Article

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Inhibitory action of antioxidants (ascorbic acid or α-tocopherol) on seizures and brain damage induced by pilocarpine in rats

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ABSTRACT

Temporal lobe epilepsy is the most common form of epilepsy in humans. Oxidative stress is a mechanism of cell death induced by seizures. Antioxidant compounds have neuroprotective effects due to their ability to inhibit free radical production. The objectives of this work were to comparatively study the inhibitory action of antioxidants (ascorbic acid or α-tocopherol) on behavioral changes and brain damage induced by high doses of pilocarpine, aiming to further clarify the mechanism of action of these antioxidant compounds. In order to determinate neuroprotective effects, we studied the effects of ascorbic acid (250 or 500 mg/kg, i.p.) and α-tocopherol (200 or 400 mg/kg, i.p.) on the behavior and brain lesions observed after seizures induced by pilocarpine (400 mg/kg, i.p., P400 model) in rats. Ascorbic acid or α-tocopherol injections prior to pilocarpine suppressed behavioral seizure episodes. These findings suggested that free radicals can be produced during brain damage induced by seizures. In the P400 model, ascorbic acid and α-tocopherol significantly decreased cerebral damage percentage. Antioxidant compounds can exert neuroprotective effects associated with inhibition of free radical production. These results highlighted the promising therapeutic potential of ascorbic acid and α-tocopherol in treatments for neurodegenerative diseases.

Key words: seizures, status epilepticus, pilocarpine, ascorbic acid, α-tocopherol.

Ação inibitória de antioxidantes (ácido ascórbico e α-tocoferol) nas convulsões e dano cerebral em ratos induzidos pela pilocarpina

RESUMO

A epilepsia de lobo temporal é a mais comum forma de epilepsia em humanos. O estresse oxidativo é um dos mecanismos de morte celular induzida pelas crises convulsivas. Os compostos antioxidantes apresentam efeitos neuroprotetores devido à sua capacidade de inibir a produção de radicais livres. Os objetivos do presente trabalho foram estudar de forma comparativa a ação inibitória de antioxidantes (ácido ascórbico e α-tocoferol) sobre as alterações comportamentais e histopatológicas no hipocampo de ratos após convulsões induzidas pela pilocarpina. A fim de determinar os efeitos neuroprotetores destas drogas, o presente trabalho estudou os efeitos do ácido ascórbico (250 ou 500 mg/kg, i.p.) e do α-tocoferol (200 ou 400 mg/kg, i.p.) sobre o comportamento e as lesões cerebrais observados após convulsões induzidas pela pilocarpina (400 mg/kg, i.p., P400), em ratos. As injeções de ácido ascórbico ou α-tocoferol antes da administração de pilocarpina reduzem o número de animais que convulsionam. Estes achados sugerem que os radicais livres podem induzir o desenvolvimento de lesão cerebral durante as crises epilépticas. No modelo P400, o ácido ascórbico e o α-tocoferol, diminuem significativamente os danos cerebrais. Os compostos antioxidantes podem exercer efeitos neuroprotetores, e esses resultados podem estar associados à inibição da produção de radicais livres. Estes resultados sugerem um promissor potencial terapêutico tanto para o ácido ascórbico quanto para o α-tocoferol no tratamento de doenças neurodegenerativas.

Palavras-chave: crises epilépticas, estado de mal epiléptico, pilocarpina, ácido ascórbico, α-tocoferol.

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Status epilepticus (SE) is a severe form of continuous seizure attacks and a medical emergency associated with brain damage and significant mortality[1]. The common sequels of SE include continuing recurrent seizures, permanent neurological deficit and brain injury. The SE can be induced by administration of pilocarpine or lithium-pilocarpine[2,3]. Systemic injection of pilocarpine induces SE in rodents associated to histopathological alterations, which are most prominent in the limbic structures[4].

