Behavioural Brain Research 312 (2016) 321–332

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Neuroinflammatory response to experimental stroke is inhibited by eriodictyol

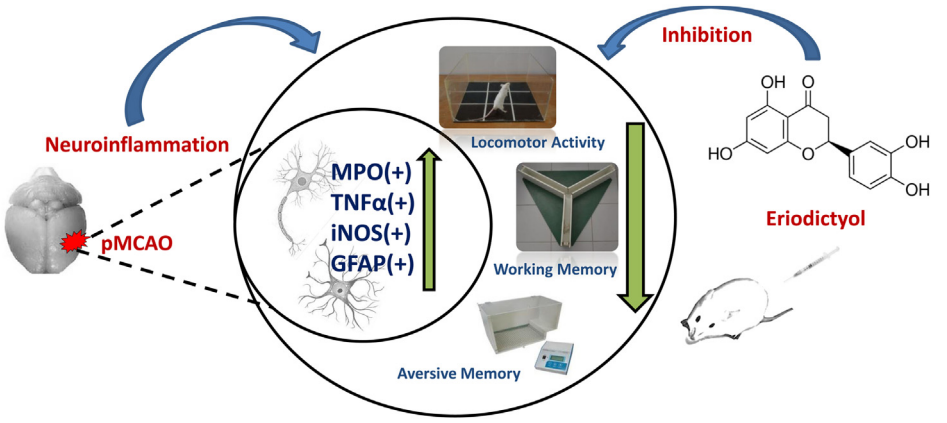
Emerson de Oliveira Ferreira[a], Mara Yone Soares Dias Fernandes[b], Neila Maria Rocha de Lima[a], Kelly Rose Tavares Neves[b], Marta Regina Santos do Carmo[b], Francisco Arnaldo Viana Lima[b], Analu Aragão Fonteles[b], Ana Paula Fontenele Menezes[a], Geanne Matos de Andrade[a,b,c,*]

[a] Post-Graduate Programme in Medical Sciences, Department of Clinical Medicine, Faculty of Medicine, Federal University of Ceará, Brazil
[b] Post-Graduate Programme in Pharmacology, Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Brazil
[c] Institute of Biomedicine of Brazilian Semi-arid, Brazil

HIGHLIGHTS

- Neuroprotective effect of eriodictyol in brain ischemia.
- Eriodictyol ameliorates memory impairment in mice.
- Anti-inflammatory activity of eriodictyol.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 March 2016
Received in revised form 21 June 2016
Accepted 24 June 2016
Available online 25 June 2016

ABSTRACT

Background: Cerebral ischemia is a common disease and one of the most common causes of death and disability worldwide. The lack of glucose and oxygen in neuronal tissue leads to a series of inflammatory events, culminating in neuronal death. Eriodictyol is a flavonoid isolated from the Chinese herb Dracocephalum rupestre that has been proven to have anti-inflammatory properties.

Abbreviations: BDNF, brain-derived neurotrophic factor; CREB, cAMP-response-element-binding protein; DAB, 3,3′-diaminobenzidine; ERK, extracellular-signal-regulated kinase; Etyol, eriodictyol; GFAP, glial fibrillary acidic protein; HTAB, hexadecyltrimethylammonium bromide; iNOS, inducible nitric oxide synthase; MAPK, mitogen-activated protein kinases; MPO, myeloperoxidase; NF-kB, nuclear factor kappa B; NO, nitric oxide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; PBS, phosphate buffered saline; PKB, protein kinase B; pMCAO, permanent middle cerebral artery occlusion; RT, room temperature; SO, sham-operated; TNFα, tumor necrosis factor alpha; t-PA, tissue plasminogen activator; TTC, 2,3,5-triphenyltetrazolium.

* Corresponding author at: Department of Physiology and Pharmacology, Faculty of Medicine, Federal, University of Ceará, Rua Cel. Nunes de Melo 1127, Porangabussu, Fortaleza, CE, 60430-270, Brazil.
E-mail addresses: emersonoliveira.shalom@hotmail.com (E.d.O. Ferreira), maraiony@hotmail.com (M.Y.S.D. Fernandes), neilamrl@hotmail.com (N.M.R.d. Lima), kelly.rose@hotmail.com (K.R.T. Neves), martacarmo@yahoo.com.br (M.R.S.d. Carmo), arnviana@hotmail.com (F.A.V. Lima), analufonteless@gmail.com (A.A. Fonteles), ap_fontenele@yahoo.com.br (A.P.F. Menezes), gmatos@ufc.br, geannecunha@yahoo.com.brr (G.M.d. Andrade).

http://dx.doi.org/10.1016/j.bbr.2016.06.046
0166-4328/

Keywords:
Eriodictyol
Flavonoids
Memory
Neuroprotection
pMCAO
Neuroinflammation

Hypothesis/Purpose: Thus, the present study was designed to explore whether eriodictyol has neuroprotective effects against the neuronal damage, motor and memory deficits induced by permanent middle cerebral artery occlusion (pMCAO) in mice.
Study Design: Animals were orally treated with eriodictyol (1, 2 and 4 mg/kg) or vehicle (saline) 30 min before pMCAO, 2 h after, and then once daily for the following five days.
Methods: The parameters studied were neuronal viability, brain infarcted area; sensorimotor deficits; exploratory activity; working and aversive memory; myeloperoxidase (MPO) activity; TNFα, iNOS and GFAP immunoreactivity.
Results: The treatment with eriodictyol prevented neuronal death, reduced infarct area and improved neurological and memory deficits induced by brain ischemia. The increase of MPO activity and TNF-α, iNOS and GFAP expression were also reduced by eriodictyol treatment.
Conclusion: These findings demonstrate that eriodictyol exhibit promising neuroprotection effects against the permanent focal ischemia cerebral injury in the mice experimental model and the underlying mechanisms might be mediated through inhibition of neuroinflammation.

