Neuroscience Letters 632 (2016) 23–32

Contents lists available at ScienceDirect

Neuroscience Letters

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

Research article

Post-ischemia mdivi-1 treatment protects against ischemia/reperfusion-induced brain injury in a rat model

Xuesong Ma[a,b,1], Yuying Xie[a,1], Yifan Chen[c], Baoqing Han[a], Jun Li[a], Sihua Qi[a,*]

[a] Department of Anesthesiology, Fourth Affiliated Hospital, Harbin Medical University, 37 Yiyuan Street, Nangang District, Harbin 150001, China
[b] School of Life Science and Technology, Harbin Institute of Technology, Harbin 150001, China
[c] Department of Nephrology, Fifth Hospital in Harbin, 27 Jiankang Road, Xiangfang District, Harbin 150001, China

HIGHLIGHTS

- Post-ischemia mdivi-1 treatment decreased infarct volume and improved neurological function.
- Post-ischemia mdivi-1 treatment reduced apoptosis in cerebral cortex tissue.
- Post-ischemia mdivi-1 treatment inhibited mitochondrial fragmentation.
- Post-ischemia mdivi-1 treatment attenuated translocation of Drp1 protein to the mitochondria.
- Post-ischemia mdivi-1 treatment enhanced I/R-induced mitochondrial biogenesis.

ARTICLE INFO

Article history:
Received 26 June 2016
Received in revised form 6 August 2016
Accepted 15 August 2016
Available online 16 August 2016

Keywords:
Ischemia/reperfusion
Mitochondrial fission
Mitochondrial division inhibitor-1 (mdivi-1)
Mitochondrial biogenesis
Peroxisome proliferator-activated receptor coactivator-1α (PGC-1α)

ABSTRACT

When given prior to brain ischemia, mitochondrial division inhibitor-1 (mdivi-1) attenuates the brain damage caused by ischemia. Here, we investigated the potential effects of post-ischemia mdivi-1 treatment (1 mg/kg, i.p., administered immediately after 2 h of ischemia and prior to reperfusion) using a MCAO rat model. Mdivi-1 treatment decreased infarct volume and improved neurological function. In addition, cytochrome C release was attenuated, and neuronal apoptosis was decreased. The mitochondrial fission protein dynamin-related protein 1 (Drp1) was decreased in the mitochondrial fraction but increased in the cytosolic fraction. Mdivi-1 treatment augmented the increases in the mRNA expression of peroxisome proliferator-activated receptor coactivator-1α, nuclear respiratory factor-1, and mitochondrial transcriptional factor A. In conclusion, when given after ischemia and prior to reperfusion, mdivi-1 can protect against brain damage by inhibiting the mitochondria-mediated apoptosis induced by mitochondrial fission. Post-ischemia mdivi-1 treatment might promote I/R-induced mitochondrial biogenesis.

1. Introduction

A variety of pharmacological treatments can attenuate tissue damage when given prior to, but not after, brain ischemia [1,2], which limits their therapeutic potential. The onset of reperfusion is more often predictable. As a result, interventions after ischemia but prior to reperfusion are more clinically relevant.

Mitochondria are a major site of energy metabolism and the primary source of reactive oxygen species. In addition, mitochondria mediate the apoptotic response to cerebral ischemia [3,4]. Mitochondrial fragmentation has been correlated with neuronal apoptosis [5]. However, the release of cytochrome C (Cyt-C) is not the result of Bax/Bak-initiated mitochondrial network remodeling [6]. Therefore, the role of mitochondrial fragmentation in neuronal apoptosis through mitochondrial-dependent pathways remains unclear.

Mitochondrial division inhibitor-1 (mdivi-1), a selective inhibitor of the mitochondrial fission protein dynamin-related protein 1 (Drp1), suppressed oxidative stress and downregulated Drp1 in cultured neurons that were subjected to hypoxia/reoxygenation

Abbreviations: mdivi, 1 mitochondrial division inhibitor-1; Drp1, dynamin-related protein 1; PGC-1α, peroxisome proliferator–activated receptor coactivator-1α; NRF-1, nuclear respiratory factor-1; TFAM, mitochondrial transcription factor A; Cyt-C, cytochrome C; COX-I, Cyt-C oxidase subunit I; COX-IV, Cyt-C oxidase subunit IV; I/R, ischemia-reperfusion; MCAO, middle cerebral artery occlusion.

* Corresponding author.
E-mail address: sihuaqi_2013@163.com (S. Qi).
[1] Contributed equally, Co-first authors.

http://dx.doi.org/10.1016/j.neulet.2016.08.026
0304-3940/

[7]. In rats with middle cerebral artery occlusion (MCAO) followed by reperfusion, mdivi-1 delivered prior to ischemia attenuated tissue damage [8]. Mdivi-1 pre-treatment also decreased the expression of Drp1 and Cyt-C. The administration of a Drp1 inhibitor before MCAO also reduced the infarct volume [9]. However, whether post-ischemia mdivi-1 treatment protects the brain against I/R injury is unknown.

Mitochondrial function is recovered by mitochondrial biogenesis, which is central to the overall restoration of cell structure and function [10]. Mitochondrial biogenesis has been shown to be protective against brain ischemia [11]. Mitochondrial biogenesis is mainly regulated by peroxisome proliferator–activated receptor coactivator-1a (PGC-1α), nuclear respiratory factor-1 (NRF-1), and mitochondrial transcriptional factor A (TFAM).

Here, we examined the effect of post-ischemia mdivi-1 treatment using a rat MCAO model for brain ischemia/reperfusion (I/R) injury. The potential effects of mdivi-1 treatment on mitochondrial biogenesis were also examined.