Pilocarpine administration induces seizures with three distinct phases: [A] an acute period, which lasts 1-2 days which is associated to repetitive seizures and SE; [B] a seizure-free (silent period) characterized by a progressive return to normal electroencephalography (EEG) and behavior that 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. 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[5]. 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[6,7]. Brain necrosis is associated with the occurrence of seizures, although studies have demonstrated that this association is not obligatory, especially in the pilocarpine model[8]. 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 well[2]. Oxidative stress mediated by free radical produces lipid peroxidation[9], increases the nitrite content in the hippocampus, striatum and frontal cortex[2] and may play a major role in the neuronal injury development after seizures induced by pilocarpine[10]. The biological effects of free radicals are controlled *in vivo* by a wide range of antioxidants, such as α-tocopherol, ascorbic acid, vitamin A, and reduced glutathione[11,12]. Acid ascorbic (ascorbic acid, AA) and α-tocopherol (α-tocopherol) have many functions in the brain and in the neuronal microenvironment. They work as neuromodulators as well as antioxidant/free radical scavengers[13-16]. It has been suggested that ascorbic acid and α-tocopherol have neuroprotective properties in some experimental models of excitotoxic neurological disorders, including seizure activity induced by pilocarpine[15-18].

The objectives of the present study were to comparatively study the inhibitory action of antioxidants (ascorbic acid or α-tocopherol) on behavioral changes and brain damage induced by high doses of pilocarpine, in order to further clarify the mechanism of action of these antioxidant compounds.

METHOD

88 adult male Wistar rats (250-280 g, 2 months old) were used. The animals were housed in cages with free access to food and tap water and were kept with standard light-dark cycling (12 h with alternate day and night cycles). The experiments were performed in accordance with the guide for the care and use of laboratory animals of the US Department of Health and Human Services, Washington, DC.

The substances used were pilocarpine hydrochloride (Sigma Chemical, USA), ascorbic acid (Sigma Chemical, USA), α-tocopherol (Sigma Chemical, USA) and atropine (Sigma Chemical, USA). All doses are expressed in milligrams per kilogram (mg/kg) and were intraperitoneally administered in a volume of 10 mL/kg. The rats were treated with saline 0.9% (control group, n=12), pilocarpine (400 mg/kg, n=16), atropine (50 mg/kg, n=12, A group), ascorbic acid (250 mg/kg, n=12, AA250 group), ascorbic acid (500 mg/kg, n=12, AA 500 group), α-tocopherol (200 mg/kg, n=12, VITE200 group) and α-tocopherol (400 mg/kg, n=12, VITE 400 group). All animals were observed for 24 h to detect any behavioral change, poisonous symptoms or death. The rats were treated with a single dose of atropine (50 mg/kg, n=12), ascorbic acid (250 mg/kg, n=12), ascorbic acid (500 mg/kg, n=12), α-tocopherol (200 mg/kg, n=12) and α-tocopherol (400 mg/kg, n=12), thirty minutes prior to intraperitoneal administration of pilocarpine 400 mg/kg (A plus P400, AA250 plus P400, AA500 plus P400, VITE200 plus P400 and VITE400 plus P400 groups, respectively). After the last administration of the drug, the animals were placed in 30 × 30 cm^2 chambers to register wild running, clonus, tonus, clonic–tonic seizures[8], the number of animals that had seizures, status epilepticus (SE) and deaths after pilocarpine administration. SE was defined as continuous seizures for a period longer than 30 min[19]. The pilocarpine group (P400 group) studied was constituted by the rats that presented seizures and SE for periods longer than 30 min and that did not die within 24 h of observation. Mortality was recorded for 24 h after pilocarpine-induced SE and corresponded to 60%. Cholinergic reactions were defined by the appearance of peripheral cholinergic reactions, such as myosis, piloerection, chromodacryorrhea, diarrhea, masticatory and other stereotyped movements. It important to clarify that the doses of ascorbic acid and α-tocopherol that were used were determined from dose-response studies and observations of the doses currently used in animal studies in the literature[14,18]. The doses used were not equivalent to those used by humans, since rats have different metabolic rates.

All groups were closely observed for 24 h for behav-

ioral changes and convulsive state. After 24 h of observation, the animals were sacrificed by decapitation 24 h after the treatment, and their brains were dissected out and fixed in 10% formalin[20,21]. After an initial coronal section at the level of the optic nerve, sections of 3-5 µm in thickness were prepared and stained with hematoxylin & eosin (H&E) for optical microscopy analysis (100x). The degree of brain damage was defined on a scale ranging from 0 (none) to 100 (total) using optical microscopy and previously defined to be reliable for morphological analysis[22]. Brain damage presence was confirmed if one or more structures showed at least 50% involvement.

