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Antioxidant Treatments: Effect on Behaviour, Histopathological and Oxidative Stress in Epilepsy Model

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1. Introduction

The aim of this chapter is to describe several studies which have attempted to measure/detect effects of antioxidant compounds (lipoic acid, ubiquinone, ascorbic acid and alpha-tocopherol) on behavioral alterations, neuronal damage and oxidative stress in hippocampus of rodents in epilepsy model induced by pilocarpine.

Epilepsy can cause an uncomfortable impact on social, educational and emotional development of affected people, especially in childhood and adolescence, but its diagnosis has had great progress over the last years. Nevertheless, among the diagnostic details requiring research, the precise localization and lateralization of epileptogenic focus remains unclear, despite the fact that it has been demonstrated that removal of cerebral cortex region may result in a state free of seizures. Moreover, epilepsy is considered a risk factor for depression and other psychological problems, whereas cognitive impairment may be related to behavioral troubles, particularly of conduct and attention deficit, hyperactivity and psychiatric disorders.

Neurotransmitter systems involved in the experimental model of epilepsy induced by pilocarpine are not fully defined yet. This seizure model in rodents is widely used to study the pathophysiology of convulsive process, since it reproduces behavioral, electroencephalographic (EEG) and neurochemical changes that are similar to those of the temporal lobe epilepsy in humans. The pilocarpine model is used to study the action mechanism of new drugs and antioxidant compounds during the installation and maintenance and/or propagation of epileptogenesis as well as to evaluate effects of new compounds isolated from medicinal plants on behavioral, histopathological, and other parameters relating neurochemical changes with epileptic activity (Costa Júnior et al, 2010).

Histopathological studies using this model have demonstrated neuronal damage in various brain regions. Some specific brain areas revealed typical histopathological changes, mainly hippocampus, striatum and frontal cortex, suggesting the involvement of these different areas during the establishment of the epileptic process. Among those areas, the cellular and structural modifications seen in hippocampus and striatum may be significantly related to the mechanisms of installation and propagation in epileptogenesis of limbic seizures.

The temporal lobe epilepsy is the most common form of epilepsy. It is characterized by spontaneous recurrent seizures that are often blocked by treatment with antiepileptic drugs.

Seizures can be characterized as clinical manifestations resulting from abnormal neuronal discharges, producing an imbalance between the mechanisms of inhibitory and excitatory neurotransmission. The mechanisms of activation, propagation and maintenance of seizures are widely studied but little understood. Many studies have been performed using the pilocarpine model to clarify the effects of new drug modulators on brain mechanisms of seizures and status epilepticus (Santos et al, 2010).

Status epilepticus is clinically defined as prolonged electrical and clinical seizure activity in which the patient does not regain consciousness to a normal alert state between repeated tonic-clonic attacks. The disorder is a neurological emergency associated with a mortality rate of 10-12% and an even greater morbidity. Status epilepticus can lead to permanent pathological damage and altered physiological function in certain brain regions and induces major changes in membrane phospholipids, massive increases in arachidonic acid concentrations, diacylglycerol-mediated activation of protein kinase C, calcium-mediated changes in calmodulin kinase II and possibly generation of free radicals that could play an essential role in mechanism of oxidative stress involved in neuronal damage. Status epilepticus can be characterized by a permanent change in neurotransmitter systems and oxidative stress that it is more facilitated in the brain rather than in other tissues because it contains large quantities of oxidizable lipids and metals (Freitas et al, 2010). Moreover, status epilepticus can produce considerable changes on the enzymatic activity of antioxidants systems according to the brain areas and the phase of the seizure studied.

The role of monoamines, amino acid and oxidative stress in pilocarpine model was investigated in hippocampus, striatum and frontal cortex of adult rats. The status epilepticus was induced by pilocarpine and the results correspond to its acute phase. The data obtained suggest that pilocarpine induced neurotransmitters and oxidative stress changes in brain regions which are similar to those found in human temporal lobe epilepsy (El-Etri et al, 1993, Freitas et al, 2003, Ferreira et al, 2009).

2. Pathophysiology of seizures in epilepsy model induced by pilocarpine

Epilepsies are complex neurobehavioral disorders resulting from increased excitability of neurons in several brain regions involving various neurotransmitters (Rauca et al, 2004). The cholinergic system plays an important role in generating EEG activity as well as regulating the vigilance states. Pilocarpine is a cholinergic agonist with a moderate affinity with M₁ muscarinic receptors and high affinity with M₅ ones. Muscarinic cholinergic agonists have effects on rapid eyes movement (REM) and slow wave sleep, playing a role in REM induction (MacGregor et al, 1997; Perlis et al, 2002). On the other hand, pilocarpine at a high dose (400 mg/kg, i.p.) makes seizures progress to a long-lasting status epilepticus (SE) within 1-2 h and induces behavioral and EEG alterations in rodents, which are similar to human temporal lobe epilepsy (TLE) (Marinho et al, 1998).

Pilocarpine-induced rodent models TLE might provide information regarding histopathological damage and oxidative stress consequences, as well as neurochemical changes associated with seizure activity in hippocampus of young and adult rats (Cavalheiro et al, 1991; Smith and Shibley, 2002, Freitas et al, 2003). TLE can be characterized by a permanent change in neurotransmitter systems and in development of the oxidative stress that is more facilitated in the brain rather than in other tissues because for several reasons, including a high consumption of oxygen, the presence of large quantities of oxidizable lipids and pro-oxidative metals, and its comparatively lower antioxidant capacity

(Frantseva et al, 2000; Naffah-Mazzacoratti et al, 2001). Neuronal cells continuously produce free radicals and reactive oxygen species (ROS) as part of their metabolic processes and during the establishment of convulsive process (Gilbert and Sawas, 1983; Halliwell and Gutteridge, 1999). The free radicals are very reactive and might produce oxidative damage in DNA, proteins and lipids (Peterson et al, 2002), leading to a sequence of outcomes that culminates in neuronal degeneration during the installation of seizures.

ROS can affect the ion transport, proteins and channels, via protein oxidation or via membrane phospholipids peroxidation, resulting in a deleterious change on the ionic homeostasis and the neuronal transmission (Rong et al, 1999; Sah et al, 2002). The ROS increment induces oxidative stress, which is defined as the excessive production of free radicals, such as superoxide (O_2^-), hydroxyl radical ($OH\cdot$), nitric oxide (NO) and their metabolites (nitrate and nitrite) and others that can dramatically alter the neuronal function. Therefore, some researches have correlated the overproduction of these compounds with seizure-induced neuronal death and status epilepticus (MacGregor et al, 1997; Ferrer et al, 2000).

Several compounds can produce free radical such as H_2O_2 , which in high concentration can react with O_2 (Haber-Weiss reaction) or iron (Fenton reaction) producing highly reactive $OH\cdot$. The conversion of H_2O_2 to H_2O and O_2 is made by catalase and glutathione peroxidase (Michiels et al, 1994; Simonié et al, 2000). The formed $OH\cdot$ radical is likely to react with non-radical molecules, transforming them into secondary free radicals. This reaction occurs during the lipid peroxidation producing hydroperoxides in brain epileptic (Vanhatalo and Riikonen, 1999). Nitric oxide (NO) can be estimated by their metabolites, which are associated with neurodegenerative diseases (Vanhatalo and Riikonen, 2001). Despite the fact that numerous studies clearly indicate the importance of antioxidant enzymatic activities in the epileptic phenomenon, the mechanisms by which these enzymes influence seizures and status epilepticus are not completely understood (Michiels et al, 1994; Simonié et al, 2000).

