracts. New AEDs (GBP, LEV, LTG, and TGB) cause no, or very little, OS as opposed to other drugs (CBZ, PHT, PB, VPA, TPM, or OXC), which have pronounced albeit heterogeneous and dose-dependent effects. It is suggested that AEDs should be administered together with free radical sweepers (vitamins, trace elements, electrolytes, melatonin) and other anti-oxidizing substances. Conclusions: (1) Epileptic seizures and AEDs cause OS. The effects vary greatly depending, among other things, on the daily drug dose. (2) The findings of research using a variety of seizure models are more unequivocal than the findings of research on patients with epilepsy. This suggests that the relations among seizures, AEDs, OS etiology, and OS consequences are complex. (3) Since existing AEDs cause OS, it is necessary to develop a new approach to AED treatment. (4) It is important to know the patient's specific characteristics, including previous history, lifestyle, age, gender, weight, diet, environment, etc. They can be valuable tools to improve the quality of life of a person suffering from epilepsy. This concept of managing the patient health is called targeted medicine or personalized medicine.

Keywords: epileptic seizures, epilepsy, oxygen stress, antioxidants, personalized medicine

1. Introduction

Within the last three decades, interest in oxygen stress and its role in the development of organ pathology has increased considerably, and the importance of this phenomenon has been increasingly recognized. Oxygen stress means that the production of free radicals and reactive forms of oxygen (RFO) has exceeded the antioxidant defense mechanism capacity [1, 2]. Bartosz [3] thinks that RFO research may be the key to a better understanding of certain biochemical, physiological, and pathological aspects of living organisms and suggests that such understanding could be applied to clinical practice. Free radicals, the product of oxygen stress, may play an important role as physiological markers that control cell process signals. However, when produced in excess, or when the antioxidant defense system is weak, they may lead to cell injury.

Excessive free radical production is related to various physiological and pathological states such as aging, epileptic seizures, or the use of xenobiotics, including fat-soluble drugs [4, 5]; this also applies to antiepileptic drugs (AEDs) [6–8]. A number of nonspecific factors [9] as well as dietary habits affect the state of antioxidants in the healthy elderly [10]. This suggests that oxidation and anti-oxidation processes are rather ubiquitous and hence nonspecific.

As far as epilepsy is concerned, when the number of free radicals in the neuron increases, this interferes with the respiratory chain in the mitochondria, destabilizes the lysosomal membranes, and lowers the convulsion threshold [11–13]. Peroxydation of the neuronal membranes modifies their electrophysiological properties and leads to an abnormal bioelectric discharge in the neurons.

Epilepsy is frequent in diseases involving dysfunction in the mitochondrial structures. It is a sign of energetic anomalies in the ATP synthesis due to ADP phosphorylation [14]. The mitochondria have vital functions such as energy production, cellular harm control, neurotransmitter synthesis, and free radical production. It is not clear yet which of these functions are affected in epileptic seizures [15]. Liang and Patel found an increase in spontaneous and evoked epileptic attacks in a subgroup of mice with a partial inherent mitochondrial SOD deficit. This effect correlated with chronic mitochondrial oxygen stress (aconitase enzyme deactivation) and reduced oxygen use. They think that oxygen stress caused by free radical peroxides increases seizure susceptibility in this subgroup of mice. This susceptibility increases with age and also with increased environmental stimulation and the use of stimulants. Oxygen stress and mitochondrial dysfunction may both cause and be caused by epileptic attacks [16]. According to Dubenko and Litovchenko [17], the application of energy metabolism activators improves the clinical and electroencephalographic course of epilepsy. This has been demonstrated experimentally by positive histological changes. According to these writers, this treatment prevents neuronal harm and the development of encephalopathy.

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Research on various seizure models, in animals and humans, has shown that not only epileptic seizures but also EEG discharges themselves may cause complex metabolic neural lesions and oxidation-antioxidant disequilibrium [18–21].

On the other hand, a number of experimental studies have shown that AEDs can also produce free radicals and significantly increase the peroxydation of neuronal membrane lipids and reduce the protective effects of antioxidants. These changes may lead to increased seizure and idiosyncratic drug effect frequency [7, 22–25]. PHT initiates an oxidation damage to proteins and fats in the maternal and embryonic liver tissue organelle in murine rodents [1].

A group of researchers have also found that oxidation stress and resistance to AEDs trigger adaptive mechanisms, i.e., production of endogenous antioxidant sweepers, which prevent the harmful effects of oxidation [26]. These researchers studied nitrogen oxide and endogenous antioxidant GSH sweepers, GSH-Px, complete (T) and superoxide dismutase (T-SOD), Mn-SOD, and catalase in the cerebrospinal fluid of children with various neurological diseases. All the antioxidant parameters were the highest in the children with bacterial meningitis compared with the other groups. In the epilepsy group, nitrogen oxide, GSH, and GSH-Px were higher than in the aseptic meningitis and control group [26]. The authors think that oxygen stress may be related to seizure pathology and that its reduction may lead to a better prognosis for the course of epilepsy.

