Behavioural Brain Research 335 (2017) 41–54

Contents lists available at ScienceDirect

Behavioural Brain Research

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

Research report

Effects of curcumin on short-term spatial and recognition memory, adult neurogenesis and neuroinflammation in a streptozotocin-induced rat model of dementia of Alzheimer's type

Taysa B. Bassani[a,*], Joelle M. Turnes[a], Eric L.R. Moura[a], Jéssica M. Bonato[b], Valentín Cóppola-Segovia[c], Silvio M. Zanata[c], Rúbia M.M.W. Oliveira[b], Maria A.B.F. Vital[a]

[a] Department of Pharmacology, Federal University of Paraná, Curitiba, PR, 81531-980, Brazil
[b] Department of Pharmacology and Therapeutics, State University of Maringá, Maringá, PR, 87020-900, Brazil
[c] Department of Basic Pathology, Federal University of Paraná, Curitiba, PR, 81531-990, Brazil

ARTICLE INFO

Keywords:
Alzheimer's disease
Curcumin
Adult neurogenesis
Recognition memory
Spatial memory
Neuroinflammation

ABSTRACT

Curcumin is a natural polyphenol with evidence of antioxidant, anti-inflammatory and neuroprotective properties. Recent evidence also suggests that curcumin increases cognitive performance in animal models of dementia, and this effect would be related to its capacity to enhance adult neurogenesis. The aim of this study was to test the hypothesis that curcumin treatment would be able to preserve cognition by increasing neurogenesis and decreasing neuroinflammation in the model of dementia of Alzheimer's type induced by an intracerebroventricular injection of streptozotocin (ICV-STZ) in Wistar rats. The animals were injected with ICV–STZ or vehicle and curcumin treatments (25, 50 and 100 mg/kg, gavage) were performed for 30 days. Four weeks after surgery, STZ-lesioned animals exhibited impairments in short-term spatial memory (Object Location Test (OLT) and Y maze) and short-term recognition memory (Object Recognition Test – ORT), decreased cell proliferation and immature neurons (Ki-67- and doublecortin-positive cells, respectively) in the subventricular zone (SVZ) and dentate gyrus (DG) of hippocampus, and increased immunoreactivity for the glial markers GFAP and Iba-1 (neuroinflammation). Curcumin treatment in the doses of 50 and 100 mg/kg prevented the deficits in recognition memory in the ORT, but not in spatial memory in the OLT and Y maze. Curcumin treatment exerted only slight improvements in neuroinflammation, resulting in no improvements in hippocampal and subventricular neurogenesis. These results suggest a positive effect of curcumin in object recognition memory which was not related to hippocampal neurogenesis.

1. Introduction

Alzheimer's disease (AD) is the most common progressive neurodegenerative disorder of the elderly, characterized by a decline in cognitive functions. Sporadic AD (sAD; *i.e.*, late-onset AD), the most frequent form of AD with an unknown etiology, presents early abnormalities in brain glucose metabolism and insulin signaling, thus resulting in an insulin-resistant state in the brain, which has been suggested to be related to etiological events in its pathogenesis [1,2]. Additionally, protein aggregates [3], mitochondrial dysfunctions [4], oxidative stress [5], neuroinflammation [6], brain cholinergic deficits [7], and dysfunctions in adult neurogenesis [8–10] are among the main features of sAD. These features may be modeled by a intracerebroventricular (ICV) injection of low, subdiabetogenic doses of streptozotocin (STZ) in rodents [11,12] and non-human primates [13].

Adult neurogenesis is a multi-step process of the formation of new neurons in the mammalian brain throughout life. Evidence suggests that newly generated neurons play a crucial role in hippocampus-dependent learning and memory (*e.g.*, spatial memory and orientation) and recovery from neuronal injury [14]. Neurogenesis starts from the proliferation of resident neural stem cells (NSC) and neural progenitor cells (NPC) in two neurogenic niches in the central nervous system (CNS): the subventricular zone (SVZ) of the lateral ventricles (LVs), and the subgranular zone (SGZ) of the dentate gyrus (DG) of the