Status epilepticus is a severe form of continuous seizure attacks and a medical emergency associated with brain damage and significant mortality (Aminoff and Simon, 1980). The common sequels of status epilepticus include continuing recurrent seizures, permanent neurological deficit and brain injury. The status epilepticus can be induced by the administration of pilocarpine or lithium-pilocarpine (Hirsh et al, 1982; Freitas et al, 2004). Pilocarpine administration induces seizures with three distinct phases: [A] an acute period, which lasts 1-2 days and is associated to repetitive seizures and status epilepticus; [B] a seizure-free (silent period) characterized by a progressive return to normal EEG and behavior, which lasts 4 to 44 days; [C] a chronic period characterized by spontaneous recurrent seizures (SRS) that starts 5 to 45 days after pilocarpine administration and persists until the animal dies. In addition, systemic injection of pilocarpine induces status epilepticus in rodents associated to histopathological alterations, which are most prominent in limbic structures (Cavalheiro et al, 1991), such as hippocampus, striatum, frontal cortex and others (Tomé et al, 2010).

Pilocarpine is a muscarinic cholinergic agonist able to elicit seizures and status epilepticus in rodents, characterizing an experimental model frequently used to study SRS (Turski et al, 1983; Freitas et al, 2005). This seizure model resembles several phenomenological features of human temporal lobe epilepsy, including a particular resistance to anticonvulsant medication (Browne and Holmes, 2001). Tissue accumulation of free radicals can occur in many metabolic disorders, such as seizures. Affected patients present a variable degree of neurological dysfunction, including mental retardation, cognitive deficit and cerebral

edema. However, the exact mechanisms involved in these alterations remain poorly understood.

It has been described that the impairments in learning, memory and behavior observed in patients with epilepsy are caused, at least in part, by changes in cholinergic system function (Bruce and Baudry, 1995), since there are consistent evidence that high levels of acetylcholine (ACh) in the brain are associated with cognitive dysfunction (Brozek et al, 2000). Cholinergic transmission is mainly terminated after ACh hydrolysis cause by acetylcholinesterase enzyme (AChE).

In the brain, the phenomena of excitotoxicity has been related to an over production of free radicals by the tissue during pilocarpine-induced seizures and status epilepticus (Simonié et al, 2000) and in human epilepsy (Vanhatalo and Riikonen, 1999). The increase in ROS levels can be responsible for this neuropathology and can activate apoptosis processes (Rong et al, 1999). The free radicals in the convulsive process can be neutralized by an elaborate antioxidant defense system consisting of enzymes such as superoxide dismutase, glutathione peroxidase, catalase, and glutathione reductase, and numerous non-enzymatic antioxidants like reduced glutathione (GSH), indicating a neuronal response (Ferrer et al, 2000). Status epilepticus induces ROS production by protein oxidation measured by tyrosine nitration (Rong et al, 1999). It can also be determined by both the end-product of lipid peroxidation, malondialdehyde (MDA) levels (Bruce and Baudry, 1995), and the effectiveness of the antioxidant enzymatic responses (Dal-Pizzol et al, 2000). The hippocampus is the most affected area by pilocarpine-induced seizures. Other authors also characterized the neuropathology associated with this convulsive process in striatum, frontal cortex, thalamus and amygdala (Perlis et al, 2002; Freitas et al, 2004).

Seizures represent one of the most severe in vivo stimulatory stress that the brain is exposed to and generalized status epilepticus represents a very severe form of seizures. The international Classification of seizures has defined it as a condition characterized by an epileptic seizure that is so frequent or so prolonged as to create a fixed and lasting condition (Krug et al, 1981). Major motor status epilepticus can lead to permanent pathological damage and altered physiological function in certain brain regions. The pathophysiological changes seen in complex partial, simple partial and absence status epilepticus are much less clear (Freitas et al, 2005). SE can cause brain damage, but can also result from it, and it has been difficult to separate the two, particularly in humans (Lapin et al, 1998).

Status epilepticus has been widely studied in animal models. In status epilepticus, glutamate, aspartate, serotonin, dopamine and acetylcholine play major roles as excitatory neurotransmitters, and GABA as the dominant inhibitory neurotransmitter (Hort et al, 2000; Costa-Lotufo et al, 2002). However, the relation among brain excitatory and inhibitory neurotransmitters and status epilepticus cannot be perfectly established yet and deserves further studies with the purpose to clarify the pathophysiology of seizures.

The pathophysiology of epilepsy is not yet fully defined. The pilocarpine model of seizures in animals are widely used to study the pathophysiology of convulsive process (Ben-Ari et al, 1980), since it reproduces the behavioral and EEG changes that are similar to humans TLE (Ben-Ari et al, 1981). These models are used to study the involvement of neurotransmitter systems as modulators of epileptogenesis, but also to observe behavioral changes, histopathologic, and other neurochemical parameters related to seizure activity (Marinho et al, 1997; Costa- Lotufo et al, 2002, Freitas et al, 2006).

In general, pilocarpine-induced seizures seem to depend on activation of muscarinic receptor, the enzymatic activity changes in some systems (Simonié et al, 2000; Naffah-

Mazzacoratti et al, 2001; Liu et al, 2002), metabolism of fosfoinositídeos (Marinho et al, 1998), as well as on the involvement of other neurotransmitter systems such as noradrenergic, dopamine (Kulkarni and George, 1996), serotonergic, GABAergic (Loup et al. 1999; Costa-Lotufo et al, 2002) and glutamatergic (Massieu et al. 1994; Chamberlain et al, 2000).

3. Behavioral alterations in epilepsy model

Status epilepticus is an emergency situation which requires prompt medical attention. If it is severe permanent brain damage or death has to be prevented with pretreatment with antioxidant compounds. Status epilepticus often occurs in individuals with a history of seizures, in whom there are neuronal substrates already predisposed towards supporting seizure activity.

The pilocarpine model is an useful animal experimental to investigate the development of acute, silent and chronic phases (Cavalheiro et al, 1991). Immediately after pilocarpine administration, all animals persistently presented behavioral changes, including initial akinesia, ataxic lurching, peripheral cholinergic signs (miosis, piloerection, chromodacryorrhea, diarrhea and masticatory automatisms), stereotyped movements (continuous sniffing, paw licking, rearing and wet dog shakes that persisted for 10-15 min), clonic movements of forelimbs, head bobbing and tremors.

These behavioral changes progressed to motor limbic seizures as previously described by Tursky et al. (1983a). Limbic seizures lasted for 30-50 min evolving to status epilepticus for a period longer than 30 min. During 1 h of acute phase of seizures, no case of fatality was observed between the adult rats. However, during the 24 h observation of this phase, 63% of adult animals died (Cavalheiro et al, 1994; Todorova et al, 2004). Similarly, these results for the behavioral alterations in pilocarpine model were described previously by Marinho et al. (1998).

According to our previous studies (Freitas et al, 2004), few minutes after pilocarpine administration, the animals exhibited stereotyped oral and masticatory movements, hypokinesia, salivation, tremor and partial or generalized limbic seizures. Approximately 30 min after pilocarpine injection, the seizures evolved to status epilepticus lasting 12-18 h. During this period, 40% of animals died due to SE. This acute phase was followed by a silent period varying from 4 to 44 days (mean of 15 days) during which the animals displayed normal behavior. A chronic period of spontaneous and recurrent seizures (SRS) (3-4 seizures/week) was also observed and all animals which survived SE, displayed the chronic phase. During the interictal period, there were no behavioral alterations in the animals.

In epilepsy model, pilocarpine induced the first seizure to occur at 34.93 ± 0.70 min. All the animals that received pilocarpine injection (at a dose 400 mg/kg, i.p.) presented generalized tonic-clonic convulsions with status epilepticus, and 60% survived the seizures (Freitas et al, 2004).

