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Curcumin pretreatment attenuates brain lesion size and improves neurological function following traumatic brain injury in the rat

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ABSTRACT

Turmeric has been in use since ancient times as a condiment and due to its medicinal properties. Curcumin, the yellow coloring principle in turmeric, is a polyphenolic and a major active constituent. Besides anti-inflammatory, thrombolytic and anti-carcinogenic activities, curcumin also possesses strong antioxidant property. The neuroprotective effects of curcumin were evaluated in a weight drop model of cortical contusion trauma in rat. Male Wistar rats (350–400 g, n = 9) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and subjected to head injury. Five days before injury, animals randomly received an i.p. bolus of either curcumin (50 and 100 mg/kg/day, n = 9) or vehicle (n = 9). Two weeks after the injury and drug treatment, animals were sacrificed and a series of brain sections, stained with hematoxylin and eosin (H&E) were evaluated for quantitative brain lesion volume. Two weeks after the injury, oxidative stress parameter (malondialdehyde) was also measured in the brain. Curcumin (100 mg/kg) significantly reduced the size of brain injury-induced lesions ($P < 0.05$). Neurological examinations (rotarod and inclined-plane tests) were performed on days 1, 3, 7 and 14 post-brain injury. Control injured rats had a significant neurological deficit during 2 weeks ($P < 0.001$). The injury increased brain levels of the malondialdehyde by 35.6% and these increases were attenuated by curcumin (100 mg/kg). Curcumin treatment significantly improved the neurological status evaluated during 2 weeks after brain injury. The study demonstrates the protective efficacy of curcumin in rat traumatic brain injury model.

1. Introduction

Traumatic brain injury is a major cause of death and disability globally. Outcome for patients with brain injury has improved over the past decades due to improved pre-hospital and neurointensive care that has focused on avoiding/reducing secondary insults in these patients (Sharma and Vavilala, 2012) rather than through the use of new pharmacological treatments for brain injury. Despite promising results from experimental drug trials, none of the clinical trials of new drugs have been able to show a significant effect on outcome for patients with head injury (Zafonte et al., 2012). It appears that further improvements in care require continued focus on pathophysiological mechanisms responsible for the enhanced vulnerability of the brain to secondary insults after trauma. Understanding the effects of different secondary insults requires multimodality monitoring to elucidate each insult's effects on the tissue and at what time point the different insults are dangerous to the patient.

The cascade of delayed or secondary pathologic events that follow an injury is extremely complex, and the relative importance of each event appears to differ in each individual case. Therefore, a great need exists for a broad-spectrum neuroprotective agent that may target several pathophysiologic processes and thus confer widespread protection to the damaged brain.

Several mechanisms have been suggested to be involved in the etiology of brain injury including NMDA receptor activation leading to excitotoxicity, excessive nitric oxide (NO) generation and free radical mediated oxidative stress (Moojen et al., 2012; Ghorbani et al., 2012). Several studies have revealed that during brain injury there is excessive generation of oxidative stress parameters such as lipid peroxides and the antioxidant defense is impaired causing more vulnerability and damage to the brain (Slemmer et al., 2008; Sadeghnia et al., 2012). Of the many biological targets of oxidative stress, the lipids are the most involved class of biomolecules. A biomarker for lipid peroxidation is malondialdehyde (MDA), it is a highly toxic molecule and it has been implicated in a range of disease pathologies by producing oxidative damage in the tissues. A number of agents, both synthetic and natural, have been screened to evaluate their preventive and therapeutic efficacy against head injury. Dietary supplementation with blueberries, spinach and spirulina reduces ischemia/reperfusion induced apoptosis

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and cerebral infarction (Singleton et al., 2010). Turmeric (Curcuma longa rhizomes) has been extensively used as an effective therapeutic agent since ages. Turmeric as well as its constituent curcumin has been shown to exhibit anti-inflammatory, anti-carcinogenic and antioxidant activities, besides several other pharmacological properties (Khurana et al., 2012). Prophylactic/therapeutic effect of curcumin in cancer chemo-prevention, multiple sclerosis and myocardial infarction has been reported (Nagaraju et al., 2012). Curcumin has been found to be effective in the treatment of anterior uveitis and cystic fibrosis (Cartiera et al., 2010). More recently, the neuroprotective efficacy of curcumin in attenuating 3-nitropropionic acid (a fungal toxin) and lead induced neurotoxicity has been reported (Kumar et al., 2007). Pari and Murugan found that tetrahydrocurcumin prevented brain lipid peroxidation in streptozotocin induced diabetic rats (Pari and Murugan, 2007). The neuroprotective effect of curcumin was associated with its antioxidant potential in these studies (Merrell et al., 2009). Curcumin has been reported to cross the blood–brain barrier and based on the potential of curcumin to inhibit the formation of amyloid beta oligomers and fibrils in mice the use of curcumin has been recommended for the clinical trials to prevent or treat Alzheimer's disease (Wang et al., 2013). The effect of curcumin was studied in rats following intraperitoneal treatment, 30 min after MCAO, indicating its neuroprotective potential in ischemia. It has been suggested to be mediated through its antioxidant activity (Thiyagarajan and Sharma, 2004). Curcumin is recognized as a promising compound with multiple pharmacological properties and the present study was undertaken in rats treated with interaperitoneal curcumin before head injury to comprehensively evaluate the potential neuroprotective effects of curcumin with respect to the brain lesion, malondialdehyde (MDA), a marker of lipid peroxidation, and posttraumatic neurologic motor deficits following an experimental model of weight drop brain injury in the rat.