Akarsu et al. [27] came to similar conclusions. They studied the state of oxidation in 21 children with febrile seizures and 21 children without febrile seizures. They assessed the level of arginase and catalase in the red blood cells, malondialdehyde—an indicator of lipid peroxidation (MDA)—and nitrogen oxide in the plasma and cerebrospinal fluid. The control group consisted of 41 children divided into three subgroups: (1) with fever, (2) without convulsions, and (3) without fever and without convulsions. Both fever and convulsions had a significant effect on the oxidation mechanism. Febrile and afebrile convulsions differed in their generation of oxygen stress. According to the authors, higher levels of oxygen stress may be a factor that protects against neuronal lesion during convulsions in afebrile convulsions.

Not only can oxygen stress initiate epileptogenesis, it can also worsen the course of epilepsy. Consistently with these conclusions, using antioxidants in conventional epilepsy therapy and hence attenuating oxygen stress could have a positive effect on the course of epilepsy [28]. Many researchers share this opinion, but few of them are clinical epileptologists. Earlier, a review was published of research on the possible role of oxygen stress in the early stages of epileptogenesis, both in animal models and humans [29]. There has also been discussion of attempts to use exogenous antioxidants which, according to many writers, have antiepileptogenic properties (which AEDs do not).

It has been reported that refractory epileptic patients may benefit from pharmacogenetic testing for variations in the genes encoding drug-metabolizing enzymes and drug transporters of AEDs [30]. Since the treatment options are limited, the use of personal medication, which could have beneficial effects in epilepsy treatment, is indicated [31]. The aim of personalized medication is to maximize the likelihood of therapeutic efficacy and to minimize the risk of drug toxicity. Specific genes have been linked to adverse drug reactions in the form of a severe rash in Stevens-Johnson syndrome [32, 33].

2. Preventing oxidation stress due to epileptic seizures

Experimental research with animal models and clinical observation have shown that epileptic seizures lead to a number of harmful activities in the brain: disturbed blood circulation, increased cerebrospinal fluid pressure, brain edema, and hypoxia, all of which lead to a sudden reduction of energy carriers (ADP, ATP, phosphocreatine) and pH neuron reduction. During seizures, arachidic acid is released in the postsynaptic membranes. This has an activating effect on presynaptic neuronal endings and leads to increased glutamate release. Arachidic acid also increases the production of free oxygen radicals, leading to increased lipid peroxidation. These in turn may activate phospholipase C and then lead to the release of arachidic acid from cellular membranes, setting a vicious circle in motion [3].

2.1. The metrazol seizure model (PTZ)

Intraperitoneally induced seizures in rats by means of PTZ rupture the blood-brain barrier. This has been demonstrated with Evans dye, used to mark the permeability of this barrier [34]. Research suggests that free radicals are involved in the permeability of the blood-brain barrier; this permeability leads to albumin extravasation to the thalamic nuclei, brain stem, frontal cortex, and occipital cortex. The animals that have been given vitamin E or selenium (Se) prior to the seizure induction had less extravasation in these structures. It has also been demonstrated that in young rats in normothermic conditions, the barrier permeability was greater in males ($p < 0.05$) [35].

In this convulsion model in mice, prior administration of erdosteine (mucoliticum), which acts as an antioxidant, leads to much weaker oxygen stress and much longer latency time from pentylenetetrazol (PTZ) administration to convulsion onset ($p < 0.05$) [36]. On the other hand, compared with the control group, the experimental group had lower levels of MDA and xanthine oxidase (oxidizers) and a higher level of SOD ($p < 0.001$). These studies show that administration of erdosteine reduces convulsion-induced oxygen stress and therefore protects the neurons.

In mice, *Nigella sativa* oil (NSO), a powerful antioxidant that has been in used in folk medicine and the kitchen for thousands of years, prevented epileptic seizures induced by PTZ kindling much more effectively than valproic acid (VPA) [20].

2.2. The kainic acid model

Kainic acid (KA) is used as a model substance in the assessment of neurotoxicity. It leads to excessive RFT production due to reduced antioxidant activity. When KA was administered to rats, lipid peroxidation of the neuronal membrane increased in proportion to seizure progression [37]. In the same model, SOD and catalase activity increased significantly on day 5 following KA administration and returned to the base level 3 weeks later; GGH-PX activity also increased significantly on day 5 but was still high 3 weeks later [38]. Lipid and protein peroxidation, assessed by MDA concentration, increased significantly 8 and 16 h later then decreased on day 2 and day 5 following KA injection. The authors attribute the rapid increase

in MDA and protein peroxidation to free radicals produced in this phase of the pathological KA effect; they think that the changes in the enzymatic scavenger activity and the reduced MDA concentration may have been caused by glia proliferation due to neuronal death.

