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Research report

Carvacrol prevents impairments in motor and neurochemical parameters in a model of progressive parkinsonism induced by reserpine

Lívia Cristina R.F. Lins[a], Marina F. Souza[a], José Marcos M. Bispo[a], Auderlan M. Gois[a], Thaís Cristina S. Melo[a], Rayr Antonio S. Andrade[b], Lucindo J. Quintans-Junior[a], Alessandra M. Ribeiro[c], Regina H. Silva[d], José R. Santos[b,*], Murilo Marchioro[a]

[a] Department of Physiology, Federal University of Sergipe, São Cristóvão, SE, Brazil
[b] Department of Biosciences, Federal University of Sergipe, Itabaiana, SE, Brazil
[c] Department of Biosciences, Federal University of São Paulo, Santos, SP, Brazil
[d] Department of Pharmacology, Federal University of São Paulo, São Paulo, SP, Brazil

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ABSTRACT

Parkinson's disease (PD) is a neurodegenerative disease characterized by a progressive degeneration of dopaminergic neurons in the substantia nigra pars compact (SNpc), with consequent depletion of dopamine in the striatum, which gives rise to the characteristic motor symptoms of PD. Although its etiology is unknown, several studies have suggested that oxidative stress plays a critical function in the pathophysiology of PD, and antioxidant agents could be helpful to slown down the dopaminergic neurodegeneration. Carvacrol (CA) is a phenolic monoterpene found in essential oils of many aromatic plants that presents antioxidant and neuroprotective effects. This study aimed to assess the effect of CA in a reserpine (RES)-induced rat model of PD. Male Wistar rats received 15 s.c. injections of 0.1 mg/kg RES or vehicle, every other day, concomitantly to daily i.p. injections of CA (12.5 or 25 mg/kg) or vehicle. Across the treatment, the animals were submitted to behavioral evaluation in the catalepsy test (performed daily), open field test (7th day) and assessment of vacuous chewing movements (12th, 20th and 30th days). Upon completion of behavioral tests, rats were perfused and their brains underwent tyrosine hydroxylase (TH) immunohistochemical analysis. Our results showed that CA (12.5 e 25 mg/kg) prevented the increase in catalepsy behavior and number of vacuous chewing movements, but failed to revert the decreased open-field locomotor activity induced by RES. In addition, CA in both doses prevented the decrease in TH immunostaining induced by RES in the SNpc and dorsal striatum. Taken together, our results suggest that CA shows a protective effect in a rat model of PD, preventing motor and neurochemical impairments induced by RES. Thus, the use of CA as a promising new strategy for the prevention and/or treatment of PD may be considered.

1. Introduction

Parkinson's disease (PD) is a progressive and age-related neurodegenerative disease and is the second most common neurodegenerative disorder in humans (Lau and Breteler, 2006; Pringsheim et al., 2014). Although multiple factors are associated with the etiology and pathogenesis of PD, its cause is still unknown. The main pathological hallmark of PD is a progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), with resulting dopaminergic deafferentation in the striatum. This outcome gives rise to cardinal motor symptoms, such as bradykinesia, muscular rigidity, resting tremor and postural instability (Wu et al., 2012; Miller and O'Callaghan, 2015).

None of the current treatments is able to stop or even slow down the neurodegeneration in PD. Therefore, only symptomatic treatment is available for PD patients (Dexter and Jenner, 2013; Rizek et al., 2016). Dopamine replacement therapy employing levodopa (L-dopa) remains the primary mode for treatment of motor symptoms of PD. However, the effectiveness of L-dopa therapy declines over time, and motor complications, such as motor fluctuations and dyskinesia, have been associated with long-term L-dopa therapy (Olanow, 2015; Dexter and Jenner, 2013). Therefore, several studies have focused on identifying new drugs able to improve the motor symptoms and to modify the course of PD.

* Corresponding author at: Department of Biosciences, Universidade Federal de Sergipe Campus Professor Alberto Carvalho, Av. Vereador Olímpio Grande S/N, CEP 49503-000, São Cristóvão, SE, Brazil.
E-mail address: joseronaldosantos@gmail.com (J.R. Santos).

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In the last years, animal models of PD induced by neurotoxins, including reserpine (RES), have been considered a helpful tool to investigate the mechanisms underlying the physiopathology of PD, as well to do screening of new drugs or interventions with neuroprotective effects (Fernandes et al., 2012; Santos et al., 2013; Sarmento Silva et al., 2015; Peres et al., 2016; Campêlo et al., 2017). Several studies have indicated that a range of pure compounds derived from herbal medicines are effective on in vivo and in vitro PD models via modulation of multiple targets involved in the pathogenesis of PD (Song et al., 2012; Li et al., 2013; Shahpiri et al., 2016).

Carvacrol (CA) is a phenolic monoterpene found in the essential oils of the *Lamiaceae* family including *Origanum, Thymus, Satureja* and *Corydothymus*. CA is the major constituent of the essential oils of Origanum species (Baser, 2008). It is considered safe for consumption and is approved by the Food and Drug Administration as a food additive (De Vincenzi et al., 2004). Studies have shown different pharmacological activities of CA on the central nervous system (CNS), including anxiolytic-like (Melo et al., 2010), antinociceptive (Melo et al., 2012; Guimarães et al., 2012), antidepressant (Melo et al., 2011), anticonvulsant (Quintans-júnior et al., 2010), antioxidant, anti-inflammatory and neuroprotective (Yu et al., 2012; Baluchnejadmojarad et al., 2014; Deng et al., 2013; Li et al., 2016; Peters et al., 2012) effects.

