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Research report

Evidence for the protective effects of curcumin against oxyhemoglobin-induced injury in rat cortical neurons

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ABSTRACT

Curcumin (CCM) is a natural polyphenolic compound in *Curcuma longa* that has been reported to exhibit neuroprotective effects. Subarachnoid hemorrhage (SAH) is a severe neurological disorder with an unsatisfactory prognosis. Oxyhemoglobin (OxyHb) plays an important role in mediating the neurological deficits following SAH. The present study, therefore, aimed to investigate the effect of CCM on primary cortical neurons exposed to OxyHb neurotoxicity. Cortical neurons were exposed to OxyHb at a concentration of 10 μM in the presence or absence of 5 μM (low dose) or 10 μM (high dose) CCM for 24 h. Morphological changes in the neurons were observed. Cell viability and lactate dehydrogenase (LDH) release were assayed to determine the extent of cell injury. Additionally, levels of superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-Px), and reactive oxygen species (ROS) were measured. Neuronal apoptosis was assayed via TUNEL staining and protein levels of cleaved caspase-3, Bax, and Bcl-2 were measured by Western blot. Levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 were measured using ELISA kits. Our results suggested that CCM at both low and high doses markedly improved cell viability and decreased LDH release. CCM treatment decreased neuronal apoptosis. Additionally, oxidative stress and inflammation induced by OxyHb were alleviated by CCM treatment. In conclusion, CCM inhibits neuronal apoptosis, and alleviates oxidative stress and inflammation in neurons subjected to OxyHb, suggesting that it may be beneficial in the treatment of brain damage following SAH.

1. Introduction

Subarachnoid hemorrhage (SAH) is a severe neurological disorder that has high rates of mortality and morbidity (Sehba et al., 2012). In approximately 85% of cases of SAH, the cause is rupture of an intracranial aneurysm (van Gijn et al., 2007). Although significant progress has been made in illustrating the underlying mechanisms of SAH and in developing treatment, the prognosis of patients with SAH remains poor and unsatisfactory. The rupture of an aneurysm reduces cerebral blood flow, leading to early brain injury (EBI), which is defined as brain injury occurring within 72 h following SAH. Recent studies have suggested that EBI is the primary cause of the poor prognosis associated with SAH (Fujii et al., 2014, 2013; Sehba et al., 2012). The mechanisms underlying EBI include inflammation and oxidative stress, leading to blood–brain barrier (BBB) disruption and neuronal apoptosis (Fujii et al., 2013; Marbacher et al., 2014). Therefore, therapeutic agents targeting inflammation, apoptosis, and oxidative stress may beneficial for SAH patients.

Oxyhemoglobin (OxyHb), a major component of blood, has been reported to be a major cause of cerebral vasospasm and neurological deficits following SAH (Luo et al., 2011). The underlying mechanisms implicating OxyHb may include the following: (1) OxyHb removes NO, leading to vasospasm (Schwartz et al., 2000); (2) OxyHb activates the Rho/Rho kinase pathway and protein kinase C, resulting in cerebrovascular constriction (Wickman et al., 2003); (3) Oxidation of OxyHb to MetHb leads to reactive oxygen species (ROS) production (Ayer and Zhang, 2008). Due to these linkages, OxyHb has been widely used in an in vitro model of SAH (Cui et al., 2015; Li et al., 2015a; Wu et al., 2010). Thus, in the present study, OxyHb was employed to mimic the effects of SAH on primary cortical neurons.

Curcumin (CCM), a natural polyphenolic compound that is primarily extracted from *Curcuma longa*, confers a wide range

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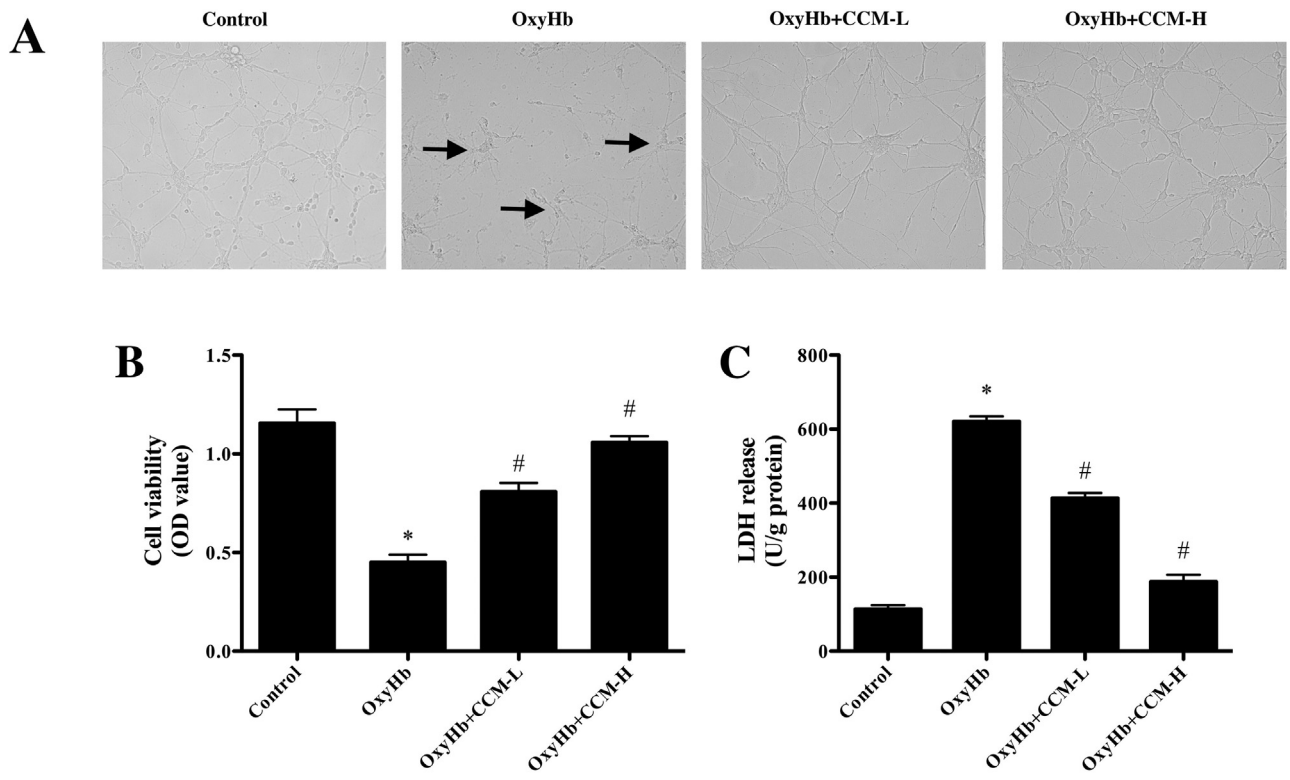

