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Review Article

FREE RADICALS, EPILEPSY AND ANTI-OXIDANT: AN OVERVIEW

Patil CD*, Ahire YS, Pathade PA, Pathade VV, Mali PR
Department of Pharmacology, K.B.H.S.S.T's institute of pharmacy, Malegaon-camp, Dist- Nasik, Maharashtra, India

*Patil Chandrashekhar D, Department of Pharmacology, K.B.H.S.S.T's institute of pharmacy, Malegaon-camp, Dist- Nasik, Maharashtra, India. E-mail: cdpatil1000@gmail.com
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ABSTRACT
Epilepsy is a common and heterogeneous disorder arising from biochemical and molecular events in the brain that are incompletely understood. Oxidative stress is emerging as a mechanism that may play an important role in the etiology of seizure induced neuronal death. In oxidative stress generation the free radical called reactive oxygen species and reactive nitrogen species (ROS, RNS) result in oxidative damage to cellular protein lipid and DNA and contribute the majority of seizure induced free radical prediction. Episodes of seizures can be prevented by eliminating causative or precipitating factors. However, the use of an antioxidant is a redox active compound that limits oxidative stress by reacting nonenzymatically with a reactive oxidant. Although oxidation reactions are critical for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Melatonin is one of the major antioxidant which is an pineal hormone, acts as a direct free radical scavenger and indirect antioxidant. It is suggested that the increase in neurodegenerative diseases is most prominent reason for decrease in the levels of melatonin with age. Unlike other antioxidants, melatonin can easily cross all morphophysiological barriers and enter in cell compartments. Evidence are accumulating to suggest the potential of melatonin in neurodegenerative conditions, but much information needs to be generated before it find place as a drug on the treatment of epilepsy.

KEYWORDS: Epilepsy, Oxidative stress, Free radical, Antioxidants

INTRODUCTION

Epilepsy is as common in adults over 60 as in children under 10, 25% of all cases develop before the age of five. The epilepsies affect more than 50 million people worldwide, incidence of epilepsy is high in children younger than 5 years of age, but precipitously rises in individuals older than 65 year. In contrast with genetic forms of epilepsy, acquired epilepsy accounts for approximately 60% of all cases and is usually preceded by injury such as an episode of prolonged seizures or status epilepticus, childhood febrile seizures, hypoxia, or trauma.

Seizure is a sudden disruption of the brain's normal electrical activity accompanied by altered consciousness and/or other neurological and behavioral manifestations. Epilepsy is a condition characterized by recurrent seizures that may include repetitive muscle jerking called "convulsions". It refers to seizures, with or without alterations in consciousness, , behavior, sensation and autonomic function. The episodes may be partial- involving only some parts of the body or generalized-involving the whole body.

Most seizures are benign, but a seizure that lasts a long time can lead to status epilepticus, a life-threatening condition characterized by continuous seizures, sustained loss of consciousness, and

respiratory distress. Non-convulsive epilepsy can impair physical coordination, vision, and other senses. Undiagnosed seizures can lead to conditions that are more serious and more difficult to manage. [1,2]

ETIOLOGY OF EPILEPSY

In order for epilepsy to occur, there must be an underlying physical problem in the brain. The problem can be so mild that an individual is perfectly normal other than seizures. There are different causes of epilepsy as follows[3,4]

- Free radical from various sources.
- Brain abscess or inflammation.
- Infectious diseases like measles, mumps, and diphtheria.
- Neurological disease.
- Degenerative disease.
- Hyperglycemia.
- Head trauma.
- Alcoholism

Prevention of Epilepsy

Episodes of seizures can be prevented by eliminating causative or precipitating factors. A normal lifestyle should be encouraged, wherever possible. Moderate exercise and participation in sports with proper safeguards may be permitted. Driving vehicles or operating heavy machinery should be avoided; if possible Counseling that includes information about the importance of taking medications regularly should be given to the patient and family members. Many cases of epilepsy can be prevented by wearing seatbelts and bicycle helmets, putting children in car seats, and other measures that prevent head injury and other trauma. Prescribing medication after first or second seizures or febrile seizures also may help prevent epilepsy in some cases. Finally, identifying the genes for many neurological disorders can provide opportunities for genetic screening and prenatal diagnosis that may ultimately prevent many cases of epilepsy prevents brain damage in the developing baby that may lead to epilepsy and other neurological diseases or disorders. [4,5]

