Review

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Role of the oxidative stress in the pathogenesis of epilepsy

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Abstract

Epilepsy is a neurological disorder marked by sudden recurrent episodes of sensory disturbance, loss of consciousness, or convulsions, associated with abnormal electrical activity in the brain. We still do not know all mechanisms involved in the pathogenesis of epilepsy. In this article we wanted to review role of the oxidative stress in the pathogenesis of epilepsy. First, we described what is oxidative stress and what is its mechanism of action. Some important reactive species are described. We also accentuated hypersensitivity of nervous system to the action of reactive oxidizing substances. In the main text, we described the oxidative stress, mitochondrial dysfunction and disorders in calcium homeostasis as epileptogenic factors. We summarized laboratory parameters that can be used as indicators of oxidative stress in epilepsy. Especially, the influence of oxidative stress on glutamate transporter activity was described. Involvement of Nrf2 in the pathogenesis of epilepsy was also explained. At the end, we presented some of clinical studies that described correlation between oxidative stress and epilepsy.

Keywords: Epilepsy, mitochondria, Nrf2, oxidative stress, radicals

INTRODUCTION

The role of free radicals in the development of numerous pathophysiological events has been studied for decades. In reaction with biomolecules, free radicals have the ability to impair cellular morphology, function, ionic homeostasis, and enzymatic activity. Free radicals are atoms, molecules or ions containing one or more unpaired electrons in the last molecular or atomic orbit. This makes this class of compounds very reactive. Due to the tendency to pair uncomparable electron(s) in the last orbit, free radicals act as extremely strong electrophiles, or strong oxidizing agents. In reaction with a substrate free radicals are reduced and lose the character of free radicals, and the substrate is oxidized and becomes the free radical of the second generation, thereby starting a chain of radical reactions.

The Most Important Reactive Species

The superoxide anion radical ($O_2^{\bullet-}$) is formed by a single-electron reduction of molecular oxygen.

The metabolic path of this radical depends on the pH of the environment and can be started in the direction of oxidation or reduction. In alkaline conditions, the pathway of reduction of $O_2^{\bullet-}$ to hydrogen peroxide (H_2O_2) is favorit, by means of compounds containing proton (ascorbate, Fe-S-clusters). Conversely, under acidity conditions, $O_2^{\bullet-}$ is oxidized to O_2 in the presence of Fe^{3+}, Cu^{2+}, ferry-cytochrome and quinone (1).

Hydrogen peroxide is formed by self-oxidation of $O_2^{\bullet-}$. This reaction can be followed by enzymatic and non-enzymatic routes. In the presence of the enzyme superoxide dismutase (SOD), the reaction proceeds 4 times faster than the non-enzymatic reaction. Hydrogen peroxide belongs to stable reactive oxygen types of non-radical type, as there are no unpaired electrons in the last orbit. However, this molecule easily passes through the cell membranes and, in addition, is the starting reactant to create a highly reactive hydroxyl radical ($HO^{\bullet}$) (1).

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The $HO^{\cdot}$ is, as has been said, the most reactive oxygen species (ROS). Extremely short-lived, which means it is highly reactive, attacks of the purine and pyrimidine bases, e.g. reacting with guanine builds an 8-hydroxyguanine radical and other products that are closely related to the incidence of cancer formation (1).

At the end of the 1980s, nitrogen (II) oxide (NO) and similar compounds endothelial-relaxing compounds played a role in inflammatory processes, thrombosis, immunity, and neurotransmission. Enzyme nitric oxide synthetase (NOS) synthesizes NO from L-arginine, with NO and citrulline produced in an equimolar relationship (2). Under certain conditions, citrulin passes back to arginine, which closes the L-arginine-NOS-citrulline-L-arginine circuit. The final degradation products of NO are nitrites (NO_2^-) and nitrates (NO_3^-). Protective and toxic effects of NO are attributed to different redox states (nitrosium ion NO^+, nitroxyl anion NO^- and nitrogen oxide radical, i.e. nitroxyl radical, $NO^{\cdot}$). NO^+ and NO^- show protective properties, as opposed to $NO^{\cdot}$. Metals with variable valence are targeted sites for the binding of $NO^{\cdot}$, whereby the NO-metal complex is built. NO binds to hemocyloxigenase, catalase, cytochrome C oxidase, hemoglobin, and NOS, and this reaction is reversible in character, as opposed to reaction where iron-bound for Fe-S in NADH (nicotinamide adenine dinucleotide)-ubihinone oxidoreductase, cis-akonitases, and succinate oxidoreductase. The nervous system has an extremely diluted anatomical and neurochemical network in which the creation and activity of $NO^{\cdot}$ take a noticeable place. It circulates from the circulation of L-arginine into astrocytes and neurons where certain types of NOS are activated, so that the created $NO^{\cdot}$ is included in the regulation of the function of neurons and other cells. In the basal ganglia it is possible to increase or decrease the activity of gamma amino-butyric acid (GABA) under the action of $NO^{\cdot}$ (2).

