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Vitamin C antioxidant effects in hippocampus of adult Wistar rats after seizures and status epilepticus induced by pilocarpine

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Abstract

Vitamin C (VIT C) is an exogenous antioxidant able to alter the brain oxidative stress. Antioxidant properties have been showed in seizures and status epilepticus (SE) induced by pilocarpine in adult rats. This present study was aimed at was investigating the VIT C effects on latency to first seizure, in percentage of seizures, mortality rate, as well as hippocampal lipid peroxidation levels and catalase activity after seizures and SE. The VIT C effects were investigated after the pretreatment with dose 250 mg/kg, i.p., 30 min before pilocarpine administration (400 mg/kg, s.c., pilocarpine group (P400)). The VIT C increase the latency to first seizure and decrease the mortality rate and lipid peroxidation levels. In P400 + VIT C and VIT C groups were observed an increase in hippocampal catalase activity. Our results suggests that the vitamin C can exert antioxidant and anticonvulsive effects in adult rats, suggesting that this vitamin can be able by reduction of lipid peroxidation content and increased of catalase enzymatic activity which cerebral compensatory mechanisms in free radical formation during SE.

Keywords: Vitamin C; Pilocarpine; Hippocampus; Lipid peroxidation; Catalase; Seizures; Status epilepticus

Status epilepticus (SE) is a neurologic emergency associated mortality rate of 10–12% [26]. This condition is characterized by prolonged or repetitive epileptic discharges, resulting clinically in persistent alterations of normal brain function and cognitive state [27]. SE has been previously reported to increase acetylcholine turnover and release in rat brain during development of epilepsy [1,18,27]. Seizures and SE induced by pilocarpine have demonstrated behavioural and electroencephalographic characteristics similar to those in human temporal lobe epilepsy [27].

Neurochemical as well as enzymatic activities studies suggest that SE alters free radical metabolism in rat brain. The oxidative stress has been associated with neuronal damage induced by seizures and SE [3]. The membrane lipid peroxidation, can be produced by increase in free radicals levels and/or decrease in activities antioxidant defense [9,10]. The brain is a preferential target for the peroxidative process because it has a high content of polyunsaturated fatty acids [19,25]. Organisms have systems that prevent hazardous effects of free radicals such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-pX) and reduced glutathione (GSH). Recent studies suggest that differences can be reported in free radical scavenging enzymatic activities during the convulsive process induced by pilocarpine [13,22].

Vitamin E can inhibit the neuronal damage produced by increase lipid peroxidation contents observed during seizures and status epilepticus induced by pilocarpine in adult brain rats [2], its results can justify the interest in study of vitamin C pharmacological effects in seizures and status epilepticus induced by pilocarpine. The pilocarpine model is an useful animal model to investigate the development of neuropathology of temporal lobe epilepsy [4]. Barros et al. [2] described the importance of catalase activity and vitamin E effects in epileptic rats. However, the mechanisms by vitamin C and catalase activity influence seizures and SE are not completely understood, as well as vitamin C effects in convulsive process remained understood.

This work was performed in order to determine vitamin C acute treatment effects on latency to first seizures, percentage of animals that presented seizures and mortality rate, as well as lipid peroxidation levels and hippocampal catalase activity after seizures and status epilepticus induced by pilocarpine in adult rats.

Male Wistar rats (250–280 g; 2-month-old) were used. Animals were housed in cages with free access to food and water.

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All animals were kept with standard light–dark cycle (lights on at 07:00 a.m.). The experiments were performed according to the Guide for the care and use of laboratory the US Department of Health and Human Services, Washington, DC (1985).

Experiments were conducted at 7:00 a.m. in an experimental room. In a set of experiments, the animals were vitamin C 250 mg/kg, i.p. (VIT C 250) or 0.9% saline, i.p., treated and 30 min later, they received pilocarpine hydrochloride, 400 mg/kg, s.c. (P400 + VIT C 250). Other three groups received alone P400 (pilocarpine group), vitamin C (VIT C 250) or 0.9% saline (control group). Animals were closely observed for behavioural changes (appearance of peripheral cholinergic reactions, such as miosis, piloerection, chromodacryorrhea, diarrhea, masticatory and stereotyped movements), latency to the development of the first seizures, status epilepticus and mortality rate, immediately after the injection of pilocarpine during 24 h. The survivors were killed by decapitation and their brains were dissected on ice to remove hippocampus for determination of lipid peroxidation level and catalase activity. The pilocarpine group (P400) was constituted by those rats that presented seizures; SE for a period longer than 30 min and that did not died during 24 h.

