

# *Acta Medica Okayama*

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*Volume 56, Issue 6*

2002

*Article 2*

DECEMBER 2002

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## A new method of inducing selective brain hypothermia with saline perfusion into the subdural space: effects on transient cerebral ischemia in cats.

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## Abstract

In this study, we tested brain surface cooling as a new method of inducing selective brain hypothermia, and evaluated its effects on focal cerebral ischemia using a cat model of transient middle cerebral artery (MCA) occlusion. Cats underwent 1 h of MCA occlusion followed by 5 h of reperfusion. Brain surface cooling was induced for 4 h during and after MCA occlusion in the hypothermia group, but not in the normothermia group. Brain surface cooling was performed using saline perfusion into the subdural space. Rectal temperature, brain surface temperature, and deep brain temperature were monitored, and regional cerebral blood flow (rCBF) and somatosensory evoked potential (SEP) were serially measured. After 5 h of reperfusion, water content was also measured. Although the rectal temperature was maintained at about 37 degrees C, the brain surface temperature decreased rapidly to 33 degrees C and was maintained at that temperature. For 3 h following reperfusion, the rCBF was lower in the hypothermia group than in the normothermia group. At 4 and 5 h after reperfusion, the recovery of SEP amplitude was significantly more enhanced in the hypothermia group than in the normothermia group. In the gray matter, the water content was significantly more diminished in the hypothermia group than in the normothermia group. These results demonstrate that our method is useful for protecting the ischemic brain from a transient MCA occlusion. This method may be adapted for neurological surgery.

**KEYWORDS:** brain hypothermia, cerebral ischemia, cerebral blood flow, somatosensory evoked potential

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\*PMID: 12685856 [PubMed - indexed for MEDLINE]

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*Original Article*

## A New Method of Inducing Selective Brain Hypothermia with Saline Perfusion into the Subdural Space: Effects on Transient Cerebral Ischemia in Cats

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In this study, we tested brain surface cooling as a new method of inducing selective brain hypothermia, and evaluated its effects on focal cerebral ischemia using a cat model of transient middle cerebral artery (MCA) occlusion. Cats underwent 1 h of MCA occlusion followed by 5 h of reperfusion. Brain surface cooling was induced for 4 h during and after MCA occlusion in the hypothermia group, but not in the normothermia group. Brain surface cooling was performed using saline perfusion into the subdural space. Rectal temperature, brain surface temperature, and deep brain temperature were monitored, and regional cerebral blood flow (rCBF) and somatosensory evoked potential (SEP) were serially measured. After 5 h of reperfusion, water content was also measured. Although the rectal temperature was maintained at about 37 °C, the brain surface temperature decreased rapidly to 33 °C and was maintained at that temperature. For 3 h following reperfusion, the rCBF was lower in the hypothermia group than in the normothermia group. At 4 and 5 h after reperfusion, the recovery of SEP amplitude was significantly more enhanced in the hypothermia group than in the normothermia group. In the gray matter, the water content was significantly more diminished in the hypothermia group than in the normothermia group. These results demonstrate that our method is useful for protecting the ischemic brain from a transient MCA occlusion. This method may be adapted for neurological surgery.

**Key words:** brain hypothermia, cerebral ischemia, cerebral blood flow, somatosensory evoked potential

**T**he cerebroprotective effects of hypothermia have been extensively studied in various animal models of ischemia. Systemic hypothermia is the most common method for inducing brain hypothermia in medical procedures, but selective brain hypothermia, which is the selective reduction of brain temperature while maintaining normal body temperature, has been utilized frequently

[14, 20, 25] because systemic hypothermia produces various complications, such as infection, arrhythmia, and coagulopathy [22, 26, 27].

In addition, mild hypothermia has become more widely used because it has been shown that small reductions in brain temperature markedly protect the brain from ischemic injury [3]. The combination of selective brain hypothermia and mild hypothermia may be more experimentally useful than systemic hypothermia in that it avoids the complications of systemic hypothermia. For these reasons, we tested a method involving saline

Received February 20, 2002; accepted June 11, 2002.

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perfusion into the subdural space as a new way of inducing selective brain hypothermia, in order to evaluate its effects on focal cerebral ischemia using a cat model of transient middle cerebral artery (MCA) occlusion.

