Brain Research Bulletin 121 (2016) 115–123

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Neuroprotective effects of penehyclidine hydrochloride against cerebral ischemia/reperfusion injury in mice

Ya Shu[a,b], Yin Yang[c], Pengbo Zhang[a,*]

[a] Department of Anesthesiology, Second Affiliated Hospital of Xi'an Jiaotong University School of Medicine, Xi'an, China
[b] Department of Pain Treatment Pain Management, First Affiliated Hospital of Xi'an Medical University, Xi'an, China
[c] The Second Department of Orthopedics, Xi'an Central Hospital, Xi'an, China

ARTICLE INFO

Article history:
Received 29 November 2015
Received in revised form 14 January 2016
Accepted 18 January 2016
Available online 21 January 2016

Keywords:
Penehyclidine hydrochloride
Cerebral ischemia/reperfusion injury
JNK/p38MAPK

ABSTRACT

Various reports have suggested that penehyclidine hydrochloride (PHC), a new cholinergic antagonist, exhibits a variety of biological actions such as anti-tumor and cardioprotective effects. This study aimed to investigate the effects of PHC on cerebral ischemia/reperfusion (I/R) injury and evaluate whether the c-Jun N-terminal kinase (JNK)/p38 mitogen-activated protein kinase (p38MAPK) pathway is involved in the protective effects of PHC. Male C57BL/6 mice were randomly assigned to Sham group, ischemia/reperfusion (I/R) group, I/R + PHC (0.1 mg/kg) group, and I/R + PHC (1 mg/kg) group. Mice were subjected to 2 h of transient middle cerebral artery occlusion, followed by 24 h of reperfusion except the mice in the sham group. Neurological deficits, infarct volume, brain water content, blood–brain barrier (BBB) integrity, and neuronal apoptosis were evaluated. The levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), superoxide production, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were measured. The expressions of the key proteins in the JNK/p38MAPK pathway were detected using the Western blot. The results suggested that compared to the I/R group, the PHC-treated group showed improved neurological deficits and BBB integrity, and reduced infarction volume, brain water content, and apoptosis. In addition, PHC significantly suppressed the levels of TNF-α, IL-1β, superoxide production, and MDA, and increased the levels of SOD and GSH-Px. Finally, PHC significantly downregulated the phosphorylation of JNK, p38MAPK, and c-Jun, indicating PHC protects against cerebral I/R injury by downregulating the JNK/p38MAPK signaling pathway.

1. Introduction

Ischemic stroke is a leading cause of death worldwide and effective treatment for it is still lacking due to the narrow therapeutic window (Hankey, 2012). The underlying mechanisms involved in ischemic stroke include a series of pathophysiological processes, such as neuroinflammation, oxidative stress, calcium overload, neuronal necrosis and apoptosis, glutamate excitotoxicity, and mitochondrial dysfunction (El-Sahar et al., 2015; Gao et al., 2015; Hadadha et al., 2015; Singh et al., 2015; Yang et al., 2015). Although the use of recombinant tissue plasminogen activator has greatly improved the outcome of stroke patients, the general prognosis is unsatisfactory (Daou et al., 2015). Therefore, it is necessary to develop new therapeutic agents for the treatment of ischemic stroke.

Penehyclidine hydrochloride (PHC) (Fig. 1), a new cholinergic antagonist, has been recently shown to exhibit various biological activities, such as anti-inflammation, anti-oxidation, cardioprotection, and neuroprotection (Cao et al., 2015; Wang et al., 2014; Wu et al., 2015; Yu and Wang, 2013). It has been reported that, in renal ischemia/reperfusion (IR) injury, PHC exerts a protective effect that is associated with attenuating oxidative stress, inflammatory response, and apoptosis (Wang et al., 2014). However, whether PHC protects against cerebral I/R injury remains to be determined.

Mitogen-activated protein kinase (MAPK) signaling pathways are involved in cerebral ischemic injury and play important roles in regulating cell death, apoptosis, and survival (Nozaki et al., 2001). MAPKs include three kinds of kinases: c-Jun N-terminal kinase (JNK), p38 mitogen-activated protein kinase (p38MAPK), and extracellular signal-regulated kinase (ERK). The JNK/p38MAPK pathway can induce apoptosis. In this study, we first assessed the effects

* Corresponding author at: Department of Anesthesiology, Second Affiliated Hospital of Xi'an Jiaotong University School of Medicine, No. 157 West Five Road, Xi'an 710004, China. Fax: +86 2983666789.
E-mail address: neuro0123@sina.com (P. Zhang).

http://dx.doi.org/10.1016/j.brainresbull.2016.01.008
0361-9230/

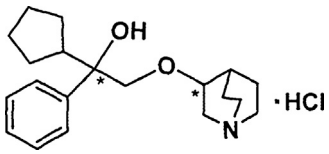

Fig. 1. The chemical structure of penehyclidine hydrochloride (PHC).

of PHC on cerebral I/R injury and then evaluated its effects on the JNK/p38MAPK signaling pathway.

2. Materials and methods

2.1. Animals and reagents

Male C57BL/6 mice (25–30 g) were purchased from the Laboratory Animal Center of the Xi'an Jiaotong University. All experiment procedures were according with the National Institutes of Health (NIH) Guidelines on the Use of Laboratory Animals, and were approved by the Xi'an Jiaotong University Committee on Animal Care. The mice were kept in a pathogen-free environment with free access to food and water in a temperature-controlled room with an alternating 12-h light–dark cycle.