It is confirmed that, under physiological conditions, acetylcholine was released under the influence of $NO^{\cdot}$. At higher concentrations of $NO^{\cdot}$, a negative feedback mechanism is initiated via the NMDA (N-methyl-D-aspartate) receptor, and the catecholamine synthesis increases. The release of dopamin is reduced at low concentrations of NO• while at high concentrations it is increased. The release of serotonin in the hypothalamus and the locus ceruleus is regulated through NMDA and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/cainate receptors activated with glutamate, released under the influence of $NO^{\cdot}$. Indirectly, $NO^{\cdot}$ controls the release of histamine and basically this regulation also lies in the release of excitatory amino acids under the effect of $NO^{\cdot}$ (2).

Control of the release of neurotransmitters by $NO^{\cdot}$ is mainly accomplished through excitatory amino acids. Although low $NO^{\cdot}$ concentrations reduce glutamate release, long-term potentiation processes are not disturbed. On the contrary, these processes are not only fully realized, but long-term release of glutamate is achieved through them. The opposite effects on glutamate are achieved at high concentrations of $NO^{\cdot}$ (2).

Antioxidant Protection

In physiological conditions, the antioxidant system (AOS) neutralizes the harmful effects of free radicals, while in pathological conditions, with increased psychophysical efforts, and in the metabolism of individual xenobiotics, the capacity of this system is insufficient, and the need for additional intake of antioxidants is indicated. High concentration of free radicals, whether due to excessive creation or inadequate removal, or inadequate response of key (direct) and secondary (indirect) AOS mechanisms, leads to the development of oxidative stress. The antioxidant protection system in humans is very extensive and consists of several levels: primary (free radical removing enzymes and non-enzymes components), secondary (specific oxidoreductases and reparative enzymes) and tertiary level (proteins that chelate metals with variable valence) (3).

Superoxide dismutase is a metalloenzyme that catalyzes the reaction of dismutation of $O_2^{\cdot-}$ to H_2O_2 and O_2, with the redox status of the metal ion (Cu or Mn) in the active center. It is present in all tissues and a specific activity has been shown in the brain (1, 3).

Glutathione peroxidase (GSH-P) is a selenoprotein found in all mammalian tissues. There are four of its isoenzymes, where GSH-Px is best studied. It is localized in cytosol and in mitochondria and plays a role in the reduction of low concentrations of H_2O_2 and organic hydroperoxides to alcohol and water (1, 3).

Glutathione reductase catalyses the reduction reaction of oxidized glutathione, with the participation of NADPH (nicotinamide adenine dinucleotide phosphate) coenzyme. This enzyme is included in the AOS, since the glutathione disulfide reduction provides sufficient amounts of GSH (glutathione) (1, 3).

Oxidative Stress in the Central Nervous System

It is known that the central nervous system (CNS) in relation to other organic systems is most sensitive to the action of reactive oxidizing substances, such as, free radicals. Given that the brain is at the highest level of development compared to other organic systems, the logical consequence is that this, still minimal explored part of the organism, has the most complex and extremely sophisticated functional organization. The functional complexity of the brain rests on certain structural, biochemical, and neuroanatomic specificities, which together make it particularly sensitive to the action of free radicals. In the structure of the brain parenchyma, the content of polyunsaturated phospholipids exhibits high levels of presence, which is the basis of a particular susceptibility to oxidative damage. Also, in the brain there is a high iron content, reaction catalysts

in which compounds with free radicals are formed. By contrast, the brain is characterized by a relatively low level of protective antioxidant enzymatic systems, such as catalase and SOD, as well as glutathione tripeptide involved in the function of GSH-P. It is important to note that although the brain accounts for only 2% of the body's average body weight, more than 20% of the energy that is generated at rest is spent precisely by this organ, primarily on synaptic transmission (4).

However, it should be emphasized that not all parts of the brain are equally sensitive to oxidative stress. In numerous experimental studies, it has been shown that certain brain structures are more sensitive than other parts of the brain in various pathophysiological events (ischemia, degenerative processes, toxic compounds). The phenomenon of selective sensitivity of the brain to different noxa is known for a very long time. The most sensitive parts of the brain include the pyramidal neurons of the CA1 and CA3 hippocampus sectors, the small pyramidal neurons of the third layer of the bark of the brain, striatum, and cholinergic neurons in nc. Basalis Meynert of the basal forward brain.

A number of studies started from the assumption that there is a common mechanism in a series of pathophysiological mechanisms that would form the basis of selective damage only to individual brain structures triggered by various causes. Studies in rats have shown that if the blood flow through the brain during the epileptic attack, the blood glucose and blood oxygen content is maintained in physiological frameworks, no energy depletion of the nerve cells occurs, but there is a characteristic selective damage to sensitive neuronal populations. This indicates that an epileptic seizure can lead to damage to the neuron unrelated to changes in energy metabolism. As a probable cause of damage only to certain groups of neurons, a specific biochemical organization of particularly sensitive brain structures is listed. The peculiarity of the biochemical organization, in conjunction with certain neuroanatomic communications of selectively sensitive structures, provides the basis for the time and space spread of toxic reactions caused by free radicals. Selectively sensitive brain parts are characterized by the presence of high density of glutamate postsynaptic NMDA receptors, mediators in the phenomenon of excitotoxic damage to the nerve cell. Excessive activation of these receptors is a key mechanism in the basis of prolonging and spreading the epileptic seizure and consequent neuronal damage. Blockade of the NMDA receptor with preparations that prevent prolonged potentiation (D-aminophosphonovalerate, D-aminophosphonoheptanoate, death-associated protein 5, death-associated protein 7, phenicic acid, ketamine) results in strong anticonvulsant activity, as well as the protection of particularly sensitive parts of the brain (5).

