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FeTPPS protects against global cerebral ischemic-reperfusion injury in gerbils

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Abstract

Neuronal damage following cerebral ischemia is mediated by various mechanisms, among which nitrosative stress plays an important role. Peroxynitrite, a powerful oxidant, contributes heavily to the neuronal damage in cerebral ischemic-reperfusion (IR) injury. In the present study, we have investigated the neuroprotective effects of a peroxynitrite decomposition catalyst, 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrinato iron(III) [FeTPPS] in global cerebral IR injury in gerbils. Neurological damage was significantly attenuated by FeTPPS treatment (1 and 3 mg kg^{-1}, i.p.) as evident from reduction in neurological symptoms, hyperlocomotion, memory impairment and CA1 hippocampal neuronal damage in IR challenged gerbils. FeTPPS treatment also attenuated the increased malondialdehyde (MDA) levels and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) positive cells after cerebral IR injury. Results of this study demonstrates the neuroprotective activity of FeTPPS in global cerebral IR injury and its neuroprotective effects may be attributed to reduction in oxidative stress and DNA fragmentation.

Keywords: FeTPPS; Bilateral carotid occlusion; Peroxynitrite; Oxidative stress; Delayed neuronal death; Gerbil

1. Introduction

Stroke is an acute neurological injury, resulting from interruption of blood supply to the brain. It is the major cause of the mortality and morbidity in the industrialized countries [1,2]. Dramatic reduction of blood to the brain results in ischemia of the whole brain (global ischemia) or of defined cerebral territories (focal ischemia) depending on the cerebral artery occluded. Global cerebral ischemia may be an outcome occurring as a consequence of conditions like cardiac arrest, coronary artery bypass surgery and other situations that deprive brain from the blood, even for a short period of time. Approximately 10–20% of cardiac arrest patients show recovery, others die, or persist in a vegetative state. Patients who recover display neurological damage, impaired learning and memory deficits because of ischemic neurodegeneration in certain areas of brain [3,4]. Pyramidal neurons of CA1 field in the hippocampus are among the most vulnerable cells to ischemia. Severe loss of hippocampal neurons has been shown to occur after brief periods of ischemia as a consequence of immediate, maturational and delayed neuronal death [5–9].

Pathophysiology of cerebral ischemia involves a complex multifactorial cascade [2,10]. Nitrosative stress, i.e. enhanced peroxynitrite formation is thought to play a key role in progressive ischemic neurodegeneration [11,12]. Peroxynitrite, a potent oxidant formed in the biological system from nitric oxide and superoxide, produces wide arrays of deleterious effects by reacting with a variety of biomolecules including protein, lipids and DNA [11,13]. Peroxynitrite can easily permeate lipid bilayer leading to peroxidation of membrane lipids as commonly indicated by measuring malondialdehyde levels [14–16].

Peroxynitrite can directly hydroxylate and nitrate the aromatic residues of amino acids and nucleotides in the cytosol and nucleus [17–19]. Peroxynitrite is also known to induce variety of other downstream consequences which ultimately contribute to its damaging effects [20,21]. Several evidence have suggested that targeting peroxynitrite toxicity in cerebral ischemic-reperfusion (IR) injuries is a promising avenue for the future experimental and clinical research [11,21–23].

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Recently, peroxynitrite decomposition catalysts (PDCs) like 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrinato iron(III) (FeTPPS) has shown protective effects against nipradilol-induced neuronal damage [24] and in many other pathological conditions [25,26]. However, its effects have not been studied in global cerebral ischemia model, therefore, in this study we have evaluated neuroprotective effects of FeTPPS in global cerebral IR injury in gerbils.

2. Materials and methods

2.1. Animals

Adult male Mongolian gerbils (50–70 g) were obtained from Central Animal Facility of National Institute of Pharmaceutical Education and Research (NIPER), S.A.S. Nagar, Punjab, India. Animals were housed in a room at controlled temperature ($22 \pm 1\,^{\circ}C$). Standard gerbil chow pellet and water were allowed *ad libitum*. All procedures used in this study were approved by Institutional Animal Ethics Committee, NIPER.

