Selective Brain Hypothermia Augmenting Neuroprotective Effects of Decompressive Craniectomy for Permanent Middle Cerebral Artery Infarction in a Rat Model

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■ OBJECTIVE: **To evaluate the combined effects of a decompressive craniectomy and prolonged selective brain hypothermia on large hemispheric infarction in a rat model.**

■ METHODS: **Permanent middle cerebral artery infarction using an endovascular occlusion technique was created in rats assigned to 4 groups. Normothermia was maintained without a craniectomy in group A ($n = 20$) as the control, prolonged (>44 hours), selective brain hypothermic treatment was performed on group B ($n = 20$), a craniectomy was performed on group C ($n = 18$), and prolonged, selective brain hypothermic treatment using a cooling coil implanted in the craniectomy site was combined with a craniectomy for group D ($n = 18$).**

■ RESULTS: **Group B and C exhibited a significantly reduced infarct volume when compared with the control. Furthermore, group D showed a significantly reduced infarct volume when compared with group C, plus a significantly improved neurologic score. These results for group D were associated with an increased neuronal cell count and reduced hyperactive microglia and hypertrophic astrocytes in the cortical penumbra ($P < 0.01$). Moreover, a greater preservation of normal-appearing axonal bundles and the blood–brain barrier was observed in the core infarct region at the caudoputamen.**

■ CONCLUSIONS: **A decompressive craniectomy reduced the infarct volume and improved the neurologic outcomes in a rat model of middle cerebral artery infarction. Furthermore, when combined with prolonged selective brain hypothermia, significant additional benefits were observed for the neurologic outcomes, infarct volume, and degree of neuroinflammation.**

INTRODUCTION

The persistence of acute occlusion of either the internal carotid artery (ICA) or the proximal middle cerebral artery (MCA) beyond the therapeutic time window for restoration of the cerebral blood flow results in large hemispheric infarction that is associated with a high mortality rate of up to 80%.[1] After 3 European randomized, controlled clinical trials and their meta-analysis revealed that early surgical decompression reduced case fatalities and increased the incidence of favorable outcomes, a decompressive craniectomy has become the standard treatment for malignant MCA infarction.[2-5] Notwithstanding, the proportion of unfavorable outcomes (moderately severe disability to mortality) remains approximately 60%–70%.[2-5]

In the meantime, the neuroprotective properties of therapeutic hypothermia already have been demonstrated in various animal

Key words
- Cerebral infarction
- Craniectomy
- Hypothermia
- Middle cerebral artery
- Rat

Abbreviations and Acronyms
BBB: Blood–brain barrier
EBA: Endothelial barrier antigen
Iba-1: Ionized calcium binding adaptor molecule 1
ICA: Internal carotid artery
ICP: Intracranial pressure
LFB: Luxol fast blue
MCA: Middle cerebral artery
PBS: Phosphate buffered saline
pMCAO: Permanent middle cerebral artery occlusion
RIV: Relative infarct volume
TTC: 2,3,5-Triphenyl-tetrazolium chloride

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stroke models and some clinical studies.[6-10] Yet, the clinical application of therapeutic hypothermia is extremely limited, since the laborious process of whole-body cooling is associated with significant systemic complications, such as arterial hypotension, thrombocytopenia, and pneumonia,[6,10,11] whereas external head cooling is slow and inefficient due to a variety of external cooling barriers covering the brain, including the skull, temporalis muscle, and scalp, along with the temperature shielding effect of the cerebral blood flow.[12]

However, a decompressive craniectomy can be used as a window to provide prolonged, selective brain hypothermia as a supplementary treatment without systemic complications. Accordingly, the current experimental study was designed to evaluate the combined effects of a decompressive craniectomy and prolonged, selective brain hypothermic treatment on cerebral infarction in a rat model.

MATERIALS AND METHODS

Animal Preparation

Male Sprague–Dawley rats weighing 375–450 g were housed in individual cages at 22°C under a 12-hour day/night cycle and allowed free access to food and water. All the procedures were in accordance with institutional guidelines.

