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Harmine mediated neuroprotection via evaluation of glutamate transporter 1 in a rat model of global cerebral ischemia

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HIGHLIGHTS

- Harmine treatment attenuated cerebral infarct volume and decreased neurons death.
- Harmine treatment elevated GLT-1 mRNA and protein expression.
- Harmine treatment attenuated astrocyte activation.

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ABSTRACT

Global cerebral ischemia (GCI) causes energy deficiency results in excessive release of glutamate from neurons. Astrocytic glutamate transporters play a predominant role in keeping extracellular glutamate concentrations below excitotoxic levels. Glutamate transporter 1 (GLT-1) may account for more than 90% of glutamate uptake in adult forebrain. Preclinical findings implicate that Harmine present neuroprotection effects in a rat model of amyotrophic lateral sclerosis disease, and the beneficial effects were specifically due to up-regulation of GLT-1. However, no experiments have explored the potential of Harmine to provide neuroprotection in the setting of GCI. The current study was designed to determine whether Harmine could attenuate cerebral infarction as well as improve neuronal survival after GCI. Furthermore, to test whether the mechanisms were associated with up-regulating of GLT-1, we used a GLT-1 specific inhibitor dihydrokainate (DHK) and analysis the expression of GLT-1 mRNA and protein in cortex of brain. We also examined whether Harmine treatment affected astrocytes activation via immunofluorescence. Our results showed that post-GCI administration of Harmine could attenuate cerebral infarct volume and decrease neurons death. It also caused significantly elevation of GLT-1 mRNA and protein and remarkably attenuation of astrocyte activation. We provide novel clues in understanding the mechanisms of which Harmine exerts its neuroprotective activity in neurological disorders.

1. Introduction

Glutamate induced ionic imbalance and excitotoxicity that affect neuronal and vascular elements in the affected brain are the major early events of global cerebral ischemia (GCI). Decreased uptake of glutamate by astrocytes causes an exposure of neurons to glutamate, leading to glutamate-mediated excitotoxicity in the affected brain [1].

In order to protect neurons from glutamate-mediated excitotoxicity, astrocytes and neurons must eliminate excessive glutamate from the extracellular space. Previous studies demonstrated that astrocytic glutamate transporters play a predominant role in keeping extracellular glutamate concentrations below excitotoxic levels [18]. The uptake of glutamate is primarily performed by a family of five subtypes of sodium dependent glutamate transporters. Among these transporters GLT-1 may account for more than 90% of glutamate uptake in adult forebrain [8]. The GLT-1 dysfunction has been demonstrated in the pathogenesis of multiple neurological disorders, including stroke, Alzheimer's disease, several forms

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of epilepsy, amyotrophic lateral sclerosis, and traumatic brain injury [7,11,13,19,20]. Thus, drugs and agents that increase glutamate transport activity would act as powerful tool for enhancing the clearance of glutamate in such pathological conditions. Several in vitro and in vivo studies have shown an up-regulation of glutamate transporters by several drugs and molecules such as beta-lactam antibiotics, arundic acid (ONO-2506), citicoline, and pituitary adenylate cyclase-activating polypeptide [6].

Harmine is a β-carboline alkaloid that was first isolated in 1847 from seeds of *Banisteriopsis caapi* and *Peganum harmala*, both of which have traditionally been used for medicinal and ritual preparations in the South America, Central Asia, and Middle East [24]. Moreover, it is also existed in common plant derived foods and human tissues [10]. Harmine has a wide spectrum of pharmacological actions, including antidepressant-like actions, antioxidative action, antiplasmodial activity, and antigenotoxic activities [2,9,15]. Preclinical findings implicate that Harmine also present neuroprotection actions in rats subjected to a model of amyotrophic lateral sclerosis disease, and the beneficial effects were specifically due to up-regulation of GLT-1 [14]. However, no experiments have explored the potential of Harmine to provide neuroprotection in the setting of cerebral ischemia/reperfusion injury.

In this study, we intraperitoneal injected Harmine (30 mg/kg) to investigate whether Harmine could attenuate cerebral infarction and improve neuronal survival in a rat model of GCI. Furthermore, to test whether any neuroprotective effect was associated with GLT-1, we used a GLT-1 specific inhibitor dihydrokainate (DHK) and analysis the expression of GLT-1 mRNA and protein in cortex of brain. We also examined astrocytes activation induced following GCI via immunofluorescence. We anticipate this study would shed light on the Harmine mediated neuroprotection in neurological disorders.

