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Research Report

Keep warm and get success: The role of postischemic temperature in the mouse middle cerebral artery occlusion model

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ABSTRACT

Intraluminal suture middle cerebral artery occlusion (MCAO) model is the most frequently used model for ischemic stroke. However, the success rate of this model is variable among different research studies. This study aimed to investigate the effect of postischemic temperature on the success rate. A total of 100 *C57BL/6* mice were randomized into two groups: control group ($n = 50$), body temperature was allowed to self-regulate after MCAO; temperature-controlled group ($n = 50$), mice were kept warm in an incubator for 12 h after MCAO. The body temperature of animals was measured before, during, and for 12 h after MCAO. Neurological deficits and infarct volumes were measured at 24 h after MCAO. There was significant difference ($P < 0.05$) of the body temperature between the two groups from 0.5 h to 3.5 h post ischemia. Moreover, there was obvious difference between the success rates of the two groups (control group: 52%, temperature-controlled group: 84%, $P < 0.05$). In the successful models, infarct volume was significantly ($P < 0.05$) higher in temperature-controlled group ($53.44\% \pm 9.83\%$, $n = 42$) than control group ($45.63\% \pm 10.24\%$, $n = 26$). There was significant difference of the modified neurological severity scores ($P < 0.05$), left adhesive tests ($P < 0.05$) between the two groups. Our data demonstrated that postischemic warming contributed to the success of mouse MCAO model.

1. Introduction

Stroke, 87% of which is ischemic stroke, is one of the leading causes of mortality and morbidity (Go et al., 2013; Liu, 2013). However, there is only one evidence-based effective treatment, intravenous tissue plasminogen activator, in current clinical practice for ischemic stroke (Abou-Chebl, 2012; Jiang et al., 2012b). To elucidate the pathophysiological mechanisms of stroke, many kinds of animal models have been developed during last decades (Jiang et al., 2012a). The intraluminal suture middle cerebral artery occlusion (MCAO) model firstly developed in rats by Koizumi et al. (1986) has become the most widely used stroke model. This model is easy to perform, minimally invasive, and does not require craniectomy (Carmichael, 2005).

The intraluminal suture MCAO model was introduced into mouse by Chan et al. (1993). Due to the genetically modified (transgenic or knockout) mouse strains which provide a unique opportunity to understand the pathophysiological mechanisms of ischemic stroke, this mouse model has attracted increasing attention. Although many efforts have been made to improve this model (Ansari et al., 2011; Belayev et al., 1999; Chen et al., 2008; Xue et al., 2001), the success rate remains unsatisfying and is diverse among different studies. This might contribute to bias in preclinical research studies which led to the failure of many neuroprotective therapeutics.

Previous research studies found that hypothermia reduced (Florian et al., 2008; Miyazawa et al., 2003; Ohta et al., 2007) and hyperthermia exacerbated (Noor et al., 2003; Noor et al., 2005) ischemic brain injury; accordingly, fluctuation in animal temperature would influence the outcome of stroke. Barber et al. (2004) found that after intraluminal suture MCAO, mild hypothermia occurred in mice and alleviated ischemic injury. However, most of research studies just kept the mouse warm during surgery by heating pad while few of them controlled temperature in the postischemic period (Barber et al., 2004). Therefore, we intended

Abbreviations: MCAO, middle cerebral artery occlusion; mNSS, modified neurological severity scores; CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; TTC, 2, 3, 5-Triphenyltetrazolium chloride; SD, standard deviation.

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to investigate whether postischemic body temperature of mice (controlled temperature or spontaneous hypothermia after MCAO procedure) had an impact on the success rate and outcome of mouse intraluminal suture MCAO model.

2. Materials and methods

2.1. Animal preparation

One hundred adult male *C57BL/6* mice weighing 23 g to 25 g (7–8 W) were used in the present study. Animals were purchased from Model Animal Research Institute of Nanjing University (Nanjing, Jiangsu, China). All protocols were approved by the animal subjects review board of Jinling Hospital. All procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1996). All efforts were made to minimize the number of mice used and their suffering.

All animals were fasted overnight before surgery, but were allowed free access to water. Mice were housed in controlled environment with ambient temperature of 25 °C, relative humidity of 65% and 12 h light/dark cycle. Mice were anesthetized with pentobarbital sodium (Sigma-Aldrich, USA).

2.2. Experimental groups

The mice used in this study were randomized into two groups, temperature-controlled group ($n = 50$), in which mice went through 90-min brain ischemia during which body temperature was controlled at 37 °C, and subsequently were kept warm at >35 °C for 12 h in an incubator (Guohua Instruments Corp., Changzhou, China); control group ($n = 50$), in which mice received 90-min MCAO during which body temperature of mice was also controlled at 37 °C, thereafter mice were allowed to self-regulate body temperature in the cages at 25 °C. The whole experiment design was shown in Fig. 1.

