Life Sciences 183 (2017) 45–49

Contents lists available at ScienceDirect

Life Sciences

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

Potential therapeutic and protective effect of curcumin against stroke in the male albino stroke-induced model rats

Yuanyuan Zhang [a], Yi Yan [b], Yi Cao [a], Yongtao Yang [a], Qing Zhao [a], Rui Jing [a], Jiayi Hu [a], Juan Bao [a,*]

[a] Department of Cerebrovascular Disease, The Second Affiliated Hospital, Kunming Medical University, Kunming 650101, China
[b] Department of Anesthesiology, Kunming General Hospital of Chengdu Military Command, Kunming 650032, China

ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form 19 June 2017
Accepted 25 June 2017
Available online 27 June 2017

Keywords:
Curcumin
Stroke
p53
Bax
TNF-α

ABSTRACT

Aims: The present study was carried out to understand the therapeutic effect of curcumin (CUR) against stroke in the experimental animal model. The study investigates the healing effect of CUR on mitochondrial dysfunction and inflammation.
Materials and methods: Male albino, Wistar strain rats were used for the induction of middle cerebral artery occlusion (MCAO), and reperfusion. Enzyme-linked immunosorbent assay (ELISA) was used for the determination of interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) in the brain region. Western blot analysis was used to determine the protein expression levels of Bax, Bcl-2, p53, and Sirt1.
Key findings: The water level was determined in brain region by using standard method. Experimental results indicated that the use of CUR significantly reduced brain edema and water content. IL-6 and TNF-α were significantly reduced in the brain region following use of CUR. Mitochondrial membrane potential (MMP) also reduced significantly after CUR treatment. Protein expression of p53 and Bax were significantly reduced, whereas Bcl-2 and Sirt1 were increased following CUR treatment.
Significance: Taking all these data together, it is suggested that the use of CUR may be a potential therapeutic agent for the treatment of stroke.

1. Introduction

Curcumin (CUR) is a principal compound of turmeric, which belongs to the ginger family. The bis-desmethoxycurcumin and desmetho xycurcumin exist as two forms of turmeric. Turmeric exists as enol and keto forms, and yellow color of turmeric is due to the presence of phenol compound. Manolova et al., [1] have reported the enol form is stronger than keto form. The anti-inflammatory and antioxidant activity of CUR has been reported in the animal cells [2–5]. Several researchers have reported the neuroprotective, anti-inflammatory, antioxidant and immunomodulatory effects of CUR in rats [6].

CUR has been well known for the reduction of symptoms of mental stress and somatic symptom disorder, and to promote liver *qi* [7]. CUR treatment reduced the development of abdominal aortic aneurysms in C57Bl/6 mice [8]. CUR was very effective against ischemia-reperfusion injury in the rat [9], and dopaminergic neuronal cell death in C57BL/6N mice [10]. Gokce et al., [11] have recently demonstrated that CUR treatment before the induction of ischemia-reperfusion injury reduced pro-inflammatory cytokine expression in albino rats. De Alcântara et al., [12] have further shown that CUR treatments increased the neuronal viability and attenuated the immunoreactivity for TNF in the cerebral ischemic injury model of rats. Miao et al., [13] have reported that CUR treatment of rats subjected to cerebral artery occlusion and reperfusion showed reduced brain levels of TNF-α, and IL-6, elevated mitochondrial membrane potential, upregulated Sirt1, and Bcl-2 expression, and downregulated p53, and Bax expression, and also demonstrated reduced infarct volumes and brain edema.

Ischemic stroke is a most common neurological disease in the world [14]. Researchers have attempted to find the remedy for stroke. However, clinical and therapeutic efficiency is very limited in the clinical field. The rapid restoration of cerebral blood flow is an available strategy. There are several agents such as plasminogen activator used as a remedy for the stroke. Even though, Mattiasson et al., [15] have reported the limited efficacy and safety measures.

Therefore, the present study was aimed to investigate the potential therapeutic effect of CUR against stroke in an experimental male albino rat model.

2. Materials and methods

2.1. Materials

CUR (≥94%) and other reagent were used in this study were purchased from Sigma-Aldrich (St. Louis, MO 63178 USA). Healthy male

* Corresponding author at: Department of Cerebrovascular Disease, The Second Affiliated Hospital, Kunming Medical University, No. 374 Dian Mian Road, Kunming 650101, China.
E-mail address: juanbao6@hotmail.com (J. Bao).

http://dx.doi.org/10.1016/j.lfs.2017.06.023
0024-3205/

albino rats were obtained from the Animal House, Shanghai, China, weighing (180–200 g). Animals were kept in polypropylene cages, at temperature 25 ± 0.5 °C, relative humidity 60 ± 5% and a photoperiod of 12 h/day. All the animals were treated according to internationally accepted ethical procedures.

2.2. Induction of middle cerebral artery occlusion (MCAO)

Male albino rats were given chloral hydrate solution (10%) through intraperitoneal injection. Monofilament nylon was used to occlude the middle cerebral artery. Ischemic induction and reperfusion were started following 30 min. The entire period of surgery was carried out at room temperature. The experimental procedure was repeated for sham control rats [16].