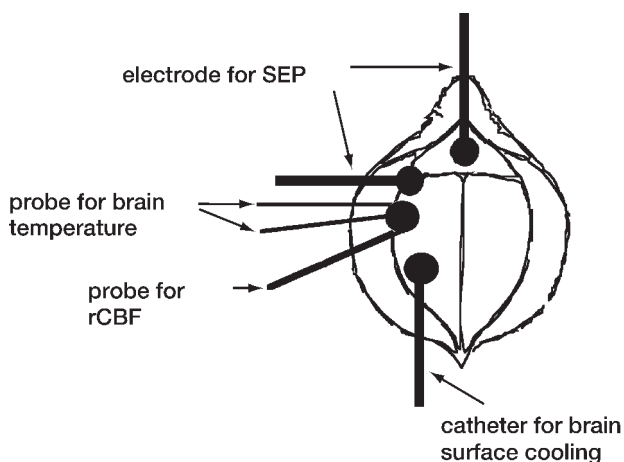
## Materials and Methods

**Preparation.** In this study, all animals were treated in accordance with institutional Policies and Guidelines for the Care and Use of Laboratory Animals. The studies were carried out in 14 adult cats of either sex, weighing 2.5 to 3.0 kg. All cats were bred with free access to food and water. After injections of ketamine hydrochloride (20 mg kg<sup>-1</sup>, i.m.), the subjects' left femoral arteries and veins were catheterized for blood pressure monitoring, blood sampling, and for administration. After tracheostomies and immobilization with pancuronium bromide (0.5 mg kg<sup>-1</sup>, i.v.), the animals were ventilated mechanically with room air and placed in stereotactic frames. During these studies, anesthesia was maintained with pentobarbital sodium (25 mg kg<sup>-1</sup>, i.v.) and pancuronium bromide (0.5 mg kg<sup>-1</sup> h<sup>-1</sup>, i.v.).

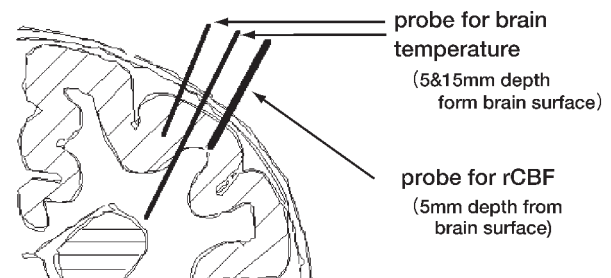
Under microscopic control, the left MCA was exposed just above the left optic nerve using a transorbital route [6]. After a midline incision was made, the skull was exposed and 2 burr holes were placed over the parietal bones using a dental drill. One hole was placed at 0.5 cm posterior and 1.5 cm lateral to the bregma, and the

other was at 2.0 cm posterior and 1.0 cm lateral to the bregma. The former was used for measuring brain temperature and regional cerebral blood flow (rCBF), while the latter was used to insert a catheter into the subdural space for saline perfusion (Fig. 1). Each probe and catheter was fixed with dental cement. Plate electrodes were placed over the left sigmoid gyruses (the forelimb projection area in the primary somatosensory cortex) for monitoring somatosensory evoked potential (SEP). A common reference electrode was placed at the forehead.

**Measurements.** The animals' rectal temperatures and brain temperatures were monitored using the Hyperthermia system (Aloka Co., Tokyo, Japan). The rectal temperature was measured with a probe inserted into the subjects' rectum at a depth of 10 cm. The brain temperatures were measured at depths of 5 mm and 15 mm from the brain surface to monitor brain surface temperature and deep brain temperature, respectively (Fig. 2). Regional cerebral blood flow was measured by means of the hydrogen clearance technique [4] and was calculated from the initial 2 min of the clearance curve using the following formula: rCBF (ml 100 g of brain<sup>-1</sup> min<sup>-1</sup>) = 69.3/T<sub>1</sub><sup>2</sup>. Neuropack Mini (Nihonkoden Co., Tokyo, Japan) was used for SEP measurement. A stimulating electrode was placed over the right distal median nerve proximal to the transverse carpal ligament, and stimuli were applied at a frequency of 2Hz. The evoked potentials resulting from 200 median nerve stimuli were measured and averaged. The SEP amplitudes were evaluated as the primary cortical responses, and were determined by measuring the difference between the second positive and the major negative peak of the potentials [10]. At the end of the study, the water



**Fig. 1** Schematic illustration of preparation. Two burr holes were placed over the parietal bone. One was used for measuring brain temperature and rCBF, and the other for brain surface cooling.