Permanent hemispheric infarction was induced based on endovascular occlusion of the MCA.[13,14] After we anesthetized the rats with isoflurane in a supine position, the left common carotid artery, external carotid artery, and ICA were all exposed through a midline neck incision. A silicon-coated monofilament suture with a diameter of 0.35–0.40 mm (Doccol Co.; Albuquerque, New Mexico, USA) was then inserted through an arteriotomy of the common carotid artery to the ICA and gently advanced to the Circle of Willis to occlude the origin of the left MCA. The procedure was performed by an experienced researcher (J.-H.K.) to ensure a reliable MCA occlusion and minimize any variations between the rats. The rats were placed on a homeothermic heat blanket (Harvard Apparatus Co.; South Natick, Massachusetts, USA) at 37°C to maintain a normal body temperature during the surgery.

Hemicraniectomy and Selective Brain Hypothermic Treatment

After we sutured the neck wound, a hemicraniectomy was performed using a midline scalp incision in a prone position. A hemicranial bone flap was created with the use of a dental drill and microscissors. A cruciate dural incision completed the decompression and no infarcted brain tissue was removed.

Selective brain hypothermia was then administered to the freely moving rats using a metal coil method.[15] The cooling coil, which was produced by bending a hypodermic needle (26 G) into a flat spiral with a diameter of 5–6 mm and thickness of 0.5 mm, was implanted between the dura and temporalis muscle, connected to a silicon tube that was passed through an overhead swivel (Instech Laboratories, Inc.; Plymouth Meeting, Pennsylvania, USA) to allow the animals to move freely, and then connected to a homeothermic device (Figure 1A–C). The exposed brain was covered by a sterilized latex sheet to avoid any focal damage adjacent to the cooling coil. The metal coil was cooled to 20°C by flushing it with 4°C saline from a homeothermic device using a peristaltic pump.

Study Design

To determine the cooling conditions including the temperature of the cooling coil and flow volume of saline through the coil, the brain temperature was monitored using a thermocouple placed deep (5 mm) in the hemispheres ipsilateral and contralateral to the permanent middle cerebral artery occlusion (pMCAO) in the craniectomized ($n = 3$) and noncraniectomized ($n = 3$) rat brains (Figure 2A). In addition, the temperature of the cooling coil also was monitored. The target hypothermic brain temperature (~33°C) in the ipsilateral hemisphere was obtained by adjusting the temperature of the cooling coil (20°C) and flow volume of 4°C saline (10 mL/min) through the coil (Figure 2B). Under these hypothermic conditions, the temperature in the contralateral hemisphere and the rectal temperature were both maintained at

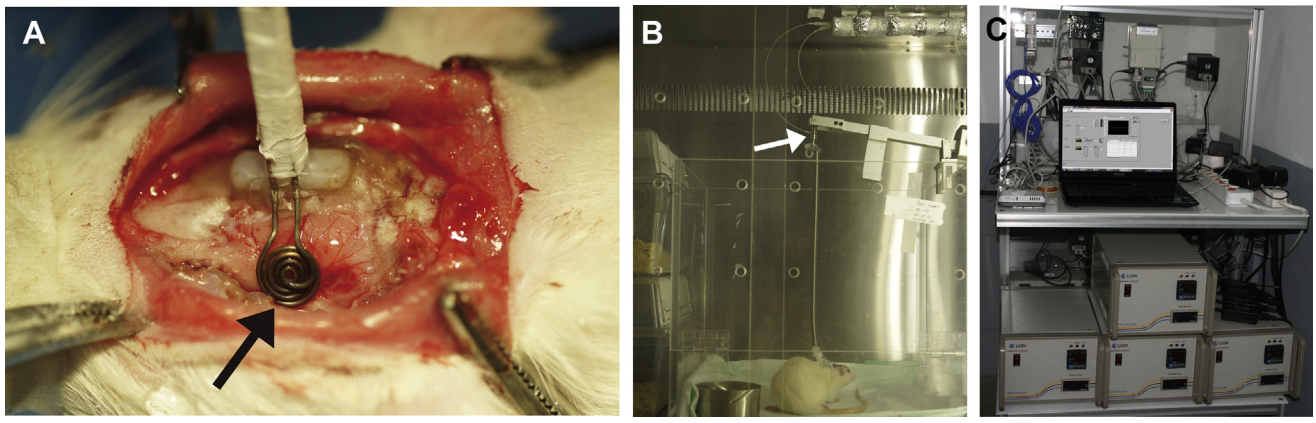

Figure 1. Selective brain hypothermic treatment. (**A**) Cooling coil (*arrow*) implanted in a craniectomized rat. (**B**) Overhead swivel (*arrow*) with silicone tubes allowing free activity of the rat. (**C**) Homeothermic system adjusting the temperature of the cooling coil to 20°C by circulating cold water.