2.3. Transient focal ischemia

The MCAO procedure has been described before (Jiang et al., 2010). Mouse was anesthetized with pentobarbital sodium (50 mg/kg i.p.) and placed in an operating table with a feedback regulated heating pad (RS Biomedtech). After cutting off hair in the cervix and sterilizing the skin, we cut open the skin along the cervical midline and dissected the paratracheal muscles bluntly under the operating microscope (Reward Technology Crop., Shenzhen, China). When the right common carotid artery (CCA), external carotid artery (ECA), and internal carotid artery (ICA) were exposed and isolated, two 6-0 sutures were tied at the proximal and distal end of the ECA. The ECA was then cut distal to the superior thyroid artery after two microclips were temporarily placed on the right CCA and ICA. Thereafter, a small hole was cut in the right ECA between the two locations tied with sutures, a silicone-coated 6-0 nylon monofilament was gently inserted from the hole into the right ECA. After the suture tied at the proximal end of the ECA was loosed, the silicone-coated 6-0 nylon monofilament was advanced from ECA into the lumen of ICA until the ipsilateral blood flow of middle cerebral artery supply territory decreased to below 30% of the baseline monitored by a laser Doppler flowmetry (LDF; Perimed PF 5000, Stockholm, Sweden). Then the suture at the proximal was tied again and skin was sewed up. During the whole procedure, rectal temperature was maintained at 37 °C. After 90 min, mouse was briefly reanesthetized and the filament was removed to allow reperfusion. At the onset of reperfusion, mouse was put into an incubator set at approximately 42 °C to keep warm or put back into the cage at 25 °C to allow self-regulate body temperature.

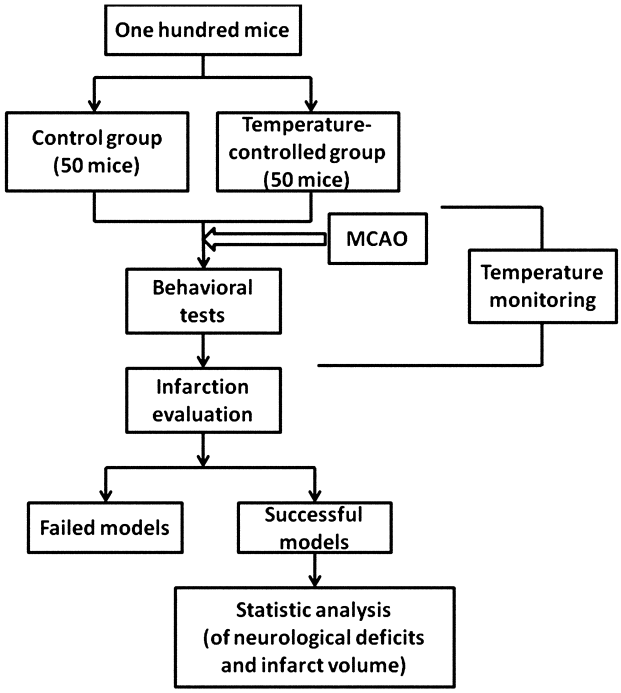

Fig. 1. Schematic of the experimental design. One hundred mice were randomized into two groups: control group ($n = 50$) and temperature-controlled group ($n = 50$). All of the mice underwent MCAO. The body temperature was monitored before, during and for 12 h after the MCAO procedure. A battery of behavioral tests were performed in all mice at 1 day after MCAO. At 24 h post ischemic onset, infarct volumes were detected by 2, 3, 5-Triphenyltetrazolium chloride (TTC). Infarction involving only the striatum but not cortex was regarded as failed model. Only data of successful models was used for the statistical analysis of neurological deficits and infarct volume.

2.4. Physiological monitoring

Animal weight was recorded before and 24 h after MCAO via a small animal balance (Jinling Technology Crop., Nanjing, China). After the ventral tail artery was isolated and cannulated, arterial blood pressure was measured during surgery. One sample of arterial blood (0.3 ml) was acquired before reperfusion for the test of PaO_2, $PaCO_2$, pH and blood glucose (Table 1).

2.5. Temperature monitoring

Rectal temperature was monitored continuously and maintained at 37.0 °C during MCAO procedure via a thermostatically feedback-regulated heating pad. Rectal temperature was monitored and recorded before surgery, and at 0 h, 5 h, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, 3.5 h, 4 h, 5 h, 6 h, 7 h, 8 h, 9 h, 10 h, 11 h, 12 h after MCAO

Table 1
Physiological data of two groups during the MCAO procedure.

	Control Group (Mean ± SD)	Temperature-controlled Group (Mean ± SD)	*P*
BP (mmHg)	103.95 ± 2.61	105.10 ± 3.09	0.211
pH	7.32 ± 0.05	7.30 ± 0.07	0.557
PCO_2 (mmHg)	44.78 ± 5.05	43.59 ± 3.26	0.381
PO_2 (mmHg)	102.45 ± 6.15	103.55 ± 3.17	0.482
Glucose (mM)	8.78 ± 0.80	8.65 ± 1.06	0.664

BP, blood pressure.