4. Antioxidant compounds effects on behavioral alterations in epilepsy model

The nervous system contains some antioxidant enzymes, including superoxide dismutase and glutathione peroxidase that are expressed in higher quantities than catalase (Shivakumar et al, 1991). This spectrum of enzymatic defense suggests that the brain may efficiently metabolize superoxide, but it may have difficulties in eliminating the hydrogen peroxide produced by this reaction. The accumulation of hydrogen peroxide is of major

concern since the brain contains large quantity of iron and copper, which may catalyze the formation of hydroxyl radical, which, in turn, can induce lipid peroxidation (Castagne et al, 1999).

The glutathione peroxidase is presented in large amounts during the Central Nervous System (CNS) development, but decreases in aged rats (Nanda et al, 1996). Nevertheless, other scavengers such as ascorbic acid and alpha-tocopherol also decrease the propagation of radical chain reaction. For these reasons, free radicals have been pointed as important molecules involved in the nervous system pathologies such as Huntington disease, Alzheimer, ischaemia and epilepsy (Jenner, 1998).

In epilepsy model induced by pilocarpine administration, we found that superoxide dismutase and catalase activities in the hippocampus are not altered during the acute phase of seizures. On the other hand, according to several authors, the augment of these enzymatic activities could decrease the O_2^- and H_2O_2 levels. Taken together, these results show that during the acute phase, the hippocampus of the adult animals in pilocarpine model after seizures is more vulnerable to oxidative stress.

In addition, high-levels of hydroperoxides were also observed in the same group of animals, which indicated that the lipid peroxidation could be dependent of disability of the antioxidant enzymatic (superoxide dismutase and catalase) activities. As the hydroperoxides are a class of compounds produced as the result of phospholipid peroxidation, its high concentration in the tissue suggests that the hippocampal cells are more vulnerable to damage during the acute period of seizures. Thus, the results described in literature about pilocarpine model suggest that the beneficial effects of antioxidants compounds in reducing the behavioral changes caused by seizures may be partly explained by their ability to remove free radicals, and prevent the formation of hydroperoxides in hippocampus of seized rats.

The need for animal models of epilepsy is driven by the constraints of studying human epileptic brain. Although a great deal has been learned through the study of human epileptic brain tissue throughout the past 100 years, and particularly based in recent experiments with pilocarpine model, our work was aimed at investigating the antioxidant effects of lipoic acid, ubiquinone, ascorbic acid and alpha-tocopherol in adult rats under pilocarpine-induced seizures. Our studies have demonstrated that all animals pretreated with the lipoic acid at the dose (10 or 20 mg/kg) during the first hour of acute phase of seizures induced by pilocarpine injection also manifested behavior alterations, such as peripheral cholinergic signs, tremors, staring spells, facial automatisms, wet dog shakes, rearing and motor seizures, which develop progressively within 1-2 h into a long-lasting status epilepticus. However, these behavioral changes occur at lower rates (Table 1). The findings also suggest that when administered 30 min before pilocarpine, lipoic acid reduces the percentage of animals that seized, increases latency to the first seizure and the survival percentage (Table 1).

Corroborating these data, other antioxidant compound evaluated in pilocarpine model when administered at the dose of 5 mg/kg before pilocarpine, ubiquinone had no effects on seized animals and survival percentage, but increased latency to the first seizure, when compared with the pilocarpine group (Table 1). On the other hand, at a dose of 10 or 20 mg/kg ubiquinone produced a higher reduction of seizures percentage, and a higher increase to that produced by dose of 5 mg/kg in latency to the first seizure and survival rate in pilocarpine model (Table 1).

Drugs	Dose (mg/kg)	Cholinergic Reactions (%)	Latency to first seizure (min)	Seizures (%)	Status epilepticus (%)	Survival rate (%)
Pilocarpine	400	100	35.00 ± 0.70	75	75	40
Ascorbic acid	250	100	80.00 ± 1.30*	33 ^a	33 ^a	100 ^a
	500	100	189.19 ± 1.15*,**	25 ^{a,b}	25 ^{a,b}	100 ^{a,b}
Alpha-tocopherol	200	100	103.00 ± 0.90*	32 ^a	32 ^a	68 ^a
	400	100	158.10 ± 1.05*,**	16 ^{a,b}	16 ^{a,b}	70 ^{a,b}
Ubiquinone	5	100	69.00 ± 0.65*	60 ^a	60 ^a	60 ^a
	10	100	89.00 ± 0.83*,**	35 ^{a,b}	35 ^{a,b}	75 ^{a,b}
Lipoic acid	10	100	106.13 ± 1.05*	00 ^a	00 ^a	75 ^a
	20	100	119.00 ± 1.02*	10 ^a	10 ^a	90 ^a

Rats were acutely treated with the drug doses shown in the table above and 30 min afterwards they received pilocarpine (400 mg/kg). Following, the animals were observed for 24 h for assessment of cholinergic reactions, motor seizures which develop progressively within 1-2 h into a long-lasting status epilepticus and survival rate. Results of latency to first seizure and latency to installation of status epilepticus are expressed in minutes (min) as mean ± S.E.M of the number of experiments shown in the experimental groups and the others in percentages *p<0.05, vs pilocarpine. **p<0.05 vs lower dose (ANOVA and *t*-Student Newman Keuls *post hoc* test); ^ap<0.05, vs pilocarpine. ^bp<0.05, vs lower dose (χ^2 test).

Table 1. Effect of pretreatment with antioxidant drugs on behavioral alterations in pilocarpine-induced seizures and lethality in adult rats.

In preclinical practice, the animals pretreated with ascorbic acid (250 mg/kg) in pilocarpine model developed cholinergic reactions, 33% had seizures, 25% built up to status epilepticus and no animal died (Table 1). Ascorbic acid administration, 30 min before pilocarpine injections, increased the latency to the onset of the first seizure in 129% and latency of the status epilepticus in 195% (Table 1). In pilocarpine model, it is also shown that when administered at the smaller dose (200 mg/kg), 30 min before pilocarpine injection, alpha-tocopherol can be decrease by 43% the percentage of animals that seized, increased (194%) latency to the first seizure, and increased survival (41%) (Table 1). Conversely, a higher dose (500 mg/kg) of the ascorbic acid in pilocarpine model, produced changes in behavior with lower intensity, such as peripheral cholinergic signs (100%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (25%) were observed, which progressively developed (1-2 h) into long-lasting status epilepticus (25%), revealing a survival rate of 75% (Table 1). Other studies have revealed that alpha-tocopherol at a dose 400 mg/kg blocks all alterations in behavior, revealing only 16% of motor seizures, which progressively developed (1-2 h) into long-lasting status epilepticus in pilocarpine model and a survival rate of 84% from the seizures (Table 1).

5. Histopathological alterations in epilepsy model

Cholinergic mechanisms play an important role in the activation of limbic seizures, and dopaminergic, serotonergic, GABAergic and glutamatergic systems are responsible for the propagation and/or maintenance of seizures and status epilepticus induced by pilocarpine (Freitas et al, 2004). Previous studies have described a model of limbic seizures followed by brain damage produced by systemic injection of a high dose of pilocarpine in rats. The

evidence included temporal correlation among free radical generation, development of seizures and neuroprotective effects of antioxidant drugs against neuronal damage caused by seizures (Kabuto et al, 1998).

Other studies have shown pilocarpine-induced seizures and brain damage in various cerebral regions and a significant hippocampal injury in this epilepsy model (Turski et al, 1991; Curia et al, 2008). The anticonvulsant effect in the absence of anticholinergic drugs subsequent to the seizure onset suggests that muscarinic receptor activation is involved directly in the beginning of seizures by pilocarpine.