CURRENT TRENDS IN ANTIEPILEPTIC THERAPY

In the recent years there have been a lot of advances in the management of childhood epilepsies. Apart from the existing anticonvulsants, newer anti-epileptics, other methods of treatment for epilepsy are now available. These aspects are considered under the following headings.[6]

- Epilepsy surgery.
- Vagal nerve stimulation.
- Recently available newer anti-epileptic Drug.
- Ketogenic diet
- Antioxidant therapy

Epilepsy surgery

The most common type of surgery for epilepsy is removal of a seizure focus, or small area of the brain where seizures originate. This type of surgery, which doctors may refer to as a lobectomy or lesionectomy, is appropriate only for focal seizures that originate in just one area of the brain. Reduction or complete cessation of seizures about 70 - 90 percent of the time. [7]

Vagal nerve stimulation

The Vagal nerve stimulation is a new nonpharmacologic alternative to traditional AED therapy. VNS has been found also to be beneficial in Lennox - Gastaut syndrome, absence epilepsy. The Vagus nerve communcates with the nucleus tractus solitarius in the brainsteam and from there may affect the brain widely. Areas possible activated by VNS include the medulla, cerebellu, parabrachial nucleus, locus coeruleus, hypothalamus, thalamus (including the intralaminar and ventroposterior parvocullular nuclei), amygdala, hippocampus, cingulate gyrus, and contralateral somatosensory cortex. Stimulation of these areas appear to be inhibitory to the epileptiform discharge.[8,9]

Ketogenic diet

Studies have shown that, in some cases, children may experience fewer seizures if they maintain a strict diet rich in fats and low in carbohydrates. This unusual diet, called the ketogenic diet, causes the body to break down fats instead of carbohydrates to survive. This condition is called ketosis. Researchers are not sure how ketosis inhibits seizures. One study showed that a byproduct of ketosis called beta-hydroxybutyrate (BHB) inhibits seizures in animals. If BHB also works in humans, researchers may eventually be able to develop drugs that mimic the seizure-inhibiting effects of the ketogenic diet.[10]

Oxidative Stress and Epilepsy

Oxidative stress is defined as imbalance between pro-oxidant and antioxidant result in damage of cell or death of cell, in oxidative stress generation the free radical e.g. ROS,RNS result in oxidative damage to cellular protein lipid and DNA and contribute the majority of seizure induced free radical prediction. Thus the epilepsy from activity results in free radical production which may one of the factors leading to cell death. The concentration of two product of free radical increase lipid peroxidation, malonaldehyde and 4-hydroxy -2methyl nonenal, lipid peroxidation was increase in both hemisphere and cell death rate and neuron loss also increased in hippocample area. Studies in this laboratory are directed toward determining whether prolonged seizure activity in animals result in the increased production of ROS and if oxidative injury contributes to seizure-induced brain damage. Two general approaches are taken to address this. Because reactive species are transient, unstable, and localized to cellular compartments, their measurement in biological system, particularly in vivo, is challenging. The selective oxidation of certain cellular macromolecules to oxidative attack renders them suitable as surrogate markers in vivo. In the first approach, seizure-induced oxidative damage to susceptible targets of oxidative damage (protein, lipids and DNA) is assessed after kainate-induced SE.[11,12]

Mitochondrial oxidative stress and dysfunction

Oxidative stress and mitochondrial dysfunction occur as a consequence of prolonged epileptic seizure and may contribute to seizure-induced brain damage. However ,as with each of the diverse signaling events activate by seizure, the crucial question is whether acute changes in seizure-induced free radical production and mitochondrial dysfunction are epileptogenic, i.e. resulting in chronic redox alteration that increase seizure susceptibility and result in the development of subsequent epilepsy. The most prominent example of mitochondrial dysfunction causing epilepsy is the occurrence of epilepsy in mitochondrial disorders arising due to mutation in mDNA or nuclear DNA .Several common neurological insults such as hypoxia, trauma, and aging and neuronal diseases such as stroke and Alzheimer disease render the brain susceptible to epileptic seizure .In fact, although epilepsy occurs in all age groups, the incidence of epilepsy is markedly increased in the elderly.The ability to produce oxidative stress and mitochondrial dysfunction is common to each of these neuronal condition.This raises an intriguing possibility that mitochondrial dysfunction initiate by free radical production could be increase seizures susceptibility.[7,13,14] Relationship between oxidative stress and neuronal damage is shown in figure 1.