2.2. Materials

FeTPPS (structure shown in Fig. 1; molecular weight 1024.3) was purchased from Calbiochem, USA. All other chemicals were of analytical grade and were purchased from Sigma, USA or local commercial suppliers.

2.3. Induction of global cerebral IR injury

Gerbils were anesthetized with 2% halothane in a mixture of 70% nitrous oxide and 30% oxygen followed by maintenance of anesthesia with 1.5% halothane. Body temperature was maintained at $37.0 \pm 0.5\,^{\circ}C$ by using feed back homeothermic blanket system (Harvard Apparatus Ltd., Kent, UK), throughout the surgical procedure. Global cerebral IR injury was induced by 5 min bilateral common carotid arteries occlusion (BCAO) followed by 96 h reperfusion [5]. During the experiment, behavioral parameters (neurological functions, locomotor activity and passive avoidance test) were monitored. After 96 h of onset of reperfusion, gerbils were sacrificed for histological and DNA fragmentation studies.

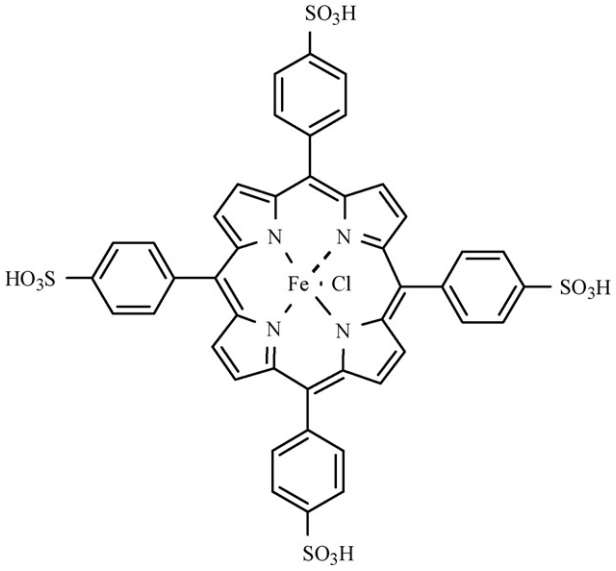

Fig. 1. Chemical structure of 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrinato iron(III) chloride (FeTPPS).

2.4. Treatment schedule

FeTPPS (1 and $3\,mg\,kg^{-1}$) was administered intraperitoneally 30 min prior to the onset of ischemia. Saline was used as vehicle for FeTPPS. Sham-operated group was subjected to the same surgical procedure without occluding the common carotid arteries. Each group consist of five to six animals, unless and otherwise stated.

2.5. Neurological deficit

Gerbils were observed for neurological symptoms 24 h after the onset of reperfusion on a five-point score: 0, no neurological symptoms; 1, hunched posture; 2, tonic seizures; 3, ptosis; 4, unconsciousness [27].

2.6. Locomotor activity

Locomotor activity was assessed 24 h after onset of reperfusion using Opto Varimex auto track system (Columbus Instruments, USA) as reported previously [16]. Animals were acclimatized to room conditions and apparatus prior to start of the experiment. Animals were placed in the plexiglass activity chamber (43 cm × 43 cm × 20 cm) and the total activity was recorded for 10 min. The locomotion was expressed in terms of total photobeam counts/10 min.

2.7. Passive avoidance test

Passive avoidance test was carried out 96 h after onset of reperfusion using passive avoidance apparatus (Ugo Basile, Italy), consisting of dark and white compartments. The experimental session was divided into three phases: habituation trial, acquisition trial and retention trial as reported by Dhar et al. [16]. Response latency (in seconds) was measured during retention trial.

2.8. Estimation of neuronal damage

The gerbils were euthanised 96 h after onset of reperfusion by decapitation. The brains were removed, fixed in 10% formalin for 24 h and paraffin embedded. Brain coronal sections (5 μm) were obtained with the help of microtome (Leica RM2145, Germany). Representative brain sections at level of 1.5 to 1.7 mm posterior to bregma were selected and stained with cresyl violet (0.5%) [16]. Images of hippocampus CA1 region were captured for histological examination at a magnification of 40× using a