2. Material and methods

2.1. MCAO and pharmacological intervention

This study was approved by the Committee of Animal Use of Harbin Medical University and performed in accordance with the National Institutes of Health's Guidelines for the Care and Use of Laboratory Animals.

MCAO was carried out based on previously described methods [12,13]. Briefly, male Wistar rats (8–9 weeks of age; 250–280 g) were anesthetized with chloral hydrate (350 mg/kg, i.p.). The middle cerebral artery was occluded mechanically using a 4-0 monofilament nylon thread with a silicon rubber–coated tip (40–3734 PK 10; Doccol Corporation, Redlands, CA, USA) for 2 h before reperfusion. The suture was removed to restore the blood flow and initiate reperfusion. Recirculation was verified using laser Doppler imaging. Neurological deficits were evaluated by a blinded observer prior to reperfusion using a crude but fast scoring system [14] as follows: (0) no neurological deficits; (1) failing to fully extend the right forepaw; (2) circling to the right; (3) falling to the right; and (4) failing to walk spontaneously and exhibiting depressed levels of consciousness. Rats with a neurological deficit score of 2 or 3 randomly received mdivi-1 (1 mg/kg, i.p.) or vehicle (5% dimethyl sulfoxide) immediately after the 2-h ischemia (and prior to reperfusion). A group of rats that received a sham operation but not MCAO were included as an additional control. Core temperature was maintained at 37.0 °C ± 0.5 °C during the procedure. The mean arterial blood pressure, pH, blood glucose, and arterial blood gas values were measured before the suture was inserted, after 30 min of MCAO, and 15 min after reperfusion.

2.2. Evaluation of infarct area

Rats were euthanized 24 h after reperfusion with 5% isoflurane and perfused with saline. Coronal brain sections (2 mm) were prepared and stained with 2,3,5-triphenyltetrazolium for 30 min in the dark. The infarct area was calculated using NIH Imagine 1.6 by an investigator blinded to the treatment conditions and is presented as a percentage of the whole brain.

2.3. Neurological scoring

Neurological deficits were evaluated 24 h after reperfusion by a blinded observer using an 18-point scoring system [15]. Spontaneous activity was assessed as each rat's ability to approach all four walls of the cage. Symmetry in the movement of all four limbs was evaluated by holding the rat by the tail. Forepaw outstretching was tested by observing the rat walking along the edge of the table on its forelimbs while being held by its tail. Symmetry in the outstretching of both forelimbs was evaluated while the rat reached for the table and the hind limbs were kept in the air. Climbing was tested by placing the rat on the wall of a wire cage and then pulling its tail. Side stroking was evaluated by observing the rats' reaction to being touched with a blunt stick on each side of the body. The response to vibrissae touch was assessed by brushing a blunt stick against the vibrissae on each side. The sum of the scores in each of the six tests was used as an overall index of neurological function.

2.4. Neuronal apoptosis

Neuronal apoptosis was detected using terminal deoxynucleotidyl transferase–mediated dUDP-biotin nick-end labeling (TUNEL) using a kit from Roche (Beijing, China). The number of apoptotic cells in the ipsilateral cortex (region of interest; Fig. 3G) was assessed under light microscopy (5 randomly chosen fields per section; 400× magnification). All TUNEL-positive cells were counted. To exclude false-positive cells, cell counting was performed by a pathologist who was blinded to the group assignments.

2.5. Electron microscopy

Mitochondrial morphology was examined using transmission electron microscopy (TEM). Representative images were obtained using previously described methods [16] and are shown in Fig. 2C. Fifteen randomly selected areas were used per animal to measure the number of mitochondria, including large neuronal-like nuclei covering approximately one-quarter of the visible image at 8200× magnification [11,17]. The proportion of "elongated" mitochondria was estimated as the number of mitochondria appearing with a length that was at least twice the width. For the quantitative analysis, random sections were studied, except the regions with swollen or less dense mitochondria. The total number of mitochondria was counted by an observer who was blinded to the treatment status to prevent observer bias [18].

2.6. Western blot

For total cell extracts, left cerebral cortices (region of interest; Fig. 2C) were homogenized in RIPA buffer. Cytosolic and mitochondrial fractions were prepared from the cerebral cortex using a Tissue Mitochondria Isolation Kit (catalog no. 3606; Beyotime, Shanghai, China) according to the manufacturer's protocol and previously described methods [19]. Briefly, the cerebral cortex of the penumbra areas (50 mg) was homogenized in 1:10 (w/v) ice-cold isolation reagent A plus 1 mM PMSF and centrifuged at 600 × *g* for 5 min at 4 °C. The supernatant was centrifuged at 11,000 × *g* for 10 min at 4 °C to yield mitochondrial preparation. The resulting supernatants were further centrifuged at 12,000 × *g* for 10 min at 4 °C to yield cytosolic preparation. The purity of the mitochondrial or cytosolic fractions was verified with mitochondrial maker voltage-dependent anion channel (VDAC) and cytosolic maker β-actin, respectively. The proteins were denatured in sodium dodecyl sulfate (SDS) gel loading buffer at 95 °C for 5 min and then separated with SDS-PAGE and transferred to polyvinylidene difluoride membranes and blocked with 5% skim milk. Blotting was carried out based on previously described methods [20] using antibodies against one of the following proteins: Cyt-C oxidase subunits I (COX-I, 1:1000; catalog no. ab14705; Abcam, Cambridge, UK) and IV (COX-IV, 1:1000; catalog no. 4850; Cell Signaling Technology, MA, USA). The membranes were probed overnight at 4 °C with primary antibodies against Cyt-C (14 kDa) (1:5000; catalog no. ab133504; Abcam), optic atrophy type 1 (OPA1, 92 kDa and 100 kDa) (1:1000; catalog no. ab42364; Abcam), and dynamin-