Cholinergic reactions, seizures and mortality rate were presented as percentages (% seizures and % death, respectively) and compared with a nonparametric test (χ^2). For statistical analyses of histopathological abnormalities, the results were compared using ANOVA and the Student-Newman-Keuls test as a *post hoc* test. In order to determine differences between groups, the results were compared by one-way analysis of variance (ANOVA) followed by Newman-Keuls ($p<0.05$) using the GraphPad program (Intuitive Software for Science, San Diego, CA).

RESULTS

Pilocarpine induced the first seizure at 35.00±0.70 min. All animals showed generalized tonic-clonic convulsions with SE leading to a survival rate of 40%. Animals treated with saline or atropine 30 min before pilocarpine injection as well as those that received saline, atropine, ascorbic acid or α-tocopherol manifested no behavior alterations (Table 1). On the other hand, in the AA250 plus P400 group, changes in behavior were observed, such as peripheral cholinergic signs (100%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (50%), which developed progressively within 1-2 h into a long-lasting SE (50%), revealing a survival rate from the seizures of 50%. In the AA500 plus P400 group, 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 SE (25%), revealing a survival rate of 75% (Table 1). In the VITE200 plus P400 group, we found changes in behavior, such as peripheral cholinergic signs (100%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (32%), which progressively devel-

Table 1. Behavioral changes in rats treated intraperitoneally with pilocarpine, atropine, ascorbic acid, or their combinations.

Groups	n	Cholinergic reactions (%)	Seizures (%)	Status epilepticus (%)	Death (%)
P400	16	100	75	75	60
A	12	0[a]	0[a]	0[a]	0[a]
A plus P400	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]
AA250 plus P400	12	100	50[a]	50[a]	50
AA250	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]
AA500 plus P400	12	100	25[a]	25[a]	25[a]
AA500	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]

Pilocarpine was administered in a single dose (400 mg/kg, P400), AA groups with ascorbic acid (250 or 500 mg/kg,) and A group with atropine (50 mg/kg). The A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400. The AA plus P400 groups were treated with ascorbic acid (250 or 500 mg/kg) and 30 min before P400. Results are expressed as percentages of the number of animals. The animals treated with saline, ascorbic acid, or atropine 30 min before P400 did not show any behavioral changes. [a]$p<0.05$ compared with P400 group (χ^2 test). [b]$p<0.05$ compared with A plus P400, AA250 plus P400 groups or AA500 plus P400 groups (χ^2 test).

Table 2. Behavioral changes in rats treated intraperitoneally with pilocarpine, atropine, α-tocopherol, or their combinations.

Groups	n	Cholinergic reactions (%)	Seizures (%)	Status epilepticus (%)	Death (%)
P400	16	100	75	75	60
A	12	0[a]	0[a]	0[a]	0[a]
A plus P400	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]
VITE200 plus P400	12	100	32[a]	32[a]	32
VITE200	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]
VITE400 plus P400	12	100	16[a]	16[a]	16[a]
VITE400	12	0[a,b]	0[a,b]	0[a,b]	0[a,b]

Pilocarpine was administered in a single dose (400 mg/kg, P400), VITE groups with α-tocopherol (200 or 400 mg/kg,) and A group with atropine (50 mg/kg). The A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400. The VITE plus P400 groups were treated with α-tocopherol (200 or 400 mg/kg) and 30 min before P400. Results are expressed as percentages of the number of animals. The animals treated with saline, α-tocopherol, or atropine 30 min before P400 did not show any behavioral changes. [a]$p<0.05$ compared with P400 group (χ^2 test). [b]$p<0.05$ compared with A plus P400, VITE200 plus P400 or VITE400 plus P400 groups (χ^2 test).

Table 3. Histopathological abnormalities in the hippocampus treated intraperitoneally with pilocarpine, atropine, ascorbic acid, or their combinations.