In the KA model in mice, prior or simultaneous administration of melatonin (a powerful hydroxyl radical scavenger) (20 mg/kg i.p.) had an anti-oxidizing effect and prevented lipid peroxidation, cerebral mitochondria DNA injury, and seizures [39].

In the KA model, prior administration of vineatrol significantly reduced the MDA level in rats' brains but had no effect on the glutation level [40]. Doses exceeding 20 and 40 mg/kg lengthened the latency time to that of the first seizures. Additional administration of vineatrol 30 and 60 min after KA administration significantly reduced seizure incidence. The authors suggest that vineatrol could potentially be administered in the epileptic state.

Sok et al. [21] studied the anticonvulsive effects of *Petasites japonicum* (BMP), a plant grown in East Asia and used for both culinary purposes and in folk medicine. Its root extracts are still used for headaches and asthma. Prolonged administration of BMP, prior to KA administration, reduced mortality in mice by one half, and administration of the BMP-I subfraction reduced convulsive seizures and also significantly reduced neuronal loss in parts CA_1 and CA_3 of the hippocampus. The authors suggest that BMP-I is the factor responsible for prevention of oxidization lesion in the mouse brain.

Hsieh et al. [41] tested a traditional Chinese herb (*Gastrodia elata* B1—GE) administered to treat epilepsy in a controlled study using the KA seizure model in rats. They found that prior administration of GE significantly reduced in vitro lipid peroxydation in the rats' brains, an effect analogous to the effect of phenytoin (PHT—20 mg/kg). The authors think that GE has an antiepileptic effect and is a free radical scavenger. This antiepileptic effect may be at least partly attributable to the GE's vanilla component [42].

2.3. The pilocarpine model

Pilocarpine, an imidazole alkaloid extracted from the leaves of the *Pilocarpus jaborandi* shrub, is a parasympathomimetic, cholinergic antagonist that acts similarly to acetylcholine. It is often used to evoke epileptic convulsions and epileptic states in animal models. The mechanisms leading to seizures or status epilepticus are unknown. It is thought that oxygen stress plays an important role, but we still do not know which brain structures are more sensitive. Studies of the activity of catalase, a free radical scavenger, have found different effects of the epileptic state on the catalase level in different brain structures [19]. The highest elevation was found in the hippocampus (36%), striatum (31%), and frontal cortex (15%); no changes in the level of catalase activity were found in the cerebellum. The authors think that the endogenous increase in the catalase activity, responsible for removal of free oxygen radicals produced during convulsions, may be a compensatory defense mechanism that counteracts the negative effects of oxygen stress in the status epilepticus. Other researchers have come to similar conclusions [26, 43]. Tejada et al. [43] evoked a pilocarpine epileptic state and found that MDA increased significantly (64%), suggesting oxygen injury. They found a simultaneous increase in the anti-oxidizing activity of catalase enzymes (28%), GSH-Px (28%) and SOD

(21%). On the other hand, vitamin E concentration in the cerebral cortex was reduced (15%) due to increased lipid peroxydation following pilocarpine administration.

Barros et al. [18] applied the same model and found that administration of vitamin E (200 mg/g i.p.) 30 min prior to the administration of pilocarpine (400 mg/kg s.c.) led to increased (214%) catalase activity in the hippocampus compared with rats given only pilocarpine (67%) or physiological saline. The authors think that increased catalase activity may be responsible for the regulation of free radicals evoked by the status epilepticus.

In this same model in rats, prior administration of vitamin C (250 mg/kg i.p.) reduced the negative effects of oxygen stress and neuronal lesion [44]. The latency time to convulsion onset following pilocarpine administration was longer, and mortality in the status epilepticus was reduced compared with the group which did not receive vitamin C or received physiological saline. This study also demonstrated that in the group receiving only vitamin C, the level of lipid peroxidation was lower than in the group that received (a) pilocarpine and (b) pilocarpine and vitamin C. In all the experimental groups, catalase activity in the hippocampus increased compared with the control group which only received physiological saline. The authors think that the neuroprotective function of vitamin C in adult rats may be due to reduced lipid peroxidation and increased catalase activity following convulsions and status epilepticus [44].

