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Original article

Curcumin reverses neurochemical, histological and immunohistochemical alterations in the model of global brain ischemia

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ABSTRACT

Curcumin, a curcuminoid from *Curcuma longa*, presents antioxidant and anti-inflammatory actions and, among pathological changes of cerebral ischemic injury, inflammation is an important one. The objectives were to study the neuroprotective action of curcumin, in a model of global ischemia. Male Wistar rats (sham-operated, ischemic untreated and ischemic treated with curcumin, 25 or 50 mg/kg, p.o.) were anesthesized and their carotid arteries occluded, for 30 min. The SO group had the same procedure, except for carotid occlusion. In the 1st protocol, animals were treated 1 h before ischemia and 24 h later; and in the 2nd protocol, treatments began 1 h before ischemia, continuing for 7 days. Twenty four hours after the last administration, animals were euthanized and measurements for striatal monoamines were performed, at the 1st and 7th days after ischemia, as well as histological and immunohistochemical assays in hippocampi. We showed in both protocols, depletions of DA and its metabolites (DOPAC and HVA), in the ischemic group, but these effects were reversed by curcumin. Additionally, a decrease seen in 5-HT contents, 1 day after ischemia, was also reversed by curcumin. This reversion was not seen 7 days later. On the other hand, a decrease observed in NE levels, at the 7th day, was totally reversed by curcumin. Furthermore, curcumin treatments increased neuronal viability and attenuated the immunoreactivity for COX-2 and TNF-alpha, in the hippocampus in both protocols. We showed that curcumin exerts neuroprotective actions, in a model of brain ischemia that are probably related to its anti-inflammatory activity.

1. Introduction

Curcumin is the major chemical component of turmeric, produced from the rhizome of *Curcuma longa*, a traditional plant belonging to the Zingiberaceae family and used in Ayurvedic medicine for over 6,000 years. Curcumin is a polyphenol that possesses anti-inflammatory, antioxidant, antidiabetic, anticarcinogenic properties, among others.

Brain ischemia is a condition that occurs when there is not enough blood flow to the brain for meeting metabolic demands. This leads to limited oxygen supply or cerebral hypoxia and often to the death of brain tissues, cerebral infarction, or ischemic stroke. Stroke is currently the second most common cause of death and major cause of disability worldwide. Because of the aging population, the burden will greatly increase during the next 20 years.[1] However, stroke recently declined in the USA from the third to the fourth leading cause of death.[2]

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Cerebral ischemia results from severe reductions in cerebral blood flow (CBF) after cardiac arrest, the occlusion of cerebral and extracerebral vessels supplying nervous tissues, or periods of prolonged systemic hypotension. Severe and/or prolonged reduction in CBF leads to deprivations of oxygen and glucose, as well as to the building up of potentially toxic substances. Because nerve cells do not store alternative energy sources, these hemodynamic reductions can result in the reduction of metabolites, as ATP, leading to metabolic stress, energy failure, ionic perturbations and ischemic injury.[3]

Cells that undergo severe ischemia may die within minutes of the insult or display a delayed vulnerability. Ischemic insults can be focal or global, as well as permanent or transient ones, leading to reperfusion in post-ischemia areas. Depending on how early reperfusion is initiated, metabolic and ionic homeostases can return and cell survival maintained.[4]

Both necrotic and apoptotic cell death mechanisms have been implicated in the pathogenesis of brain ischemia injury.[5–8] The brain is vulnerable to oxidative stress, due to its high rate of oxidative metabolic activity.[9] Oxidative stress, leading to calcium accumulation, mitochondrial dysfunction and the production of reactive oxygen radicals, is an important mechanism of cell death, following brain ischemia.[10,11]

Inflammation is a host defense mechanism initiated by injury, through which blood leukocytes and soluble factors, as cytokines, chemokines, complement and lipid by-products attempt to restore tissue homeostasis.[12] Inflammation plays an important role in the pathogenesis of ischemic brain injury. Experimental and clinical studies have shown that the brain responds to ischemic injury with an acute and prolonged inflammatory process, characterized by rapid activation of resident cells, as microglia, production of inflammatory mediators and infiltration of inflammatory cells into the brain ischemic tissue.[13]

Considering that curcumin presents anti-inflammatory and antioxidative properties, as shown by us[14] and others,[15,16] and the importance of inflammation and oxidative stress in brain injury, the objectives of the present work were to evaluate the neuroprotective effects of curcumin on neurochemical (striatal DA and DOPAC) determinations and on histological (fluoro-jade staining) and immuno-histochemical (COX-2 and TNF-alpha) assays in the hippocampus, in the model of global ischemia in rats.