**Fig. 2** Schematic illustration of the location of each probe in brain coronal section. The probes used for measuring brain surface temperature and rCBF were in the gray matter, and the probe used to measure deep brain temperature was in the white matter.

content was measured in the gray matter of the ectosylvian gyrus (the ischemic core) and in the white matter by the specific gravimetric technique [13].

**Brain surface cooling with saline perfusion.** The drip-method, of the type used for intravenous drips in humans, was used for brain surface cooling. With the catheter inserted through the left parietal burr hole, the saline, maintained at 20 °C and placed at a height of 1 m, was dripped into the subdural space of the left cerebral hemisphere, and flowed out the left orbit, which had been opened at a transorbital exposure of the MCA. The dripping speed was controlled to maintain the brain surface temperature at about 33 °C.

**Experimental protocol (Fig. 3).** After the control values for rCBF and SEP were determined while maintaining the rectal temperature at about 37 °C using a heating pad, the left MCA was occluded using a Sugita mini clip for 1 h followed by 5 h of reperfusion. The rectal temperature, the brain surface temperature, and the deep brain temperature were monitored continuously, while rCBF and SEP were measured once every hour. At the end of each protocol, the cats were sacrificed with intravenous potassium chloride. Immediately after that, the brain was removed and cut so the water content could be measured. Following MCA occlusion, brain surface cooling was induced for 4 h (during the occlusion period and 3 h of reperfusion) in the hypothermia group (33 °C) ( $n = 7$ ), but was not induced in the normothermia group (37 °C) ( $n = 7$ ).

**Statistical analysis.** Data are presented as

mean  $\pm$  standard error of mean. Data from these studies were analyzed by using the unpaired *t*-test. A value of 0.05 or less was considered significant.

## Results

Throughout these studies, the mean arterial blood pressure was kept at 90–130 mm Hg, and arterial blood gas parameters were kept within the normal range (PCO<sub>2</sub>: 30–40 mmHg, PO<sub>2</sub>: 100–120 mmHg).

**Rectal and brain temperature (Fig. 4).** Rectal temperature was maintained between 36.5 and 37.5 °C in both groups during the studies. In the hypothermia group, after the brain surface cooling began, the brain surface temperature decreased rapidly to 32–33 °C and was maintained at that temperature. On the other hand, deep brain temperature decreased to about 35 °C (1–1.5 °C lower than rectal temperature). A temperature gradient existed between the brain surface and the deep brain during the brain surface cooling. When the brain surface cooling was stopped, the brain surface and deep brain temperatures increased rapidly to about 36 °C. In the normothermia group, the brain surface and the deep brain temperature were maintained at about 36 °C.

**Regional CBF (Fig. 5).** In both groups, rCBF markedly diminished during MCA occlusion, and recovered rapidly during reperfusion. The rCBFs measured in the first 2 h of reperfusion were significantly lower in the hypothermia group ( $29.4 \pm 3.1$  and  $24.5 \pm 2.6$  ml 100 g of brain-1 min-1) than in the normothermia