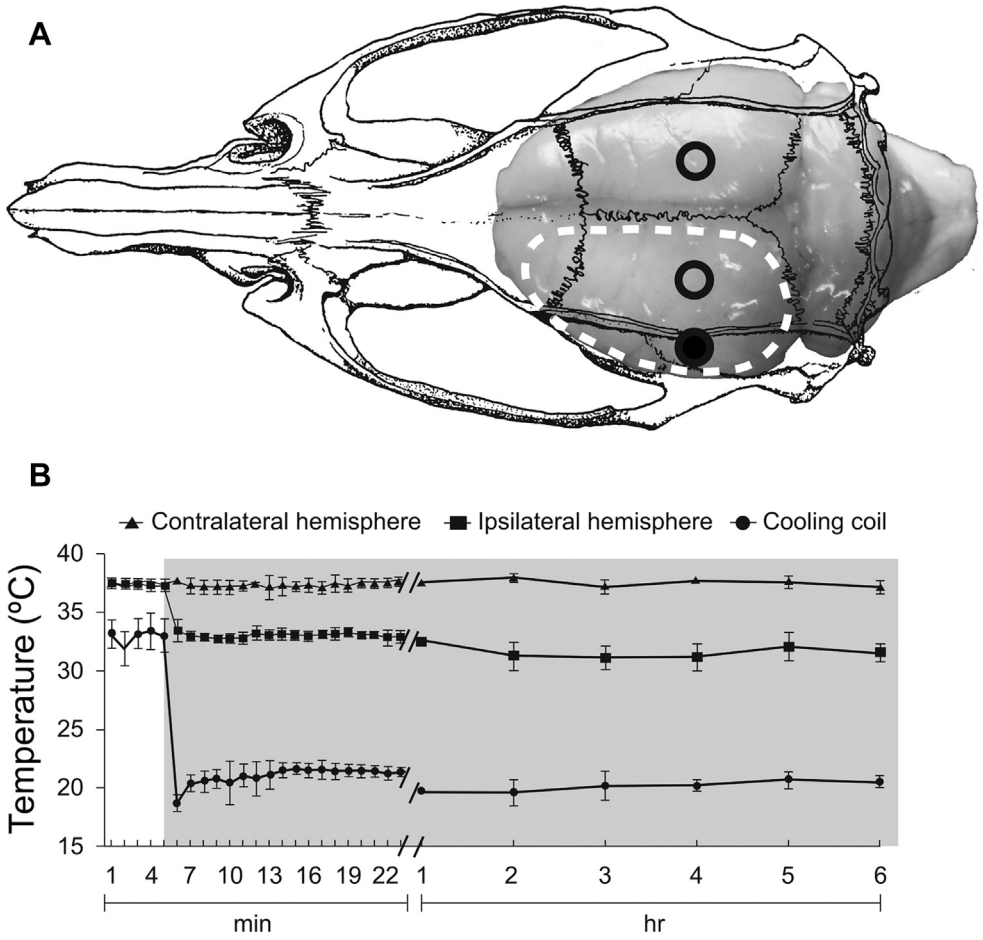

Figure 2. Brain temperature monitoring during selective brain hypothermic treatment in craniectomized rats. (**A**) Locations of thermocouples. The brain temperature was measured using thermocouples (*open circle*: 5 mm posterior to coronal suture, 2 mm lateral to sagittal suture, and 5 mm deep) placed in the cerebral hemispheres ipsilateral and contralateral to the permanent middle cerebral artery occlusion. The temperature of the cooling coil also was measured using another thermocouple (*filled circle*). Note the boundary of the craniectomy (*dotted line*). (**B**) Graph showing brain temperature. The target temperature (~33°C) for the infarcted hemisphere was reached within a few minutes after starting the hypothermic treatment within the craniectomized rats. Results are means ± standard deviations.

