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Research article

Therapeutic hypothermia attenuates global cerebral reperfusion-induced mitochondrial damage by suppressing dynamin-related protein 1 activation and mitochondria-mediated apoptosis in a cardiac arrest rat model

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HIGHLIGHTS

- The underline mechanism of therapeutic hypothermia is proposed.
- Cardiac arrest animal model which induced by transoesophageal cardiac pacing is more close to the clinical situation.
- The hypothesis is confirmed by histopathology and protein expression.

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ABSTRACT

Therapeutic hypothermia is effective to attenuate brain ischemia/reperfusion (I/R) injury after cardiac arrest, and multiple mechanisms have been proposed. Dynamin-related protein 1 (Drp1), a large GTPases of dynamin superfamily, predominantly controls mitochondrial fission and is related to IR-induced Cyt C release and apoptosis. However, the effect of therapeutic hypothermia on Drp1 and mitochondrial fission after cardiac arrest remains still unclear. In this study, non-cardiac arrest and post-cardiac arrest rats received 6-h normothermia (37–38 °C) or therapeutic hypothermia (32–34 °C), and the hippocampus was harvested at 6 h and 72 h after cardiac arrest. Results showed the expression of Drp1 and Cyt C increased after cardiac arrest, but therapeutic hypothermia partially reversed this increase at 6 h after cardiac arrest. Transmission electron microscopy (TEM) also showed a change in morphology following therapeutic hypothermia after cardiac arrest. Moreover, therapeutic hypothermia could decrease the histopathological damage, inhibit the apoptosis of CA1 neurons and improve the survival and neurological outcomes at 72 h after cardiac arrest. Taken together, our study demonstrates that therapeutic hypothermia is neuroprotective against global cerebral I/R injury, which is, at least partially, ascribed to the inhibition Drp1 and Cyt C expression and the protection of mitochondrial structure.

1. Introduction

Cardiac arrest causes high disability and high mortality, threatens human health and brings heavy burden to the family and society. Advanced cardiopulmonary resuscitation techniques enable physicians to have more chance to rescue cardiac arrest patients and improve their quality of life [1–3]. Global cerebral ischemia/reperfusion (I/R) injury during cardiac arrest has been known to cause neuron damage [4–6]. Although the duration of ischemia affects the outcome of cardiac arrest patients, the harmful consequence of cardiac arrest is mainly observed in the reperfusion phase. I/R injury may cause the release of inflammatory mediators, oxidative stress, intracellular calcium influx, release of reactive oxygen species (ROS), and opening of the mitochondrial permeability transition pore (mPTP), leading to cell apoptosis and necrosis [4].

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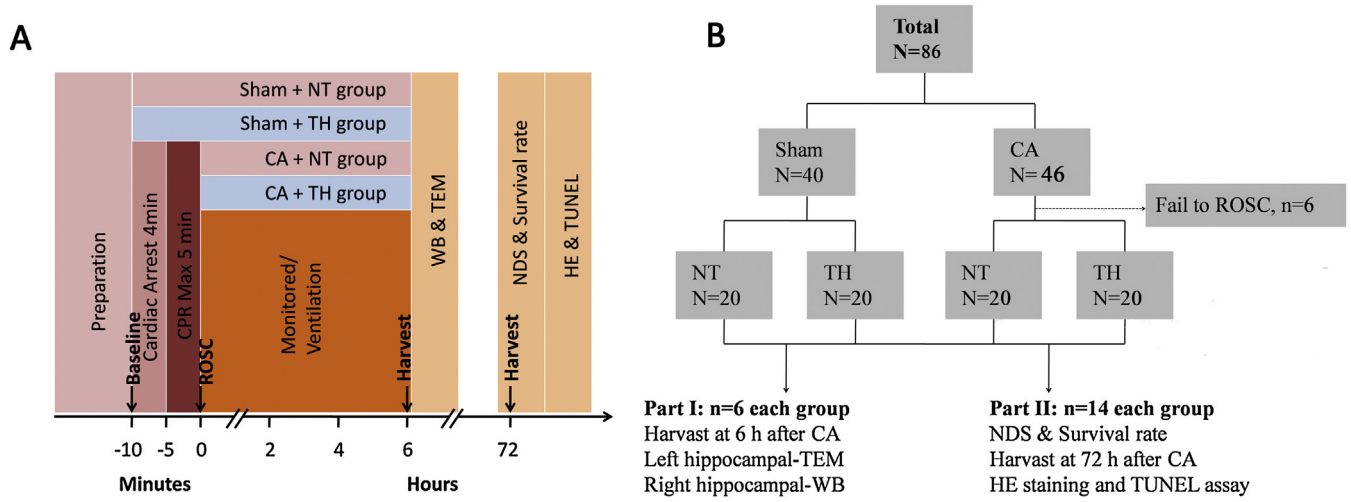

Fig. 1. (A) Schematic of experimental protocol. Animals were anesthetized and intubated intratracheally, and receive darteriovenous catheterization, then restored calm during the preparation period. After 4-min cardiac arrest induced by electrical stimulation, cardiopulmonary resuscitation (ventilation, chest compression, administration of epinephrine at 100 μg/kg and sodium bicarbonate at 1 mEq/kg and defibrillation) was performed. Cardiopulmonary resuscitation sustained for 5 min. Animals achieving ROCS were monitored, and the body temperature was controlled for 6 h under anesthesia. NDS and survival rate were evaluated at 72 h. (B) Flow diagram of the experiments.