However, the oxidative stress might also play an essential role in the production of neuronal damage, which can be justified by neuroprotective actions of antioxidant compounds according to previous studies (Freitas et al, 2004; Xavier et al, 2007; Ayyildiz et al, 2007a). Previous research indicates that anticonvulsant effects of noradrenergic antagonist drugs have a fundamental role in the mechanisms responsible for beginning, severity and duration of seizure. In fact, the reduction of severity and duration of seizures are protective against neurotoxicity caused by seizures induced by chemical convulsants (e.g. pilocarpine, kainic acid and others). These data, in spite of confirming a pivotal role of anticonvulsant drugs in modulating seizure threshold and neuronal death, offer a novel target, which may be used to develop anticonvulsant and neuroprotective agents (Pizzanelli et al, 2009).

There are several indications that free radical plays a role in epileptogenesis. During seizures, the ROS concentration and brain lipid peroxidation increase (Curia et al, 2008). It is currently hypothesized that any pathological process such as status epilepticus, which releases dopamine and glutamate, activates D₂ and NMDA receptors.

This may lead to neuronal necrosis by elevating intracellular calcium and activating potentially destructive calcium-dependent enzymes, augmenting the production of free radicals during seizures induced by pilocarpine (Michotte et al, 1997).

The available experimental data suggest that convulsion generally accelerate brain damage. Limbic status epilepticus causes neuronal necrosis in hippocampus, amygdala, pyriform cortex, entorhinal cortex, thalamus, neocortex, striatum and substantia nigra (Ayyildiz et al, 2007b). The neuronal damage depends on synaptic activation (Vanin et al, 2003), probably via a glutamatergic calcium-mediated mechanism (Marinho et al, 1998).

In the epilepsy model induced by a high dose of pilocarpine, we can observe neuronal loss in some brain areas, namely the hippocampus, striatum, amygdala, pyriform cortex, entorhinal cortex, lateral septum, thalamus and substantia nigra, suggesting the involvement of these different areas during the establishment of the epileptic process (Honchar et al, 1983; Turski et al, 1983; Clifford et al, 1987; Marinho et al, 1997; Borelli et al, 2002; Freitas et al, 2006).

Among the areas in which neuronal damage occurs, the striatum and fronto-parietal cortex, besides being the most affected areas, may be related in important ways with the mechanism of propagation and/or maintenance (epileptogenesis) limbic seizures (Marinho et al, 1998). Barone and collaborators (1991) demonstrated that through intracerebral administration in the striatum of D₂ dopamine agonists, there was a protection with respect to the development of seizures in adult rats, suggesting the participation of this brain region in limbic seizures.

Histopathological examinations during the acute phase of seizures induced by pilocarpine show extensive hippocampal brain damage, pyriform, entorhinal, frontal, temporal and parietal cortices and in the striatum and amygdaloid nucleus (Marinho et al, 1997).

Cerebral lesions during the acute period are characterized by neuronal loss, gliosis and vacuolation, although there are contradictory data with respect to the severity and relative

distribution of brain damage (Mello et al, 1993). Brain necrosis is associated with the occurrence of seizures, although studies have demonstrated that this association is not obligatory, especially in the pilocarpine model (Peredery et al, 1992). The seizures induced by pilocarpine can be blocked by atropine, pointing towards involvement of the cholinergic system. On the other hand, atropine did not act after seizure onset, suggesting that others neurotransmitters and oxidative stress may participate in the maintenance and/or propagation of seizures and brain damage as well (Hirsh et al, 1982). Oxidative stress mediated by free radical produces lipid peroxidation, increases the nitrite content in the hippocampus, striatum and frontal cortex (Freitas et al, 2004) and may play a major role in the neuronal injury development after seizures induced by pilocarpine.

6. Antioxidant compound effects on histopathological alterations in epilepsy model

Hence, it could be expected that antioxidant drugs such as ascorbic acid and alpha-tocopherol, can be used as scavengers of free radicals, reducing brain injury induced by pilocarpine. In previous histopathological analyses, ascorbic acid and alpha-tocopherol antioxidants protected animals against seizures, status epilepticus and brain damage induced by pilocarpine (Figure 1) by decreasing the percentage of seizures, status epilepticus and death in relation to both doses tested.

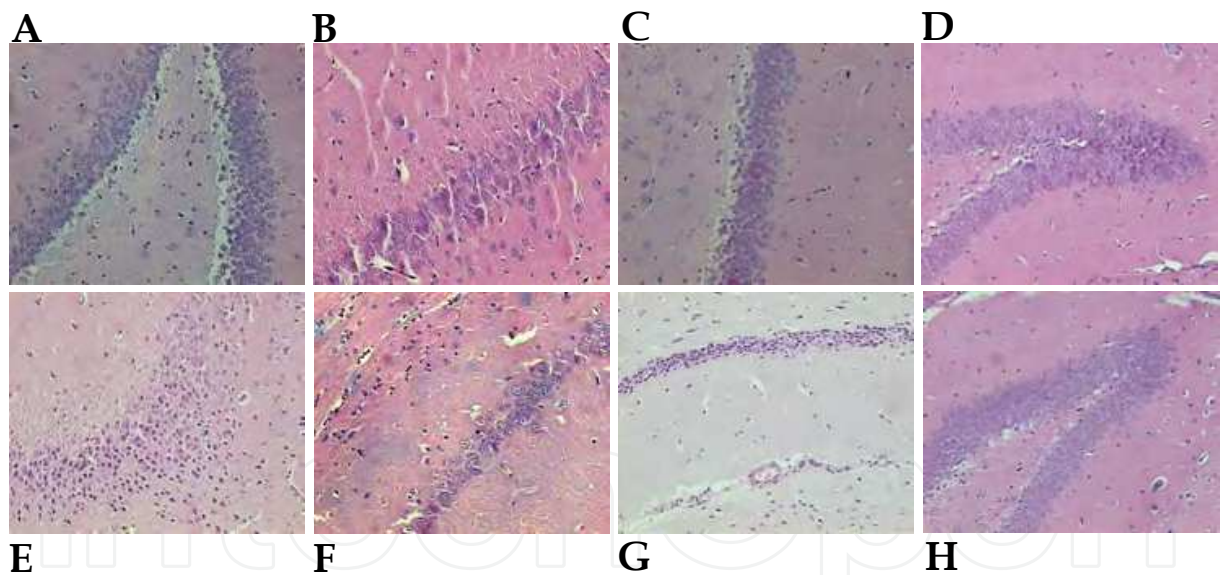


Fig. 1. Histopathological alterations in rat hippocampus treated with pilocarpine, ascorbic acid or their combinations. [A] Control group; [B] Pilocarpine group; [C] ascorbic acid 250 group; [D] ascorbic acid 250 plus Pilocarpine groups was treated with ascorbic acid (250 mg/kg) and 30 min before Pilocarpine; [E] ascorbic acid 500 group; [F] ascorbic acid 500 plus Pilocarpine group was treated with ascorbic acid (500 mg/kg) and 30 min before Pilocarpine. Severity of lesion was expressed as mean \pm S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement. Hematoxylin & Eosin staining (H&E). Magnification, 100 X. One representative experiment with n=6 is shown.

A variety of epilepsy models reflect the effects of acid ascorbic and alpha-tocopherol and specify their action (Koza et al, 2007; Gaby, 2007). Previously, it had been demonstrated that these compounds reduced the frequency of penicillin-induced epileptiform activity (Ayyildiz et al, 2006; Ayyildiz et al, 2007b). In recent years, many roles of alpha-tocopherol have been discovered, including not only an antioxidant function, but also pro-oxidant, cell signaling, and gene regulatory functions. Some studies have reported that alpha-tocopherol is considered to be the main antioxidant substance in the human body, interfering with the production of hydroxyl radical and also with the oxygen in cell membranes, thereby reducing lipid peroxidation (Barros et al, 2007).

