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Original Contribution

Protective effects of cyclosporine A and hypothermia on neuronal mitochondria in a rat asphyxial cardiac arrest model☆

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ABSTRACT

Background: Cyclosporine A (CsA) was neuroprotective in the settings of traumatic brain injury and stroke. We sought to investigate the protective effects of CsA and hypothermia on neuronal mitochondria after cardiac arrest.
Methods and Results: Five groups were included: sham (S), normothermia (N), CsA (C), hypothermia (H), and CsA plus hypothermia (C + H). Cardiac arrest was induced by 10 min of asphyxia. CsA (10 mg/kg) was administered immediately after return of spontaneous circulation in the CsA groups. Temperature of the rats was maintained at 33 ± 0.5 °C after return of spontaneous circulation in the hypothermia groups. Hippocampal mitochondria were measured after 2 h of resuscitation. Mitochondrial transmembrane potential was significantly higher in the C, the H, and the C + H groups than in the N group and was higher in the C + H group than in the C and the H groups. Cytosolic cytochrome c was significantly higher in the N group. Superoxide dismutase activity was significantly lower in the N group than in the other groups and was higher in the C and the C + H groups than in the H group. Malondialdehyde concentration was significantly higher in the N group.
Conclusions: CsA or hypothermia used immediately after resuscitation enhanced mitochondrial transmembrane potential, kept cytochrome c from releasing out of the mitochondria, increased superoxide dismutase activity, and decreased malondialdehyde concentration in hippocampus. Moreover, the protective effects of CsA were reinforced by hypothermia. One of the mechanisms that hypothermia protected neuronal mitochondria from damage was inhibiting the opening of mitochondrial permeability transition pore.

1. Introduction

The high morbidity and mortality associated with cardiac arrest remains a difficult problem. Though many cardiac arrest victims are successfully resuscitated initially, only a small number of them survive to hospital discharge. Post-resuscitation syndrome is an important factor that affects survival after resuscitation. It is a complex state characterized by myocardial dysfunction, brain injury, global ischemia–reperfusion (I/R) injury and systemic inflammatory response [1]. Most patients die during the post-resuscitation period due to neuronal damage, which develops as a consequence of global cerebral ischemia during cardiac arrest [2]. Furthermore, as many as 40% to 50% of the surviving patients suffer from permanent cognitive impairment [3,4]. Therefore, improving the long-term health of cardiac arrest patients and optimizing the treatment of post-resuscitation syndrome are required.

The energy imbalance due to mitochondrial dysfunction leads to neuronal damage. The main mitochondrial damage after resuscitation is the opening of mitochondrial permeability transition pore (mPTP). mPTP, a non-specific channel, allows molecules as large as 1500 Da to enter the mitochondrial matrix, as well as water and other substances, resulting in mitochondrial swelling, cytochrome c release into the cytosol, activation of the apoptotic cascade, loss of mitochondrial transmembrane potential ($\Delta\Psi m$), ion balance disruption, oxidative phosphorylation uncoupling, increased reactive oxygen species (ROS) production, and reduced energy generation. Robust bursts of ROS further induce mPTP opening, creating a vicious cycle [5–7]. Cyclosporine A (CsA), an inhibitor of mPTP, inhibits activation of mPTP and pore formation by inhibiting matrix CypD interaction with pore proteins [8,9]. CsA demonstrated a significant increase in $\Delta\Psi m$, accompanied by lower levels of intramitochondrial Ca^{2+} and reactive oxygen species production in traumatic brain injury [10]. The neuroprotective properties of CsA were mediated through modulation of the mPTP and maintenance of mitochondria homeostasis [10]. CsA administration reduced infarct size, DNA fragmentation and apoptotic bodies, and inflammatory responses in a stroke model in the rat brain [11]. CsA treatment at onset of resuscitation decreased the mitochondrial swelling of the heart and improved both post-cardiac arrest myocardial dysfunction and survival [12]. The

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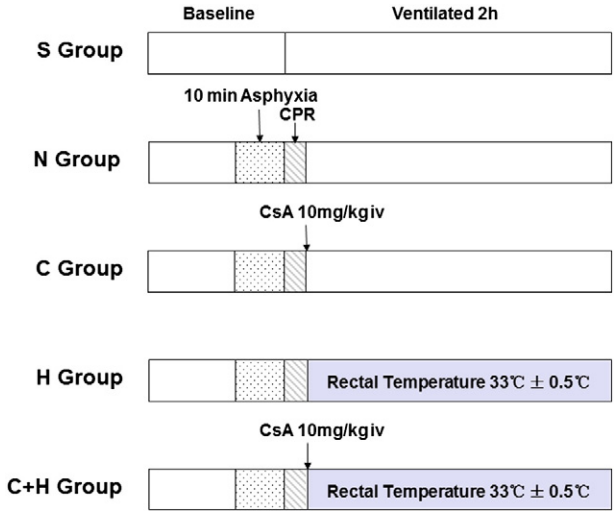

Fig. 1. The experimental protocol. S group, sham group; N group, normothermia group; C group, CsA group (10 mg/kg, i.v.); H group, mild hypothermia group; C + H group, CsA + mild hypothermia group.

mitochondrial integrity and respiration function of the heart are ameliorated under CsA treatment at onset of resuscitation [12]. These reports indicate CsA might be a promising agent in treating neuronal mitochondrial dysfunction after cardiac arrest [10–12]. Therapeutic mild hypothermia is a well-documented method that has been proven effective during cerebral resuscitation by many studies [13–21]. However, few studies have attempted to determine what the influence of CsA and hypothermia on the opening of neuronal mPTP and the protection of mitochondrial function following cardiac arrest. In this study, we sought to find out the protective effects exerted by CsA and mild hypothermia on neuronal mitochondria after resuscitation in a rat asphyxial cardiac arrest model.