Role of Antioxidant in Epilepsy

An antioxidant can be defined as “any substance that when present in low concentration compared to that of an oxidisable substrat significantly delays or inhibit the oxidation of that substrat”. ‘An antioxidant is a redox active compound that limits oxidative stress by reacting nonenzymatically with a reactive oxidant’; an antioxidant enzyme is a protein that limits oxidative stress by catalyzing a redox reaction with a reactive oxidant.

Protection based on following criteria:

1) Inhibition of generation and scavenging capacity against reaction oxygen species.
2) Increasing levels of endogenous antioxidant.
3) Metal chelating capacity (E.g. - Cu/Zn SOD, MnSOD).
4) Activity as anti-oxidant enzymes (e.g. SOD, Catalase, Glutathione Peroxidase.)
5) Inhibition of oxidative enzymes (Xanthine oxidase and Cyclo-oxygenase.)
6) Proteins that minimize the availability of peroxidase such as iron ions, copper ions.

The increase in oxygen free radical activities initiate lipid peroxidation and stimulate the glycation of the proteins. Alteration of structure and function of collagen basement membrane, inactivation of the enzymes plays role in epileptic complication which leads to epilepsy.[15,16] An antioxidant is substance that offer protection to cell membranes and prevent oxidative stress to the tissues of the body by neutralizing toxic oxygen molecules and free radicals are molecules that slow or prevent the oxidation of other chemicals. Oxidation is a redox chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can involve the production of free radicals, which can form dangerous chain reactions. Antioxidants can terminate these chain reactions by removing radical intermediates and can inhibit other oxidation reactions by being oxidized themselves. As a result, antioxidants are often reducing agents such as thiols or phenols. Although oxidation reactions are critical for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Low levels of antioxidant molecules or inhibition of these antioxidant enzymes causes oxidative stress and may damage or kill cells. As oxidative stress has been implicated in the pathogenesis of many human diseases, the use of antioxidants in pharmacology, particularly as treatments for epilepsy and neuronal diseases. However, it is unknown whether oxidative stress is the cause or the consequence of such diseases. Antioxidants are also widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as epilepsy and neuronal diseases. Although some studies have suggested antioxidant supplements have health benefits, other large clinical trials did not detect any benefit for the formulations tested, and excess supplementation may occasionally be harmful. In addition to these uses in medicine, antioxidants have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline.[7]Antioxidants are exogenous (natural or synthetic) or endogenous compounds acting in several ways including removal of O_2, scavenging reactive oxygen species or their precursors, inhibiting ROS formation and binding metal ions needed for catalysis of ROS generation. The natural antioxidant system can be classified into two major groups: enzymes and low molecular weight antioxidants (LMWA). The enzymes include SOD, catalase, peroxidase, and some supporting enzymes. The LMWA group of molecules can be further classified into directly acting antioxidants (e.g., scavengers and chain breaking antioxidants) and indirectly acting antioxidants (e.g., chelating agents). The former are extremely important in defense against OS. This subgroup currently contains several hundred compounds. Most of them, including ascorbic and lipoic acids, polyphenols, and carotenoids, are derived from dietary sources. The cell itself synthesizes a minority of these molecules, such as glutathione and NADPH. The distribution of protective antioxidants in the body has some interesting features. For instance, there is a relatively high concentration of the water-soluble antioxidant vitamin C in the brain. However, vitamin E concentrations in CNS are not remarkably different from those in other organs. The concentrations of antioxidants also vary within the different regions of the brain itself. For instance, the lowest concentration of vitamin E is found in the cerebellum. It was also shown that enzymatic antioxidants, such as catalase, are in lower concentrations in the brain than in other tissues.[12]

- Vitamin E
- Ascorbic acid
- Coenzyme Q10
- Melatonin
- Lipoic acid
- Ebselen
- N-acetylcysteine
- Glutathione
- Metal ion chelators
- Uric acid

Possible anticonvulsant effect of antioxidant is shown in figure 2. Antioxidant enzymes catalyse the breakdown of free radical species, usually in the intracellular environment. Transition metal binding

proteins prevent the interaction of transition metals such as iron and copper with hydrogen peroxide and superoxide producing highly reactive hydroxyl radicals. Chain breaking antioxidants are powerful electron donors and react preferentially with free radicals before important target molecules are damaged. In doing so, the antioxidant is oxidised and must be regenerated or replaced. By definition, the antioxidant radical is relatively unreactive and unable to attack further molecules. [15,17]