2.4. The audiogenic seizure model

Prolonged melatonin administration to rats congenitally predisposed to audiogenic convulsions (the Krushinsky-Molodkina model) had no effect on seizures evoked by a 20 times more powerful auditory stimulus [45]. VPA administration significantly reduced convulsions, but VPA and melatonin combination had a significantly larger anti-seizure effect—it lengthened the latency time and reduced seizure severity. However, the rats receiving the combined treatment displayed a much more rapid onset of myclonia than the rats receiving either VPA or melatonin [45].

3. Counteracting AED-evoked oxygen stress

3.1. AEDs in animal models of epileptic seizures

Researchers using animal models have found a variety of effects of AEDs, administered in various doses, on oxidant and antioxidant processes in an astrocyte culture in rats [46]. Here is a selected list of studied variables: LDH and GS levels, RFT production, lipid peroxidation, and DNA fragmentation. Drugs such as CBZ, TPM, and OXC caused oxygen stress whatever their dose. GBP, LEV, LTG, and TGB, on the other hand, caused no significant metabolic changes whether given in large or small doses. Cortical astrocytes seem to tolerate this latter group of AEDs better than the former group.

In a similar model of rat cortical cell culture, VPA was found to protect against the negative effects of oxygen stress [47]. Administration of VPA for 7 days prevented lipid and protein

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oxidization anomalies. The authors think that by preventing the accumulation of free radicals, VPA affects one or more of the neuroprotective processes.

VPA is a relatively safe drug, but it can sometimes be related to allergic idiosyncratic hepatopathy, a rare condition but more frequent in children less than 2 years of age taking more than one type of AED. The mechanism of toxic hepatopathy is unknown, but it has been suggested that it is caused by oxygen stress which leads to excessive RFT production and reduction of total antioxidant capacity [48, 49]. Therefore, therapeutic strategies or specific medicines that reduce oxygen stress may protect against toxic hepatopathy in patients taking VPA. Sabayan et al. [49] have hypothesized that garlic (allium) preparations may prevent this liver damage by removing free radicals and preventing the reduction of glutathione activity which accompanies the treatment with VPA.

TPM with its many mechanisms of action has undoubted effectiveness in the treatment of epilepsy in children. However, TPM administered in rat stomachs for 3 months may lead to such adverse effects as toxic liver dysfunction [50]. In a study of young rats, it was found that small doses of TPM (40 mg/kg a day) might reduce total antioxidant capacity in the organism and lead to a minor liver pathology. Large doses of TPM (80 mg/kg a day) or a combination of TPM (40 mg/kg) and VPA (300 mg/kg a day) significantly increased the risk of such adverse effects. Glutathione levels in the liver were significantly lower in the rats given large doses of TPM and in the rats on the TPM + VPA regime than that in the rats taking small doses of TPM and the controls given only distilled water. Histopathological examination also revealed disseminated punctual necrosis as well as lipid and degenerative changes in some hepatocytes.

In this same model, TPM (40 and 80 mg/kg i.p.) had no effect either on rats' status epilepticus or mortality, but larger doses significantly reduced KA-evoked lipid peroxidation [51].

LEV (2000 mg/kg i.p.) administered prior to pilocarpine administration (400 mg/kg s.c.) in mice prevented peroxidation increase in the hippocampus (but did not increase the nitrate level or reduce catalase activity in the hippocampus or cortical glutation) [52]. Perhaps the anti-oxidizing, neuroprotective effect of LEV and the consequent reduction of oxygen stress can be attributed to a different mechanism than the one which is active in the case of other AEDs.

In the KA convulsion model in rats, pre-convulsion administration of zonisamide led to an increased antioxidant level in the hippocampus [53]. The authors think that zonisamide has neuroprotective properties against free radicals.

LTG does not lead to detectable increases in lipid peroxydation in rats in vivo [54]. The antiepileptic effectiveness of LTG in the partial complex epilepsy model (stimulation of the dentate gyrus) in rats was in reverse proportion to the level of activity of nitrogen oxide [55].

3.2. AEDs in human epilepsy

When used in human epilepsy, AEDs have various but equivocal effects on the oxidization processes [7, 22]. The authors studied the effects of epilepsy and prolonged AED treatment (CBZ, PHT, and VPA) on the levels of trace elements, electrolytes, and oxidization and

anti-oxidization activity in the blood serum in 70 patients with epilepsy and 14 untreated patients with epilepsy (controls) [7, 22]. They found increased Zn, Ca, Na, MDA, and GSH-Px and reduced copper, ceruloplasmin, and total antioxidant capacity in the treated patients (especially in those treated with VPA); treatment had no effect on the levels of Se, Mg, and K. In the untreated patients with epilepsy, uric acid (a powerful free oxygen radical scavenger) was elevated but the total antioxidant capacity in the serum was reduced, suggesting that different antioxidants had different activities in this epileptic group.