It has been reported that inflammation and oxidative stress are involved in the pathogenesis of dopaminergic neurodegeneration in PD (Uttara et al., 2009; Janda et al., 2012; Rocha et al., 2015; Le et al., 2016). Thus, anti-inflammatory and antioxidant agents could be alternative strategies for prevention or treatment of PD (Hwang, 2013; Wang et al., 2015). In this respect, previous studies have shown that CA has anti-inflammatory (Li et al., 2016) and antioxidant (Samarghandian et al., 2016) effects on rat's brain. Interestingly, the intraperitoneal administration of CA showed a protective effect on the behavioral impairments induced by 6-hydroxydopamine (a hemi-parkinsonism rat model) that was related to an antioxidant action (Baluchnejadmojarad et al., 2014). Moreover, CA can modulate neurotransmitters systems in CNS, including the dopaminergic system (Zotti et al., 2013). These results suggest that the natural compound CA has valuable effects on CNS, which could provide protective action against physiopathological alterations of PD.

In this context, the aim of the present study was to investigate the possible protective effect of CA in a RES-induced rat model of Parkinson's disease, with emphasis on behavioral and immunohistochemical effects.

2. Materials and methods

2.1. Animals

Seven-month-old male Wistar rats (n = 46) were used in this study. All animals were housed in groups of four or five per plastic cage (30 cm × 37 cm × 16 cm), under controlled conditions of ventilation, temperature (22 ± 1 °C) and a 12/12 h light/dark cycle (lights on 6:00 a.m.), with free access to water and food. Animals used in this study were handled according to the Brazilian law for the use of animals in scientific research (Law number 11.794) and all the procedures were approved by the local Animal Ethics Committee (Protocol number 33/2014). All efforts were made to minimize animal pain, suffering or discomfort and reduce the number of animals used.

2.2. Drugs

Carvacrol (5-isopropyl-2-methylphenol, ≥98% purity) and reserpine were purchased from Sigma-Aldrich (St. Louis, MO, USA). Carvacrol (CA) was emulsified with 0.2% Tween 80 and dissolved in saline (0.9% isotonic saline) at the concentrations of 12.5 and 25 mg/mL. Vehicle of carvacrol (VC) consisted of 0.2% Tween 80 dissolved in saline. Reserpine (RES) was dissolved in glacial acetic acid and then diluted in distilled water at the concentration of 0.1 mg/mL. Vehicle of reserpine (VR) consisted of the same amount of glacial acetic acid diluted in distilled water as in the RES solution. CA and VC were freshly prepared before administration, and RES and VR were prepared every 48 h and kept at 4 °C between administrations.

2.3. General procedures and experimental design

Before the beginning of experimental procedures, animals were handled daily during five minutes for five consecutive days. Afterwards, the rats were randomly assigned to the following groups: Vehicle of carvacrol + vehicle of reserpine (VC + VR; n = 9); vehicle of carvacrol + reserpine (VC + RES; n = 9); Carvacrol (12.5 mg/kg) + vehicle of reserpine (CA12.5 + VR; n = 7); Carvacrol (25 mg/kg) + vehicle of reserpine (CA25 + VR; n = 7); Carvacrol (12.5 mg/kg) + reserpine (CA12.5 + RES; n = 7) and Carvacrol (25 mg/kg) + reserpine (CA25 + RES; n = 7). The animals received 15 s.c. injections of RES (0.1 mg/kg) or VR every 48 h, and they were concomitantly treated with a daily i.p. injection of CA (12.5 or 25 mg/kg) or VC. The volume of injection was 1 ml/kg of body weight in all cases. The carvacrol injection was given first and reserpine injection was given after a ten-minute delay.

Throughout the treatment, the animals were submitted to the following behavioral tests (from 8:00 a.m. to 4:00 p.m.): (1) catalepsy test performed daily; (2) open field test on 7th day (24 h after the 4th injection of RES or VR); (3) assessment of vacuous chewing movements on 12th, 20th and 30th days (48 h after the 6th, 10th and 15th injections of RES or VR, respectively, before the injections). The day of first injection of RES or VR was considered as 0 day. All apparatuses were cleaned with 10% ethanol before behavioral testing to eliminate odor traces. All behavioral tests were conducted before the injections in the respective treatment day. All behavioral analysis were conducted by a trained rater blind to treatment. At the end of the behavioral tests, the animals were transcardially perfused and their brain removed and

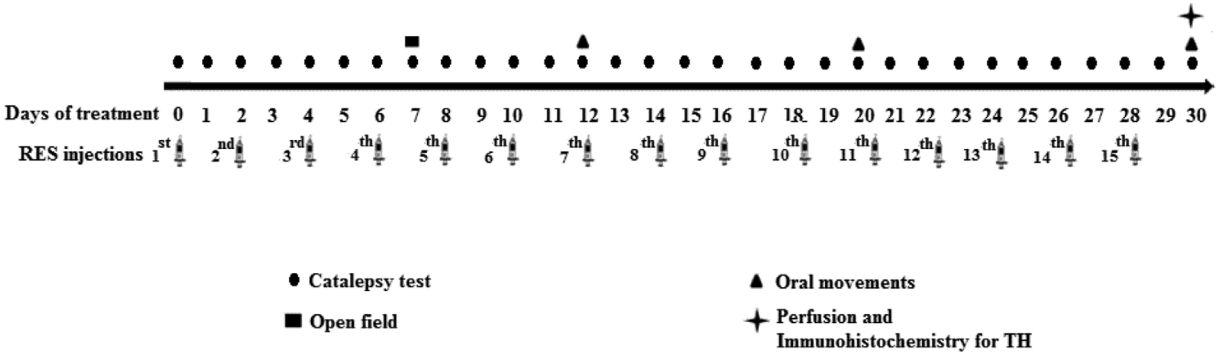

Fig. 1. Schematic illustration of the experimental design.