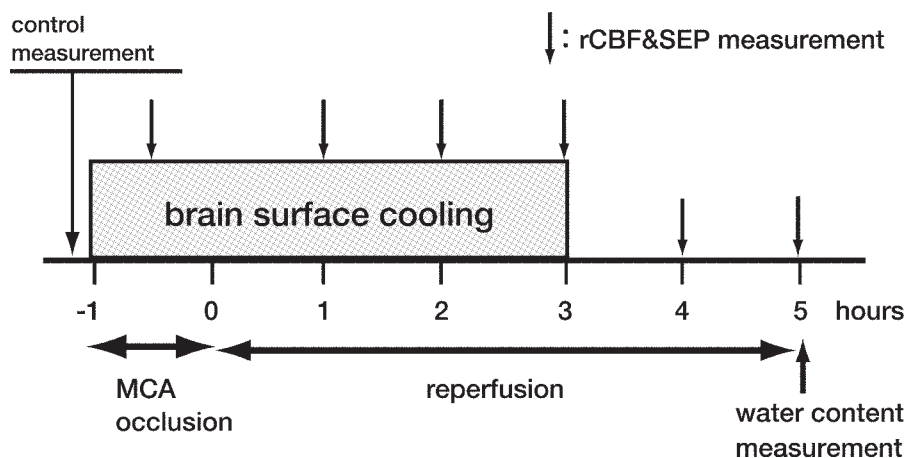


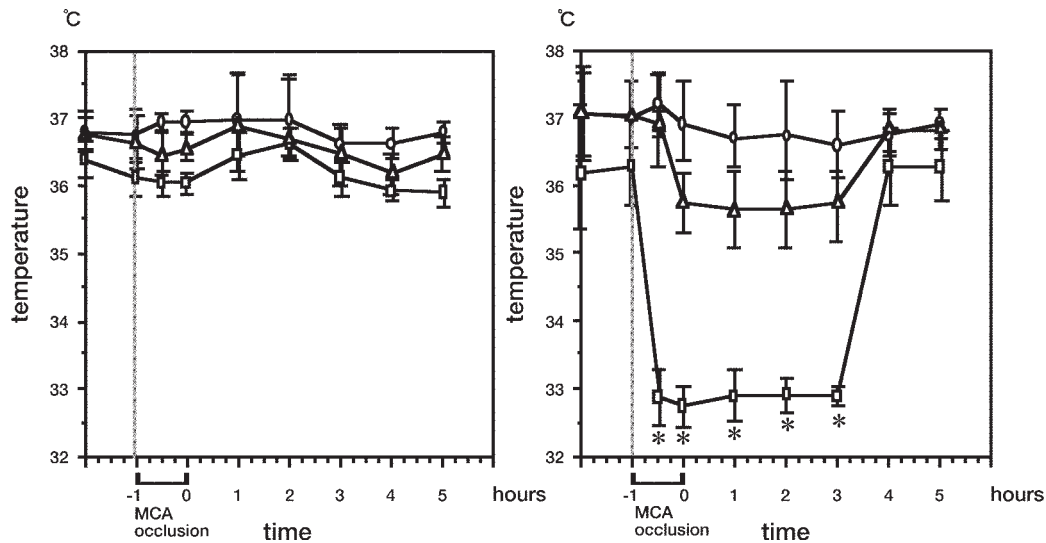
Fig. 3 Experimental protocol. Brain surface cooling was performed in the hypothermia group, but not in the normothermia group.

group ( $53.5 \pm 8.4$  and  $41.8 \pm 3.8$  ml 100 g of brain $\cdot$ min $^{-1}$ ). Brain surface cooling prevented early postischemic hyperemia.

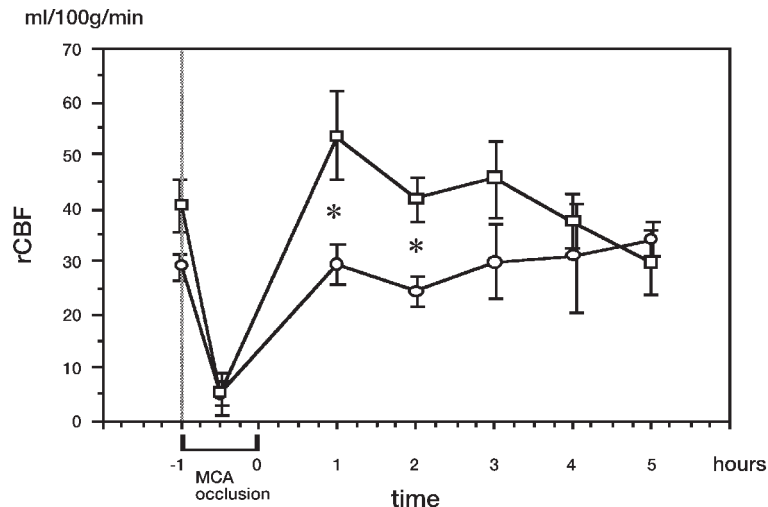
**SEP (Fig. 6).** The change of SEP amplitude was evaluated by calculating the percentage change with respect to the control amplitude of SEP. The recovery of

SEP amplitude was significantly more enhanced in the hypothermia group than in the normothermia group at 4 and 5 h after reperfusion. At 5 h after reperfusion, the SEP amplitude in the hypothermia group showed almost full recovery ( $92.5 \pm 2.6\%$ ).