According to these authors, some nutrients may have a positive effect on the reduction of seizure frequency (vitamin B_6, vitamin E, Mg, Mn, taurine, glycine, omega-3 fatty acids; vitamin B_1 may improve cognitive functioning in patients with epilepsy).

In order to prevent the negative effects of LPP, prophylactic or therapeutic replenishment of folic acid, vitamin B_6, vitamin D, and L-carnitine may be advisable. Vitamin K is recommended toward the end of pregnancy in women taking AEDs. In some cases melatonin may reduce seizure frequency. However, supplementation can very seldom substitute AEDs completely [56].

Mahle and Dasgupta [1] found that PHT monotherapy significantly increased lipid hydroperoxidase in blood serum concentration compared with the control group. The total blood serum antioxidant capacity was lower in patients than in healthy controls. These researchers found a weak correlation between lipid hydroperoxidase concentrations, trygliceridemia, and cholesterol level in the serum of patients with epilepsy.

The negative consequences of oxygen stress in the serum were significantly larger in women with epilepsy treated with PHT monotherapy than in healthy women and women with untreated epilepsy [57]. According to the authors, as an addition to glutathione to PHT treatment, modification of the activity of CuZn-SOD enzymes and reduction of copper absorption during pregnancy may prevent the incidence of the aforementioned, albeit somewhat controversial, fetal phenytoin syndrome [58]. PHT is very rarely administered to patients with epilepsy in Poland (1–2%) and probably only exceptionally to epileptic women in reproductive (childbearing) age.

Comparative studies of the effects of PHT and CBZ monotherapies found a significant increase in the blood serum level of MDA and CuZn-SOD and a significant reduction of glutathione in patients treated with PHT compared with a healthy control group and a group with untreated epilepsy [59]. No differences were found for CBZ except for a slight increase in CuZn-SOD activity. All in all compared with PTH, CBZ caused fewer interferences with antioxidant activity, lipid peroxidation, and level of trace elements (Cu, Zn).

VPA used in monotherapy for 60 days in 50 children with epilepsy (mean age 8.5 ± 3.6 years) led to liver dysfunction, free radical production, and DNA oxidation injury in the liver cells and neurons. The general oxidation state, measured by the level of 8-hydroxy-2-deoxyguanosine (8-OHdG), depended on the drug dose [60]. A linear relation was found between the VPA serum level and the lipid peroxidation magnitude. In a group of children with a VPA concentration 114 ± 9.7 μg/ml, peroxidation was significantly higher than in a control group

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of children with a VPA concentration 81.0 ± 8 μg/ml. Free radicals caused DNA oxygen injury due to a significant increase in the serum level of 8-OHdG, which may be a good biological indicator of increased risk of VPA-evoked degeneration.

Other researchers have also found a linear relation between lipid peroxidation and the VPA level in the plasmas of patients with epilepsy [4]. They measured lipid peroxidation spectrofluorometrically, before and after Fenton reaction evocation, in 75 patients and 4 healthy controls. Interestingly, lipid peroxidation was higher in patients with partial epilepsy than in patients with generalized epilepsy and higher in women than men. Gender differences in oxygen stress effects have also been found in PHT-treated patients with epilepsy [57], PTZ rat models [35], and hippocampal sections in patients [61].

A comparative study of the effect of 2-year VPA and CBZ monotherapies on changes in the antioxidant system in children with epilepsy found significant differences in the effects of both AEDs [62]. The researchers measured the level of glutation, GSH-Px, red blood cell SOD, and serum lipid peroxidation. They studied two groups: (1) 25 healthy children and (2) 27 children with epilepsy untreated prior to the study onset, 14 of whom were treated with VPA and 13 with CBZ. The treatment lasted 2 years. Laboratory tests were conducted in treatment months 13 and 24. The antioxidant systems in the children taking VPA for 2 years were more altered than the antioxidant systems of the children taking CBZ.

Another comparative study of the effects of CBZ and VPA on epileptic children found no differences in the serum concentrations of Cu, Zn, Mn, Se, and Mg [24]. The only difference was found for the GSH-Px activity which was significantly higher in the VPA group. There were no differences in the SOD levels.

A more recent comparative study of the effect of VPA, CBZ, and PB monotherapies on the oxidation and anti-oxidation systems in children with epilepsy yielded slightly different results [6]. The control groups consisted of children with untreated epilepsy and healthy children. The researchers found that the level of total antioxidant capacity in the serum was significantly reduced in the group with untreated epilepsy compared with the healthy group. The level of peroxidation was significantly elevated in both the untreated group with epilepsy and the CBZ treatment group compared with the healthy controls. The pattern of results was similar for the children treated with PB and for the control group. According to the authors, children with epilepsy are at risk of oxygen stress due to seizures and AEDs. Their oxidation and anti-oxidation processes are unbalanced. VPA restores this balance more effectively than CBZ or PB.