As during the seizure and brain ischemia, the process of neuronal extinction is mediated by excitatory amino acids. Early or immediate excitotoxicity is the result of prolonged depolarization caused by glutamate. It is characterized by the rapid formation of neuronal swelling, and it is dependent on the extracellular concentration of sodium and chlorine ions. The primary effect of glutamate, acting on all three types of receptors (NMDA, cisquatic and kainate), is to open membrane channels for sodium, which leads to its intense infusion, depolarization of the membrane and the accompanying entry of chlorine and water (6).

Late or delayed cell death of neurons in ischemia is also a consequence of excitatory neurotransmission and mediated by excessive calcium accumulation. It is distinguished by delayed and gradual disintegration of neurons, it is dependent on extracellular calcium concentration and mediated by its transmembrane entry into the neuron. Excessive entry of calcium into the cell is considered one of the general mechanisms of damage to different types of cells in conditions of action of toxins, ischemia and immune mediators. The increased concentration of free cytosolic calcium, which extends beyond physiological limits, triggers more harmful biochemical reactions in the cell, primarily the formation of free radicals (7).

Oxidative Stress and Epilepsy

Epilepsy is a neurological disorder marked by sudden recurrent episodes of sensory disturbance, loss of consciousness, or convulsions, associated with abnormal electrical activity in the brain. The first experimental evidence of the correlation between oxidative stress and epilepsy originate in 1989 when a group of scientists demonstrated an increased activity of SOD in experimental animals treated with biculins (competitive GABA antagonist, inhibitor neurotransmitter) (8). Since then, a large number of studies have been published to explain the relationship between oxidative stress and epilepsy.

Although it is still unknown whether oxidative stress is the cause of epileptic events or oxidative stress is the result of epileptic seizures, it is clear that prolonged excitation of neurons caused by epileptic seizures leads to an increase in the concentration of ROS, which then contribute to brain damage (9). Additionally, hyperproduction of free oxygen radicals leads to disorders of intracellular calcium homeostasis, which modulates the excitability of neurons and synaptic transmission, making neurons more vulnerable and causing energy crash and neuronal loss (10). It is also known that the accumulation of free radicals due to repeated epileptic seizures leads to prolonged neuronal damage, which then creates predispositions for the development of other neurological diseases.

The Role of Oxidative Stress, Mitochondrial Dysfunction and Calcium Homeostasis in Epilepsy

The correlation of oxidative stress and epilepsy has been demonstrated both in experimental and clinical studies. The parameters followed in the studies are different: F2-isoprostenes derived from the peroxidation of arachidonic acid; ad-

vanced oxidation protein products (AOPP) that are an indicator of protein oxidation; non-protein bound iron (NPBI) which is a prooxidant substance capable of catalyzing the Fenton reaction; 8-hydroxy-2'-deoxyguanosine (8-oxo-dG) which is a key marker of deoxyribonucleic acid (DNA) damage, malondialdehyde (MDA), nitrogen (II) oxide (NO) and etc. Antioxidant markers that are monitored in the studies are: glutathione reductase, GSH-P, SOD, catalase and many other non-essential AOS components.

The use of adequate animal models in these studies is of great importance in order to understand the mechanisms underlying epileptogenesis. The most commonly used models are based on the use of hemoconvulsants, such as pilocarpine and kainic acid. Kainic acid is an analog of glutamate that causes depolarization and then epileptic seizures, primarily stimulating hypokampal neurons. Pilocarpine is a muscarinic receptor agonist capable of inducing epilepticus status. Other used compounds include: strychnine, pentylentetrazole, quinoline, N-methyl-D, L-aspartate, and all are NMDA receptor agonists. Also, there is not a small number of studies in which epileptic seizures in experimental animals are caused by electrostimulation. In comparison with humans, the brain of experimental rats proved to be the most suitable for the above research (11).

Indicators of Oxidative Stress in Epilepsy

The results of numerous studies demonstrate an increased concentration of biomarkers of oxidative stress in epilepsy: in some regions of the rat brain after injection of pentylentetrazole or kainic acid, increased lipid peroxidation has been shown while the same change has been demonstrated after electrostimulation in the entire rat brain (12, 13). In addition, a statistically significant increase in the concentration of 8-oxo-dG was detected in both cases (14).

We will first mention the role of $O_2^{\cdot-}$ in the pathogenesis of epilepsy. The central mechanism of action of this radical is direct oxidation and consequent inactivation of Fe-S protein, such as, for example, akonitase, one of the Krebs cycles enzymes, which catalyzes the reversible reactions of stereospecific isomerization of citrate, cis-akonite, and isocytes in mentioned cycle. This oxidation of akonitase, or the release of Fe^{2+} from the complex $[4Fe\text{-}4S]^{2+}$ stimulates the Fenton reaction, i.e. the production of Fe^{3+} and $HO^{\cdot}$ which then triggers a further series of oxidation of biomolecules, among others, mitochondrial DNA, proteins, lipids, and the like. In addition, the blocking of akonitase blocks the Krebs cycle, which is the main source of reduced coenzymes, and thus blocks the synthesis of ATP. The combined energy depletion of neurons, the increased concentration of reactive Fe^{3+} as well as the abundant production of free radicals are clear causes of inadequate neuronal functioning and increased tendency for both the development of an epileptic attack and its death (15).