Groups	Histopathological abnormalities in the hippocampus			
	(%) Rats with brain lesion	(%) Severity of lesion	Number of animals with brain damage	Total number of animals
P400	85	59.92±0.23	5	6
A	0[a]	0[a]	0	6
A plus P400	0[a,b]	0[a,b]	0	6
AA250 plus P400	50[a]	20.00±0.32[a]	3	6
AA250	0[a,b]	0[a,b]	0	6
AA500 plus P400	33[a,b]	17.66±0.33[a]	2	6
AA500	0[a,b]	0[a,b]	0	6

Pilocarpine was administered in a single dose (400 mg/kg, P400), AA groups with ascorbic acid (250 or 500 mg/kg,) and A group with atropine (50 mg/kg). The A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400. The AA plus P400 groups were treated with ascorbic acid (250 or 500 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. [a]$p<0.05$ compared with P400 group (χ^2 test). [b]$p<0.05$ compared with A plus P400, AA250 plus P400 groups or AA500 plus P400 groups (χ^2 test).

Table 4. Histopathological abnormalities in the hippocampus treated intraperitoneally with pilocarpine, atropine, α-tocopherol or their combinations.

Groups	Histopathological abnormalities in the hippocampus			
	(%) Rats with brain lesion	(%) Severity of lesion	Number of animals with brain damage	Total number of animals
P400	85	59.92±0.23	5	6
A	0[a]	0[a]	0	6
A plus P400	0[a,b]	0[a,b]	0	6
VITE200 plus P400	33[a]	13.66±0.33[a]	2	6
VITE200	0[a,b]	0[a,b]	0	6
VITE400 plus P400	17[a,b]	10.50±0.50[a]	1	6
VITE400	0[a,b]	0[a,b]	0	6

Pilocarpine was administered in a single dose (400 mg/kg, P400), VITE groups with α-tocopherol (200 or 400 mg/kg,) and A group with atropine (50 mg/kg). The A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400. The VITE plus P400 groups were treated with α-tocopherol (200 or 400 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± S.E.M. of scores of damage based in 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. [a]$p<0.05$ compared with P400 group (ANOVA and Student-Newman-Keuls test). [b]$p<0.05$ compared with VITE200 plus P400 groups or VITE400 plus P400 groups (ANOVA and Student-Newman-Keuls test).

oped (1-2 h) into a long-lasting SE (32%), revealing a survival rate of 68%. The VITE400 plus P400 group manifested alterations in behavior, such as peripheral cholinergic signs (100%), staring spells, facial automatisms, wet dog shakes, rearing and motor seizures (16%), which progressively developed (1-2 h) into long-lasting SE (16%), revealing a survival rate of 84% from the seizures (Table 2).

Brain tissue examinations of the control (saline 0.9%) (Figs 1A and 2A), atropine (50 mg/kg) (Figs 1C and 2C), atropine plus pilocarpine (A plus P400) (Figs 1D and 2D), AA (250 or 500 mg/kg) and α-tocopherol groups (VITE 200 or 400 mg/kg) did not reveal hippocampal histopathological changes (Tables 3 and 4). On the other hand, pilocarpine-treated animals (400 mg/kg) presented neuronal loss, gliosis, and typical vacuolar degeneration in hippocampus region (Figs 1B and 2B). Histopathological damage was observed in three (50%), two (33%), two (33%) and in one animal (17%) in the AA250 plus P400, AA500 plus P400, VITE200 plus P400 and VITE400 plus P400 groups, respectively (Tables 3 and 4).

Brain tissue examination of AA 250 (Fig 1E), AA 500 (Fig 1G), VITE200 (Fig 2E), and VITE400 (Fig 2G) did not show any hippocampus histological alterations. However, the AA250 plus P400 (Fig 1F), AA500 plus P400 (Fig 1H), VITE200 plus P400 (Fig 2F), and VITE400 plus P400 groups (Fig 2H) showed typical histopathological chang-