Immediately after decapitation of the animals, the hippocampus was dissected for the preparation of homogenates 10% (w/v). The formation of lipid peroxides during lipid peroxidation process was analysed by measuring the thiobarbituric-acid-reacting substances (TBARS) in cells, as previously described by Draper and Hadley [8]. Briefly, the samples were mixed with 50 mM potassium phosphate monobasic buffer pH 7.4 and catalytic system of formation of free radicals ($FeSO_4$ 0.01 mM and ascorbic acid 0.1 mM), and then incubated at 37 °C for 30 min. The reaction was stopped with 0.5 ml of trichloroacetic acid 10%, then the samples were centrifuged (3000 rpm/15 min), the supernatants were retrieved and mixed with 0.5 ml of thiobarbituric acid 0.8%, then heated in a boiling water bath for 15 min and after this period, immediately cold in bath of ice. Lipid peroxidation was determined by the absorbance at 532 nm. The results above were expressed as μmol of malondialdehyde (MDA)/g wet tissue.

Immediately after decapitation of the animals, the hippocampus was dissected and ultrasonically homogenized in 1 ml of 0.05 M phosphate buffer, pH 7.0; the protein concentration was measured according to the method described by Lowry et al. [20] and used for catalase activity determinations. Catalase activity was measured by the method that employs hydrogen peroxide to generate H_2O and O_2 [5]. The activity was measured by degree of this reaction. The standard assay substrate mixture contained 0.30 ml of hydrogen peroxide in 50 ml of 0.05 M phosphate buffer, pH 7.0. The sample aliquot (20 μl) was added to 980 μl of substrate mixture. After 1 min, initial absorbance was recorded and final absorbance was read after 6 min. The reaction was followed at 230 nm. A standard curve was established using purified catalase (Sigma, MO, USA) under identical conditions. All samples were diluted with 0.1 mmol/l phosphate buffer (pH 7.0) to provoke an inhibition 50% of diluent rate (i.e. the uninhibited reaction). The results were expressed as μM/min/μg protein [21].

The drug dosages were determined from both dose–response studies, including pilocarpine (data not shown), and observations of the doses currently used in animal studies in the literature. The doses used are not equivalent to those used by humans because rats have higher metabolic rates.

Results of the latency to first seizures were compared using ANOVA and the Student–Newman–Keuls test as post hoc test because these results show a parametric distribution. The number of animals that seized and the number that survived were calculated as percentages (%seizures and %survival, respectively) and compared with a nonparametric test (χ^2). In both situations statistical significance was reached at p less-than-or-equals, slant 0.05. The statistical analyses were performed with the software GraphPad Prism, Version 3.00 for Windows, GraphPad Software (San Diego, CA, USA).

All animals treated with P400 presented peripheral cholinergic signs (miosis, piloerection, chromodacriorrhea, diarrhea, masticatory), and stereotyped movements (continuous sniffing, paw licking and rearing) followed by motor limbic seizures in 75% (60/80) of the tested animals ($p < 0.0001$). The convulsive process persisted and built up to a status epilepticus in 75% (60/80) of these rats, leading to death of 63% (50/80) of the animals ($p < 0.0001$) (Table 1). The animals pre-treated with VIT C and pilocarpine group (P400) developed cholinergic reactions, 33% (04/12) had seizures, 25% (03/12) built up to status epilepticus ($p < 0.0001$) and no one animal died (Table 1). VIT C administration, 30 min before P400, increased the latency to the onset of the first seizure in 129% (P400 = 35.00 ± 0.70; VIT C = 80.01 ± 1.28) ($p < 0.05$) and latency of the status epilep-

Table 1
Vitamin C effects on latency to first seizures, seizures and status epilepticus induced by pilocarpine in adult rats

Pharmacological class					
Drugs	Dose (mg/kg)	Latency to first seizures (min)	%Seizures	%Survival	Number of animals/group
P400 group	400	35 ± 0.7	75	27	80
P400 + VIT C 250	250	80 ± 1.3[a]	33[b]	100[b]	12
VIT C 250	250	00	00[b,c]	100[b]	12

Animals were pretreated acutely, intraperitoneally, with drugs listed above and 30 min after receiving pilocarpine 400 mg/kg, s.c. Results for latency to first seizure are expressed as means ± S.E.M. of the number of experiments shown in the table. Result for %seizures and %survival are expressed as percentages of the number of animals from each experimental group.

[a] $p < 0.05$ as compared with P400 + VIT C 250 (ANOVA and Student–Newman–Keuls test).

[b] $p < 0.0001$ as compared with pilocarpine group (χ^2 test).

[c] $p < 0.0001$ as compared with P400 + VIT C 250 (χ^2 test).