Special evidence of the influence of oxidative stress on the pathogenesis of epilepsy comes from a study with transgenic animals. Rats with genetically determined hyperexpression of SOD activity are resistant to epileptic seizures induced neurodegeneration (i.e., loss of neurons), whereas those with partial deficiency of this enzyme exhibit a tendency to mentioned changes (16).

Although the brain is an organ in which, in comparison with other organs of the human organism, there is a significantly lower catalase activity, it is sufficient to metabolize a large amount of hydrogen peroxide. Significant increase in catalase activity has been demonstrated in experimental epilepsy models. This increased activity of catalase is thought to be the consequence of the enzymatic antioxidant response to increased production of free radicals (17).

The activity of NADPH oxidase (NOX) has been proven in numerous tissues. This enzyme has seven isoenzymes: NOX1, NOX2, NOX3, NOX4, NOX5, DUOX1 and DUOX2 (18), with the largest activity in the neurons being NOX1, NOX2, NOX3 and NOX4. Experimental studies have proven the role of NOX2 in the onset of epileptic seizures. Namely, a statistically significant increase in the activity of this isoenzyme has been demonstrated during epileptic seizures (19). Also, in various experimental models, inactivation of the activity of this enzyme has been shown to be accompanied by a reduction in the incidence of attacks (20). In addition, NOX2 is involved in the pathogenesis of hypotension after the status of the epilepticus, because the increased activity of this enzyme is detected in the rostral ventrolateral medulla, the key nucleus of the baroreceptor reflex (21).

The following evidence of the correlation between oxidative stress and epilepsy is derived from studies in which the consequences of the lack of 3-hydroxyisobutyl-CoA hydrolysis, one of the mitochondrial enzymes, were investigated. Mutation in the gene for this enzyme is presented as one serious neurodegenerative disease with associated frequent seizures. The explanation lies in the fact that 3-metaacryl-CoA, a metabolite accumulated in the absence of this enzyme, is a good source of free radicals that oxidize cysteine residues in numerous enzymes involved in the process of oxidative phosphorylation, which disrupts the structure of the mitochondria (22).

Another evidence of the effect of oxidative stress on the occurrence of epilepsy is the increased incidence of epileptic seizures in experimental animals, which in some studies were locally in the cortex of the large brain administered iron in others, certain mitochondrial toxins (3-nitropropionic acid) were injected and in third were exposed to increased partial pressure of oxygen (hyperbaric hyperoxia) (23-25). In all three groups of studies there was an increased production of ROS, and consequently an increased incidence of epileptic seizures, in up to then fully healthy animals.

The Influence of Oxidative Stress on Glutamate Transporter Activity

We will now look more closely at the role of ROS on the activity of the glutamate transporter. It is known that glutamate is an excitatory neurotransmitter, and as discussed earlier its role in the pathogenesis of epilepsy is clear. Transporters of neurons and glial cells are responsible for preventing excessive accumulation of glutamate in synapses. Glial transporters for glutamate GLAT (EAAT-1) and GLT-1 (EAAT-2) as well as three glutamate transpoters on neurons EAAT-3, EAAT-4 and EAAT-5 are plasma membrane proteins. Among the above, it is considered that the most important of these are glial transporters, especially GLT-1, which is particularly expressed in the hippocampus and cerebral cortex. The aforementioned transpoter has two properties: it is particularly sensitive to low concentration of ATP (adenosine triphosphate), but also to an increased concentration of ROS. From here, a clear conclusion is drawn that oxidative stress induces reduced ATP synthesis and oxidative stress will induce a disorder of the activity of mentioned transporter, resulting in an increased concentration of glutamate, thereby increasing the excitability of neurons (26). In addition, experimental evidence suggests that the use of glutamate transporter inhibitors (eg, trio-β-benzyloxyaspartate) prolongs epileptiform activity in young rats (27). Also, reduced GLT-1 expression has been demonstrated in the cortex of rats with genetically predisposed epilepsy (28).

The extracellular glutamate taken by said glial cell transporter is rapidly converted into glutamine, non-cytotoxic amino acid, by the action of glutamine synthetase. Glutamine can then be transported again to the neuron where the glutamate will be synthesized. Reduction of the indicated cycle was demonstrated in vivo by magnetic resonance spectroscopy in the hippocampus of patients suffering from temporal lobe epilepsy (29). The results of glutamine synthetase expression in material obtained by surgical resection of these patients are contradictory. The decreased activity of the enzyme was demonstrated in patients whose pathoanatomic substrate was hypocampal sclerosis, while in others the increased activity of the enzyme was detected (30). However, it is known that glutamine synthetase is subject to oxidation and nitrosylation by reactive oxygen and nitrogen species by the fact that ONOO (peroxynitrite)-translation of the tyrosine residue into inactive forms. In support of this is illustrated by the fact that inhibition of NOS results in an increase in the activity of glutamine synthetase (31).