PHC injection (1 mg/1 ml) was purchased from List Pharmaceutical (Chengdu, China). 2,3,5-triphenyltetrazolium chloride (TTC) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Antibodies against phosphorylated-p38MAPK (p-p38MAPK), p38MAPK, p-JNK, and JNK were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against c-Jun, p-c-Jun, and β-actin were purchased from Cell Signaling Technology (Beverly, MA, USA). Terminal deoxynucleotidyl transferase dUTP nick end-labeling (TUNEL) kits were purchased from Roche (Mannheim, Germany).

2.2. Model of cerebral ischemia/reperfusion

Middle cerebral artery occlusion (MCAO) was performed as previously described (Kilic et al., 2008). Briefly, mice were anesthetized with sodium pentobarbital (40 mg/kg i.p.). A 6–0 rounded tip nylon monofilament was gently advanced from the right common artery to the internal carotid artery until a faint resistance was felt, signifying MCAO. Focal cerebral ischemia for 2 h was induced by MCAO and reperfusion was produced by withdrawing the suture. The same surgical procedures were performed on sham animals without MCAO. The rectal temperature was maintained at 37 ± 0.5 °C with the use of a thermostat-controlled heating blanket and a lamp from the start of the surgery until the animals recovered from anesthesia. The mice were allowed to recover from surgery in a cage with free access to food and water in a temperature-controlled and air-ventilated room during the experiment.

2.3. Experimental groups and drug treatment

All the mice were randomly assigned to the following groups (n = 30 for each group): Sham, I/R, I/R + PHC (0.1 mg/kg), I/R + PHC (1 mg/kg). PHC was diluted in normal saline and administered twice (immediately after induction of ischemia and at the beginning of reperfusion) to the mice in the I/R + PHC groups (0.1 mg/kg and 1 mg/kg) by intraperitoneal injections. The mice in the sham group and I/R groups were administered the same volume of normal saline as that of the PHC diluted in normal saline administered to the mice in the I/R + PHC groups.

2.4. Neurological score evaluation

Neurological deficits based on a four-tiered grading system was assessed 24 h after I/R by a blinded investigator as previously described (Hara et al., 1997): 0, no apparent deficits (normal); 1, failure to extend forepaw (mild); 2, circling to the left side (moderate); and 3, loss of walking or righting reflex (severe).

2.5. Measurement of infarct volume

Infarct volume was evaluated by 2,3,5-triphenyltetrazolium chloride (TTC) at 24 h after reperfusion. Animals were euthanized and the brains were quickly removed. Then the brain was sliced into five coronal sections (3 mm thick each) and stained with 2% solution of TTC at 37 °C for 20 min, followed by fixation in 4% paraformaldehyde. TTC-stained sections were photographed and the digital images were analyzed using image analysis software (Image-Pro Plus 5.1). The lesion volume was calculated by multiplying the area by the thickness of slices. The percentage hemisphere lesion volume (%HLV) was calculated by the following formula (Tatlisumak et al., 1998): %HLV = ([total infarct volume – (the volume of intact ipsilateral hemisphere – the volume of intact contralateral hemisphere)])/contralateral hemisphere volume × 100%.

2.6. Measurement of brain water content

According to the methods previously reported (Zhao et al., 2015), the mice were deeply anesthetized by sodium pentobarbital (60 mg/kg i.p.) and decapitated after 24 h of reperfusion. The brains were rapidly removed and divided into 2 hemispheres along the midline and the cerebellum on the ice. The brain samples was immediately weighed on an electronic analytical balance to achieve the wet weight and were weighed again to obtain the dry weight after 24 h dried in an oven at 100 °C. Water content was calculated as follows: (wet weight – dry weight)/(wet weight) × 100%.

2.7. Evaluation of Blood brain barrier (BBB) permeability

Evans blue (EB) dye was used to evaluate the BBB permeability as the method previously described (Hu et al., 2014). After 24 h of reperfusion, mice were injected with EB (2% in saline, Sigma) in a dose of 3 ml/kg through tail vein and allowed to circulate for 2 h. Under deep anesthesia, they were transcardially perfused with saline and sacrificed. The brain in each group was removed and weighted, homogenized in formamide (1 ml) and incubated at 37 °C for 48 h. After centrifugation, the optical density of the supernatant was measured at OD 625 nm by a microplate reader (Multiskan Spectrum, Thermo Scientific, USA). According to a linear standard curve, the amount of EB (mg/g wet weight) was quantified and expressed as relative amount.

2.8. TUNEL staining

After 24 h of reperfusion, the mice were anesthetized and perfused transcardially with 0.9% saline followed by icy 4% paraformaldehyde solution, and fixed in 4% paraformaldehyde for 24–48 h for paraffin embedding. TUNEL staining was performed according to the manufacturer's protocol, and slices were labeled with streptavidin-horseradish peroxidase and TUNEL-positive cells emitted a green fluorescent color. TUNEL-positive cells were quantified using light microscopy at 400× magnification, and 8–10 fields for each section were selected from the ischemic adjacent cortex, the ischemic penumbra area. The average percentage of TUNEL-positive cells were determined and expressed through a scale calibration.