Bolayir et al. [63] studied the effect of OXC on anti-oxidation processes in 13 adult patients with epilepsy prior to monotherapy and after 1 year of monotherapy. They also studied 15 healthy controls. They measured lipid peroxidation activity, SOD, GSH-Px, and catalase in the red blood cells. The patients had significant differences in the levels of GSH-Px and SOD after 1 year of treatment compared with the pre-treatment levels. The MDA level was also significantly different from the level of the control group and from that assessed before the treatment. These findings suggest that the anti-oxidation systems in patients treated with OXC are negatively affected after 1 year of treatment.

3.3. Dietary management in epilepsy

The idea that a specific way of eating can affect epilepsy was (recognized) first postulated by Hippocrates, who noticed that fasting could prevent convulsions [64]. All forms of dietary therapy that can be used for epilepsy involve ketogenic [65–68] medium-chain triglyceride, modified Atkins, and low-glycemic index diets that restrict carbohydrates and increase fat in the diet. However, most of these metabolic treatments for epilepsy can cause some side effects and nutritional deficiencies such as diarrhea, constipation, nausea, vomiting, and increased acid reflux. There is growing interest in ketogenic diet and it is available in many countries. The reasons why ketogenic diet prevents seizure are not fully understood. One hypothesis is that the ketones produced by the diet are able to enter the brain and reduce the levels of reactive oxygen species and make the brain use energy more efficiently, resulting in fewer seizures [69, 70]. It has been shown that ketogenic diet can produce a significant reduction in seizure frequency in the elderly as well [66, 71, 72].

3.4. Vitamins and minerals

Long-term use of antiepileptic drugs can affect the vitamin and mineral status in epilepsy patients. Antiepileptic drugs have been shown to decrease the levels of the B group vitamins such as folate and vitamins B_6 and B_{12} [73, 74], which are important for controlling the metabolism. For example, the low folate levels caused by AEDs lead to high levels of homocysteine, a risk factor for heart disease [74–76]. Epileptic patients have reduced folic acid levels due to the use of AEDs [77]. It has been reported that epileptic patient using AEDs should be supplemented with B vitamins, especially with the metabolically active form of folic acid, L-methylfolate, to reduce the homocysteine levels [78].

Significantly lower levels of vitamin D are found in the blood of patients taking antiepileptic drugs. The explanation is that the use of AEDs increases the liver enzyme activity of cytochrome P450, which is involved in breaking down of vitamin D [79–83]. Therefore, patients who are taking AEDs may need to take vitamin D and calcium supplements [84].

Pyridoxine-dependent epilepsy is a rare autosomal recessive disorder characterized by a combination of various seizure types that usually occurs during the first hours of life and is unresponsive to standard anticonvulsants, responding only to immediate administration of pyridoxine hydrochloride (vitamin B_6) [85–87]. However, not all types of seizures can be treated with pyridoxine, but a potentially effective option is the biologically active form of vitamin B_6 (pyridoxal-5-phosphate) [88–90].

Other antioxidants that have been reported to have the capacity to mitigate mitochondrial oxidative stress in the brain and lower seizure frequency in epilepsy include vitamin E, vitamin C, and selenium [91–97]. Vitamin E is shown to prevent several types of seizures in animal models [98, 99]. Epileptics are also more likely to have low vitamin E levels, though this may be a result of taking antiepileptic drugs [100].

Magnesium is essential for enzyme function including ATP-generating reactions [101]. It stimulates the production of prostacyclin and nitric oxide [102], supports mitochondrial

integrity, and modulates ion transport [103, 104]. Magnesium has been shown to be associated with many health conditions; for example, it is essential for brain function and development [105, 106]. Epileptics have significantly lowered serum magnesium levels, and the seizure activity correlates with the level of hypomagnesemia [107–109].

3.5. Melatonin

Numerous studies on melatonin conducted over the last 30 years have confirmed that this neurohormone is susceptible to circadian rhythms, has antioxidant properties, and modulates immunological activity [110]. Melatonin affects the blood platelets and prolongs their life. It is transported by platelets to all body tissues. Thanks to its lipophilic function, it crosses the cell membranes easily, regulates blood-tissue exchange, and interacts with the endothelial cells. Platelets can behave like mobile and wandering serotonergic and/or melatonergic elements, comparable with cerebral neurotransmitter release [111].

Melatonin was found to be a potent free radical scavenger, and therefore it reduces oxygen stress and prevents excessive arousal from injured neurons as demonstrated with various animal models and humans.