The Role of Mitochondria and Calcium Metabolism in Epileptogenesis

Mitochondria were discovered at the end of the XIX century, and only seventy years later they would describe their role in the production of ATP (hemiosmotic hypothesis). Later, the importance of mitochondria in the maintenance of calcium homeostasis was confirmed later, and in the end, their role in apoptosis-programmed cell death, mediated by the release of intramithohondrial cytochrome c. The earliest experiments have shown that during an epileptic seizure, especially if prolonged, there is a decrease in the concentration of available glucose molecules by neurons, resulting in energy depression of neurons.

There is clear evidence that mitochondrial dysfunction affects the excitability of neurons and synaptic transmission. Decreased intracellular concentration of ATP and intracellular calcium homeostase disorder are contributing factors in the development of epilepsy. On the other hand, epileptic seizures are a trigger for mitochondrial damage and their secondary dysfunction, from where we conclude that there is circulus vitiosus: mitochondrial damage - epileptic seizure - mitochondrial damage.

The use of previously mentioned experimental models has demonstrated the role of calcium in epileptogenesis. Excessive calcium entry into neurons during an epileptic seizure has been demonstrated in rat experiments in which the bipolar disorder is caused by bactericidal (competitive GABA-A receptor antagonist) or L-allylglycine. It has been proven that this sudden and excessive Ca^{2+} entry leads to neuronal death, especially in the CA1 and CA3 regions. At least two important biochemical pathways Ca^{2+} in neurons have been described. The first is mediated by a mitochondrial unibody mitochondrial uniporter (MCU) which represents the main site of Ca^{2+} entry into the neuron mitochondrial. The whole process is dependent on the circulation of hydrogen ions (H^+) and sodium (Na^+). After passing the mitochondrial membrane, calcium is precipitated as calcium phosphate or activates the enzyme complex of oxidative phosphorylation, resulting in increased synthesis of ROS. Inhibition of MCU activity in certain experiments significantly reduced the intracellular amount of ROS (32).

Opening the mitochondrial permeability transition pore is another way to pass Ca^{2+} through the mitochondrial membrane. This protein is located in the inner mitochondrial membrane, and recently it has been discovered that it is actually an ATP synthase dimer (complex V respiratory chain). An epileptic seizure induced damage to the neuron is accompanied by an increased production of ROS, which then stimulates the opening of this transporter, the release of calcium from the mitochondria to the cytosol, and the launch of well-known mechanisms of programmed cell death (activation of hydrolytic enzymes - e.g., lipase, caspase, etc.) (33).

There are various calcium transporters on the plasma cell membrane. The NMDA receptor is a heterodimer composed of two GluN1 (glycine-binding subunit) and two GluN2 subunits and is permeable primarily for Na^+ and Ca^{2+} ions. The role of this receptor in the pathogenesis of epilepsy is supported by the fact that the hyperactivity of this receptor has

been found in focal cortical dysplasia, highly epileptogenic lesion (34). The proof is also the fact that in the NMDA-receptor encephalitis, a disease in which there are activating antibodies to the N-terminal domain of the GluN1 subunit of this receptor, the main clinical symptomology is just epileptic seizures (35). Also, some mutations in the subunits of this receptor (GRIN (Glutamate Ionotropic Receptor NMDA)1 and GRIN2B) are the cause of epileptic encephalopathies with clinically manifest epileptic seizures (36). On the other hand, there are AMPA receptors for calcium, which also play a role in epilepsy, but above all in the pathogenesis of status epilepticus.

The Role of Nrf2 in the Onset of Epilepsy

In the past few years, researchers have focused attention on the molecular pathways of oxidative stress in epilepsy, especially considering the role of the transcription factor Nrf2 (nuclear factor erythroid 2-related factor 2), which is presumed to play a central role in the regulation of the AOS. One of the negative cytoplasmic regulators of this transcription factor is KEAP1 (Kelch-like ECH associated protein 1) responsible for ubiquitination and proteasomal degradation of Nrf2 to control the activity of this transcription factor (37). There is experimental evidence that Nrf2 could be a potential target for new drugs for chronic neurological diseases, including epilepsy. Potential drugs bind to KEAP1, which stabilizes the Nrf2 molecule and enables its translocation into the nucleus where it binds as a heterodimer together with a Maf transcription factor for a specific DNA sequence that stimulates the transcription of the gene for certain antioxidant proteins: glutathione-S-transferase, NADP(H): quinone oxidoreductase 1, but also for enzymes involved in glutathione biosynthesis and its reparation, which significantly improves the antioxidant protection system in the cell. An example is the small molecule RTA408, which is currently used in clinical studies to treat mitochondrial myopathies and Friedrich's ataxia. Also, dimethylfumarate, a drug that is approved for the treatment of multiple sclerosis, acts exactly like the Nrf2 inducer (38).

