Life Sciences 122 (2015) 65–71

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Antiepileptogenic, antioxidant and genotoxic evaluation of rosmarinic acid and its metabolite caffeic acid in mice

Vanessa Rodrigues Coelho [a], Caroline Gonçalves Vieira [a], Luana Pereira de Souza [a], Felipe Moysés [b], Carla Basso [b], Débora Kuck Mausolff Papke [c], Thienne Rocha Pires [c], Ionara Rodrigues Siqueira [b], Jaqueline Nascimento Picada [c], Patrícia Pereira [a,*]

[a] Laboratório de Neurofarmacologia e Toxicologia Pré-Clínica, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Laboratório de Neuropsicofarmacologia, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Laboratório de Genética Toxicológica, Universidade Luterana do Brasil, Canoas, RS, Brazil

ARTICLE INFO

Article history:
Received 1 August 2014
Accepted 17 November 2014
Available online 11 December 2014

Keywords:
Caffeic acid
Comet assay
Kindling
Oxidative stress
Pentylenetetrazole
Rosmarinic acid

ABSTRACT

Aims: Antioxidant compounds have been extensively investigated as a pharmacological alternatives to prevent epileptogenesis. Rosmarinic acid (RA) and caffeic acid (CA) are compounds with antioxidant properties, and RA has been shown to inhibit GABA transaminase activity (*in vitro*). Our aim was to evaluate the effect of RA and CA on seizures induced by pentylenotetrazole (PTZ) using the kindling model in mice.
Main methods: Male CF-1 mice were treated once every three days during 16 days with RA (1, 2 or 4 mg/kg; i.p.), or CA (1, 4 or 8 mg/kg; i.p.), or positive controls diazepam (1 mg/kg; i.p.) or vigabatrin (600 mg/kg; p.o.), 30 min before PTZ administration (50 mg/kg; s.c.). After the last treatment, animals were sacrificed and the cortex was collected to evaluate free radicals (determined by 2′,7′-dichlorofluorescein diacetate probe), superoxide dismutase (SOD) and genotoxic activity (Alkaline Comet Assay).
Key findings: Rosmarinic acid 2 mg/kg increased latency and decreased percentage of seizures, only on the 4th day of observation. The other tested doses of RA and CA did not show any effect. Rosmarinic acid 1 mg/kg, CA 4 mg/kg and CA 8 mg/kg decreased free radicals, but no dose altered the levels of enzyme SOD. In the comet assay, RA 4 mg/kg and CA 4 mg/kg reduced the DNA damage index.
Significance: Some doses of rosmarinic acid and CA tested showed neuroprotective action against oxidative and DNA damage produced in the kindling epilepsy model, although they did not produce antiepileptogenic effect *in vivo*.

Introduction

Epilepsy is characterized by unprovoked episodes of aberrant synchronous excitation of brain regions that disrupt normal functioning and cause successive seizures [7,47]. According to the World Health Organization (WHO), about 50 million people are affected worldwide, and approximately 70–80% of patients with new-onset epilepsy enter remission when they are treated with antiepileptic drugs currently prescribed. Antiepileptic drugs, now commonly referred to as antiseizure drugs (ASDs), provide symptomatic benefits by preventing the occurrence of seizures in patients. In spite of these benefits, ASDs fail to control seizures in 20–30% of patients, or present troubling side effects [14,20,27]. The development of therapeutic strategies to prevent the recurrent seizures and the establishment of *status epilepticus* has been the main goal of the contemporary epilepsy research [20,43].

The mechanisms underlying seizures are complex, and vary across the numerous seizure types that have been characterized. A failure is believed to occur in the ability to maintain the balance between brain excitation and inhibition process. Thus, neurotransmitters involved in neuronal inhibition, such as gamma aminobutyric acid (GABA), or neuronal excitation such as glutamate and aspartate, have attracted the interest of researchers aiming to elucidate the mechanisms involved in epilepsy pathogenesis [7,33]. Furthermore, it is known that neural tissues are especially sensitive to oxygen levels, and oxidative stress is thought to be involved in epileptogenesis. Levels of reactive oxygen species (ROS) increase in response to sustained neuronal electrical activity and seizures. Therefore, antioxidants have been suggested as therapeutic design strategies for the treatment and modulation of epilepsy [43].

Rosmarinic acid (α-*O*-caffeoyl-3,4-dihydroxyphenyl lactic acid; RA) and its major metabolite caffeic acid (CA) are compounds that occur in many plants, and present several biological activities ([3,18,35,36,37,53]), among which antioxidative activity [12,21,28,37,39,55]. Furthermore, RA was able to inhibit the enzyme GABA transaminase

* Corresponding author. Tel.: +55 51 33083183; fax: +55 51 3121.
E-mail address: patipere@yahoo.com.br (P. Pereira).

http://dx.doi.org/10.1016/j.lfs.2014.11.009
0024-3205/

in vitro [4], which would increase the levels of GABA *in vivo*. This finding makes these compounds interesting targets in investigations about the treatment of epilepsy.

The aim of this study was to evaluate the possible antiepileptogenic activity of the RA and CA using the chemical kindling induced by pentylenetetrazole (PTZ) in mice. We also investigated the effects of RA and CA on the production of free radicals, on the activity of antioxidant enzyme superoxide dismutase (SOD), and on deoxyribonucleic acid (DNA) damage in total cortex of mice after the kindling model.