**Brain tissue water content (Fig. 7).** In



**Fig. 4** Sequential changes of temperature in each location. Normothermia ( $n = 7$ ) (in the left) and hypothermia ( $n = 7$ ) (in the right) group are shown.  $*P < 0.001$ , compared to deep brain and rectal temperature.  $\circ$ , rectal temperature;  $\triangle$ , deep brain temperature;  $\square$ , brain surface temperature.



**Fig. 5** Sequential changes of rCBF in both groups.  $*P < 0.05$ , compared to the normothermia group.  $\square$ , normothermia group ( $n = 6$ );  $\circ$ , hypothermia group ( $n = 6$ ).

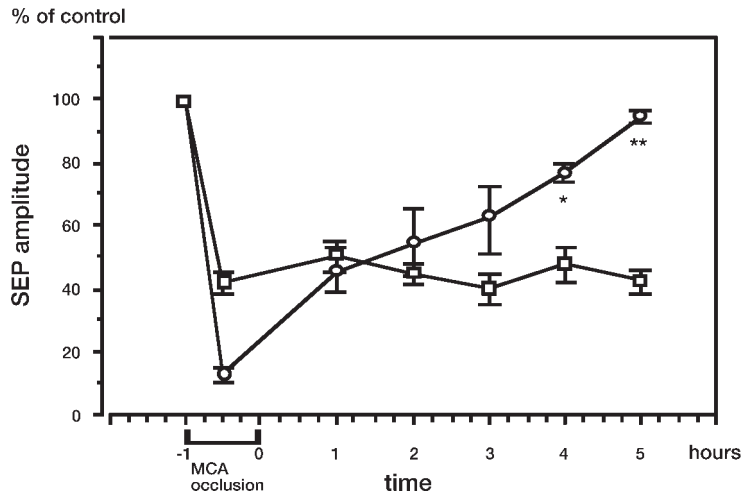
the gray matter, specific gravity was significantly higher in the hypothermia group ( $1.0392 \pm 0.0004$ ) than in the normothermia group ( $1.0327 \pm 0.0017$ ). However, no significant difference was observed in the white matter. Brain edema was diminished only in the gray matter. Accordingly, brain surface cooling was effective in the gray matter, but not in the white matter regarding the brain edema that resulted from MCA occlusion.

**Discussion**

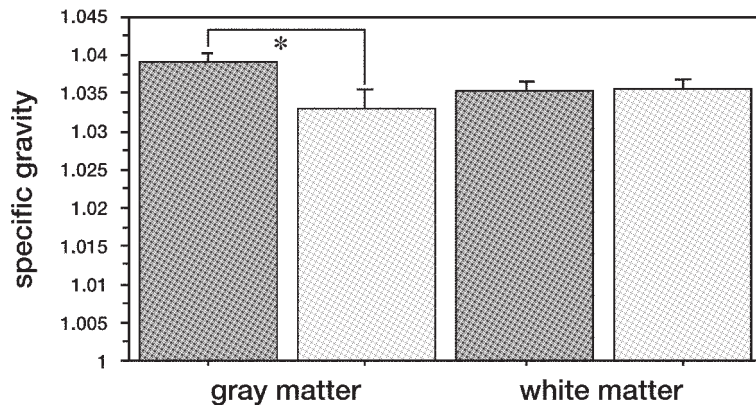
*History and complications of hypothermia.*

It is generally agreed from Busto's report [3] that mild hypothermia has marked cerebroprotective effects in cerebral ischemia. Many reports have shown that mild hypothermia is effective in improving outcomes in experimental stroke models [29, 30] and in humans [9, 11, 23, 24].

Systemic hypothermia is the most common method of hypothermia employed in surgical procedures, but many



**Fig. 6** Sequential changes of SEP amplitude in both groups. \* $P < 0.05$ , \*\* $P < 0.01$ , compared to the normothermia group.  $\square$ — $\square$ , normothermia group (n = 5);  $\circ$ — $\circ$ , hypothermia group (n = 5).



**Fig. 7** Specific gravity in the gray and white matter. In the gray matter (left), specific gravity is significantly higher in the hypothermia group compared to the normothermia group (\* $P < 0.05$ ).  $\blacksquare$ , hypothermia group (n = 7);  $\square$ , normothermia group (n = 7).

studies have reported that it carries a risk of causing complications [22, 26, 27]. For example, a decrease of cardiac output and whole-body oxygen consumption [26], conduction abnormalities, blood coagulopathies [8], myocardial infarctions [18], and infection [28] have been reported. These reports suggest that systemic hypothermia can produce fatal complications, especially in the cardiovascular system and immune functions.