Involment of Microglia and Astrocytes in the Pathogenesis of Epilepsy

Microglia are known as brain's main immune cells that regulate neurogenesis, promote survival of neurons, modulate axonal wiring, induce synapse formation and contribute to the maintenance of synaptic function. Some recent studies showed that after acute seizures or status epilepticus induced by convulsive drugs microglia are rapidly activated in the brain regions affected by the convulsive stimuli (39). Activated microglia release proinflammatory citokines and ROS, which may lead to neuronal hyperexcitability and neurodegeneration. The microglial activation together with astrocytic activation contributes to the process of epileptogenesis in animal models of epilepsy. Those processes are described in temporal lobe epilepsy in most recent researches.

The role of microglia in the epileptogenesis has been studied by using minocycline, an inhibitor of microglial activation which blocks the proliferation of microglia and the expression of Cluster of Differentiation 68, a member of lysosomal/endosomal – associated membrane glycoprotein. Minocycline treatment 12 h prior to kainate-induced epileptic seizure reduced neuronal damage in CA3 and CA1, which indicate that activated microglia may have a neurodegenerative role following seizures (40). As we said earlier, inflammatory citokines (IL-1β, IL-6 and TNF-α) have important role in the pathogenesis of epilepsy – their expression is elevated in the hippocampus within 1 day of seizure induced by electrostimulation. They can be produced by several cells, including microglia, especially M1 phenotype (41). The proof of their role in epileptogenesis is that specific inhibition of IL-1β have been shown to be anti-convulsant in rodent models and in some children with drug-resistant epilepsy (42, 43).

Some recent studies showed that also noninflammatory changes of microglia are sufficient to cause epilepsy (44). Elevated mammalian target of rapamycin signaling in mouse microglia leads to phenotypic changes, including an ameboid-like morphology, increased proliferation, and robust phagocytosis activity, but without a significant induction of proinflammatory cytokines. There are evidences that these noninflammatory changes in microglia disrupt homeostasis of the CNS, leading to reduced synapse density, moderate neuronal degeneration, and massive proliferation of astrocytes.

Clinical Studies on the Correlation between Oxidative Stress and Epilepsy

In clinical trials, there are still controversies about the relationship between oxidative stress and epilepsy. The largest limiting factor is the inhomogeneity of the investigated groups, especially when it comes to the role of oxidative stress in the onset of epileptic seizures in genetic diseases and syndromes. The parameters of oxidative stress and febrile convulsions are examined. Akarsu and colleagues designed a study in which they examined the activity of arginase and catalase in erythrocytes, the concentration of MDA and NO in cerebrospinal fluid (liquor) and plasma, and have shown that oxidative stress is induced in both afebrile and febrile convulsions (45).

In patients suffering from epileptic encephalopathies, which is defined as a condition in which epileptic activity contributes to the incidence of severe cognitive and behavioral disorders, but not in those suffering from idiopathic epileptic syndromes that relate to states of unknown etiology with possible genetic predisposition plasma levels of F2-IsoPs and AOPP are significantly higher, while there is no statistically significant difference in NPBI concentrations, suggesting that iron is not involved in generating free radicals in these patients (46).

The role of mitochondria in epileptogenesis has been particularly proven in epileptic encephalopathies caused by mitochondrial DNA (mtDNA) mutations. Some of the syndromes are: Leigh's syndrome, mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes, MERRF (myoclonic epilepsy with ragged-red fibers), Kearns-Sayre syndrome and etc. We will give some examples. MERFF is a rare disease characterized by myoclonus, muscle weakness, cerebellar ataxia, heart-pulse blocks, and dementia. It is caused by mutation in the gene for tRNALys in mtDNA (mutation A to G on 8344 nucleotide vapor to mtDNA) resulting in abnormal activity of the complex I of the electron transport chain. In cells isolated from patients suffering from this disease, increased production of ROS has been demonstrated, reduced production of ATP and calcium homeostasis disorder (47). Similar disorders were also found in Leigh's syndrome causing over 35 mutations in mtDNA (48). Part of the mitochondrial protein is encoded in the nucleus, so some of the mutations in these genes have been identified as the cause of mitochondrial dysfunction. An example is the POLG gene encoding the catalytic subunit of mtDNA polymerase (49). The range of the disease and syndrome is associated with mutations in this gene: Alpert-Huttenlocher Syndrome, mitochondrial recessive ataxia syndrome, spinocerebellar ataxia with epilepsy, myoclonic epilepsy, myopathy and sensory ataxia and the like. In children suffering from mitochondrial encephalopathy, tests of the cytochrome components of the respiratory chain were performed in the biopath m. vastus lateralis. It has been shown that in this population the incidence of defects in the structure and function of complex I is significantly higher, which is at the same time the largest transport chain, made up of 44 subunits, and therefore the largest source of free radicals (50).

CONCLUSION

In this study we have presented well-known mechanisms for the effect of oxidative stress on epilepsy so far. From all of the above, it is clear that free-radical induced neuronal damage is not the only one, but certainly it is a significant pathophysiological support in the onset of epilepsy.