The neuroprotective effect of melatonin was confirmed in a randomized, double-blind trial with epileptic children receiving VPA monotherapy [112]. The researchers administered VPA + melatonin to 15 children and VPA + placebo to 14 children for 14 days. The posttest GSS-R level was significantly higher ($p = 0.05$) in the VPA + melatonin group, and the percentile difference in the value of this enzyme was also significant ($p = 0.005$).

Gupta et al. [113] found that CBZ and VPA administered in monotherapy to 22 children with epilepsy had differential effects on the blood serum levels of melatonin. In both groups the researchers measured endogenous and exogenous melatonin 30 min after administration. The serum level of melatonin was higher in the CBZ group (165 pg/ml ± 50–350) than in the VPA group (78 pg/ml ± 13–260). The authors think that these differences in the level of melatonin can be attributed to different effects of these two AEDs, additional epilepsy and CBZ-dependent RFT increase, or differences in melatonin kinetics in conditions of oxygen stress. In a study by the present author [29], adding melatonin to the patients' regular AED for several weeks did not affect seizure frequency in cases in which the course of the epilepsy was severe.

3.6. Selenium

The neuroprotective effect of selenium, one of the trace elements, is related to selenoproteins which are antioxidants [114]. Selenium insufficiency has been found in young children with severe mental retardation and drug-resistant epilepsy [115]. Oral administration of selenium (3–5 μg/kg m.c.) reduced seizure frequency, improved EEG recordings, and normalized liver activity. In another study, the serum level of selenium in 30 patients with intractable epilepsy was also lower (66.88 ng/ml ± 17.58) than in healthy controls matched for age, socioeconomic status, and place of residence (85.93 ng/ml ± 13.93) ($p < 0.05$) [116]. However, the low selenium level in the blood serum did not correlate with the measured risk factors for drug-resistant epilepsy: age of onset, infant seizures, neurological disorder, or etiology of epilepsy.

The clinical implications of these results, and those quoted above, should be interpreted carefully because epilepsy is such a complex and heterogeneous disease, as suggested by the findings reviewed in this article.

3.7. Drug-resistant epilepsy: polytherapy

Drug-resistant seizures force the physician to use polytherapy with various AEDs. Polytherapy increases the production of free radicals and disturbs mineral balance to a greater extent than monotherapy, leading to increased oxygen stress. Both increased free radical production and inhibition of the enzymes that remove scavengers lead to adverse states and aggravation of the morbid process [22, 117].

Patients with epilepsy and on long-term AED therapy are at greater risk of atherosclerotic changes in the arteries [118]. Metabolic dysfunctions in these patients have been attributed to altered homocysteine, lipid and lipoprotein metabolism, and uric acid. According to the authors, these dysfunctions are indications for routine antioxidant multivitamin supplementation (folic acid, B_{12}, B_6, C and E, and beta-carotene). The protective, anti-atheromatic effect of vitamins is based on their antioxidant and anti-inflammatory properties. However, in the other research quoted above, increased lipid hydroperoxidase concentration had only weak correlations with the risk factors for vascular changes (triglyceridemia, cholesterolemia) [1].

Tupeev et al. [119] found a positive effect of prolonged vitamin E treatment (600 mg/day) in patients with generalized seizures: seizure frequency was reduced, EEG improved, and antioxidant activity increased.

Assuming that AEDs can trigger free radical production and lipid peroxydation, Hung-Ming et al. [23] studied the effects of TW970, a modified version of the Chinese herbal specific *chaihu-longu-muli-tang* which has antiepileptic and antioxidant properties. They administered it for 4 months to 3 groups of patients: (1) 20 patients with drug-resistant epilepsy (at least 4 attacks a month), (2) 20 patients with mild epilepsy (fewer than 4 attacks a month), and (3) a control group of 20 healthy adults matched for age. The patients were tested prior to the introduction of TW970 and 4 months after the introduction. In the resistant group, seizure frequency dropped from 13.4 ± 3.4 to 10.7 ± 2.5 a month, but the difference was not significant ($p = 0.084$). Prior to the TW970 introduction, the resistant epilepsy group had significantly higher lipid peroxidation and increased MDA and CuZn-SOD activity, including reduced glutathione, compared with the healthy control group. After 4 months of TW970 treatment, the levels of MDA and CuZn-SOD normalized in the resistant epilepsy group, whereas no significant changes in the parameters were found in the mild epilepsy group, either prior to or following TW970 therapy. The authors suggest that TW970 may reduce seizure frequency in resistant epilepsy and that antioxidants may be responsible for this effect.

Many Native American plants are valued by local medical practitioners for their positive effects on health and a number of diseases, including epilepsy: *Celastrus paniculatus* L. (CP), *Picrorhiza kurroa* (PK), and *Withania somnifera* L. (WS). It has been found that extracts of these plants are dose-dependent free radical scavengers and that they prevent DNA injury due to oxygen stress. PK extract had a more powerful effect than CP or WS. These favorable biological

properties have been attributed to anti-stress, immune-modulating, anti-inflammatory, and antiaging effects [120].