Our results demonstrated that seizure pattern and brain damage observed in pilocarpine-treated animals differ from those pretreated with alpha-tocopherol (400 mg/kg) plus pilocarpine (400 mg/kg). The latter reproduced the syndrome with lower intensity of histopathological changes and mortality rate, in comparison with the alpha-tocopherol (200 mg/kg) plus pilocarpine, corroborating the outcomes obtained by Ribeiro and collaborators (2005) and Ayyildiz and collaborators (2006). The percentage of status epilepticus (75%) that was found further corroborated prior investigations (Clifford et al, 1987; Marinho et al, 1997).

Ascorbic acid is probably the most important water-soluble antioxidant in the brain extracellular fluid, and it is essential in regenerating reduced alpha-tocopherol in membranes (Niki, 1991). Despite the fact that ascorbic acid has an antioxidant role to counter oxidative stress, ascorbic acid also form reactive oxidants, especially in the presence of transition metals. The evidence suggests that ascorbic acid participates in pro-oxidant reactions under certain conditions (Layton et al, 1998).

The outcomes confirm that ascorbic acid (250 or 500 mg/kg) decreased the frequency of pilocarpine-induced seizures, status epilepticus and brain lesions in rats. In addition, ascorbic acid decreases the severity of hippocampal lesions and mortality rate caused by pilocarpine. Yamamoto and collaborators (2002) demonstrated that the injection of ascorbate, 60 min before FeCl_3 administration, prevented the occurrence of epileptic discharges. Since there are wide variations of alpha-tocopherol and ascorbic acid doses used in different models of seizure, more detailed investigations are necessary before an ultimate conclusion on the effects of those compounds on pilocarpine-induced seizures can be achieved.

In conclusion, there is an accumulation of free radicals after status epilepticus induced by pilocarpine, and oxidative changes in other parameters during the acute phase. This finding suggests that seizures, status epilepticus and deaths induced by pilocarpine have a large participation in brain oxidative stress, which is closely related to the mechanism of propagation and/or maintenance of the epileptic focus by pilocarpine. These results suggest that free radicals as well as the muscarinic receptor activation seem to be involved in the genesis of seizures and brain damage obtained with pilocarpine. On the other hand, the muscarinic activation seems to play a major role in the neuronal damage produced by pilocarpine. Antioxidant compounds can exert neuroprotective function during acute phase of seizures, thereby decreasing the severity of hippocampal lesions. All these outcomes indicate the promising therapeutic potential of ascorbic acid and alpha-tocopherol in treatments for neurodegenerative diseases.

Brain tissue examinations of the animals pretreated with ascorbic acid (250 or 500 mg/Kg; Figure 1), alpha-tocopherol (200 or 400 mg/kg Figure 2), lipoic acid (10 or 20 mg/kg, Figure 3) or ubiquinone (5 or 10 mg/kg; Figure 4), did not reveal hippocampal and striatal

histopathological changes. Then again, pilocarpine-treated animals presented neuronal loss, gliosis, and typical vacuolar degeneration in hippocampus and striatum regions. Histopathological damage in hippocampus was observed in 50, 33, 33 and 17% of the animals co-administered with ascorbic acid (250 or 500 mg/kg) or alpha-tocopherol (200 or 400 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg), respectively (Table 2). In addition, the analyses of histopathological damage in hippocampus of rats pretreated with lipoic acid (10 or 20 mg/kg) or ubiquinone (5 or 10 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg) revealed a reduction of 52, 68, 52 and 100% in the number of animals with neuronal damage, respectively (Table 2).

Drugs	Dose (mg/kg)	Rats with lesion (%)	Severity of lesion (%)	Number of animals with lesion per group
Pilocarpine	400	83	59.92 ± 0.23	5
Ascorbic acid	250	00	00	0
	500	00	00	0
Ascorbic acid plus P400	250	50 ^a	20.00 ± 0.32 ^a	3
	500	33 ^{a,b}	17.66 ± 0.33 ^{a,b}	2
Alpha-tocopherol	200	00	00	0
	400	00	00	0
Alpha-tocopherol plus P400	200	33 ^a	13.66 ± 0.33 ^a	2
	400	17 ^{a,b}	5.97	1
Ubiquinone	5	00	00	0
	10	00	00	0
Ubiquinone plus P400	5	33 ^a	12.00 ± 0.25 ^a	2
	10	00	00	0
Lipoic acid	10	00	00	0
	20	00	00	0
Lipoic acid plus P400	10	33 ^a	11.96 ± 0.12 ^a	2
	20	17 ^{a,b}	5.97	1

Pilocarpine was administered in a single dose (400 mg/kg, P400, n=6), ascorbic acid groups with ascorbic acid (250 or 500 mg/kg), alpha-tocopherol (200 or 400 mg/kg, n=6), ubiquinone (5 or 10 mg/kg, n=6) and lipoic acid group with lipoic acid (10 or 20 mg/kg; n=6). The ascorbic plus P400 groups were treated with ascorbic acid (250 or 500 mg/kg, n=6) and 30 min before P400. The alpha-tocopherol plus P400 group was treated with alpha-tocopherol (200 or 400 mg/kg) and 30 min before P400. The ubiquinone plus P400 group was treated with ubiquinone (5 or 10 mg/kg) and 30 min before P400. The lipoic acid plus P400 group was treated with lipoic acid (10 or 20 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based on a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was defined as present if there was at least 50% hippocampal involvement. Results for % rats with brain lesion and % severity of lesion are expressed as percentages of the number of animals inside in parenthesis. ^ap<0.05 compared with P400 group (χ² test). ^bp<0.05 compared with ascorbic acid 250 plus P400 group or alpha-tocopherol 200 mg/kg plus P400 group or ubiquinone 5 mg/kg plus P400 group or lipoic acid 10 mg/kg plus P400 group (χ² test).

Table 2. Histopathological alterations in hippocampus of rats pretreated with antioxidant compounds after 24 h of phase acute of pilocarpine-induced seizures.

Histopathological damage in striatum was observed only in 33, 17, 17 and 17% of the animals co-administered with ascorbic acid (250 or 500 mg/kg), ubiquinone (5 mg/kg) and lipoic acid (10 mg/kg) and that 30 min after pretreatment received pilocarpine (400 mg/kg), respectively (Table 2). Moreover, the analyses of histopathological damage in striatum of rats pretreated with lipoic acid (20 mg/kg), alpha-tocopherol (200 or 400 mg/kg) or ubiquinone (10 mg/kg), and that 30 min after pretreatment received pilocarpine (400 mg/kg) revealed no neuronal damage (Table 2).

