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Moderate hypothermia ameliorates enterocyte mitochondrial dysfunction in severe shock and reperfusion

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ABSTRACT

Background: Hypothermia can ameliorate ischemia–reperfusion-induced intestinal injury; however, whether the therapeutic mechanism of hypothermia on hemorrhagic shock, a severe condition of ischemia–reperfusion, is associated with mitochondrial protection in enterocytes is rarely reported. We aimed to evaluate the effects of hypothermia on mitochondria after shock-induced intestinal injury.

Materials and methods: A severe hemorrhagic shock model was constructed in Sprague–Dawley rats at induced hypothermic (32°C or 34°C) or normothermic temperatures (37°C), followed by resuscitation with whole shed blood and Ringer lactate (15 mg/kg body weight). After 2 h, 24 rats were killed and their intestinal tissue was collected; the remaining animals were returned to the normothermic environment to observe the survival time.

Results: There was severe mitochondrial dysfunction in the normothermia group, as well as increased oxidative stress and terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling apoptotic index. As expected, hypothermia treatment decreased mitochondrial permeability transition pore opening and restored the mitochondrial membrane potential and intracellular adenosine triphosphate content. Furthermore, hypothermia elevated mitochondrial-reduced glutathione and decreased mitochondrial malondialdehyde; consistent with the restored mitochondrial function, intestinal cell apoptosis and intestinal histopathologic injury were attenuated, the systemic inflammatory response was mitigated, and survival time was significantly prolonged. Additionally, moderate-induced hypothermia (32°C) had better therapeutic effects than mild hypothermia (34°C).

Conclusions: The results suggest that moderate hypothermia resuscitation is an effective treatment for shock-induced intestinal injury, and its therapeutic mechanism may be related to mitochondrial protection.

1. Background

Hemorrhagic shock (HS) caused by trauma is a major cause of death. HS causes a series of pathophysiologic changes, including disturbed microcirculation, local reduction in blood perfusion, lack of oxygen to tissues, and metabolic disorder. Recovery of blood perfusion and maintaining tissue oxygen supply are the major goals of resuscitation after HS [1]. Even

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so, patients often die of systemic inflammation and multiple-organ dysfunction syndrome even when the traditional resuscitation goal is achieved [2]. In severe shock conditions, the stomach and intestine undergo continuous vasoconstriction and hypoperfusion. Notably, the intestine plays a key role in the pathophysiologic changes that follow severe HS and intestinal ischemia–reperfusion (I/R) injury [3,4].

It is well known that the mitochondria mediate I/R injuries through complex events involving reactive oxygen species generation, alteration of electron transfer activity, mitochondrial permeability transition pore (mPTP) opening, and cytochrome c release. Mitochondrial dysfunction has mostly been investigated during the post-I/R phase [5]. Targeting the mitochondria is therefore often considered a relevant approach for preventing reperfusion injury. Direct inhibition of mPTP opening ameliorates mitochondrial dysfunction in vascular smooth muscle cells [6] and neuronal cells [2].

It has been widely reported that hypothermia is a rescue therapy in both experimental and clinical applications [7]. Through moderate cooling, induced hypothermia can protect the brain [8–10], heart [5,11], lung [12], and intestine [13–16] against I/R injury or other diseases. To date, the explored mechanisms of hypothermia treatment are related to mitochondrial protection, free radical reduction, and immune response suppression [17]. However, most of the focus on mitochondrial protection by hypothermia relates to its cardiac effect [5,11,18,19]. The exact involvement of hypothermia in relation to enterocyte mitochondria after pre-I/R, especially shock conditions, remains unclear. In addition, the optimal target temperature remains controversial [7]. To resolve the previously mentioned questions, we used a severe HS and reperfusion (HS/R) model and compared the effect of two hypothermia treatments (32°C and 34°C) on general survival time, mean arterial pressure (MAP), intestinal tissue injury, and, in particular, the mitochondrial function of scraped enterocytes.

2. Materials and methods

2.1. Animals and chemicals

Male Sprague–Dawley rats, weighing 180–220 g, 8 weeks old, were used for the study. They were obtained from the Laboratory Animal Center (Southern Medical University, Guangzhou, China). 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethylimidacarbocyanine iodide (JC-1) and calcein acetoxymethyl ester (calcein-AM) were from Molecular Probes (Invitrogen, Carlsbad, CA). The CellTiter-Glo Assay and terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling (TUNEL) assay kit DeadEnd Fluorometric TUNEL System were from Promega (Madison, WI). The mitochondrial/cytosolic protein extraction kit was from BestBio (Beijing, China). Antibodies against cytochrome c were from BD Biosciences (San Jose, CA). The reduced glutathione/oxidized glutathione (GSH/GSSG) ratio assay kit was from Beyotime Biotech (Beijing, China). The myeloperoxidase (MPO), malondialdehyde (MDA), and diamine oxidase assay kits and mitochondrial protein extraction kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α enzyme-linked immunosorbent assay kits were purchased from Wuhan Boster Bio-Engineering (Wuhan, China).

2.2. Experimental design

This study was carried out in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and was approved by the Committee on Ethics in Animal Experiments of the Southern Medical University. Preliminary experiments revealed that female rats were more resistant to decreased blood pressure (data not shown), which was consistent with previous reports [20–22]. Thus, to avoid the gender impact, only male rats were used. In total, 88 rats were anesthetized by intraperitoneal injection of 3% pentobarbital sodium (65 mg/kg body weight), and 0.2 mL anesthetic was added when needed. The left femoral artery was cannulated to monitor the MAP. The right femoral artery was cannulated for exsanguination; the right femoral vein was cannulated for drug administration and fluid resuscitation. After establishing arterial and venous passage, the MAP was recorded using PowerLab equipment (ADInstruments, Sydney, Australia) until the animals were killed or 10 h after autologous blood reinfusion. After a 30-min resting stage, the rats were bled using a syringe to produce 40 ± 5 mm Hg of MAP within 10 min, which was maintained for the next 2 h by blood drawing or the reinfusion of stored blood. Subsequently, the shed blood was intravenously administered within 10 min. After autologous blood reinfusion, fluid resuscitation (Ringer solution) was performed at 15 mL/kg/h and maintained for 2 h. The animals were randomly divided into the following groups using different control temperatures (using ice packs on the abdomen or a thermal mattress) in the 2-h resuscitation periods: (1) control (sham), rats were anesthetized and operated on without any other treatment; (2) 32°C resuscitation, rats were subjected to HS, resuscitated, and were maintained at 32°C for the next 2 h; (3) 34°C resuscitation, rats were subjected to HS, resuscitated, and were maintained at 34°C for the next 2 h; and (4) 37°C resuscitation, rats were subjected to HS, resuscitated, and were maintained at 37°C for the next 2 h. The temperature of the animals in each group was monitored rectally [12] (ADInstruments Shanghai Trading Co, Shanghai, China).

When the fluid resuscitation was completed, 24 rats ($n = 6$ per group) were killed for mitochondrial function determination, oxidative stress tests, and analysis of apoptosis. The remaining 64 rats ($n = 16$ per group) were used for observation of MAP and survival time analysis.

2.3. Preparation of small intestinal tissue and serum samples

Laparotomy was performed as follows: 10 cm of ileum 10 cm distal to the ligament of Treitz was carefully removed, placed on ice, rinsed thoroughly with normal saline, refilled with 10-mM dithiothreitol in enterocyte isolation buffer (17-mM HEPES, 25-mM $NaHCO_3$ in phosphate-buffered saline, pH 7.4), and tied off at both ends. The segment was gently massaged to remove the mucus. After the luminal contents