Neuroscience 311 (2015) 67–74

INHIBITION OF DRP1 BY MDIVI-1 ATTENUATES CEREBRAL ISCHEMIC INJURY VIA INHIBITION OF THE MITOCHONDRIA-DEPENDENT APOPTOTIC PATHWAY AFTER CARDIAC ARREST

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Abstract—Mitochondrial fission is predominantly controlled by the activity of dynamin-related protein1 (Drp1), which has been reported to be involved in mitochondria apoptosis pathways. However, the role of Drp1 in a rat model of cardiac arrest remains unknown. In this study, we found that activation of Drp1 in the mitochondria was increased after cardiac arrest and inhibition of Drp1 by 1.2 mg/kg of mitochondrial division inhibitor-1 (Mdivi-1) administration after the restoration of spontaneous circulation (ROSC) significantly protected against cerebral ischemic injury, shown by the increased 72-h survival rate and improved neurological function. Moreover, the increase of the vital neuron and the reduction of cytochrome c (CytC) release, apoptosis-inducing factor (AIF) translocation and caspase-3 activation in the brain indicate that this protection might result from the suppression of neuron apoptosis. Altogether, these results indicated that Drp1 is activated after cardiac arrest and the inhibition of Drp1 is protective against cerebral ischemic injury in a rat of cardiac arrest model via inhibition of the mitochondrial apoptosis pathway.

Key words: cardiac arrest, cardiopulmonary resuscitation, Drp1, Mdivi-1, apoptosis.

INTRODUCTION

Cardiac arrest is a leading cause of death worldwide. After the long-term efforts by the American Heart Association and related organizations to update and disseminate resuscitation guidelines, the in-hospital mortality among successfully resuscitated patients remains up to 70% (Neumar et al., 2008; Nichol et al., 2010). Moreover, nearly 60% of survival patients had moderate to severe cognitive deficits due to cerebral ischemia–reperfusion injury at three months after cardiac arrest (Roine et al., 1993). Neurological injury after cardiac arrest is a major contributor to morbidity and mortality in survivors of resuscitation (Neumar et al., 2008). Although therapeutic hypothermia was the first recommended protection demonstrated to improve neurologic outcomes in comatose survivors after cardiac arrest (Bernard et al., 2002; Hypothermia after Cardiac Arrest Study Group, 2002), it is limited by practical difficulties in implementation. Alternative approaches should be thus developed to further improve neurologic outcomes in patients after cardiac arrest.

Mitochondria are important organelles in all cell types, but they are particularly important in the nervous system. Mitochondria are essential to neuronal processes such as energy production, $Ca2+$ regulation, maintenance of plasma membrane potential, protein folding by chaperones (Chan, 2006). There is mounting evidence that the mitochondria-dependent apoptosis is involved in the neuron damage during the cerebral ischemia–reperfusion (Mattson et al., 2001; Chen et al., 2013). Mitochondria exist in dynamic networks that continuously undergo fusion and fission, which play an important role in maintaining their function in neurons (Knott et al., 2008). Mitochondrial fission is predominantly controlled by the activity of dynamin-related protein1 (Drp1), which has recently been demonstrated to be an intrinsic component of multiple mitochondria-dependent apoptosis pathways (Martinou and Youle, 2011). Drp1 usually resides in an inactive form in the cytosol and on activation translocates to the mitochondria. Inhibition of Drp1 translocation onto the mitochondria by mitochondrial division inhibitor-1 (Mdivi-1) prevents mitochondrial fission (Tanaka and Youle, 2008). Mdivi-1 is a derivative of quinazolinone

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Abbreviations: AIF, apoptosis-inducing factor; CA-1, cornu ammonis 1; CPR, cardiopulmonary resuscitation; CytC, cytochrome c; DMSO, dimethyl sulfoxide; Drp1, dynamin-related protein1; HE, Hematoxylin–eosin; MAP, mean aortic pressure; Mdivi-1, mitochondrial division inhibitor-1; NDS, neurologic deficit score; OPC, overall performance category; ROSC, restoration of spontaneous circulation; TUNEL, TdT-mediated dUTP nick-end labeling.

http://dx.doi.org/10.1016/j.neuroscience.2015.10.020
0306-4522/

and serves as a selective inhibitor of mitochondrial fission protein Drp1 (Cassidy-Stone et al., 2008).

Recent studies have shown that Mdivi-1 could protect the heart against ischemia/reperfusion injury (Ong et al., 2010) and block apoptotic cell death and increase retinal ganglion cell survival in ischemic mouse retina (Park et al., 2011). In addition, pretreatment with Mdivi-1 could provide neuroprotection against transient ischemic brain damage *in vivo* (Grohm et al., 2012). Therefore, we hypothesize that inhibition of Drp1 could represent a potential therapeutic strategy in a cardiac arrest model.