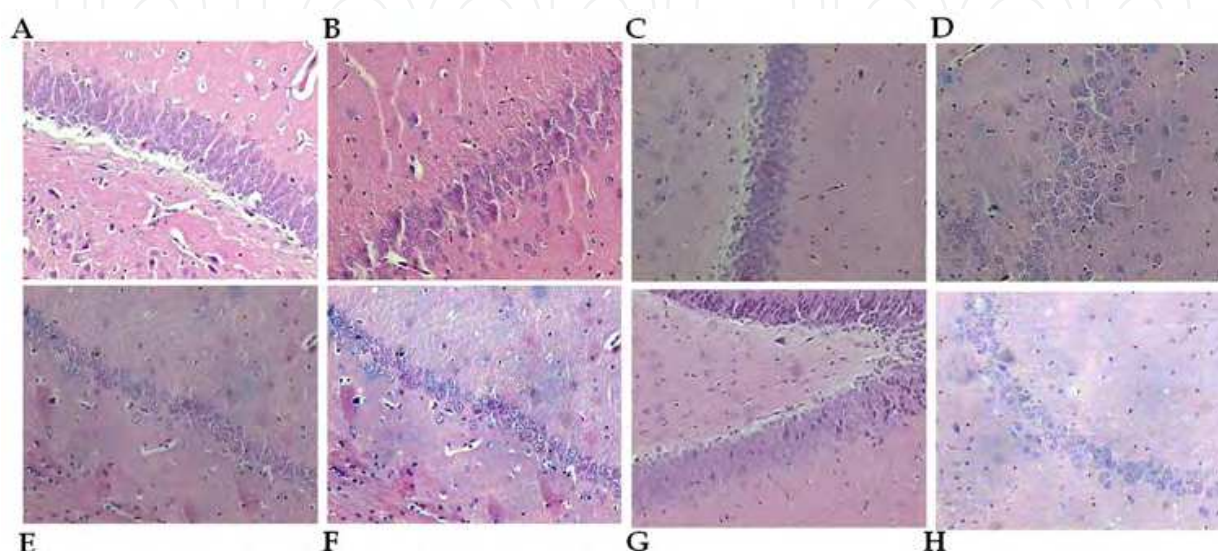


Fig. 2. Histopathological alterations in rat hippocampus treated with pilocarpine, atropine, alpha-tocopherol or their combinations. [A] Control group; [B] Pilocarpine group; [C] alpha-tocopherol 200 group; [D] alpha-tocopherol 200 plus Pilocarpine group was treated with alpha-tocopherol (200 mg/kg) and 30 min before P400; [E] alpha-tocopherol 400 group; [F] alpha-tocopherol 400 plus Pilocarpine group was treated with alpha-tocopherol (400 mg/kg) and 30 min before Pilocarpine. Severity of lesion was expressed as mean \pm S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement. Hematoxylin & Eosin staining (H&E). Magnification, 100 X. One representative experiment with $n=6$ is shown.

7. Oxidative stress in epilepsy model

The lipid peroxidation level in the brain homogenates are increased in this model. During the acute phase of seizures induced by pilocarpine, increases in lipid peroxidation level, nitrite concentration and GSH content in striatum, frontal cortex and hippocampus have been verified in the same way (Freitas et al, 2004). The improved review demonstrates that status epilepticus induces different changes in superoxide dismutase activity according to the brain region, as that enzymatic activity remained unaltered in striatum and hippocampus but increased in frontal cortex. After the first hour of acute phase of seizures an increase is detected in several regions (striatum, hippocampus and frontal cortex). In addition, catalase activity was increased in striatum, hippocampus and frontal cortex in this epilepsy model (Freitas et al, 2003).

Drugs	Dose (mg/kg)	Rats with lesion (%)	Severity of lesion (%)	Number of animals with lesion per group
Pilocarpine	400	67	55.39 ± 0.52	4
Ascorbic acid	250	00	00	0
	500	00	00	0
Ascorbic acid plus P400	250	33 ^a	25.42 ± 0.25 ^a	2
	500	17 ^{a,b}	13.46	1
Alpha-tocopherol	200	00	00	0
	400	00	00	0
Alpha-tocopherol plus P400	200	00	00	0
	400	00	00	0
Ubiquinone	5	00	00	0
	10	00	00	0
Ubiquinone plus P400	5	17 ^a	14.31	1
	10	00	00	0
Lipoic acid	10	00	00	0
	20	00	00	0
Lipoic acid plus P400	10	17 ^a	12.96 ± 0.22 ^a	1
	20	00	00	0

Pilocarpine was administered in a single dose (400 mg/kg, P400, n=6), ascorbic acid groups with ascorbic acid (250 or 500 mg/kg), alpha-tocopherol (200 or 400 mg/kg, n=6), ubiquinone (5 or 10 mg/kg, n=6) and lipoic acid group with lipoic acid (10 or 20 mg/kg; n=6). The ascorbic plus P400 groups were treated with ascorbic acid (250 or 500 mg/kg, n=6) and 30 min before P400. The alpha-tocopherol plus P400 group was treated with alpha-tocopherol (200 or 400 mg/kg) and 30 min before P400. The ubiquinone plus P400 group was treated with ubiquinone (5 or 10 mg/kg) and 30 min before P400. The lipoic acid plus P400 group was treated with lipoic acid (10 or 20 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based on a scale from zero (none) to 100 (total) percent of structural involvement. Brain damage was defined as present if there was at least 50% straital involvement. Results for % rats with brain lesion and % severity of lesion are expressed as percentages of the number of animals inside in parenthesis. ^ap<0.05 compared with P400 group (χ² test). ^bp<0.05 compared with ascorbic acid 250 plus P400 group or alpha-tocopherol 200 mg/kg plus P400 group or ubiquinone 5 mg/kg plus P400 group or lipoic acid 10 mg/kg plus P400 group (χ² test).

Table 3. Histopathological alterations in striatum of rats pretreated with antioxidant compounds after 24 h of phase acute of pilocarpine-induced seizures.

Lipid peroxidation in a tissue is an index of irreversible biological damage of the cell membrane phospholipid, which in turn leads to inhibition of most of the sulphydryl and some nonsulphydryl enzymes (Gilbert and Sawas, 1983). Lipid peroxidation level increase and reduce, whereas glutathione decrease can be induced by many chemicals (e.g. kainic acid and pilocarpine) and by many tissue injuries, and has been suggested as a possible mechanism for the neurotoxic effects of epileptic activity (Sah et al, 2002). Our findings demonstrated that lipid peroxidation levels increase after the first hour and during 24 h of the acute phase of seizures induced by pilocarpine in hippocampus, striatum and frontal cortex.

In normal conditions, there is a steady state balance between the production of ROS and their destruction by cellular antioxidant system. It is demonstrated that nitrite content in striatum and frontal cortex is augmented after seizures and status epilepticus in adult rats, suggesting a possible increase in ROS level, which can be involved in neuronal damage induced status

epilepticus. Other studies have shown that nitrite and nitrate levels were not elevated in patients with cryptogenic west syndrome (Vanhatalo and Riikonen, 2001), but it is tempting to speculate that the seizure activity per se did not account for the whole increment observed in nitrite and nitrate levels, and other mechanisms may be associated with this parameter in this epilepsy model, as well as neuronal degeneration observed in human beings. However, new studies using antioxidants drugs during status epilepticus induced by pilocarpine can indicate whether lipid peroxidation, nitrite and glutathione reduced (GSH) concentrations are involved in the pathophysiology of status epilepticus in this model.