2. Experimental procedure

2.1. Animals

Male Wistar rats (n = 40) weighing 350 to 400 g were housed individually in polycarbonate cages in a standard animal house maintained at 21 ± 2 °C and 50 ± 10% humidity with a 12-hour light:dark cycle. Rats were fed with standard laboratory chow and water. The experimental protocol was approved by an Institutional Review Committee of Mashhad Medical University for the use of Human or Animal Subjects and also the studies were carried out in accordance with the official regulations approved by the Animal Ethics Committee of Mashhad Medical University, Mashhad, Iran. The animals were also acclimatized to the laboratory conditions prior to experimentation. The rats were maintained in accordance with the National Institutes of Health guidelines for the care and use of laboratory rats.

2.2. Experimental groups

The rats were randomly allocated into four groups (n = 9) as follows; a control (sham-operated) group, a trauma group, and curcumin groups (50 and 100 mg/kg). Curcumin was administrated i.p. once daily for 5 days at the dose of 50 and 100 mg/kg/day. The dose of curcumin was chosen based on our previous experiments. The control group underwent craniotomy alone and received no medication. The trauma group underwent craniotomy followed by brain injury and received the vehicle. The curcumin groups underwent craniotomy followed by brain injury and received curcumin (50 and 100 mg/kg) 5 days before injury.

2.3. Drug treatment

Curcumin, (Sigma Chemicals, St, Louis, MO, USA), was suspended with dimethyl sulfoxide (DMSO) in double-distilled water. The vehicle given to the control animals had the same % of DMSO and had also similar osmolality and pH with the curcumin group. The solutions were sterilized through 0.22-mm filters and administered to animals by i.p. injection at the doses of 50 and 100 mg/kg once daily for 5 days. The final dose of curcumin was administered 30 min before the brain injury. The dose of curcumin was chosen based on previous experiments.

2.4. Surgical procedures and weight drop brain injury model

For the production of brain injury, we used the weight drop technique modified by Marklund et al. (2001) after Feeney et al. (1981). All rats were weighed before undergoing anesthesia. The animals were anesthetized by an intraperitoneal injection of 10 mg/kg xylazine (Sigma, St. Louis, USA,) and 50 mg/kg ketamine hydrochloride (Sigma, St. Louis, USA). Body temperature was monitored with a rectal probe and kept between 37.0 and 37.5 °C with a heating pad. After catheter preparation the animals were placed in a stereotaxic frame. All surgery was done under sterile conditions. A craniotomy (6×9 mm^2), centered over the right parietal cortex at bregma −3.5 and 3.5 mm lateral to the midline, was done using a dental drill. An 11 g weight was dropped from a height of 35 cm onto 4.5 mm diameter piston resting on the exposed dura. The device is constructed to prevent bouncing of the weight thus allowing only a single compression.

2.5. Locomotor activity

Rotarod, beam balance, and angel board tests were performed to evaluate the behavioral effect of curcumin on sensory-motor dysfunction after trauma. Neurological deficits in the vehicle- and drug-treated groups were determined after 1st, 3rd, 7th and 14th days after the brain injury.

2.5.1. Rotarod test

Rotarod testing took place over two consecutive days before injury. On day 1, the animals were trained until they were able to remain on the rod (speed = 16 RPM) for 120 s for two trials. The animals were given as many trials as required to reach this performance criterion, after which they were returned to their home cage until testing the following day. Animals that did not reach this criterion were eliminated from the present study. The rotarod test was conducted at 1st, 3rd, 7th and 14th days after brain injury. The latency time for each rat was determined from five separate trials; the lowest and highest outlier data were excluded and the remaining three data were averaged for the final result. On the test day, the animals were randomly assigned to a dose group (0, 50, 100 mg/kg/day; n = 9) and tested for three trials on the rod rotating at 16 rpm. The animals were allowed a minimum of 30 s rest period between trials. The dimensions of the rat rotarod were 8.5 cm in width and 7 cm in diameter. In order to assess the effect of curcumin on motor skill acquisition, the rats were transferred to the procedure room.

2.5.2. Inclined-plane test

We evaluated motor performance in rats, using a sliding apparatus two days before traumatic brain injury in rats and the following 2 weeks after trauma. The sliding apparatus had a 60 × 40-cm wood plane that could be inclined at an angle of 0° (horizontal) to 60°. Angel board testing took place over fourteen days. On day 1(before injury), each rat was placed on the 60°-angled inclined plane. The animals were trained until they were able to remain on the board for 120 s for two trials. The animals were given as many trials as required to reach this performance criterion, after which they were returned to their home cage until testing the following day. Animals that did not reach this criterion were eliminated from the study. The inclined-plane test was conducted at 1st, 3rd, 7th and 14th days after brain injury. The time for standing on an inclined angle board for each rat