3.8. The effects of surgery on AED-resistant temporal lobe epilepsy

López et al. [121] studied the activity of antioxidant enzymes (SOD, catalase, and GSH-Px) and markers of oxygen stress-induced molecular neuronal injury (MDA and RFT) before and at various moments after epileptic focus resection in 9 patients and a control group of 32 healthy individuals. All the studied variables normalized postoperatively except the SOD activity. Several other interesting observations seem to be somewhat related to these findings. Turkdogan et al. [122] found that increased lipid peroxidation in the plasma may be causally related to the presence of abnormal structural changes of the brain, as assessed by magnetic resonance (MR) rather than to the treatment of epilepsy, focal or generalized epileptic discharges in the EEG, duration of epilepsy, or to seizure frequency (more or fewer than 1 seizure a month). The authors found an increase in serum lipid peroxidation in 52 children with epilepsy, treated with one or more AEDs and with an abnormal brain MR, compared with 16 healthy children (the difference was significant, $p < 0.05$). No significant differences in antioxidant enzymes were found in either group. The children with well-controlled seizures and the ones with drug-resistant seizures but normal MRs had higher SOD activity than the children in the control group ($p < 0.05$). GSH-Px (an antioxidant) activity was not significantly different among the children with epilepsy and the control group [123]. This interesting and heterogeneous picture of enzymatic activity in children with epilepsy and control children suggests that the relations between various laboratory tests and numerous variables associated with the heterogeneity and treatment of epilepsy are very complex. Although the authors took seizure frequency into consideration, they did not specify when the blood tests were taken relative to the experienced or imminent seizure nor did they report EEG recording of epileptic activity prior to the blood test. This makes it very difficult to interpret the causal relations among the results of the various tests and their epileptic correlates.

4. Conclusions

Epilepsy is a brain disease that has been linked with abnormal brain oxidation processes. Despite a vast spectrum of anticonvulsant therapies toward halting abnormal electrical activity in the brain, it may be suggested that antioxidants can perhaps in the future play a role in controlling seizures. Therefore, further study is necessary, in order to display whether widely accessible antioxidants such as vitamin C or vitamin E in fact possess the ability to synergistically act with anticonvulsant medications and whether this combination can result in improved control of epilepsy.

1. Research on animal models and patients with epilepsy suggests that both epileptic seizures and AEDs (especially polytherapy), as well as other factors evoking oxygen stress, have a negative effect on the oxidation-anti-oxidation balance.

2. AEDs and drug dosage have a differential effect on oxygen stress. The research findings are equivocal or even contradictory, however, with respect to the different AEDs. New AEDs usually have a more favorable effect on the oxidation and anti-oxidation enzyme balance and trace element and electrolytic homeostasis.
3. Neuroprotectors (trace elements, vitamins, and other antioxidants) help to reduce seizure-induced oxygen stress, and therefore it is suggested that they should be used to supplement AED treatment.
4. The fact that AEDs can lead to oxidation-anti-oxidation imbalance suggests that we need to adopt a new approach to the treatment of epilepsy and AED synthesis. We need to take the negative effects of oxygen stress into consideration.
5. No drug is completely safe and effective, and considering the complex and heterogeneous nature of epilepsy, it is evident that the optimal treatment of each individual case requires a carefully performed diagnosis and the application of research-based therapy, which includes the use of personalized medicine as well as drugs. Genes are influenced by the environment and therapy. The value of food as medicine was acknowledged several centuries ago. Therefore, monitoring genomic information, pharmacogenomics, and the food-drug interactions is important as it helps to personalize the treatment to meet the patient's needs.
6. It is important to know the patient's specific characteristics including previous history, lifestyle, age, gender, weight, diet, environment, etc. They can be valuable tools to improve the quality of life of the epileptic. Targeted medicine/personalized medicine is the concept of managing the patients' health. It is believed that Hippocrates (c. 460 BC–c. 370 BC) was the one who applied this idea to medicine. He is best remembered today for his famous Oath "It's far more important to know what person the disease has than what disease the person has."

Author details

Jerzy Majkowski[1]*, Tuomas Westermarck[2] and Faik Atroshi[3†]

*Address all correspondence to: fundacja@epilepsy.pl

1 Epilepsy Diagnostic and Treatment Centre, Foundation of Epileptology, Warsaw, Poland

2 Rinnekoti Research Centre, Espoo, Finland

3 Department of Pharmacology and Toxicology, University of Helsinki, Finland

†Deceased.

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