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Research report

Rosmarinic acid prevents against memory deficits in ischemic mice

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HIGHLIGHTS

- Rosmarinic acid prevents memory deficits induced by permanent focal cerebral ischemia.
- Rosmarinic acid induced synaptogenesis in ischemic mice.
- Increased BDNF were observed in ischemic mice treated with rosmarinic acid.
- Rosmarinic acid diminished MPO activity and astrogliosis in ischemic mice.

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ABSTRACT

Polyphenols have neuroprotective effects after brain ischemia. It has been demonstrated that rosmarinic acid (RA), a natural phenolic compound, possesses antioxidant and anti-inflammatory properties. To evaluate the effectiveness of RA against memory deficits induced by permanent middle cerebral artery occlusion (pMCAO) mice were treated with RA (0.1, 1, and 20 mg/kg/day, i.p. before ischemia and during 5 days). Animals were evaluated for locomotor activity and working memory 72 h after pMCAO, and spatial and recognition memories 96 h after pMCAO. In addition, in another set of experiments brain infarction, neurological deficit score and myeloperoxidase (MPO) activity were evaluates 24 h after the pMCAO. Finally, immunohistochemistry, and western blot, and ELISA assay were used to analyze glial fibrillary acidic protein (GFAP), and synaptophysin (SYP) expression, and BDNF level, respectively. The working, spatial, and recognition memory deficits were significantly improved with RA treatment (20 mg/kg). RA reduced infarct size and neurological deficits caused by acute ischemia. The mechanism for RA neuroprotection involved, neuronal loss suppression, and increase of synaptophysin expression, and increase of BDNF. Furthermore, the increase of MPO activity and GFAP immunireactivity were prevented in MCAO group treated with RA. These results suggest that RA exerts memory protective effects probably due to synaptogenic activity and anti-inflammatory action.

1. Introduction

Stroke is the major cause of disability and the fourth leading cause of death worldwide. Approximately 100,000 deaths occurs annually due to stroke [1,2,]. Ischemic injury is associated with vascular leakage, inflammation, tissue injury, and cell death [3]. Cellular changes associated with ischemia include impairment of metabolism, energy failure, free radical production, excitotoxicity, altered calcium homeostasis, and protease activation; all these events affect brain function and contribute to long term disabilities [4]. It has been confirmed that stroke can result in cognitive impairment, and the prevalence of post-stroke cognitive impairment ranges from 20% to 80% [5] and more than a third of the patients have cognitive impairment after transient ischemic attack [6]. A mechanism involved in cognitive deficits after stroke is synaptic protein loss [7]. The disappearance of synaptic activity is one

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of the first consequences of ischemia [8]. A blood flow less than 20% leads to a loss of axons and dendrites in minutes [9,10]. Moreover, inflammation plays a significant role in the pathogenesis of ischemic stroke [11]. The inflammation responses after brain ischemia include astrocyte and microglial activation, followed by peripheral inflammatory cell infiltration in the core and penumbra zone of ischemia [12,13]. Ischemia evokes strong reactive astrogliosis that activates induced nitric oxide synthase (iNOS) and NADPH oxidase to produce NO and superoxide, which are involved in lipid peroxidation, and the release of cytokines, such as TNF and IL-1 [12]. These cytokines, depending on the concentrations, may inhibit synaptic transmission or act as neuromodulators [14]. After brain ischemia, astrogliosis has both beneficial and deleterious effects and astrocyte activation is an important proliferative component during synaptogenesis [8,15]. Studies show that astrocytes release mediators that influence from the genesis of synapses until their stabilization [16,17]. In addition to astrogliosis, neurotrophic factors such as BDNF play a pivotal role in synaptic plasticity [18], neuronal survival, and growth [19]. Studies show that systemic administration of BDNF promotes recovery after stroke [20].

Rosmarinic acid (RA) is a phenolic compound with potent antioxidant and anti-inflammatory activities [21]. RA has neuroprotective action in animal models of neurodegenerative diseases such as Alzheimer's disease [22–24] and, Parkinson's disease [25], as well as in ischemia/reperfusion models [26]. However, currently, there are no reports regarding the protective effects of rosmarinic acid on memory deficits following focal cerebral ischemia. Thus, the aim of the present work was to investigate the effect of RA on memory deficits induced by permanent focal cerebral ischemia, thereby investigating the mechanism of action.