Herein, we showed that cardiac arrest-induced global cerebral ischemia result in mitochondrial fission protein Drp1 activation *in vivo*. Importantly, we found that inhibition of Drp1 by Midvi-1 could protect against cerebral ischemic injury after cardiac arrest probably via the suppression of cytochrome c (CytC) and apoptosis-inducing factor (AIF)-dependent mitochondrial apoptosis pathway.

EXPERIMENTAL PROCEDURES

Animals and drug preparation

Male Sprague–Dawley rats (weight, 350–450 g) were purchased from the Laboratory Animal Center of Sun Yat-Sen University. Animals were housed light and temperature controlled environment. Food and water were supplied ad libitum. Animal experiments were followed the Guide for the Care and Use of Laboratory Animals and approved by the Animal Care and Use Committee of Sun Yat-Sen University.

All drugs used were purchased from Sigma–Aldrich (St. Louis, MO, USA) unless otherwise specified. Mdivi-1 was dissolved in dimethyl sulfoxide (DMSO) and used at a concentration of 0.24 and 1.2 mg/kg individually. DMSO vehicle was used as a control for cardiac arrest-induced mice.

Cardiac arrest model

All rats were fasted on the night prior to the experiment. After the induction of anesthesia by an intraperitoneally injection of 45 mg/kg of pentobarbital sodium, rats were intubated with a 14-gauge cannula mounted on a blunt needle with a 145° angled tip. A 23-guage catheter (PE-50) was advanced through the left femoral artery into the aorta to measure mean aortic pressure (MAP). Another 23-guage catheter (PE-50) was inserted into the left femoral vein for intravenous infusion. End-tidal $PaCO_2$ ($P_{ET}CO_2$) was measured with a side-stream infrared CO_2 analyzer (CAPSTAR-100, CWE Inc., Ardmore, PA, USA) interposed between the tracheal cannula and the respirator. All hemodynamic data include MAP and electrocardiogram (ECG) lead II was continuously monitored via a WinDaq data-acquisition system (DataQ, Akron, OH, USA). Rectal core temperature was monitored and maintained at 36.5 ± 0.5 °C with a heat lamp.

Anesthetized rats were paralyzed with 2 mg/kg vecuronium bromide and baseline measurements were accomplished. Asphyxia was induced by clamping the endotracheal tube. After a short induction of apnea, cardiac arrest was determined by absent pulsation of aortic artery, defined as MAP < 20 mmHg. Precordial compression was begun and mechanical ventilation with 100% FiO_2 was performed 6 min after the onset of cardiac arrest. Precordial compression at a rate of 250/min was synchronized to conduct a compression/ventilation ratio of 5:1. Adrenaline (20 μg/kg) was injected after 2 min of cardiopulmonary resuscitation (CPR). In unsuccessfully resuscitated animals, the 30 s of CPR and adrenaline administrations were repeated and maintained until restoration of spontaneous circulation (ROSC) or 10 min after cardiac arrest. ROSC was defined as the return of supraventricular cardiac rhythm with mean aortic pressure (MAP) over 60 mmHg for ⩾5 min. Animals were observed for 1 h after successful resuscitation.

Experimental design

After ROSC, the animals were randomized into the following three groups: (1) vehicle group; (2) Mdivi-1 low-dose group (Mdivi-1 dose1 group); (3) Mdivi-1 high-dose group (Mdivi-1 dose2 group). Animals in the vehicle group were given an intravenous injection of 0.1% DMSO after 1 min of ROSC. Rats in Mdivi-1 dose1 group and Mdivi-1 dose2 group were intravenously infused with Mdivi-1 at doses of 0.24 and 1.2 mg/kg, respectively. Animals underwent identical anesthetic and surgical procedures except cardiac arrest were used as the sham group.

Survival study and neurological outcome evaluation

The survival rate after cardiac arrest was observed until 72 h. At 24, 48 and 72 h after ROSC, the neurologic function scores were performed blindly, using an overall performance category (OPC) scoring system (OPC: 1 = normal; 2 = mild disability; 3 = moderate disability; 4 = severe disability/coma; 5 = death/brain death) and neurologic deficit score (NDS, 0–10%, normal; 100%, brain death) (Neumar et al., 1995). All survived animals were sacrificed at 72 h after surgery and the brain tissues were removed for the following examinations.

HE staining and TUNEL (TdT-mediated dUTP nick-end labeling) assay

Separated left hemisphere of rats fixed in 4% paraformaldehyde and the paraffin-embedded brain tissues were cut into sections of 4-μm. Hematoxylin–eosin (HE) staining (Beyotime, Hangzhou, China) and TUNEL assay (Roche, Boston, MA, USA) were performed for in situ detection of neuronal injury, according to the manufacture's instruction. Six visual fields from each brain tissue sample, in the hippocampal cornu ammonis 1 (CA-1) region were randomly selected and the HE-stained neuronal cells were counted. The immunohistochemical score (IHS) is determined by the percentage of positive-stained cells and the intensity of staining for TUNEL staining. All analyses were performed blinded.