1. Introduction

Stroke is a debilitating disease that accounts for behavioral and cognitive disturbances, especially those involving learning, motor and memory deficits [1,2]. Stroke occurs after a sudden block of blood flow to the brain, depriving the tissue of oxygen and glucose [3,4]. Clearly, glutamate excitotoxicity, calcium overload, oxidative stress, inflammation and apoptosis are important contributing factors in the pathophysiology of cerebral ischemia [5]. Inflammation is responsible for much of cerebral ischemic tissue injury [6,7]. Together with microglia, astrocytes contribute to the production of inflammatory mediators, such as TNF-α and iNOS, which are triggered by pro-inflammatory genes, and NF-kB, which is driven by astrogliosis and microgliosis [8]. Anti-inflammatories constitute an important therapeutic strategy for stroke [2,9] because currently, the only available treatment to reduce brain damage after stroke is the "clot-buster," tissue plasminogen activator (t-PA). Thus, there is a great need to develop novel therapies for cerebrovascular diseases [10].

A diet rich in vegetables and fruits with polyphenols is known to promote functional benefits for health [11]. Among the polyphenols, flavonoids are compounds that are common in naturally occurring plants and vegetables [12]. Eriodictyol (Etyol) is a flavonoid, "flavanone", (3′,4′,5,7-tetrahydroxyflavanone) isolated from the Chinese herb (*Dracocephalum rupestre*) and citrus fruits. There is evidence for its anti-inflammatory, anti-allergenic, antimicrobial, anti-cancer [13] and antioxidant properties [14,15]. Eriodictyol reduced nitric oxide (NO) and pro-inflammatory cytokines in LPS-stimulated Raw 264.7 cells and suppressed the phagocytic activity of activated macrophages. This anti-inflammatory effect of eriodictyol was related to the blockade of nuclear factor kappa B (NF-κB) [13]. In a rat model of transient focal cerebral ischemia, eriodictyol reduced brain damage and neurological deficits [9]. However, the effects of eriodictyol on neuroinflammation and memory deficits after focal cerebral ischemia have not been reported. Thus, the objective of the present study was to investigate the effects of eriodictyol on the memory deficits induced by permanent middle cerebral artery occlusion (pMCAO) in mice and the probable mechanisms of action involved.

2. Material and methods

2.1. Drugs

Eriodictyol (Sigma, USA), xylazine (2%, Kensol®, König, Argentina) and ketamine (5%, Vetanarcol®, König, Argentina). All other reagents were of analytical grade.

2.2. Animals

Male Swiss mice weighing 25–30 g obtained from the Central Animal House of the Physiology and Pharmacology Department of the Federal University of Ceará were used. Animals were housed under a 12-h light, 12 h-dark cycle and allowed access to food and water *ad libitum*. All procedures in this study were in agreement with the Guide for the Care and Use of Laboratory Animals from the US Health and Human Services Department and were approved by the ethics committee on animal experimentation of the Federal University of Ceará, under the registration number 90/2013.

2.3. Induction of permanent middle cartery occlusion (pMCAO)

pMCAO was produced by electrocoagulation of the left middle cerebral artery as reported previously [16]. Briefly, animals were anaesthetized with xylazine (10 mg/kg, i.p.) and ketamine (90 mg/kg, i.p.), an incision was made on the left temporo-parietal 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 blood flow was confirmed by visual inspection. Body temperature was kept near 37 °C. Sham-operated animals (SO group) underwent the same procedure with the exception of cauterization of the middle cerebral artery.

2.4. Experimental protocols

The animals were divided in groups and submitted to oral administration of 5% Tween 80 and saline (pMCAO group) or eriodictyol and saline (1, 2, and 4 mg/kg, by mouth, orally p.o.) thirty minutes before pMCAO and two hours after; the treatment continued once daily for the following five days. At 24 h post-ischemia, a sub-group of animals (n = 6/group) were tested for neurologic deficits and euthanized for ischemic damage evaluation. At 72 and 96 h post-ischemia, another sub-group of animals (n = 8/group) were tested for locomotor activity and working and aversive memories, immunohistochemistry and histology. At 24 h post-ischemia, a third sub-group, animals were evaluated for the MPO (n = 6/group).

2.5. Neurological evaluation

Neurological evaluation was performed 24 h after ischemia by an investigator who was blind to the experimental group design. The neurologic findings were scored using a scale previously