2.3. Experimental groups

Male albino rats were divided into three groups containing 6 each. CUR was dissolved in normal saline and administered through intraperitoneal mode

Group I: Sham

Group II: MCAO (Middle Cerebral Artery Occlusion)

Group III: MCAO + CUR (25 mg/kg bwt,)

2.4. Preparation tissue cytosolic and mitochondria fraction

Rats were sacrificed following anesthetized with intramuscular injection of anesthesia ketamine hydrochloride (100 mg/kg body weight) + Xylazine (11 mg/kg body weight). Brain tissues were surgically removed and homogenized with a suitable buffer (10 mM Tris-HCl, 1 mM K-EDTA, 0.25 mM sucrose). The brain homogenate was centrifuged for 5 min at 10000 ×*g*, and the supernatant was removed and resuspended in isolation buffer. The suspension centrifuged again for 15 min at 10000 ×*g*, and the brown sediment pellets were collected and suspended in isolation buffer. The supernatant was taken as cytosolic fractions, and both mitochondrial and cytosolic fractions were stored at −20 °C until use [13].

2.5. Western blot analysis

Cell homogenate was washed with PBS, and lysed with 10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1% NP-40, 50 mM NaF, 2 mM EDTA (pH 8.0), 1 mM PMSF, 10 μg/ml leupeptin, and 10 μg/ml aprotinin. The protein which is present in the lysate was run on SDS-PAGE. Polyvinylidene fluoride (PVDF) membrane was used for transferring protein. Tris Buffered Saline with Tween (TBST) was used for the non-specific blocking proteins. The membrane probed with an antibody against p53, Bax, Bcl-2, and Sirt1 for overnight. Membranes were washed twice with TBST and incubated with HRP-conjugated goat anti-rabbit IgG (St. Louis, MO 63178 USA) for 60 min. The protein levels of p53, Bax, Bcl-2 and sirt1 were determined by using enhanced chemiluminescence method [17].

2.6. Determination of brain water content

Brain water content was determined according to Yang et al., [18]. White and red matter of brain was desiccated at 100 °C for 48 h to get constant weight. The net weight of dried 2,3,5-triphenyltetrazolium chloride stained brain was obtained through the determination of desiccated white and red matter together. The brain water content was measured by using following formula. (Wet weight-dried weight) / wet weight × 100%.

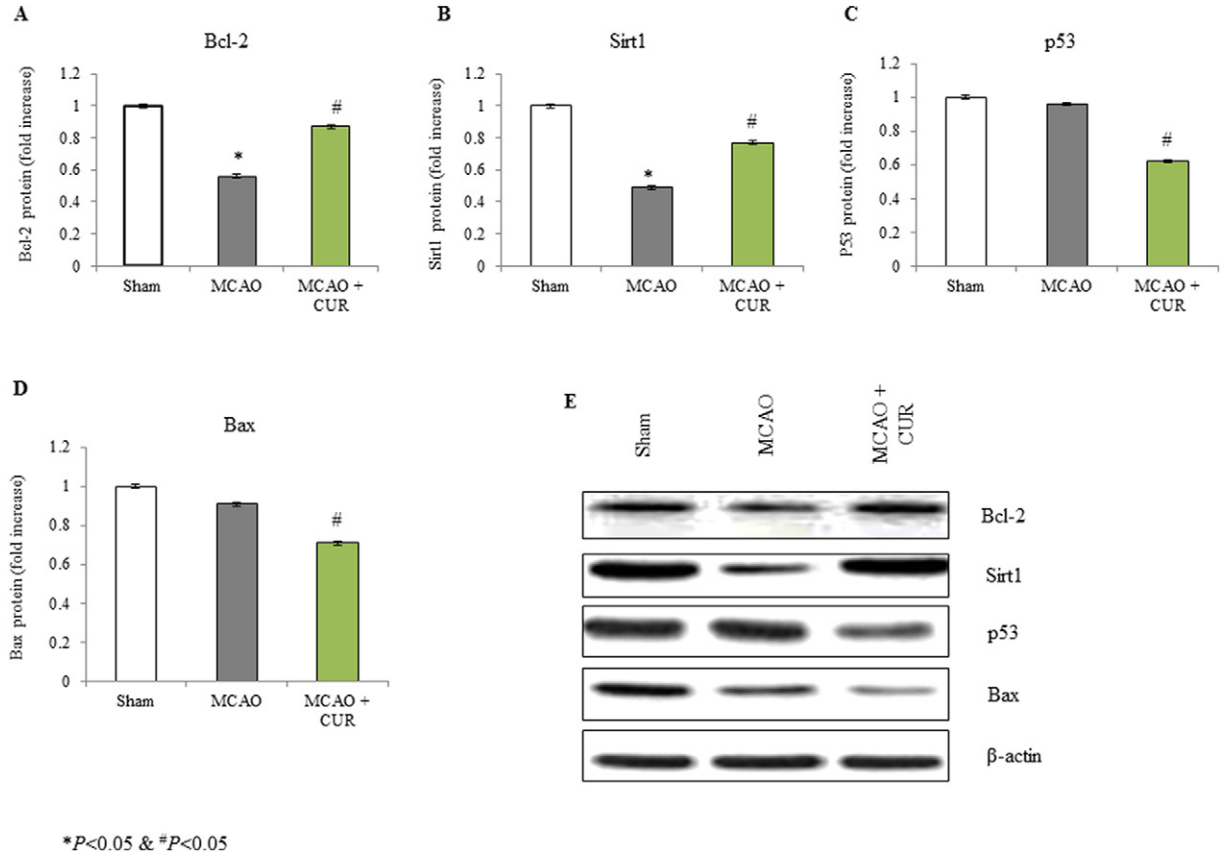

Fig. 1. The effect of CUR on Bcl-2, Sirt1, p53 and Bax expression of MCAO model rats. The western blot (E) and presentative images of Bcl-2 (A), Sirt1 (B), p53 (C) and Bax (D) are shown in the figure. The results are expressed as +SEM, $N = 6$, $^{*}P < 0.05$ vs. MCAO group, $^{\#}P < 0.05$ vs. MCAO + CUR group.