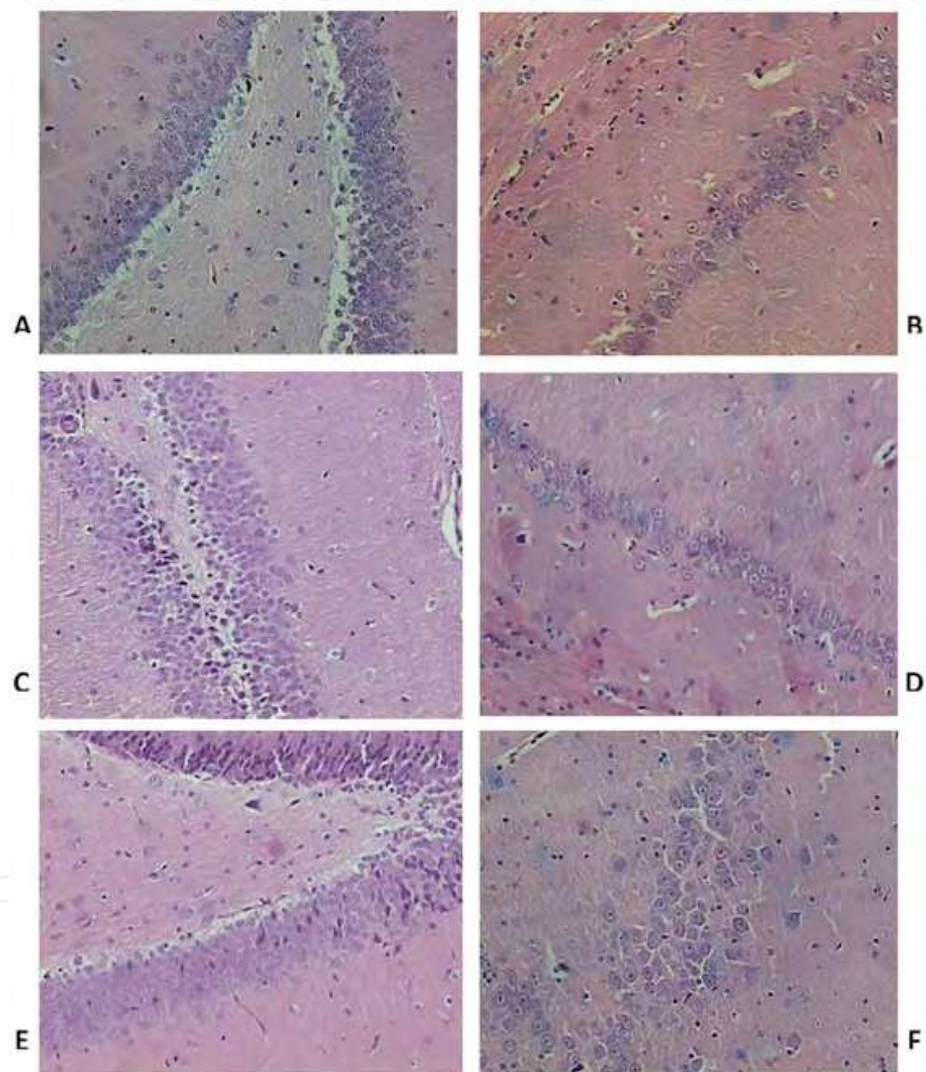


Fig. 3. Histopathological alterations in rat hippocampus pretreated with lipoic acid prior to pilocarpine-induced seizures. Severity of lesion was expressed as a mean \pm S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percentage of hippocampus involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement showed by Hematoxylin & Eosin staining (HE). Pictures (100 X) shown are from one representative experiment of $n=8$. [A]: Control group; [B]: Pilocarpine group; [C]: lipoic acid 10 group; [D]: lipoic acid 10 plus pilocarpine group; [E]: lipoic acid 20 group; [F]: lipoic acid 20 plus pilocarpine group.

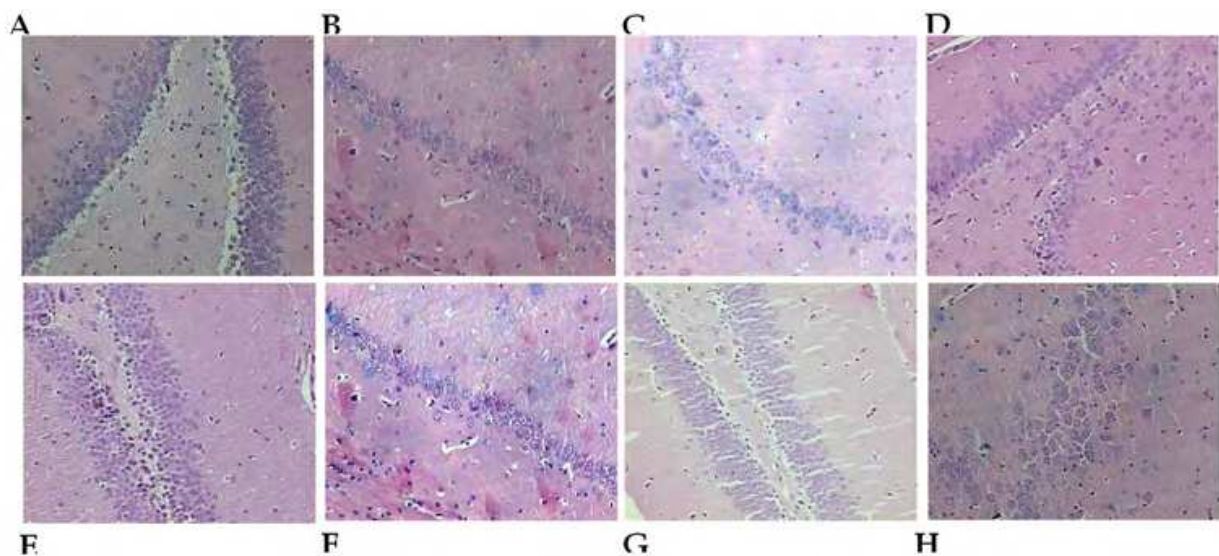


Fig. 4. Histopathological alterations in rat hippocampus pretreated with ubiquinone prior to pilocarpine-induced seizures. Severity of lesion was expressed as a mean \pm S.E.M. of scores of damage based in a scale from zero (none) to 100 (total) percentage of hippocampus involvement. Brain damage was considered positive if there was at least 50% hippocampal involvement showed by Hematoxylin & Eosin staining (HE). Pictures (100 X) shown are from one representative experiment of $n=8$. [A]: Control group; [B]: Pilocarpine group; [C]: Ubiquinone 5 group; [D]: Ubiquinone 10 group; [E]: Ubiquinone 20 group; [F]: Ubiquinone 5 plus P400 group [G]: Ubiquinone 10 plus P400; [H]: Ubiquinone 20 plus P400.

Although there were no selective brain regions particularly vulnerable to oxidative stress, there were some regional variations in the amount of oxidative damage observed. In the regions studied, there were nearly equal elevations in lipid oxidative, nitrite content and GSH markers that persisted during the acute phase of seizures.

All living organisms can suffer oxidative damage, yet the animal brain is often said to be especially sensitive (Gilbert and Sawas, 1983; Bruce and Baudry, 1995). The experimental data demonstrate that pilocarpine administration and its resulting status epilepticus produce significant alterations in hippocampus, striatum and frontal cortex. We recorded alterations in superoxide dismutase activity in frontal cortex during the seizures, however, no alterations were observed in striatal superoxide dismutase activity of rats under the same conditions. It is likely that the unaltered superoxide dismutase activity in striatum might not be related to the mechanisms involved in installation and propagation of seizures and status epilepticus induced by pilocarpine, which produces several changes in parameters related to generation and elimination of oxygen free radicals in adult rats (Sawas and Gilbert, 1985). An increase in free radical formation can be accompanied by an immediate compensatory increase of free radical scavenging enzymatic (superoxide dismutase and catalase) activities and this action was observed during status epilepticus in brain regions. Nevertheless, a similar compensatory mechanism of scavenging was observed in catalase activity after status epilepticus, suggesting that the enzymatic function of different systems can be modified either during the acute phase of seizures or according to cerebral area investigated. Literature reports the involvement of catalase activity in hippocampus, striatum and frontal cortex after status epilepticus. An increase in catalase activity in these brain areas can be related to a long-term compensatory mechanism including modulation activity of enzymes from the ROS catabolism.

Moreover, the catalase activity might be one of the mechanisms capable to avoid the development of neurotoxic effects mediated by SE, indicating that basal-oxygen radical production can damage the cell and that its control is necessary (McCord, 1989; Naffah-Mazzacoratti, 2001).

Evidences for the role of free radicals in seizures has been found by using exogenously administered enzymatic and non-enzymatic antioxidants for protection against seizures and status epilepticus-induced neuronal damage (Kulkarni and George, 1996; Freitas et al, 2005). A steady state level of O_2^- and H_2O_2 is always present within cells as a result of a normal metabolism. Superoxide dismutase and catalase are responsible for degradation of O_2^- and H_2O_2 , respectively. The balance between antioxidants enzymes, superoxide dismutase and catalase can be important during seizures and status epilepticus induced by pilocarpine. The present data indicate that pilocarpine treatment and its resulting status epilepticus induce neurochemical changes such as an increase in nitrite content and lipid peroxidation level, a decrease in GSH content as well as an activation of brain antioxidant mechanisms. The anatomic distribution of alterations observed in the enzymatic activities (superoxide dismutase and catalase) can suggest that the frontal cortex can be extensively involved in the propagation of epileptic activity and further studies should be carried out to ascertain that the catabolism of nitrite, ROS and GSH can be involved in the pathogenesis of status epilepticus.

