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Original Article

Protective effect against focal cerebral ischemia injury in acute phase of a novel invasive device for regional hypothermia

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Abstract

Background: Systemic hypothermia is considered beneficial to stroke patients. However, many complications ensue. The aim of this study was to evaluate the effect of a new invasive regional cooling device in cerebral ischemic injury in a rat model.
Methods: After a pilot study confirming the efficiency of the cooling device, 15 adult male Sprague–Dawley rats, weighing 300–400 g, were randomly assigned into three groups: cooling device applied at 14°C and at 26°C, and a sham group. Focal cerebral ischemic injury was achieved by electrocauterization of the left middle cerebral artery through craniectomy and temporal occlusion of both common carotid arteries for 3 hours. Within 30 minutes after the end of ischemic injury, the cooling device was inserted into the rat brain through a stereotactic frame to provide regional hypothermia for 2 hours. The rats were sacrificed immediately after the 2-hour regional hypothermia.
Results: Although triphenyltetrazolium chloride staining showed smaller ischemic lesions in both the 26°C and 14°C groups compared to the control group, Fluoro Jade C staining showed no neuroprotective effects in the rostrum cerebral cortex in both groups. However, both triphenyltetrazolium chloride and Fluoro Jade C staining indicated significant beneficial effects in the caudal cerebral cortex in rats with cooling device applied at 26°C compared to the 14°C and control groups.
Conclusion: Our findings indicated that the device can effectively achieve regional hypothermia and could be beneficial for patients with cerebral ischemia during the acute phase.

Keywords: brain ischemia; hypothermia; middle cerebral artery infarction; stroke

1. Introduction

Cerebral ischemic injury is one of the major causes of human stroke.[1] Although tissue plasminogen is currently the only drug approved by the United States Food and Drug Administration, several therapeutic measures, such as neuroprotective reagents, anticoagulants, and thrombolytic drugs, have been widely recognized around the world, with promising effects.[2] However, the narrow recommended 3-hour[3] to 4.5-hour[4] therapeutic window largely confines the treatment options and impairs the prognosis of stroke patients.

Conflict of interest: Dr. Ein-Yiao Shen and Taiwan Advanced Sterilization Technology, Inc. have filed an application for a U.S. patent for the design of the "Tissue Cooling Apparatus", while the Taiwanese patent has been approved (Patent 098139852, Taiwan, R.O.C.). The other authors (P.T.L., Y.P.C., and I.H.L.) declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

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In addition to the above-mentioned options, systemic hypothermia has long been known to provide stroke patients certain neuroprotective effects in experimental settings.[5,6] However, whether systemic hypothermia is beneficial or not is still controversial in clinical settings due to several side effects of systemic hypothermia, including arrhythmia, pneumonia, and thrombocytopenia, that may increase morbidity and mortality of the patients and the supportive care burden of health care personnel.[7,8] Accordingly, methods and devices that provide regional hypothermia have been developed to cool through blood vessels,[9−12] nasal cavity,[13−18] meninges[19−21] and the head.[22−27] Unfortunately, the cooling efficiency through brain parenchyma has not been measured in most of those devices, and only a few studies have explored the neuroprotective effects of regional hypothermia.[21,28−31] In addition, the optimal temperature range for regional hypothermia is still controversial.[28,29,32]

This study tested a newly designed invasive device that provides regional hypothermia via direct contact with brain parenchyma at the ischemic center. We explored its cooling efficiency and evaluated its neuroprotective effect in a rat model of permanent middle cerebral artery occlusion.

2. Methods

2.1. Cooling device

Our novel invasive cooling device was designed with a structure of concentric cylinders constituted of a 23-gauge and 18-gauge needle with the tip welded to be sealed. Hence, water could run through the 23-gauge needle and flow out through the 18-gauge needle (Fig. 1A). The hubs of both needles were kept intact to facilitate connection to extension tubes. A digital thermometer was attached to the extension tube close to the entry of water in order to monitor input water temperature (t1, Fig. 1B). The water came from two sets of infusion bags wrapped by an infusion bag pressor that provided the driving force of water at a constant pressure of 300 mmHg.

