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Nitric oxide mechanism in the protective effect of naringin against post-stroke depression (PSD) in mice

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ABSTRACT

Aim: The present study has been designed to explore the nitric oxide mechanism in the protective effect of naringin against I/R induced neurobehavioral alterations, oxidative damage and mitochondrial dysfunction in mice.

Main methods: Laca mice (25–30 g) were subjected twice to BCCAO occlusion (5 min) at the interval of 10 min, followed by 96 h reperfusion. Naringin (50 and 100 mg/kg) was administered for 10 days, starting 7 days before the animals were subjected to I/R injury. On day 10, various neurobehavioral parameters followed by biochemical parameters and mitochondrial enzyme complex activities were assessed.

Key findings: Ischemia reperfusion injury caused significant (increased immobility period, neurological score and decreased locomotor activity) oxidative damage (increased lipid peroxidation and nitrite concentration and depleted reduced glutathione, glutathione-S-transferase, superoxide dismutase and catalase) and altered mitochondrial enzyme complex activities (complex I to IV) as compared to sham treatment. Naringin (50 and 100 mg/kg) treatment significantly attenuated neurobehavioral alterations, oxidative damage and restored mitochondrial enzyme complex activities as compared to control (ischemia reperfusion) group. Further, protective effect of naringin (50 mg/kg) was attenuated by L-arginine (100 mg/kg) or sildenafil (5 mg/kg) pretreatment. Further, L-NAME (10 mg/kg) or 7-NI (10 mg/kg) pretreatment with naringin (50 mg/kg) significantly potentiated their protective effect as compared to their treatment alone.

Significance: The present study suggests the involvement of nitric oxide mechanism in the protective effect of naringin against post-stroke depression induced neurobehavioral, biochemical and cellular alterations in mice.

Introduction

Stroke is the third leading cause of death in the major industrialized countries following cardiovascular disease and cancer (Green 2008). Post-stroke depression (PSD) is a well known complication among the majority of stroke survivors. Post-stroke depression affects approximately one third of ischemic stroke survivor (Berg et al. 2009). Several factors contribute to PSD that include psychosocial stress, loss of independence and worsening of quality of life, in addition to the neurobiological alterations such as site of infarcts and brain atrophy (Juranek and Baciak 2009; Cramer 2008). The cell death in the affected brain regions results to loss of the functional activities that depend on the region and extent of cellular damage within the brain (Helps and Sims 2007).

During stroke, cerebral blood flow decreases resulting in glucose and oxygen depletion. This further causes deprivation of adenosine triphosphate (ATP) and initiates a series of cellular processes that include activation of nitric oxide and mitochondrial dysfunction. The free radicals generated during stroke eventually cause neuronal cell damage (Jeyaseelan et al. 2008). Neurotoxic free radicals produced during I/R injury are unpaired electron molecules (nitric oxide, superoxide and hydroxyl anion) that are chemically unstable and reactive, which distort neuronal tissue and cause secondary cell death. Reperfusion is a requisite to refurbish glucose, oxygen supply to the ischemic area, generating ATP and recuperating normal physiological process. However, reperfusion partially causes some ischemic injury, due to sudden release of excessive reactive oxygen species (ROS), a phenomenon known as reperfusion injury (Johansen et al. 2005).

Studies on stroke suggest that free radical scavengers and nitric oxide synthase inhibitors may be used as beneficial neuroprotectants (Gaur et al. 2009; Gaur and Kumar 2010). Agents that can inhibit/reduce the generation of free radicals may retard neuronal damage. Several preclinical studies using free radical scavengers have been shown to have beneficial effects (Johansen et al. 2005; Sugawara and Chan 2003). Study showed that free radicals generated during ischemic reperfusion impair mitochondria functions (Brooks et al. 2008). Mitochondria are the core to neuronal energy metabolism and cell death. Damage to the mitochondrial respiratory chain is central to neurodegenerative condition. NO is an important brain messenger

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released upon activation of the glutamate N-methyl-aspartate (NMDA) receptor and subsequent Ca^{2+} dependent activation of nNOS. NO has been accredited with both pro-oxidant and antioxidant actions (Lipton 1993; Wink et al. 1999). NO has also been implicated in several neurological and cellular cascades and compromises cellular energy metabolism by inhibiting components of the mitochondrial respiratory chain (Brown and Bal-Price 2003). Although, generation of NO leads to vascular dilation, an enhancement of blood flow and mitigation of hypoxic injury in endothelial cells. However, it aids glutamate excitotoxicity and free radical-induced injury by forming peroxynitrite in the neurons (Helps and Sims 2007; Brown and Bal-Price 2003). Naringin, (4′, 5, 7-trihydroxy flavonone 7-rhamnoglucoside), is a flavanone found in grapefruit citrus species, roots of *Cudrania cochinchinensis* and fruits of *Poncirus* (Swaider and Zarawaska 1996). Reports suggest that naringin possesses diverse biological and pharmacological properties including anti-inflammatory, anticarcinogenic, lipid-lowering, and antioxidant activities (Choi et al. 2001). Neuroprotective effect of naringin has been documented in neurodegenerative disorders (Gaur et al. 2009; Kumar and Kumar 2010). However, its exact status and mechanism of action has not been well understood so far.