The pilocarpine model is essential to investigate the mechanisms for initiation and propagation of seizures and status epilepticus. Additionally, it may be assumed that the increased generation of nitrite and lipid peroxidation levels after status epilepticus is not primary caused by an exhaustion of both the enzymatic and non-enzymatic defense systems measured. Adaptive mechanisms, as the induction of catalase activity, may be taken into consideration to counteract oxidative stress mediated by status epilepticus. However, the relation among brain structures, antioxidant systems, lipid peroxidation, nitrite concentration and status epilepticus cannot be perfectly established and deserves further investigation.

8. Antioxidant compound effects on oxidative stress in epilepsy model

Neurochemical alterations are observed in pilocarpine-induced seizures (Freitas et al, 2005), whose physiopathology is still poorly understood. However, there is data in literature suggesting that elevated reactive oxygen species concentrations and/or its metabolites are potentially neurotoxic (Freitas et al, 2004). We have demonstrated that lipoic acid reduces brain oxidative metabolism (Militão et al, 2010) and cannot inhibit Na^+ , K^+ -ATPase activity in rat hippocampus.

Animal models are useful to better understand the pathophysiology of seizures. In this context, the antioxidants compound effects in pilocarpine model were recently investigated (Mudd et al, 2001), revealing that they might produce a decrease in nitrite levels in rat hippocampus (Koçak et al, 2000; Augoustides-Savvopoulou et al, 2003). Animals exposed to lipoic acid treatment presented no differences in physical growth and brain, suggesting that lipoic acid ameliorates metabolic parameters in pilocarpine model (Mesulam et al, 2002).

By using this model, we investigated the effect of lipoic acid on spatial navigation tasks in the Morris water maze. Results have shown that seized rats did not present performance impairment neither in the acquisition phase nor on the time spent in target quadrant and in platform location nor in the latency to cross over the platform location in the reference memory task session. However, lipoic acid significantly impaired working memory

performance, since there was a significant effect with the 9-day interaction group and significant differences after days 2 and 5.

The biological effects of free radicals are controlled *in vivo* by a wide range of antioxidants, such as alpha-tocopherol, ascorbic acid, vitamin A, and glutathione reduced (Halliwell and Gutteridge, 1990; Ayyildiz et al, 2006). Acid ascorbic and alpha-tocopherol have many functions in the brain and in the neuronal microenvironment. They work as neuromodulators as well as antioxidant/free radical scavengers (Koza et al, 2007; Devi et al, 2008). It has been suggested that ascorbic acid and alpha-tocopherol have neuroprotective properties in some experimental models of excitotoxic neurological disorders, including seizure activity induced by pilocarpine (Gaby, 2007; Barros et al., 2007).

Systemic injection of pilocarpine, a cholinergic muscarinic agonist, induces status epilepticus in rodents, which is associated to histopathological and neurochemical changes as well as oxidative stress (Turski et al, 1983; Cavaleiro et al, 1994; Freitas et al, 2005). Elevated free radical products were observed during the status epilepticus. Free radicals are highly reactive chemical compounds due to the tendency of electrons to pair, which are normally associated with oxidative damage (Castagne et al, 1999).

Free radicals can be generated in the brain by several mechanisms such as inefficiency of the electron-carrying components of the mitochondrial transport chain, monoamines degradation, xanthine oxidase reaction or by metabolism of arachidonic acid. Nevertheless, the free radicals produced could be metabolized especially by antioxidant enzymes such as superoxide dismutases, catalase and glutathione peroxidase (Hussain et al, 1995; Meister, 1995; Frantseva et al, 2000; Ferreira et al, 2009). Then, the resulting free radicals are very likely to react with non-radical molecules and transform them into secondary free radicals, which are normally observed during the lipid peroxidation producing hydroperoxides (MacDonald et al, 1989). The lipid peroxidation and nitrite are increased in hippocampus rats during pilocarpine-induced seizures (Freitas, 2009; Militão et al, 2010). Thus, it is worthwhile assessing the role of antioxidant compounds in the prevention of these neurochemical alterations on oxidative stress during seizures. The excitotoxicity has been shown to be related to over-production of free radicals in hippocampus of adult rats. The excessive release of excitatory amino acids such as glutamate may kill neurons via excessive activation of their receptors facilitating the installation and/or propagation of seizures (MacDonald et al., 1989; Cavaleiro et al., 1994; Meldrum, 1994).

Depending on neuronal maturity, the glutamate may induce either apoptotic or necrotic types of death (Ferrer et al, 1995) by impairing the Ca^{2+} homeostasis and inducing the oxidative stress (Leite et al, 1990; Murphy and Baraban, 1990). Based on this fact, it is important to evaluate the role of ubiquinone on oxidative stress in rat hippocampus during seizures since ubiquinone is a powerful antioxidant that prevents oxidative damage caused by free radicals, including oxidation of lipids within the mitochondrial membrane (Geromel et al., 2002).

Several antioxidant compounds, such as acid ascorbic (Xavier et al, 2007), lipoic acid (Freitas, 2009) and alpha-tocopherol (Barros et al, 2007) can protect the brain against oxidative stress in rat hippocampus caused by pilocarpine-induced seizures. Studies have suggested that ubiquinone (UQ) serves as an antioxidant by activating and increasing expression of mitochondrial uncoupling proteins which have antiapoptotic and antioxidant properties (Shults and Haas, 2005; Chaturvedi and Beal, 2008). Ubiquinol, a reduced form of ubiquinone, decreases lipid peroxidation directly by acting as a chain-breaking antioxidant

and indirectly by recycling alpha-tocopherol. Thus, it is important to investigate the neuroprotective effect of ubiquinone against hippocampal damage caused by oxidative stress observed during seizures.

In addition to preventing lipid peroxidation, UQ, as an effective antioxidant, also reacts with ROS (James et al., 2004). This study implies that ubiquinone may alter the oxidative stress in rat hippocampus caused by the seizures. To further attest this hypothesis, this study was aimed at evaluating the effects of ubiquinone on superoxide dismutase, catalase and glutathione peroxidase activities as well as in hydroperoxide concentration in the rat hippocampus during acute phase of seizures induced by pilocarpine.

The pilocarpine model could prove to be useful to delineate and understand the development of behavioral and neurochemical changes associated with temporal lobe epilepsy. Pilocarpine status may provide a model for studying the basic mechanisms responsible for refractory status epilepticus, amino acids and oxidative stress in humans and evaluating new drugs. The pilocarpine model may prove useful in the study of status epilepticus for number of reasons. First, these seizures accurately model human generalized epilepsy as it is seen from the anticonvulsant profile drugs. Secondly, the severe and refractory nature of this model indicates that it should be valuable in the development of new anticonvulsant agents. Finally, the prolonged and uniform degree of status epilepticus is useful for metabolic, neurochemical and neuroanatomical studies of the sequelae of prolonged seizure activity.

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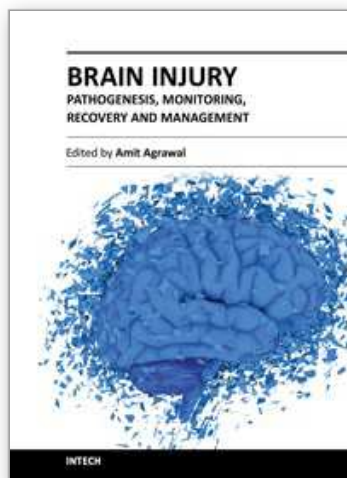
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