Endogenous Antioxidant Substances in Brain

In brain, an array of cellular defense systems exists to counterbalance the ROS. These include enzymatic and nonenzymatic antioxidants that lower the concentration of free radical species and repair oxidative cellular damage. Glutathione functions as a major antioxidant in tissue defense against free radicals in the brain. Brain is known to synthesize molecules like glutathione and NADPH. But, the concentration of glutathione is relatively in lesser quantities in the brain as compared to the rest organs of the body.[18] The natural antioxidant system present in brain can be in form of enzymes like catalase, peroxidase, superoxide dismutase or low molecular weight antioxidants. Low molecular weight antioxidants can be ascorbic and lipoic acids, carotenoids or indirectly acting, like chelating agents.[19] Free radical scavengers or antioxidants function as biological bodyguards for essential molecules by either neutralizing reactive species before them multilate a molecule or they repair damage that has been inflicted.[18] Antioxidant defenses against free radical attack is shown in figure 3.

Role of Melatonin Acts as an Antioxidant in Epilepsy

Classically, research into pathophysiology of epileptic seizures has primarily been focused on factors responsible for seizure initiation, but seizures arrest spontaneously and abruptly, and brain remains seizure free for some time thereafter. Thus, the possibility that some endogenous anticonvulsant substances is/are involved was suggested. Numerous animal studies have suggested an anticonvulsant role for the pineal hormone melatonin against various convulsive stimuli.[20-23] In gerbils, pinealectomy causes seizures, an effect which is reversed with exogenous melatonin.[24] In rats, melatonin has been reported to inhibit amygala kindled seizures[25] and antibodies to melatonin induced seizures[26] Pinealectomy, which results in absence of melatonin secretion, induces seizures in certain animals within a few hours.[27] Melatonin also exerted an anticonvulsant activity against seizures induced by several chemoconvulsants. E.g. quinolinate, kainate, glutamate, NMDA and pentylenetetrazol in rodents.[28] Melatonin Recently, a lot of endogenous substances with free radical scavenging properties have been explored for neuroprotection, of which melatonin and adenosine have gained attention. Both melatonin and adenosine are ubiquitously present endogenously in brain. Their concentrations have been found to be raised after seizures and altered in neurological conditions.[29, 30] Both melatonin as well as adenosine has a wide safety margin. Both are known to cross the blood brain barrier.

Melatonin, the pineal hormone, was a regular of biological rhythms controlling the phase and amplitude of circadian rhythm by acting both on suprachiasmatic nucleus (SCN), the biological clock that resides in the hypothalamus as well as on various other cells and tissues of the body. [31]

Because of this action the hormone has been named as ‘chronobiotic’ by Armstrong. It was Ianas, who first suggested that melatonin may have a role in scavenging free radicals.[32] Melatonin likely works via electron donation to directly detoxify free radicals. In in-vitro and in-vivo experiments, melatonin has been found to protect cells, tissues and organs against oxidative damage induced by a variety of free radical generating agents and processes, including cyanide poisoning, glutathione depletion, ischemia reperfusion, kainic acid induced excitotoxicity, and 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP).[33] Melatonin as an antioxidant is not only effective in protecting nuclear DNA, membrane lipids and possibly cytosolic proteins from oxidative damage but is also reported to alter the activities of enzymes that improve the total antioxidative defense capacity of the organism. [34]

CONCLUSION

Although oxidation reactions are critical for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidase. Melatonin is one of

the major antioxidant which is an pineal hormone, acts as a direct free radical scavenger and indirect antioxidant, but much information needs to be generated before it find place as a drug on the treatment of epilepsy.

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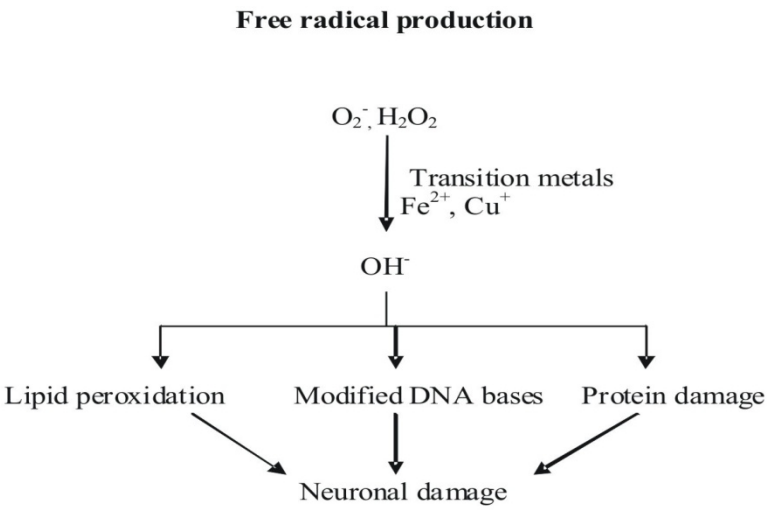