2. Materials and methods

2.1. Animals and global cerebral ischemia (GCI) model

Adult female Sprague-Dawley rats weighing 250–300 g, aged 3 months, were used in this study. All procedures were approved by the local legislation for ethics of experiments on animals. All rats were allowed free access to food and water before the operation under optimal conditions (12 h light: 12 h darkness cycle, 22 °C). Female rats were bilaterally ovariectomized, and 1 week later, GCI was induced by 4-vessel occlusion as described previously [26]. Briefly, the rats were anesthetized with 10% chloral hydrate (350 mg/kg, i.p.), the vertebral arteries were electrocauterized and the common carotid arteries (CCA) were exposed. After 24 h, the rats were anesthetized using isoflurane anesthesia and the CCA were re-exposed and clipped by artery clips for 10 min followed by reperfusion. Rats that lost their righting reflex within 30 s and whose pupils were dilated and lost response to light during ischemia were selected for the experiments. Rectal temperature was maintained at 37 ± 0.5 °C using a thermal blanket during ischemia. Sham-operated animals underwent the same surgical procedures without occlusion of the CCA.

2.2. Group and drugs administration

Rats were randomly divided into four groups: sham-operated group (Sham, $n = 30$); GCI group (GCI, $n = 30$); GCI + Harmine group (Harmine, $n = 30$) and GCI + Harmine + DHK group (DHK, $n = 30$). Harmine (30 mg/kg) was administered by intraperitoneal injection 60 min after induction of GCI as previously described [14]. The DHK was prepared at the concentration of 2 mg/ml solution in 0.9% saline. This amounts to a volume of approximately 10–12 μl depending upon the body weight of animals and was given slowly over a period of nearly 5–6 min in the left ventricle of the brain 60 min after induction of GCI as previously described [25].

2.3. Measurement of the cerebral infarct area and volume

24 h after GCI, the brains were quickly removed and sliced into coronal sections at 2 mm intervals. Each slice was immersed in a 1.0% solution of 2,3,5-triphenyltetrazolium chloride (TTC) for 30 min at 37 °C and then fixed in 10% buffered formaldehyde solution. The stained brain slices were digitally photographed and the infarct area, outlined in white, was measured on posterior surface of each section using Biovis Image Plus software version 1.5. The percentage infarct area was calculated by subtracting the outlined white area from total area of the hemisphere of affected side of the brain divided by hundred. Infarct volume, expressed in mm^3, was calculated by a linear integration of the infarct area of each slice multiplied by thickness of brain section [3].

2.4. Behavioral recovery

At 24 h post-GCI, the Bederson's test for neurological deficits was performed as previously described [4].

2.5. Immunofluorescence

Coronal sections were incubated with 10% normal donkey serum for 30 min at room temperature in PBS, containing 0.1% Triton X-100 followed by incubation with appropriate primary antibodies overnight at 4 °C in the same buffer. The frozen sections were incubated with mouse anti-neuron-specific nuclear protein (NeuN) polyclonal antibody (Santa Cruz Biotechnology; Santa Cruz, CA, USA, diluted 1:100) and mouse anti-glial fibrillary acidic protein (GFAP) polyclonal antibody (Santa Cruz Biotechnology; Santa Cruz, CA, USA, diluted 1:100) overnight at 4 °C. The next day, sections were incubated with an anti-mouse IgG (Santa Cruz Biotechnology; Santa Cruz, CA, USA, diluted 1:1000) for 2 h at 37 °C in the dark. Photographs were taken in a laser scanning confocal microscope (OLYMPUS FV1000). Primary antibodies were replaced with PBS in the negative control group.

2.6. RNA isolation and PCR studies

Total RNA was isolated from cortex of brains collected in Sham, GCI, Harmine, and DHK group using the Tri Reagent (Sigma) according to the manufacturer's instructions. Total RNA (2 μg) was subsequently transcribed into cDNA using omniscript RT kit. cDNA was amplified separately with specific primer for GLT-1, GAPDH using Taq PCR core Kit (Qiagen USA). The PCR products were resolved in 1.2% agarose gel containing EtBr 5 μg/ml and the intensity of each band was analyzed by National Institutes of Health Image 1.41 software (Bethesda, MD, USA).

2.7. Western blot analysis

Rats were anesthetized and underwent intracardiac perfusion with 0.1 mol/L phosphate-buffered saline (PBS; pH 7.4). The cortex region of brain was rapidly isolated, total proteins were extracted and protein concentration was determined by the BCA reagent (Solarbio, Beijing, China) method. Samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Separated proteins on the gel were transferred onto PVDF membranes (Roche Diagnostics, Mannheim, Germany). Blots were blocked with 5% fat-free dry milk for 1 h at room temperature. Following blocking, the membrane was incubated with indicated