2.2. Animals

Eighteen adult male Sprague−Dawley rats (BioLasco, Taipei, Taiwan) weighing 300−400 g were used in this study. All rats were housed in 12/12-hour light and dark circadian cycles with free−access to food and water. All management and procedures were approved by the Institutional Animal Care and Use Committee of National Taiwan University, Taipei, Taiwan (NTU-99-EL-1).

2.3. Anesthesia and preparation

Anesthesia was induced using 800 mL/min 5% isoflurane (Baxter, Deerfield, IL, USA) mixed with 100% oxygen within an enclosed cage. After intubation with a 16-gauge intravenous catheter, anesthesia was maintained with a small animal ventilator (SAR-830/P; CWE Inc., Ardmore, PA, USA). Surgical areas were shaved and sterilized. In order to monitor the arterial pressure continuously, the right femoral artery was catheterized with a polyethylene tube (PE-50) and connected to a digital blood pressure probe (BP-100; iWorx Systems, Dover, NH, USA). Arterial blood was sampled from the right femoral artery for blood gas analysis prior to and 30 minutes after the common carotid arteries (CCAs) were occluded. In the ischemic study, blood gas was also evaluated 1 hour after the cooling process had started. Anesthetic depth was adjusted to maintain mean arterial pressure between 90 mmHg and 120 mmHg. A digital thermometer (TM-100; iWorx Systems) was placed 3 cm deep into the rectum to monitor the body temperature continuously (214 Data Recorder; iWorx Systems). The body temperature was maintained at 37 ± 0.5°C throughout the experiment by applying alcohol or a heat pad.

2.4. Cooling efficiency

After anesthesia and preparation as described above, three rats were placed in a stereotactic frame. A midline incision and blunt dissection was made to expose the frontal bone, and three holes were drilled by electric drill (Fig. 1C). The cooling center (c, Fig. 1C) was located 6 mm left of the bregma, while two thermometer-probing points were 3 mm caudal and 5 mm left of the bregma (t2, Fig. 1C) and 5 mm rostral and 2 mm left of the bregma (t3, Fig. 1C). After the dura was cut through using a 27-gauge needle, the cooling device was placed 7 mm below the skull surface at the cooling center and digital thermometers were placed 3 mm below the skull surface in the two thermometer-probing points. The temperatures were recorded for 20 minutes, and the final temperatures were marked as static brain temperatures. After the static brain temperature was acquired, 10°C, 14°C, 20°C, and 26°C cold water was perfused through the cooling device serially for 20 minutes, and the final brain temperatures were also recorded at thermometer-probing points t2 and t3 to evaluate the efficiency of the cooling device. The brain was allowed to return to the static brain temperature between each water temperature.

2.5. Brain ischemic model

Fifteen rats were randomly allocated to three groups (14°C, 26°C, and sham control) of five rats each, and were anesthetized and prepared as described above. Permanent middle cerebral artery occlusion (MCAO), which was modified from a previous study,[33] was performed. A ventral midline incision was made to expose the bilateral CCAs and vagosympathetic trunk. After the skin incision, the soft tissue at the surgical site was immersed in 0.5% bupivacaine (Marcaine; AstraZeneca, London, UK) before the CCAs were isolated. Both CCAs were prepared with a snare composed of 6/0 nylon suture and a 1-mm long PE-50 tube for temporary occlusion after the middle cerebral artery was cauterized. To cauterize the middle cerebral artery, the left eyelid was first closed by 6/0 nylon suture and a 1.5-cm incision was made above the zygomatic bone. The left zygomatic bone was removed to expose the temporomandibular junction and the joint capsule, and the ligament was cut. Ventral retraction of the left mandible