**Introduction of selective brain hypothermia.** To avoid the complications of hypothermia, some examples of selective brain hypothermia, which is the selective reduction of brain temperature while maintaining normal body temperature, have been introduced. Kuluz *et al.* cooled the head with an ice pack, and maintained the brain at 30 °C [10]. They reported the effect on the ischemic brain in rats, but Mellergard *et al.* showed that this method was not effective in lowering the temperature of the human brain [14]. Ohta *et al.* presented a new method that involved administering cold Ringers perfusion into the vertebral artery to induce selective brain hypothermia [20]. This method, however, requires a complicated system that includes specialized machines, such as a thermostat and a hemofiltrator, and also requires the administration of a diuretic. Schwartz *et al.* presented another method of selective brain hypothermia [25]. They infused cooled blood, which was continuously withdrawn from the femoral artery, through the common carotid artery in baboons and showed that moderate and deep selective brain hypothermia could be accomplished, but they did not test the effect of this hypothermia on the ischemic brain.

Since there is no method that can induce brain hypothermia simply and is clinically effective for the ischemic brain, we attempted a new method of local brain hypothermia that did not involve intra-arterial injection of drugs and other substances. This new method aimed to induce and maintain mild hypothermia in the ischemic brain. Brain surface cooling, as a new method of inducing local brain hypothermia, was performed by saline perfusion into the subdural space.

With this method, the brain surface temperature decreased rapidly below 33 °C and was maintained at that temperature, while the rectum maintained a temperature of about 37 °C.

**CBF and neuronal function in brain surface cooling.** In our experiment, early postischemic rCBF significantly decreased as a result of hypothermia. This means that the suppression of early postischemic

hyperemia can be achieved by mild hypothermia. Also, the specific gravity of the gray matter was significantly higher and the recovery of SEP amplitude was significantly more enhanced in the hypothermia group than in the normothermia group. This means that mild hypothermia may be effective for reducing cortical edema and recovering the SEP amplitude. Morikawa *et al.* reported that after MCA occlusion, the CBF during early recirculation was positively correlated to the intras ischemic brain temperature, and a significant positive correlation was observed between the increase in cortical infarct volume and the increase in early postischemic CBF [17]. According to these reports and our results, our method may introduce selective brain hypothermia, and would be useful for reducing neurological deficits and infarct volume. The suppression of early postischemic hyperemia may play a role in the recovery of SEP amplitude observed in our study. SEP is used to monitor cortical electrophysiological function [1, 2, 15], and some studies have reported a correlation between changes in SEP and neurological deficits [5, 16, 21]. Because the SEP amplitude was recovered with our method, we can conclude that our method may be useful for improving neurological function.

On the other hand, significant differences were seen only in the specific gravity of the gray matter. In our study, the brain surface temperature decreased to 32–33 °C, but the deep brain temperature remained around 35 °C. This means that hypothermia was introduced only in the gray matter with our cooling method, and that cortical edema was reduced. Kuluz *et al.* reported that the cortex is more vulnerable to ischemia than the white matter, and that this difference in vulnerability to ischemia may have influenced the recovery of SEP amplitude [10]. Therefore the introduction of cortical hypothermia may be able to improve the SEP amplitude.

**Advantage and disadvantage of brain surface cooling.** In the future, we would like to introduce the use of this method in neurological surgery, especially surgery that requires temporary arterial occlusion. The advantages of applying our method during surgery are as follows. First, the method uses only a subdural drip of the type used for intravenous drips in human applications and saline bottles, and requires no special equipment. In the second place, it carries no risk of postoperative bleeding or changing blood characteristics, because heparin and other drugs are not needed. On the other hand, our method has a disadvantage. Selective



brain cooling was performed with saline perfusion in the subdural space, but if the subdural space is small, sufficient saline cannot fit within it. In our experiment, in fact, when the brain was swollen, the subdural space was so small that saline perfusion became incomplete, and the brain surface temperature did not decrease to the target temperature. However, during neurosurgical operations, the skull is removed and the subdural space is generally so wide that this method could be performed without difficulty.

Further studies are necessary to determine what temperature can be achieved with brain surface cooling, what temperature is best for protecting the ischemic brain, and how to monitor brain temperature before this method can be applied in humans.

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