Abbreviations: BDNF, brain-derived neurotrophic fator; CC, corpus callosum; CNS, central nervous system; DCX, doublecortin; DG, dentate gyrus; EPM, elevated plus maze; GFAP, glial fibrillary acidic protein; Iba-1, ionized calcium binding adaptor molecule-1; ICV, intracerebroventricular; LVs, lateral ventricles; NSC, neural stem cells; NPC, neural progenitor cells; OFT, open field test; OLT, object location test; ORT, object recognition test; sAD, sporadic Alzheimer's disease; SGZ, subgranular zone; STZ, streptozotocin; SVZ, subventricular zone; TNF-α, tumor necrosis factor alfa

* Corresponding author.
E-mail address: taysa_bassani@yahoo.com.br (T.B. Bassani).

http://dx.doi.org/10.1016/j.bbr.2017.08.014
Received 11 July 2017; Received in revised form 1 August 2017; Accepted 5 August 2017
Available online 08 August 2017

hippocampus. The process of adult neurogenesis can be influenced by many intrinsic and extrinsic factors. Growth factors and neurotrophins, such as brain-derived neurotrophic factor (BDNF), increase differentiation, maturation, and survival of proliferating NSC/NPC, stimulating neurogenesis. In contrast, microglial activation and release of proinflammatory cytokines (*e.g.*, interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α)) have been demonstrated to inhibit neurogenesis by reducing the proliferation of NSC/NPC. The consequence of inhibiting neurogenesis is cognitive deficit, reflected by impairments in spatial and recognition learning and memory [15]. Several studies have reported an increase in neuroinflammation in the STZ model of sAD, reflected by reactive gliosis and an increase in proinflammatory markers that are associated with cognitive decline [16–19]. Other studies reported a decrease in neurogenesis associated with oxidative stress [20] or amyloid pathology [21].

Current therapeutic options for AD are limited because most agents provide only symptomatic relief from cognitive deficits. A neuroprotective agent able to improve cognitive function along with slowing neuronal loss, decrease neuroinflammation and improve neurogenesis is needed. *Curcuma longa* (turmeric) is a rhizomatous native plant from South and Southeast Asia which belongs to the ginger family Zingiberaceae [22]. Traditionally curcumin, the main active ingredient in turmeric, has been used as a common food additive and herbal medicine [23]. Epidemiological studies have suggested that societies that widely use curcumin have a reduced incidence of AD and improved cognitive function [24,25].

The natural polyphenol curcumin possesses pleiotropic properties. It has been reported to provide neuroprotection in cellular and animal models of neurodegenerative and neuropsychiatric disorders including AD, Parkinson's disease, Huntington's disease, multiple sclerosis, depression, and schizophrenia [26]. Neuroprotection provided by curcumin may be due to its antioxidant properties, by upregulation of the transcription factor Nrf2 [27], and anti-inflammatory properties, by suppression of NF-κB activation [28]. Curcumin also inhibits amyloid beta (Aβ)-oligomerization and tau-phosphorylation [29] and increases BDNF levels in corticosterone-treated rats [30], chronically stressed rats [31] and Wistar Kyoto rats [23].

In addition, curcumin potentiates spatial and non-spatial memory in aged mice [32] and rats [33], in Aβ-induced [34] and STZ-induced models of AD [35–38]. The positive effects of curcumin on cognition may be related to its capacity to enhance neurogenesis. Curcumin has shown to increase neurite outgrowth and proliferation of NSC both *in vitro* and *in vivo* through the activation of the ERK and MAP kinase pathways [39,40]. Curcumin treatment increases neurogenesis in chronically stressed rats [41], aged rats [33] and in Aβ-induced model of AD [26]. Nevertheless, the effects of prolonged curcumin treatment on adult neurogenesis were not studied in the STZ-ICV model of sAD. Therefore, we hypothesized that curcumin treatment would be able to preserve cognitive functions of STZ-ICV injected rats by increasing adult neurogenesis and decreasing neuroinflammation.