Material and methods

Animals

Male CF1 mice (2–3 months of age, 30–40 g) were obtained from State Foundation for Health Research and Production (FEPPS). The animals were divided into ten groups: 9 groups were used in the kindling experiment (N = 8–11) and one group Sal/Sal (N = 7) was used as negative control in other measurements, totaling 82 animals. Mice were housed in plastic cages (5 per cage), with water and food *ad libitum*, under a 12-h light/dark cycle (lights on at 8:00 AM), and at a constant temperature of 23 ± 2 °C. All experimental procedures were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23) (revised, 1996). This work was approved by the Committee on the Ethical Use of Animals of UFRGS (N. 23616). Appropriate measures were taken to minimize pain or discomfort of the animals.

Drugs and pharmacological procedures

Rosmarinic acid (RA), caffeic acid (CA) and pentylenetetrazole (PTZ) were acquired from Sigma-Aldrich, vigabatrin (VGB) (Sabril®) from Sanofi Aventis, and diazepam (DZP) (Compaz®) was purchased from Cristália Prod. Quim.Farm. All drugs were dissolved in saline (NaCl 0.9%) solution. The doses of RA, CA, PTZ, VGB and DZP were chosen based on previous studies [2,9,31,35,36,49]. The injection volume used was 0.1 mL/10 g of weight of animal.

PTZ-kindling model

The procedure was carried out as described by Oliveira et al. [33]. The animals were divided into nine groups. Each treatment consisted of two repeated administrations once every three day, totaling six treatments (16 days). Animals were given intraperitoneal injections of RA (1, 2 or 4 mg/kg), CA (1, 4 or 8 mg/kg), DZP (1 mg/kg) or saline, 30 min before the subconvulsive stimuli (PTZ 50 mg/kg, s.c.). Vigabatrin (600 mg/kg) was administered by gavage (p.o) 4 h before subconvulsive stimuli. Immediately after PTZ injection, mice were placed individually in acrylic observation chambers for 30 min. The behavior observed was the latency to the first seizure and the occurrence of clonic forelimb seizures as long as or lasting longer than 3 s.

Samples preparation

After 1 h of the last PTZ administration, mice were sacrificed, brain was removed, and total cerebral cortex was dissected out on ice. Part of the fresh samples was analyzed in the comet assay (N = 5 per group). The other part of samples was immediately frozen in liquid nitrogen and stored at −80 °C. These samples were used to evaluate oxidative parameters (N = 7 per group).

Assessment of oxidative stress parameters

For the analysis of oxidative parameters, brain tissue was homogenized in ice-cold phosphate buffer (0.02 M, pH 7.4) containing EDTA (0.002 M) and phenylmethylsulfonyl fluoride (PMSF, 0.1 M) in a Teflon-glass homogenizer. After that, the homogenate was centrifuged at 1000 ×*g* for 10 min at 4 °C and the supernatant was used for the assays.

Free radical levels

We used 2′-7′-dichlorofluorescein diacetate (DCFH-DA) as a probe [24] to evaluate the formation of free radicals in total cortex. An aliquot of the sample was incubated with DCFH-DA (100 μM) at 37 °C for 30 min. The reaction was terminated by chilling the reaction mixture in ice. The formation of the oxidized fluorescent derivative (DCF) was monitored at excitation and emission wavelengths of 488 nm and 525 nm, respectively, in a fluorescence spectrophotometer. The free radical content was quantified using a DCF standard curve and results were expressed as DCF formed/mg protein. All procedures were performed in the dark, while the blanks containing DCFH-DA (no homogenate) were processed for measurement of autofluorescence [13,50].

Superoxide dismutase (SOD) activity

Superoxide dismutase activity was assessed in total cortex as described in Moysés et al. [30]. Superoxide dismutase activity was determined using a RANSOD kit (Randox Labs, USA). This method employs xanthine and xanthine oxidase to generate $O_2^{\cdot-}$, which reacts with 2-(4-iodophenyl)-3-(4-nitrophenol)-5 phenyltetrazolium chloride to form a red formazan dye that is spectrophotometrically assayed at 505 nm and 37 °C. The inhibition of chromogen production is proportional to the activity of SOD in the sample.

Protein determination

Total protein concentrations were determined according to the method described by Bradford [6] using bovine serum albumin standard.

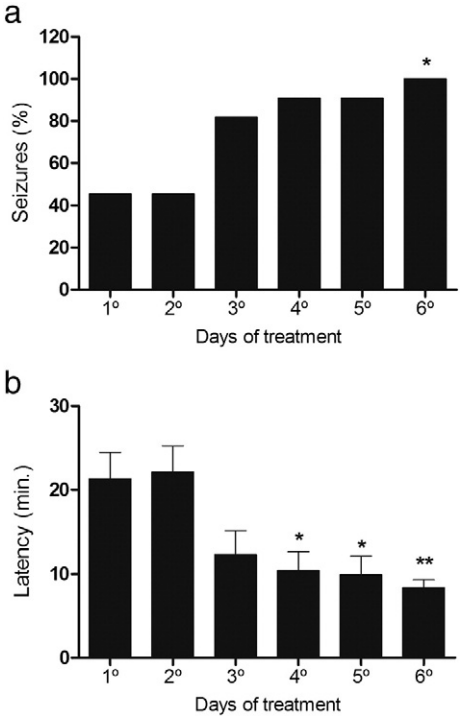

Fig. 1. Effect of PTZ administration (50 mg/kg) in mice submitted to kindling protocol. a) Percentage of mice presenting seizures as long as or longer than 3 s (*$p < 0.05$, compared to the 1st day of treatment; Fisher's Exact Test). b) Latency to the occurrence of the 1st seizure as long as or longer than 3 s (*$p < 0.05$, **$p < 0.01$ compared to the 1st day of treatment; ANOVA followed by the Bonferroni test, results were expressed as mean ± SEM). N = 11 animals per group.