Therefore, the present study has been designed to investigate the possible nitric oxide mechanism in the protective effect of naringin in experimental model post-stroke depression in mice.

Experimental procedures

Animals

Albino mice (Laca strain) 8–10 weeks old, weighing between 25 and 30 g and bred in Central Animal House facility of the Panjab University, Chandigarh, India were used. The animals were kept under standard laboratory conditions, maintained on 12-h light/dark cycle with food and water ad libitum in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 0900 and 1500 h in order to minimize the circadian changes and inter-group variations. In all experimental protocols, animals were properly randomized based on their body weights. Experimental protocol was approved by the Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs and treatment schedule

Animals were randomly divided into different groups, consisting of 12 animals each. Group 1 was vehicle treated sham group (surgically exposed, but did not receive an occlusion); Group 2 treated as vehicle control (I/R); Group 3 and 4 received naringin (50 and 100 mg/kg) treatment followed by I/R; Group 5 to 6 received L-arginine (100 mg/kg) and sildenafil (5 mg/kg) per se treatment followed by I/R injury respectively; Group 7 to 8 received L-NAME (10 mg/kg) and 7-NI (10 mg/kg) per se treatment followed by I/R respectively; Group 9 to 10 involved L-arginine (100 mg/kg) or sildenafil (5 mg/kg) pretreatment with naringin (50 mg/kg) followed by I/R injury; Group 11 to 12 received L-NAME (10 mg/kg) and 7-NI (10 mg/kg) pretreatment with naringin (50 mg/kg) followed by I/R injury.

All drug treatments were given for 10 days starting from 7 days before I/R surgery. Subsequently, after 96 h I/R injury, mice were assessed for neurobehavioral activity and then sacrificed for biochemical and cellular estimations. All drugs were administered intraperitoneally. In the present study, nitric oxide (NO) modulators were administered 1 h prior to naringin treatment. Dose of these drugs was selected based on published reports and or similar ongoing studies in our laboratory (Kumar et al. 2009; Gaur et al. 2009; Kumar et al. 2010).

Induction of cerebral ischemia/reperfusion (I/R) injury in mice

Induction of cerebral ischemia/reperfusion was carried as modified by the method of Kelly and co workers (Kelly et al. 2001; Gaur and Kumar 2010). The animals were anaesthetized with chloral hydrate (360 mg/kg, i.p.) at the time of experiment and further supplemented as needed. Both common carotid arteries were exposed over a midline incision and dissection was made between sternocleidomastoid and sternohyoid muscles parallel to the trachea. Each carotid artery was freed from its adventitial sheath and vagus nerve, which was carefully separated and maintained. The induction of ischemia was performed by occluding bilateral common carotid arteries (BCCAO) (for 5 min) with clamps for 5 min at the interval of 10 min. At the end of the occlusion, cotton thread was completely removed, arteries were visually inspected for reflow and midline incision was sutured with stitches using waxed silk suture.

During BCCAO, animals were observed for the following criteria: maintenance of dilated pupils, absence of a cornea reflex when exposed to strong light stimulation, and maintenance of rectal temperature at (37 °C ± 0.5). The animals that did not match these criteria and showed seizures were excluded from the study.

Behavioral assessments

Inclined beam-walking test

Inclined beam-walking test (neurological score) was employed to evaluate fore and hind limb motor coordination (Feeney et al. 1981). Each animal was individually placed on a wooden bar, inclined at an angle of 60° from the platform. The motor performance of mice was scored on a scale ranging from 0 to 4. A score of 0 was assigned to animal that could readily traverse the beam; score 1, 2 and 3 were to animal demonstrating mild, moderate and severe impairment, respectively. Score 4 was assigned to the animals completely unable to walk on the beam. The test was performed 96 h after global cerebral ischemia and reperfusion.

Forced-swim test (FST)

The test procedure was the same as validated earlier in our laboratory (Kulkarni and Mehta 1985). In brief, mice were individually forced to swim inside a rectangular glass jar ($25 \times 12 \times 25$ cm^3 containing 15 cm of water maintained at 23–25 °C). After the initial 2–3 min of vigorous activity, the animals showed period of immobility by floating with minimum movements. An animal is considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. The total immobility period for 6 min was recorded with the help of a stop-watch.

Assessment of gross behavioral activity (locomotor activity)

The locomotor activity (ambulatory activity) was monitored using an actophotometer (IMCORP, India). Before locomotor task, animals were placed individually in the activity meter for a 3 min acclimation period before starting actual activity tasks. Each animal was observed over a period of 5 min and activity was expressed as counts per 5 min (Reddy and Kulkarni 1998).

Dissection and homogenization

Animals were randomized into different groups for biochemical and mitochondrial complex estimations. For the biochemical analysis, animals were killed by decapitation immediately after behavioral assessments. The brains were removed and 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4).