2. Material and methods

2.1. Drugs

Commercial curcumin was purchased from Sigma-Aldrich (MO, USA) and presented ≥94% of curcuminoid content and ≥80% of curcumin. Ketamine and xylazine were from Konig Laboratory (Santana de Parnaíba, São Paulo, Brazil). Antibodies for immunohistochemistry assays were from Santa Cruz Biotechnology (Dallas, TX, USA) or Merck-Millipore (Darmstadt, Germany). All other reagents were of analytical grade.

2.2. Animals and experimental protocols

Male Wistar rats from the Animal House of the Faculty of Medicine Estácio of Juazeiro do Norte, Brazil, were maintained under standard conditions and at a controlled temperature (23 ± 1 °C), with a 12 h dark/12 h light cycle, and food and water *ad libitum*. The animals (4–10 per group) were divided into four groups: controls treated with distilled water (SO and ischemic untreated with curcumin) or treated orally with curcumin (from Sigma-Aldrich, USA), at the doses of 25 or 50 mg/kg. Two protocols were used. In the 1st one, the animals were subjected to ischemia and treated 1 h before ischemia and 24 h later, and they were euthanized, 1 h after the drug second administration. In the 2nd protocol, the animals were subjected to ischemia, but daily treatments began 1 h before ischemia and continued, at the next day, daily for 7 days. For the experimental procedure, the rats were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (20 mg/kg), then submitted or not (SO groups) to transient global brain ischemia by the occlusion of the left common carotid artery, for 30 min, followed by reperfusion. The sham-operated groups (SO) were submitted to the entire procedure, except for the artery occlusion. Twenty four hours after the last drug administration, the animals were euthanized for dissection of striata and hippocampi. Neurochemical alterations (DA and DOPAC determinations in striata) were assessed, at the 1st and 7th days after ischemia. Besides, immunohistochemical assays in hippocampi were also performed, at those same periods. The study had the approval of the Animal Experimentation Committee of the Federal University of Ceará and the experiments were carried out in accordance with the current law and the NIH Guide for the Care and Use of Laboratory Animals, 2011.

3. Neurochemical assays

3.1. Concentrations of striatal monoamine (NE, DA, DOPAC, 5-HT and HVA) by HPLC

The striata from all groups, at different post-ischemia times, were used for the preparation of 10% homogenates in 0.1 M perchloric acid. This mixture was sonicated for 30 s, centrifuged at 4 °C for 15 min, at 15,000 rpm. The supernatants were filtered (0.2 μm, Millipore) and 20 μL injected into the HPLC column (Shim-Pack CLC-ODS, 25 cm) for electrochemical detection (Shimadzu, model LCD-6A, Japan), with a 0.6 mL/min flux. The mobile phase was prepared in 0.163 M citric acid, pH 3.0, containing 0.02 mM EDTA and 0.69 mM sodium octanosulfonic acid, 4% acetonitrile (v/v) and 1.7% tetrahydrofuran (v/v). Monoamine concentrations were determined by comparison to standards and the values expressed as ng/mg tissue.

3.2. Histological study for neuronal viability (fluoro-jade staining)

Fluoro-jade is an anionic fluorescein derivative, useful for the histological staining of neurons undergoing degeneration. After paraffin removal (by immersion in xylol), sections (5 μm) from hippocampi were mounted on slides surrounded by gelatin. The tissue was rehydrated by immersion in ethanol for 3 min, followed by immersions in 70 and 50% ethanol solutions and distilled water. The slices were placed into a 0.06% potassium permanganate solution, for 15 min, washed in distilled water and transferred to a fluoro-jade solution where they stayed for 30 min (with gentle stirring). After staining, the slices were washed in distilled water (3 times, 2 min each time). The excess of water was discarded and the dry slices mounted in Fluoromount® media and examined with a fluorescence microscope. The data were quantified with the Image J software (National Institute of Health, USA).

3.3. Immunohistochemical assays for COX-2 and TNF-alpha in rat hippocampi

Sections were fixed in 10% buffered formol, for 24 h, followed by immersion in a 70% alcohol solution. They were embedded into paraffin wax, for slices processing on appropriate glass slides. These were placed into the oven at 58 °C, for 10 min, followed by deparaffinization in xylol, rehydration in alcohol at decreasing concentrations, washing in distilled water and PBS (0.1 M sodium