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Neuroprotective effects of FeTMPyP: A peroxynitrite decomposition catalyst in global cerebral ischemia model in gerbils

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Abstract

Peroxynitrite involvement has been implicated in the neuronal damage. In the present study, we have investigated the neuroprotective effects of peroxynitrite decomposition catalyst (FeTMPyP) on global cerebral ischemia. Global cerebral ischemia-reperfusion (IR) injury was produced by 5 min occlusion of both common carotid arteries followed by reperfusion of 96 h in the adult male Mongolian gerbils. The extent of injury was assessed behaviorally by measuring neurological functions, locomotor activity, passive avoidance test and by histopathological evaluation of extent of damage to CA1 hippocampal pyramidal region. FeTMPyP (1 and 3 mg kg^{-1}, i.p., administered 30 min prior to ischemia) treatment improved the neurological functions, reduced the hyperlocomotion and memory impairment in IR challenged gerbils. The loss of neurons from the pyramidal layer of the CA1 region caused by global IR injury was attenuated with FeTMPyP. FeTMPyP also inhibited lipid peroxidation as evident from reduction in brain malondialdehyde levels. These results suggest that peroxynitrite decomposition catalyst may be effective neuroprotective agent for global cerebral ischemia.

Keywords: FeTMPyP; Peroxynitrite decomposition catalyst; Peroxynitrite; Global ischemia; Oxidative stress

1. Introduction

Stroke is the third leading cause of death and a major cause of long-term disability in industrialized countries. Dramatic reduction of oxygen in stroke may lead to ischemia of the whole brain (global ischemia) or of defined cerebral territories (focal ischemia) depending on the cerebral artery occluded. Ischemia damages brain tissue, which is accompanied by biochemical alterations and neurological sequelae [1,2]. The pathophysiological mechanisms leading to neuronal injury in ischemic stroke are complex and multifactorial. There is substantial experimental evidence that free radicals are produced in the brain during ischemia-reperfusion (IR) injury [1–3]. Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, hydrogen peroxide, nitric oxide and peroxynitrite contributes to ischemic brain damage [3,4]. Superoxide can react with nitric oxide to form peroxynitrite, a potent oxidant. It has been proposed that many of the toxic effects of nitric oxide are due to the generation of peroxynitrite [3,4]. Peroxynitrite is cytotoxic via a number of mechanisms including initiation of lipid peroxidation, direct inhibition of mitochondrial respiratory chain enzymes, inactivation of membrane sodium channels, modifications of oxidative protein and reduction in antioxidant enzymes [4–6]. Moreover, peroxynitrite can also cause DNA damage resulting in the activation of the nuclear enzyme poly-(ADP-ribose) polymerase (PARP). The excessive activation of PARP in turn results in depletion of NAD and ATP and ultimately neuronal death [4,7].

Several antioxidants (LY231617, LY178002 and melatonin) showed neuroprotection in cerebral IR injury and associated conditions like oxidative stress and inflammation [2,8–11]. The metalloporphyrin class of decomposition catalysts is found to be highly potent because the concentration needed for activity is minimal as compared to other group of compounds [11,12]. Many of them are reported to mimic superoxide dismutase (SOD) activity and decompose various components of ROS depending on the metallic ion present in the co-ordination complex. Neuroprotective effects of the metalloporphyrin class of SOD mimetics such as MnTE-2-PyP (5+), AEOL 10150, M40403 and M40404 have been demonstrated in animal models of ischemia [13]. Peroxynitrite