Fig 1. Oxidative stress and neuronal damage[9]

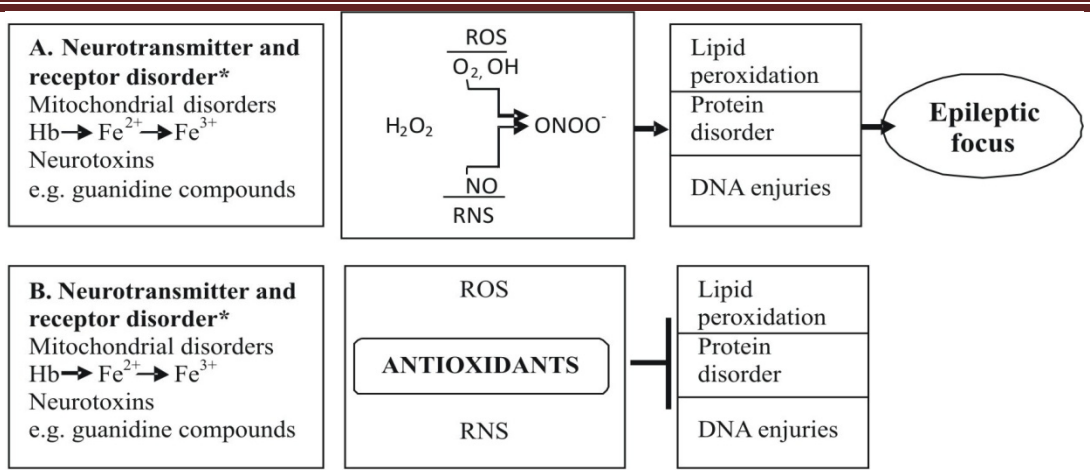

Fig. 2: Possible anticonvulsant effect of antioxidant

A, Involment of ROS and RNS in seizure mechanism: ROS and RNS, Induced by neurotransmitter and receptor disorder or neurotoxins result in neuronal disorder, which lead to epileptic focus formation. Neurotransmitter and receptor disorder may be in a vicious cycle, coupling with ROS and RNS. **B,** Anticonvulsant effect of antioxidant: antioxidant inhibits ROS and RNS, Induced neuronal damage and prevents epileptic formation.

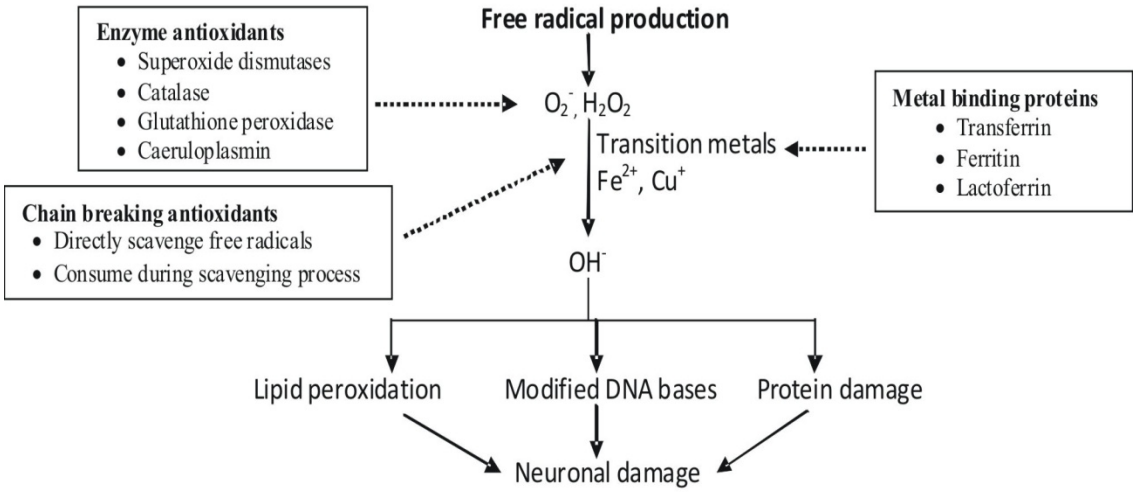

Fig 3. Antioxidant defenses against free radical attack[9]