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REFERENCES

1. Schmitt FJ, Renger G, Friedrich T, et al. Reactive oxygen species: re-evaluation of generation, monitoring and role in stress-signaling in phototrophic organisms. Biochimica Biophys Acta 2014; 1837: 835-848. [CrossRef]
2. Knowles RG, Moncada S. Nitric oxide synthases in mammals. Biochem J 1994; 298: 249-258. [CrossRef]
3. Das DK, Maulik N. Conversion of death signal into survival signal by redox signaling. Biochemistry (Mosc) 2004; 69: 10-17. [CrossRef]
4. Ranshoff RM, Benveniste EN. U: Citokines and CNS. Eds. RM Ranshoff, EN Benveniste. CRC Press, Boca Raton, New York, London, Tokyo, 1996.
5. Suzuki R, Yamaguchi T, Inaba Y, Wagner HG. Microphysiology of selectively vulnerable neurons. Prog Brain Res 1985; 63: 59-68. [CrossRef]
6. Rothman SM. Glutamate and anoxic neuronal death in vitro. Adv Exp Med Biol 1986; 203: 687-695. [CrossRef]
7. Stanimirovic D, Micic D, Ivanovic L. Prevention of ischaemia-induced disturbances of brain oxido-reductive processes by selective antagonist of NMDA-receptors. Iugoslav Physiol Pharmacol Acta 1989; 25: 437-441.
8. Armstead WM, Mirro R, Leffler CW, Busija DW. Cerebral superoxide anion generation during seizures in newborn pigs. J Cereb Blood Flow Metab 1989; 9: 175-179. [CrossRef]
9. Méndez-Armenta M, Nava-Ruíz C, Juárez-Rebollar D, Rodríguez-Martínez E, Gómez PY. Oxidative stress associated with neuronal apoptosis in experimental models of epilepsy. Oxid Med Cell Longev 2014; 2014: 293689. [CrossRef]
10. Puttachary S, Sharma S, Stark S, Thippeswamy T. Seizure-induced oxidative stress in temporal lobe epilepsy. Biomed Res Int 2015; 2015: 745613. [CrossRef]
11. Löscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy Res 2002; 50: 105-123. [CrossRef]
12. Patel M, Liang LP, Roberts LJ 2nd. Enhanced hippocampal F2-isoprostane formation following kainate-induced seizures. J Neurochem 2001; 79: 1065-1069. [CrossRef]
13. Rola R, Swiader M, Czuczwar SJ. Electroconvulsions elevate the levels of lipid peroxidation products in mice. Pol J Pharmacol 2002; 54: 521-524.
14. Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. Free Radic Biol Med 2004; 37: 1951-1962. [CrossRef]
15. Keyer K, Imlay JA. Superoxide accelerates DNA damage by elevating free-iron levels. Proc. Natl Acad Sci U S A 1996; 93: 13635-13640. [CrossRef]
16. Liang LP, Patel M. Mitochondrial oxidative stress and increased seizure susceptibility in Sod2(-/+) mice. Free Radic Biol Med 2004; 36: 542-554. [CrossRef]
17. Barros DO, Xavier SM, Barbosa CO, et al. Effects of the vitamin E in catalase activities in hippocampus after status epilepticus induced by pilocarpine in Wistar rats. Neurosci Lett 2007; 416: 227-230. [CrossRef]
18. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. Physiol Rev 2007; 87: 245-313. [CrossRef]
19. Brennan AM, Suh SW, Won SJ, et al. NADPH oxidase is the primary source of superoxide induced by NMDA receptor activation. Nat Neurosci 2009; 12: 857-863. [CrossRef]
20. Kim JH, Jang BG, Choi BY, et al. Post-treatment of an NADPH oxidase inhibitor prevents seizure-induced neuronal death. Brain Res 2013; 1499: 163-172. [CrossRef]