Fig. 1. CCM attenuates neuronal injury induced by OxyHb. With or without CCM treatment, the primary rat cortical neurons were exposed to OxyHb at a concentration of 10 μM for 24 h. (A) Morphological changes of primary rat cortical neurons. Neurons exposed to OxyHb showed a loss of condensation of the soma, a shrinkage of the neuron and neuronal arborization (black arrow), which were alleviated by CCM administration at both high and low doses. (B) Cell viability. Neurons exposed to OxyHb exhibited a decrease in cell viability, which was increased by CCM administered at both high and low doses. (C) LDH release. Neurons exposed to OxyHb exhibited an increase in LDH release, which was decreased by CCM administered at both high and low doses. The values are expressed as the mean ± SEM, $n = 6$. $^{*}P < 0.05$ versus control group, $^{\#}P < 0.05$ versus OxyHb group. CCM, curcumin; OxyHb, oxyhemoglobin; LDH, lactate dehydrogenase.

of pharmacological activities, such as cardioprotection, anti-inflammation, anti-oxidation, and anti-tumor (Li et al., 2015b; Lopresti et al., 2012; Pari et al., 2008; Ye et al., 2015). Additionally, CCM has been suggested to confer protection in neurological disorders, such as neurodegenerative diseases, cerebral ischemia, anxiety, and depression (Andrade, 2014; Haider et al., 2015; Li et al., 2015b; Lim et al., 2001; Yu et al., 2015, 2012). However, the effect of CCM on SAH remains poorly understood. This study, therefore, aims to investigate the role of CCM in SAH in primary cortical neurons treated with OxyHb.

2. Materials and methods

2.1. Reagents

Oxyhemoglobin (Ruibio, O7109) was purchased from Bomei Biotechnology Company (Hefei, China). 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and dimethyl sulfoxide (DMSO) were purchased from Beyotime (Shanghai, China). LDH, GSH-Px and ROS kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). SOD and MDA kits were purchased from Sigma–Aldrich (St. Louis, MO, USA). TNF-α, IL-1β, and IL-6 ELISA kits were purchased from Jiancheng Bioengineering Institute (Nanjing, China). Terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) kits were purchased from Roche (Mannheim, Germany). Antibodies against cleaved caspse-3, Bax, Bcl-2, and β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). The appropriate secondary antibodies were purchased from Beyotime (Shanghai, China).

2.2. Primary culture of rat cortical neurons and treatment

All animal studies were performed according to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (National Institutes of Health Publication No. 85-23, revised 1996) and were approved by the Ethics Committee of the Fourth Military Medical University. The pregnant Sprague–Dawley rats were purchased from the animal center of the Fourth Military Medical University. Primary rat cortical neurons were obtained from 17-day-old Sprague–Dawley rat embryos as reported previously (Chen et al., 2012). Briefly, the cortical tissues were carefully dissected out and placed in ice-cold Hank's balanced salt solution (HBSS; 10 mM HEPES, pH 7.3), the tissues were minced using microscissors and digested in 0.25% trypsin at 37 °C for 15 min. The minced tissues were balanced by Dulbecco's Modified Eagle's Medium (DMEM; Gibco-BRL, GrandIsland, NY, USA) with 10% FBS, and were filtered through a sterile mesh filter (40 mm). The cell suspension was centrifuged at 200 × *g* for 5 min and gently resuspended in neurobasal medium (Gibco-BRL) supplemented with 2% B27 (Gibco-BRL), 0.5 mM L-glutamine (Gibco-BRL), 100 U/mL penicillin and 100 μg/mL streptomycin (Gibco-BRL). Cultures were maintained in a 5% CO_2 humidified incubator at 37 °C. After 72 h, cytosine arabinoside (10 mM, Sigma) was added to the medium described above. The cortical neurons were cultured to day 7 for treatment. To establish an in vitro SAH model, the neurons were exposed to OxyHb (Ruibio, O7109) at a concentration of 10 μM in the presence or absence of 5 μM (low dose) or 10 μM (high dose) CCM for 24 h. The concentration of OxyHb was chosen based on the previous study (Li et al., 2015a). The sample size for each treatment was six ($n = 6$).