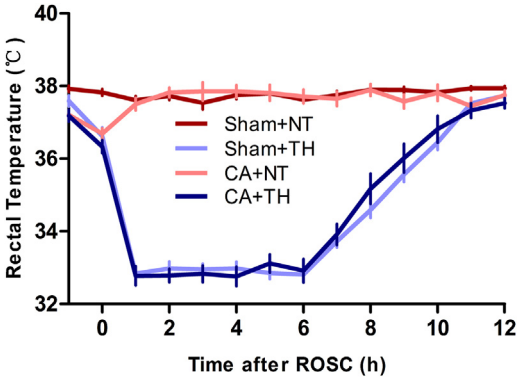

Fig. 2. Changes of rectal temperature during the experiment.

Therapeutic hypothermia has been found to be effective to attenuate I/R injury secondary to cardiac arrest *in vivo* and *in vitro* [7–9] and may improve human survival and neurological outcomes in clinical practice. In the 2015 American Heart Association Guideline for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care, therapeutic hypothermia is recommended for cardiac arrest patients [2]. It has been reported that multiple pathways and molecules are involved in the neuroprotective effect of therapeutic hypothermia, including the reduction in metabolism, inhibited production of ROS and inflammatory mediators and regulation of cell apoptosis [7]. Although therapeutic hypothermia has been widely used after cardiac arrest, the specific molecular mechanism underlying its neuroprotective effects remains to be further elucidated.

Mitochondria are essential for the normal neurological function because they not only play a crucial role in the energy production [10] but also are important in the regulation of calcium, ROS generation [11], metabolic substrates and T cells [12,13]. These processes are regulated, in part, by the morphological changes of mitochondria [14]. Studies have proven that mitochondrial-dependent apoptosis is involved in the neuronal damage during the cerebral I/R injury [15–17]. The finding that structural changes of mitochondria might be relevant to I/R induced neuron death deepens the understanding of mitochondrial regulation in apoptosis [15–17].

Generally, mitochondria undergo persistent fission and fusion, and any stimulation that may break this balance will cause cellular dysfunction or even cell death [18]. Mitochondrial fission involving the constriction and cleavage is predominantly controlled by the dynamin-related protein 1 (Drp1) which is recently demonstrated to be an intrinsic component of mitochondria-mediated apoptosis pathway [19–21].

Drp1 is a large GTPases of dynamin superfamily. During the mitochondrial fission, Drp1 accumulates on the surface of the mitochondria, then promotes its fission and Cyt C release, which has been recognized as a key component of the mitochondrial apoptotic pathway [19]. Recent studies showed that short-term inhibition of Drp1 expression and translocation by using mitochondrial division inhibitor-1 (Mdivi-1) is able to improve the neurological outcome after cardiac arrest [15,16,22].

In our previous studies, results showed therapeutic hypothermia was able to improve the neurological function when it was initiated at the time of reperfusion in a rat cardiac arrest model, which was ascribed to the inhibition of endoplasmic reticulum stress and cold-induced RNA-binding protein (CIRP) expression [8]. However, the specific role of Drp1 in the neuroprotective effects of therapeutic hypothermia following cardiac arrest remains unknown.

In this study, we hypothesize that global cerebral I/R injury secondary to cardiac arrest in a rat model may result in elevated Drp1 expression and lead to subsequent mitochondrial fragmentation and Cyt C release in the hippocampal neurons, which contributes to the neuronal damage after cardiac arrest, while this can be attenuated by therapeutic hypothermia.

2. Materials and methods

2.1. Animals and reagents

Male Sprague-Dawley rats, weighing 280–320 g, were purchased from the Animal Center of Jinling Hospital Affiliated to Medical College of Nanjing University, China. Animals were housed in a temperature-controlled room on a 12 h light-dark cycle. Animals were given *ad libitum* access to food and water. This study was approved by the Ethics Committee of Jinling Hospital and performed in accordance with the Guideline for the Use of Experimental Animals of the National Institute of Health. All reagents used were purchased from Abcam Inc (Abcam, Combridge, MA) unless otherwise specified.