2. Materials and methods

The experimental protocol for the study was approved by the Institutional Animal Care and Use Committee of The Third Affiliated Hospital, Harbin Medical University. Male Wistar rats (260–310 g) purchased from the Changchun City Billion Adams Laboratory Animal Technology Co., Ltd. were allowed to acclimate for 6 days before beginning the experiment.

2.1. Experimental groups

The rats were divided into five groups (n = 12 each, Fig. 1): sham group (S group); normothermia group (N group), in which the rats were subjected to cardiac arrest induced by 10 min of asphyxia, and the rectal temperature was maintained at 37 ± 0.5 °C after the return of spontaneous circulation (ROSC); CsA group (C group), in which CsA (10 mg/kg) [12,22] was administered intravenously immediately following ROSC in the setting of normothermia; mild hypothermia group (H group), in which rectal temperatures were maintained at 33 ± 0.5 °C following ROSC; CsA plus mild hypothermia group (C + H group), in which CsA was administered intravenously in combination with mild hypothermia. Six animals in the each group were decapitated to determine ΔΨm of the hippocampus after 2 h of resuscitation. The remaining six animals in the each group were also decapitated for the measurement of the expression of cytoplasmic cytochrome c and caspase-3, the activity of superoxide dismutase (SOD), and the concentration of malondialdehyde (MDA) of the hippocampus after 2 h of resuscitation.

2.2. Animal preparation

The rats were anesthetized with 4% isoflurane. Mechanical ventilation was initiated using a volume rodent ventilator (model 683, Harvard Apparatus, South Natick, MA, USA) at the rate of 40/min, I:E = 1:1, FiO_2 = 0.5. The tidal volume (8–12 ml/kg) was regulated by end-tidal CO_2 maintained between 35 and 45 mmHg. During surgical preparation, the rectal temperature of the rats was maintained between 37.0 ± 0.5 °C. Anesthesia was sustained with 2.5% isoflurane.

2.3. Asphyxia-induced cardiac arrest

After balancing to maintain normal mean arterial pressure (MAP), heart rate (HR), and blood gas values, the experimental animals were given vecuronium (2 mg/kg, intravenously) 5 min before asphyxia. Cardiac arrest was induced via asphyxia by turning off the ventilator and clamping the endotracheal tube. Cardiac arrest was confirmed by a MAP <20 mmHg. The rectal temperature was maintained at 37 ± 0.5 °C during the entire asphyxia period.

2.4. Resuscitation and ventilation

Following 10 min of asphyxia, chest compressions were performed at a rate of 200~300/min in combination with 0.01 mg/kg epinephrine and 2 mEq/kg $NaHCO_3$. Ventilation was resumed at the same time. Body temperature was maintained according to different groups via either heating or surface cooling following ROSC. The experimental animals were ventilated for 2 h and then hippocampus was isolated.

2.5. Isolation of hippocampal mitochondria

Hippocampal mitochondria were extracted using a tissue mitochondria isolation kit (Beyotime Institute of Biotechnology, China). The homogenate of hippocampus was centrifuged at 1000 g for 5 min at 4 °C to remove nuclei and any unbroken cells. The supernatant was collected and centrifuged at 35,000 g for 10 min at 4 °C to obtain the mitochondrial fraction (deposition).

Table 1
The characteristics of the each group at baseline.

	S group	N group	C group	H group	C + H group
Weight (g)	280.0 ± 15.6	290.3 ± 16.4	279.3 ± 15.3	292.9 ± 12.9	277.7 ± 14.2
MAP (mmHg)	98.3 ± 7.2	102.2 ± 11.6	99.8 ± 11.1	101.3 ± 11.7	92.1 ± 5.5
HR (bpm)	321.7 ± 20.0	330.2 ± 8.6	333.1 ± 13.0	321.5 ± 15.2	335.8 ± 14.5
Temp (°C)	36.9 ± 0.3	36. 9 ± 0.2	37.0 ± 0.4	37.1 ± 0.3	37.0 ± 0.4
pH	7.39 ± 0.05	7.39 ± 0.05	7.39 ± 0.04	7.38 ± 0.04	7.36 ± 0.02
P_aCO_2 (mmHg)	41.9 ± 7.1	41.5 ± 8.0	46.5 ± 9.5	42.8 ± 6.7	41.0 ± 8.8
PaO_2 (mmHg)	248.2 ± 98.7	276.0 ± 95.1	320.4 ± 56. 5	290.2 ± 93.5	225.9 ± 77.7
Na^+ (mmol/L)	135.3 ± 10.1	138.3 ± 8.3	140.4 ± 5.0	146.0 ± 10.3	136.3 ± 11.2
K^+ (mmol/L)	4.3 ± 1.1	3.8 ± 0.6	3.9 ± 0.7	3.7 ± 0.4	3.6 ± 0.6
Cl^- (mmol/L)	110.3 ± 10.8	114.8 ± 7.3	112.6 ± 5.1	117.7 ± 7.4	109.5 ± 12.2

MAP, mean arterial pressure; HR, heart rate; Temp, temperature.