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decomposition catalysts such as 5,10,15,20-tetrakis(*N*-methyl-4-pyridyl)porphyrinato iron(III) (FeTMPyP) and 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron(III) (FeTPPS) have been shown to protect cytokine-induced cytotoxicity in hippocampal culture and methamphetamine-induced neurotoxicity in rats [12]. Recently, we have demonstrated neuroprotective effect of FeTMPyP, a peroxynitrite decomposition catalyst in focal cerebral ischemia in rat [14]. According to the Stroke Therapy Academic Industry Roundtable recommendations [15], without rigorous, robust and detailed preclinical evaluation in atleast two species, it is unlikely that novel neuroprotective prove to be effective when treated in large time consuming and expensive clinical trials. In addition to above roundtable discussion, Lestage et al. have recommended that preclinical neuroprotective activity for cerebral ischemia should be tested in multiple cerebral ischemia model in order to get ideal neuroprotective agent [16]. Considering these recommendations, in the present study, we have investigated the neuroprotective effect of FeTMPyP in global cerebral ischemia in gerbils.

2. Materials and methods

2.1. Animals

Adult male Mongolian gerbils (50–70 g) were obtained from Central Animal Facility, National Institute of Pharmaceutical Education and Research (NIPER). Animals were housed in a room at a temperature of 22 ± 1 °C. Standard gerbil chow pellets and water were allowed *ad libitum.* All the procedures performed in the study were approved by Institutional Animal Ethics Committee, NIPER.

2.2. Materials

FeTMPyP 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 ischemia

Gerbils were anaesthetized with 2% halothane in a mixture of 70% nitrous oxide and 30% oxygen followed by maintenance with 1.5% halothane. Temperature was controlled at 37 °C throughout the experiment with the use of homeothermic blanket (Harvard, USA). Global cerebral IR injury was induced by 5 min bilateral common carotid arteries occlusion (BCO) followed by reperfusion of 96 h [17]. During the experiment, gerbil's behavioral parameters (neurological functions, locomotor activity and passive avoidance test) were monitored. After 96 h of reperfusion, gerbils were sacrificed for histological studies.

2.4. Treatment schedule

FeTMPyP (1 and 3 mg kg^{-1}) was administered intraperitoneally 30 min prior to the onset of ischemia. Saline was used as vehicle for FeTMPyP. Sham-operated group was subjected to the same surgical procedure without occluding the common carotid arteries. Each group consists of 6–7 animals.

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; 1, hunched posture; 2, tonic seizures; 3, ptosis; 4, unconsciousness [18].

2.6. Locomotor activity

Locomotor activity was assessed 24 h after the onset of reperfusion using Opto Varimex auto track system (Columbus Instruments, USA). 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 using passive avoidance apparatus (Ugo Basile, Italy) according to the method of Catania et al. [19] with slight modification. The experimental session was divided into three phases: habituation trial, acquisition trial, and retention trial. During the habituation trial, the gerbils were placed in the white and illuminated compartment. In this phase, the sliding doors are initially closed and open after 3 s. The gerbil can now explore both compartments for 90 s and, after this period; gerbils were taken off the apparatus. After 10 min, gerbils are placed again in the white compartment. The sliding doors open after 3 s and successively close when the gerbil crosses the cage, entering the dark room, where it remain for 10 s, then is removed from the cage. The acquisition trial was performed 60 min after the habituation trial. In this phase, the gerbil was replaced in the white compartment and when it crosses the sliding doors entering the dark compartment, it receives an electric shock (3 mA for 6 s), released from the grid. Twenty-four hours later, the retention trial was performed. The gerbil was replaced in the white room and the sliding doors open as in the previous phases after 3 s. During this phase, a timer measures the response latency as the period, in seconds, between the time when the animal placed in the white compartment and the moment when the animal crosses in the dark compartment. The cut-off time was set at 250 s.

2.8. Estimation of neuronal damage

After 96 h of reperfusion, animals were sacrificed and brain sections (5 μm) were cut and fixed with egg albumin. Sections were stained with cresyl violet (0.5%) [20]. Images of hippocampus CA1 region were captured for histological examination at a magnification of 20× using a CCD camera attached microscope (Leica, Germany) [21]. CA1 neurons with blue staining, intact round shaped nuclei were viable neurons, and while dark blue