2. Methods

2.1. Animals

In this study, 3–4 months old male Wistar rats weighing 300–340 g at the beginning of the experiment were used. All animals were obtained from our breeding colony. The animals had free access to food and water, were maintained in a temperature-controlled room (22 °C ± 2 °C) on a 12 h/12 h light/dark cycle (lights on at 7:00 AM), and were randomly housed in groups of 3–4 in polypropylene cages with wood shavings as bedding. The studies were performed in accordance with the guidelines of the Federal University of Paraná and were approved by the University Ethics Committee (protocol no. 735).

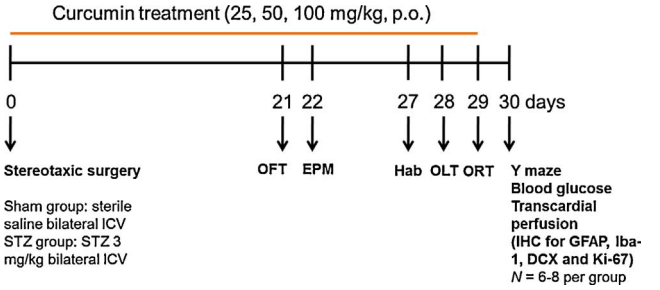

Fig. 1. Experimental design. The present study was designed to assess the effects of prolonged curcumin treatment in three different doses (25, 50 and 100 mg/kg) on short-term spatial and recognition memory, neuroinflammation and adult neurogenesis in rats that received a single bilateral ICV injection of STZ. IHC, immunohistochemistry; OFT, open field test; EPM, elevated plus maze; OLT, object location test; ORT, object recognition test; Hab, habituation.

2.2. Drugs

Streptozotocin (*N*-[methylnitrosocarbamoyl]-α-D-glucosamine) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and curcumin (from *Curcuma longa*) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.3. Experimental design

The rats were randomly divided into five groups: sham (n = 7), STZ (n = 7), STZ + curcumin 25 mg/kg (STZ + cur 25; n = 6), STZ + curcumin 50 mg/kg (STZ + cur 50; n = 8), and STZ + curcumin 100 mg/kg (STZ + cur 100; *n* = 7). The STZ groups received a single bilateral ICV injection of STZ (3 mg/kg total dose) dissolved in sterile 0.9% saline (4.5 μl per injection site). The sham group received a single bilateral ICV injection of sterile 0.9% saline (4.5 μl per injection site; Fig. 1). Treatment with curcumin (25, 50 and 100 mg/kg, per os) or its vehicle (0.5% carboxymethylcelulose in distilled water with 1% Tween 80) was performed over 30 days, once a day in the afternoon, and started 1 h before the beginning of stereotaxic surgeries.

The animals were allowed to recover for 3 weeks following surgery before behavioral evaluations. These animals were assessed in the open field test (OFT) to evaluate spontaneous locomotor activity on day 21 after surgery and in the elevated plus maze (EPM) on day 22 to assess anxiety-like behavior. Cognitive performance was evaluated in the object location test (OLT) on day 28, in the object recognition test (ORT) on day 29, and in the spatial version of the Y maze on day 30. Right after the last behavioral analysis, blood glucose levels were measured using a G-Tech Free device using blood samples that were collected by tail prick.

Afterward, animals were deeply anesthetized with chloral hydrate (400 mg/kg, i.p.) and intracardially perfused for posterior immunohistochemical evaluation of Doublecortin (DCX; a marker of newborn and migrating neurons), Ki-67 (a marker of cell proliferation), ionized calcium binding adaptor molecule (Iba-1; a marker of microglia), and glial fibrillary acidic protein (GFAP; a marker of astrocytes).

2.4. Stereotaxic surgery

The animals were deeply anesthetized with sodium thiopental (30 mg/kg, i.p.) and chloral hydrate (150 mg/kg, i.p.) and placed in a stereotaxic frame (David Kopf, USA). The skull was exposed, and the following stereotaxic coordinates for the LVs, according to Paxinos and Watson [42], were calculated: anterior/posterior, −0.8 mm from bregma; medial/lateral, ± 1.5 mm from the midline; dorsal/ventral, −3.8 mm from the skull. A hole was drilled through the skull, and a 28-gauge stainless steel needle was manually lowered into each LV. An electronic pump (Insight, Ribeirão Preto, SP, Brazil) was used to control