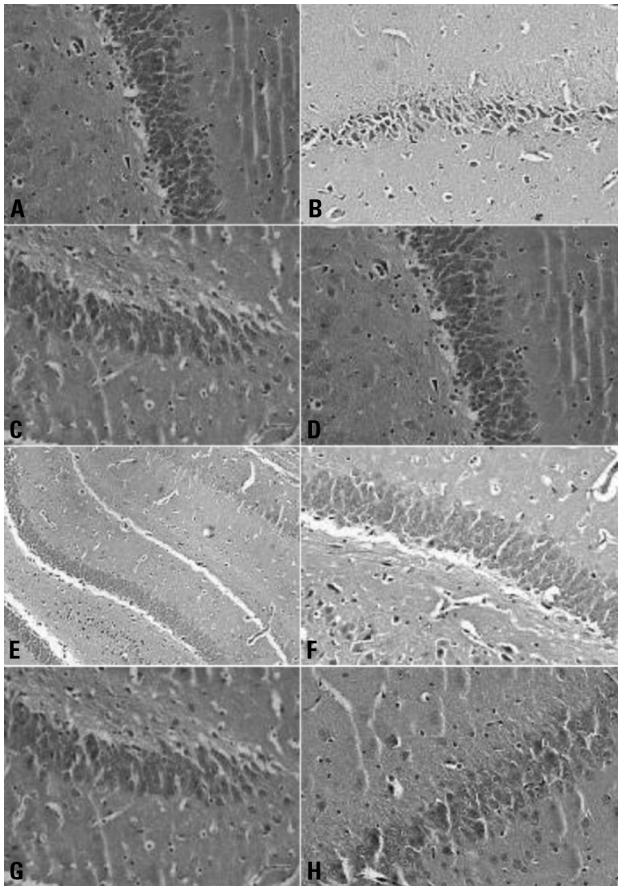

Fig 1. Histopathological alterations in rat hippocampus treated with pilocarpine, atropine, ascorbic acid or their combinations. [A] Control group; [B] P400 group; [C] Atropine group; [D] A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400; [E] AA plus 250 group; [F] AA250 plus P400 groups was treated with ascorbic acid (250 mg/kg) and 30 min before P400; [G] AA500 group; [H] AA500 plus P400 group was treated with ascorbic acid (500 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± 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.

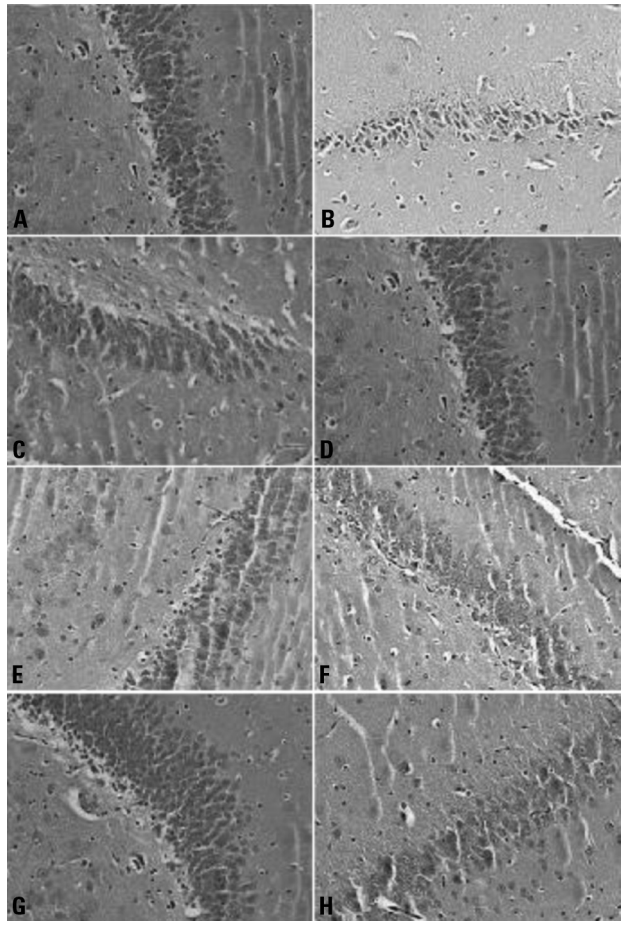

Fig 2. Histopathological alterations in rat hippocampus treated with pilocarpine, atropine, α-tocopherol or their combinations. [A] Control group; [B] P400 group; [C] Atropine group; [D] A plus P400 group was treated with atropine (50 mg/kg) and 30 min before P400; [E] VITE200 group; [F] VITE200 plus P400 group was treated with α-tocopherol (200 mg/kg) and 30 min before P400; [G] VITE400 group; [H] VITE400 plus P400 group was treated with α-tocopherol (400 mg/kg) and 30 min before P400. Severity of lesion was expressed as mean ± 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.

es characterized by neuronal loss, gliosis and vacuolation affecting 50, 33, 33 and 17% of the rats, respectively (Tables 3 and 4).