2. Methods

2.1. Subjects

Male Swiss mice weighing 25–30 g obtained from the Central Animal House of Physiology and Pharmacology Department of Federal University of Ceará were used. Animals were housed under a 12-h-light, 12-h-dark cycle and allowed free access to food and water. All procedures in this study were in agreement with the Guide for the Care and Use of Laboratory Animals by the Institute for Laboratory Animal Research of the National Research Council published by the National Academies Press (Washington, District of Columbia, USA) and were approved by the ethics committee on animal experimentation of the Federal University of Ceará.

2.2. Drugs

The following drugs were used: rosmarinic acid (SIGMA, USA); xylazine (2%, Kensol®, König, Argentina) and ketamine (5%, Vetanarcol®, König, Argentina). All other reagents were of analytical grade.

2.3. Induction of permanent media cerebral artery occlusion (pMCAO)

Permanent middle cerebral artery occlusion (pMCAO) was produced by electrocoagulation of the left middle cerebral artery as reported previously [27]. Briefly, animals were anaesthetized with xylazine (10 mg/kg, intraperitoneally) and ketamine (90 mg/kg, intraperitoneally), an incision was made on the left temporoparietal region, and the temporalis muscle was partially removed. A burr hole was drilled into the skull over the middle cerebral artery and the vessel was occluded directly, proximal to the lateral lenticulostriate branches, using electrocoagulation with a micro-unipolar coagulator. The complete interruption of the blood flow was confirmed by visual inspection. Body temperature was kept close to 37 °C. Sham-operated animals (SO group) were subjected to the same procedure, with the exception of cauterization of the middle cerebral artery. Thirty minutes before and 1 h after pMCAO, the animals received either NaCl 0.9% (pMCAO group) or RA (0.1, 1 and 20 mg/kg/day, intraperitoneally) for 5 consecutive days. There were two groups of sham-operated animals: one group received NaCl 0.9% and the other group was treated with RA (20 mg/kg/day). In the first set of experiments, animals were tested for neurological evaluation and euthanized for ischemic damage evaluation 24 h

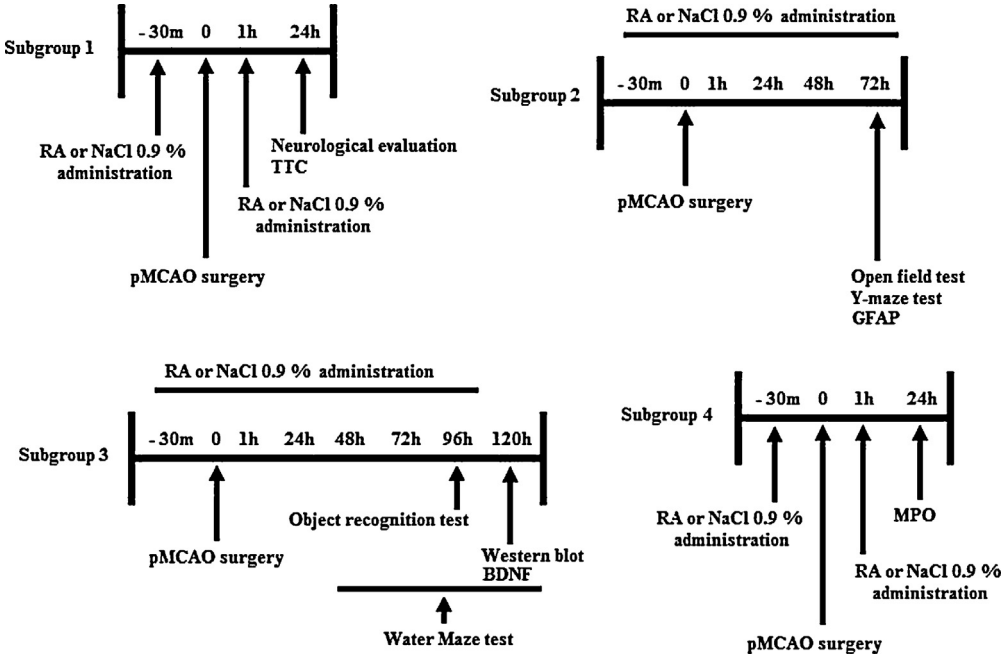

Fig. 1. Experimental design showing the treatment of subgroups. RA: rosmarinic acid; pMCAO: permanent middle cerebral artery occlusion; TTC: 2,3,5-triphenyltetrazolium salt; GFAP: glial fibrilary astrocity protein; MPO: myeloperoxidase.