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Moderate hypothermia increased the incidence of delayed paralysis through activation of the spinal microglia in an aortic cross-clamping rat model

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ABSTRACT

Background: Hypothermia reduces immediate paralysis during surgical repair of aortic aneurysms. However, it is unknown what the impact of hypothermia is on delayed paralysis, a serious complication of this type of surgery.
Methods: Sprague-Dawley rats were subjected to occlusion of the descending aorta at different duration under normothermia (38.0 ± 0.5) or hypothermia (33.0 ± 0.5°). Neurologic function was assessed. Motor neuron number, glial activation, and cytokine expression in the spinal cord were examined. Minocycline was administered perioperatively by intraperitoneal injection in the rats subjected to the aorta occlusion.
Results: In contrast to normothermia conditions at which immediate paralysis occurred when the duration of aorta occlusion exceeded 11.5 min, hypothermia did not induce immediate paralysis if the duration of aorta occlusion was less than 41 min. However, delayed paralysis was developed when the duration of aorta occlusion exceeded 18 min, and reached peak level when the duration of aorta occlusion was 40 min at hypothermia condition. The number of motoneurons was significantly decreased ($P < 0.05$) at 30 h postoperation. In addition, microglia was activated, and interleukin-1β and interleukin-6 levels were upregulated, both of which were co-localized in microglia at 24 h postoperation in the hypothermia group. Minocycline treatment attenuated the incidence and degree of paralysis but did not decrease the mortality.
Conclusions: Hypothermia, a neuroprotective strategy in cardiothoracic surgery, increased the incidence of delayed paralysis through activation of spinal microglia and cytokines. Blocking the activated microglia may be a potential intervention to prevent the incidence of delayed paralysis.

1. Introduction

During surgical repair of thoracic and thoracoabdominal aortic aneurysms, the aorta has to be occluded, and this causes spinal cord ischemia (SCI) which consequently results in spinal cord injury and even paralysis in some patients after surgery [1,2]. Clinically, hypothermia is an effective neuroprotective strategy to increase the tolerance of SCI and reduce the incidence of immediate paralysis during the interruption of spinal cord blood flow [3]. However, in clinical setting, hypothermia is always accompanied by the rewarming procedure, which may result in different clinical consequences. In particular, the impact of hypothermia/rewarming procedure on delayed paralysis is still unknown.

The rate of delayed paralysis ranges between 4% and 11% in the surgical repair of aorta aneurysm, and this incidence is becoming increasingly common [4]. Delayed paralysis may occur at several hours up to several days after operation [5]. Though many methods have been used to reduce the incidence of delayed paralysis after aortic surgery, including drainage of cerebrospinal fluid, regional cooling of the spinal cord and reattachment of the intercostal arteries [6,7], but the effectiveness of these methods remains uncertain [8]. The lack of effective strategies to treat delayed paralysis is largely due to the unclear mechanisms of delayed paralysis and no ideal delayed paralysis experimental models simulating the aortic surgery [9]. A recent study has established the delayed paralysis mouse model and showed that the spinal microglia was activated in this type of SCI [10].

Microglia are regarded as macrophages inhabiting the central nervous system. These are normally in resting state and would be activated upon spinal cord damage by trauma, ischemia or other injuries [11]. When ischemia/reperfusion injury occurs in the spinal cord during the operation, microglia are activated and release inflammatory cytokines, reactive oxygen species, and other hazardous substances that may damage the neurons [12]. It has been reported that an inhibitor

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of microglia activation, minocycline, affords neuroprotection through its high solubility and easy penetration to the central nervous system through the blood brain barrier [13].

In clinical practice, hypothermia may directly protect the neuron from the ischemia/reperfusion injury and decrease the incidence of immediate paralysis. However, the impact of hypothermia and the following rewarming of the microglia remains undetermined. The present study hypothesized that hypothermia exerted neuroprotection and reduced immediate paralysis in response to SCI, but the hypothermia/rewarming procedure also activated the spinal microglia, which would induce the secondary spinal cord injury and contribute to the development of delayed paralysis. In a rat model of delayed paralysis, we showed that moderate hypothermia could prolong the duration of aorta occlusion form 12 min to 40 min inducing immediate paralysis. However, when the duration of aorta occlusion was over 18 min, delayed paralysis developed and reached maximum when the duration was 40 min. Blocking the activated microglia by minocycline could greatly attenuate the occurrence of delayed paralysis. Thus, the neuroprotective effects of moderate hypothermia may be compromised due to the increased incidence of delayed paralysis, and minocycline may be a potential adjunct treatment to prevent delayed paralysis in aortic surgery.

2. Materials and methods

2.1. Animals and group assignment

After the approval of the experimental protocol by the Hospital Ethics Committee of the Second Xiangya Hospital of Central South University, 156 clean SD male rats (Hunan Silaike Jingda Experimental Animal Co., Ltd. License Number: SCXK (Xiang) 2011-0003), weighing 300–350 g, were used to establish the animal model of delayed paralysis. The rats were placed in SPF grade feeding chamber at 7 days before the experiment and at the following conditions: feeding chamber temperature at 22.0 ± 1.0 °C, humidity at 50%–70%, light at 150–200 Lx, at 12/12 h dark/light intervals. The rats were allowed free access to water (filtered and sterilized tap water in sterilized bottles). Complete nutritional pellet feed for rats (Hunan Silaike Jingda Experimental Animal Co., Ltd) was used as basal feed.