DISCUSSION

Cholinergic mechanisms play an important role in activation of limbic seizures and dopaminergic, serotonergic, GABAergic and glutamatergic systems are responsible for the propagation and/or maintenance of seizures and SE induced by pilocarpine[2]. Previous studies described a model of limbic seizures followed by brain damage produced by systemic injection of a high dose of pilocarpine (400 mg/kg) in rats[5,23]. The evidence included temporal correlation among free radical generation, development of seizures and neuroprotective effects of antioxidant drugs against neuronal damage caused by seizures[24]. Others studies showed that pilocarpine-induced seizures and brain damage in various cerebral regions[21,25,26].

In our work, a significant hippocampal injury was observed in the P400 group. The anticonvulsant effect in the absence of anticholinergic drugs subsequent to the seizure onset suggests that muscarinic receptor activation is involved directly on the onset 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[2,14,27,28]. Previous research indicates that anticonvulsant effects of noradrenergic antagonist drugs have a fundamental role in the mechanisms responsible for seizure beginning, severity and duration. In fact, the reduction of severity and duration of seizures are protective against neurotoxicity caused by seizures induced by hemoconvulsants (e.g. pilocarpine, kainic acid and others). These data, although 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[29].

There are several indications that free radical plays a role in epileptogenesis[2,10,26]. During seizures, the reactive oxygen species (ROS) concentration and brain lipid peroxidation increase[2]. It is currently hypothesized that any pathological process such as SE, which releases dopamine and glutamate, activates D_2 and NMDA receptors. This may lead to neuronal necrosis by elevating intracellular calcium and activating potentially destructive calcium-dependent enzymes[31], augmenting the production of free radicals during seizures induced by pilocarpine[10,31]. Thus, it could be expected that antioxidant drugs such as ascorbic acid and α-tocopherol, can be used as scavengers of free radicals, reducing brain injury induced by pilocarpine. In the present work, we showed that the antioxidants ascorbic acid and α-tocopherol protected animals against seizures, SE and brain damage induced by pilocarpine, thus decreasing the percentage of seizures, SE and death in relation to both doses tested.

A variety of epilepsy models reflect the effects of acid ascorbic and α-tocopherol and specify their action[13,15]. Previously, it had been demonstrated that these compounds reduced the frequency of penicillin-induced epileptiform activity[12,27]. In recent years, many roles of α-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 α-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[9].

Our results demonstrated that seizure pattern and brain damage observed in pilocarpine-treated animals differ from those treated with α-tocopherol plus pilocarpine (VITE400/P400). They reproduced the syndrome with lower intensity of histopathological changes and mortality rate, in comparison with the VITE200 plus P400 and P400 groups, thus corroborating the outcomes obtained by Ribeiro et al.[32] and Ayyildiz et al.[12]. The percentage of SE (75%) that was found further corroborated prior investigations[5,33,34].

Ascorbic acid is probably the most important water-soluble antioxidant in the brain extracellular fluid, and it is essential in regenerating reduced α-tocopherol in membranes[35]. Although 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 suggest that ascorbic acid participates in pro-oxidant reactions under certain conditions[36]. In the present work, the outcomes confirm that ascorbic acid (250 and 500 mg/kg) decreased the frequency of pilocarpine-induced seizures, SE and brain lesions in rats. In addition, ascorbic acid decreases the severity of hippocampal lesions and mortality rate caused by pilocarpine. Yamamoto et al.[37] demonstrated that injection of ascorbate, 60 min before $FeCl_3$ administration, prevented the occurrence of epileptic discharges. Since there are wide variations of α-tocopherol and ascorbic acid doses used in different models of seizure, more detailed investigations are necessary before an ultimate conclusion on the effects of ascorbic acid and α-tocopherol on pilocarpine-induced seizures can be achieved.

In conclusion, we suggest that there is an accumulation of free radicals after SE induced by pilocarpine, and oxidative changes in other parameters during the acute phase. This finding suggests that the seizures, SE and deaths induced by pilocarpine have a large participation of brain oxidative stress, which is closely related to the mechanism of propagation and/or maintenance of the epileptic focus by pilocarpine. The results from the present work 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 α-tocopherol in treatments for neurodegenerative diseases.

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