21. Tsai CY, Chan JY, Hsu KS, Chang AY, Chan SH. Brain-derived neurotrophic factor ameliorates brain stem cardiovascular dysregulation during experimental temporal lobe status epilepticus. PLoS One 2012; 7: e33527. [CrossRef]
22. Ferdinandusse S, Waterham HR, Heales SJ, et al. HIBCH mutations can cause Leigh-like disease with combined deficiency of multiple mitochondrial respiratory chain enzymes and pyruvate dehydrogenase. Orphanet J Rare Dis 2013; 8: 188. [CrossRef]
23. Willmore LJ, Sypert GW, Munson JV, Hurd RW. Chronic focal epileptiform discharges induced by injection of iron into rat and cat cortex. Science 1978; 200: 1501-1503. [CrossRef]
24. Zuchora B, Turski WA, Wielosz M, Urbańska EM. Protective effect of adenosine receptor agonists in a new model of epilepsy--seizures evoked by mitochondrial toxin, 3-nitropropionic acid, in mice. Neurosci Lett 2001; 305: 91-94. [CrossRef]
25. Balentine, J. D. Experimental pathology of oxygen toxicity. In: Jobsis, FF., ed. Oxygen and physiological function. Dallas: Professional Information Library; 1977: 311-378.
26. Trotti D, Danbolt NC, Volterra A. Glutamate transporters are oxidant-vulnerable: a molecular link between oxidative and excitotoxic neurodegeneration? Trends Pharmacol Sci 1998; 19: 328-334. [CrossRef]
27. Shimamoto K, Lebrun B, Yasuda-Kamatani Y, et al. DL-threo-beta-benzyloxyaspartate, a potent blocker of excitatory amino acid transporters. Mol Pharmacol 1998; 53: 195-201. [CrossRef]
28. Dutuit M, Touret M, Szymocha R, Nehlig A, Belin MF, Didier-Bazès M. Decreased expression of glutamate transporters in genetic absence epilepsy rats before seizure occurrence. J Neurochem 2002; 80: 1029-1038. [CrossRef]
29. Petroff OA, Errante LD, Rothman DL, Kim JH, Spencer DD. Glutamate-glutamine cycling in the epileptic human hippocampus. Epilepsia 2002; 43: 703-710. [CrossRef]
30. Eid T, Thomas MJ, Spencer DD, et al. Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. Lancet 2004; 363: 28-37. [CrossRef]
31. Rose C, Felipo V. Limited capacity for ammonia removal by brain in chronic liver failure: potential role of nitric oxide. Metab Brain Dis 2005; 20: 275-283. [CrossRef]
32. Wang C, Xie N, Wang Y, Li Y, Ge X, Wang M. Role of the mitochondrial calcium uniporter in rat hippocampal neuronal death after pilocarpine-induced status epilepticus. Neurochem Res 2015; 40: 1739-1746. [CrossRef]
33. Kovac S, Dinkova Kostova AT, Herrmann AM, Melzer N, Meuth SG, Gorji A. Metabolic and homeostatic changes in seizures and acquired epilepsy-mitochondria, calcium dynamics and reactive oxygen species. Int J Mol Sci 2017; 18: 1935. [CrossRef]
34. Finardi A, Colciaghi F, Castana L, et al. Long-duration epilepsy affects cell morphology and glutamatergic synapses in type IIB focal cortical dysplasia. Acta Neuropathol 2013; 126: 219-235. [CrossRef]
35. Dalmau J, Tüzün E, Wu HY, et al. Paraneoplastic anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 2007; 61: 25-36. [CrossRef]
36. Epi4K Consortium; Epilepsy Phenome/Genome Project; Allen AS, Berkovic SF, Cossette P, et al. De novo mutations in epileptic encephalopathies. Nature 2013; 501: 217-221. [CrossRef]
37. Cullinan SB, Gordan JD, Jin J, Harper JW, Diehl JA. The Keap1-BTB protein is an adaptor that bridges Nrf2 to a Cul3-based E3 ligase: oxidative stress sensing by a Cul3-Keap1 ligase. Mol Cell Biol 2004; 24: 8477-8486. [CrossRef]
38. Wild AC, Moinova HR, Mulcahy RT. Regulation of gamma-glutamylcysteine synthetase subunit gene expression by the transcription factor Nrf2. J Biol Chem 1999; 274: 33627-33636. [CrossRef]
39. Vezzani A, Lang B, Aronica E. Immunity and inflammation in epilepsy. Cold Spring Harb Perspect Med 2016; 6: a022699. [CrossRef]
40. Heo K, Cho YJ, Cho KJ, et al. Minocycline inhibits caspase-dependent and -independent cell death pathways and is neuroprotective against hippocampal damage after treatment with kainic acid in mice. Neurosci Lett 2006; 398: 195-200. [CrossRef]
41. Colton CA. Heterogeneity of microglial activation in the innate immune response in the brain. J Neuroimmune Pharmacol 2009; 4: 399-418. [CrossRef]
42. Vezzani A, Moneta D, Conti M, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Natl Acad Sci U S A 2000; 97: 11534-11539. [CrossRef]
43. Marchi N, Granata T, Freri E, et al. Efficacy of anti-inflammatory therapy in a model of acute seizures and in a population of pediatric drug resistant epileptics. PloS One 2011; 6: e18200. [CrossRef]
44. Zhao X, Liao Y, Morgan S, et al. Noninflammatory changes of microglia are sufficient to cause epilepsy. Cell Resp 2018; 22: 2080-2093. [CrossRef]
45. Akarsu S, Yilmaz S, Ozan S, Kurt A, Benzer F, Gurgoze MK. Effects of febrile and afebrile seizures on oxidant state in children. Pediatr Neurol 2007; 36: 307-311. [CrossRef]
46. Grosso S, Longini M, Rodriguez A. et al. Oxidative stress in children affected by epileptic encephalopathies. J Neuro Sci 2011; 300: 103-106. [CrossRef]
47. Wu SB, Ma YS, Wu YT, Chen YC, Wei YH. Mitochondrial DNA mutation-elicited oxidative stress, oxidative damage, and altered gene expression in cultured cells of patients with MERRF syndrome. Mol Neurobiol 2010; 41: 256-266. [CrossRef]
48. Ruhoy IS, Saneto RP. The genetics of Leigh syndrome and its implications for clinical practice and risk management. Appl Clin Genet 2014; 7: 221-234.
49. Zsurka G, Kunz WS. Mitochondrial dysfunction in neurological disorders with epileptic phenotypes. J Bioenerg Biomembr 2010; 42: 443-448. [CrossRef]
50. Khurana DS, Salganicoff L, Melvin JJ, et al. Epilepsy and respiratory chain defects in children with mitochondrial encephalopathies. Neuropediatrics 2008; 39: 8-13. [CrossRef]