A total of 156 SD male rats were randomly divided into normothermia group that had their rectum temperature maintained at normal temperature (38.0 ± 0.5 °C) during occlusion of the descending aorta (the NT group, n = 75) and hypothermia group that had their rectum temperature maintained at 33.0 ± 0.5 °C during occlusion of the descending aortas (the Hypo group, n = 81). The NT group was subdivided based on different duration of descending aortas occlusion: Sham group (NorSham, n = 10), 10 min group (Nor10, n = 10), 10.5 min group (Nor10.5, n = 10), 11.5 min group (Nor11.5, n = 16), 12 min group (Nor12, n = 14) and 15 min group (Nor15, n = 15). Similarly, the Hypo group was divided based on different duration of descending aortas occlusion: Sham group (HypoSham, n = 10), 18 min group (Hypo18, n = 15), 21 min group (Hypo21, n = 16), 30 min group (Hypo30, n = 16), 40 min group (Hypo40, n = 15) and 41 min group (Hypo41, n = 9).

2.2. Anesthesia and surgical approach

The rats were anesthetized by sevoflurane inhalation, and the rat model of SCI was generated as previously described by Awad et al. with minor modification [10]. Briefly, rats were anesthetized by inhaling 3% sevoflurane with 100% O_2 for induction followed by 2% sevoflurane for maintenance. Endotracheal cannula was intubated and a rat ventilator [Harvard Apparatus Minivent (Hollinston, MA); tidal/stroke volume, 2 ml; rate, 80 ventilations/min]. After intubation, rats were placed in the horizontal right lateral position and the left forelimb was positioned laterally beneath the mandible and secured to the surgical platform. A small transverse (dorsal to ventral) incision was made between the second and third ribs. The lateral pleura were exposed, and then blepharostats were used to completely expose the descending thoracic aorta. A non-traumatic bulldog clamp was inserted just under the left subclavian artery origin for blocking the descending thoracic aorta.

2.3. Hemodynamic monitoring and measurements of blood gases

In a small cohort of the rats subjected to the aorta occlusion at normothermia (n = 5), hemodynamic monitoring and blood gas analysis were performed by cannulation of femoral and carotid arteries. Both arteries were cannulated by threading a 0.3-mm diameter cannula in 26-gauge needle toward the heart around 1 cm. The cannula was then secured using a silk suture. Both cannulas were connected to the blood pressure/HR monitor (Columbus Instruments Physiomex, Columbus, OH).

2.4. Postoperative management

After surgery, the rats were singly caged and intramuscularly injected with 800,000 units of penicillin 50 mg/kg for 5 days to prevent infection. The rats were deprived of food but given Ringer's Lactate the first day after surgery, then allowed free access to food and water during the following days. Bladder massage was performed for the rats to help urination during the 5 days after surgery.

2.5. Behavioral scores

Immediately after surgery up to three day postoperation, the lower extremity motor functions of the rats were observed at 6 h intervals and scored according to the BBB rating scale (Basso, Beattie, and Bresnahan Locomotor Rating Scale) [14]. Subsequently the rats were scored every day until one week after surgery, then one time a week. The survival time of the rats was recorded. Both BBB scoring and rat survival time recording were completed by another two investigators who were not involved in the surgeries, and the average score of the two investigators was taken as the BBB score.

2.6. Histology and immunofluorescence

Rats in the HypoSham and those with delayed paralysis in Hyp40 group were sacrificed and perfused with 4% paraformaldehyde for Nissl staining and immunofluorescence assays. Frozen spinal cord samples were subjected to Nissl staining using standard protocols. This was followed by immunofluorescent staining using antibodies to Iba-1, Interleukin (IL)-1β, and IL-6 (Cell Signaling Technology, Boston, diluted in 1:1000, respectively) as described previously [15]. The secondary antibodies used were donkey anti-rabbit (Alexa Fluor 594) or donkey anti-mouse (Alexa Fluor 488) IgG H&L(Abcam, Cambridge, UK, diluted in 1:1000). The sections stained by immunofluorescence were cover-slipped with mounting medium (Vector Laboratories, Inc., Burlingame, CA) and visualized by fluorescence optical microscope.

2.7. RNA extraction, reverse transcription and real-time PCR

Total RNA was isolated using TRIzolR reagent based on the company protocol (Invitrogen, USA). Reverse transcription was conducted as described by our recent study using Molony Murine Leukemia Virus Reverse Transcriptase (MMLV, Promega, USA) [16]. Real-time PCR assay protocol was performed using *SYBR®Green* Real-Time PCR Master Mixes (Roche, Germany). PCR primers were; CCTCTGGTCTTCTGGAGT ACC and ACTCCTTCTGTGACTCCAGC (IL-6); ACCTTCCAGGATGAGGAC ATGA and CTAATGGGAACGTCACACACCA (IL-1β); GCC GAC AGG ATG CAG AAG GAG ATC A and AAG CAT TTG CGG TGG ACG ATG GA (β-