~37.5°C. In the craniectomized rats, the target temperature for the lesional brain was reached within 2 minutes, whereas it took 20 minutes in the noncraniectomized rats.

Eighty-eight rats were allocated randomly to 4 treatment groups and were excluded from the study in the case of a fever ($n = 3$), subarachnoid hemorrhage in the harvested brain suggesting a suture-induced rupture of intracranial vessels ($n = 3$), remarkable infection associated with implanting the metal coil in the operative wound ($n = 4$), or experimental errors ($n = 2$). As a result, 76 rats were allocated to 4 treatment groups as follows: group A ($n = 20$), no craniectomy or no hypothermia after pMCAO as the controls; group B ($n = 20$), delayed (4 hours after pMCAO) and prolonged (44 hours) brain hypothermic treatment; group C ($n = 18$), a craniectomy following pMCAO; and group D ($n = 18$), delayed and prolonged brain hypothermic treatment combined with a craniectomy following pMCAO (Figure 3).

For these freely moving rats in the 4 treatment groups, no direct invasive brain temperature monitoring was performed to avoid any brain damage during the 44-hour brain hypothermic treatment. Instead, the temperature of a cooling coil was monitored continuously and the rectal temperature checked twice a day.

Neurologic Score

The neurologic state of the animals was evaluated blindly using an established scoring system at 48 hours after pMCAO.[9,16] The neurologic scoring system was as follows: score 0, no apparent deficits; score 1, contralateral forelimb flexion; score 2, decreased grip of the contralateral forelimb while pulled by the tail; score 3,

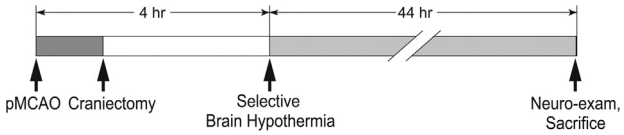

Figure 3. Schematic drawing of current experimental procedures. When combining the craniectomy and selective brain hypothermia, the experiment started with permanent middle cerebral artery occlusion (pMCAO) using an endovascular technique, followed by a hemicraniectomy. Delayed (4 hours after pMCAO) and prolonged (for 44 hours) brain hypothermic treatment was then applied up to sacrifice.

spontaneous movement in all directions and contralateral circling only while pulled by the tail; score 4, spontaneous contralateral circling; and score 5, death. Thus, the neurologic state was graded on a scale of 0–5.

Quantification of Infarct Volume

Five 2-mm thick coronal sections per brain were incubated with 1% 2,3,5-triphenyl-tetrazolium chloride (TTC) in saline for 30 minutes at 37°C. The infarcted area in each slice was delineated and measured using the Image J program (Scion Corporation, Frederick, Maryland, USA). The infarct volume was then calculated using an indirect method to minimize the effect of edema on the measurement of the brain infarct volume and expressed as a percentage of the lesion volume relative to the contralateral hemisphere.[17,18] The relative infarct volume (RIV) was determined using the following equation: RIV = 100 × (Vc − Vi)/Vc, where Vc is the volume of the contralateral hemisphere and Vi is the volume of the nonischemic brain tissue of the ipsilateral hemisphere.

Histologic Analysis

The sacrificed rats were subjected to intracardiac perfusion fixation using 0.9% saline and 4% paraformaldehyde dissolved in 0.1 M phosphate-buffered saline (PBS) (pH 7.4). The brains were removed and immediately fixed with 4% paraformaldehyde diluted in 0.2 M PBS for 7 days and cryoprotected with 30% sucrose diluted in 0.2 M PB for 3 days. Thereafter, the brains were embedded in an optimal cutting temperature compound (Tissue-Tek; Sakura Finetek, Tokyo, Japan) and sectioned at a thickness of 20 μm. The sections were then stained with a Cresyl violet solution for 5 minutes, decolorized with an acid formalin solution, washed, and mounted.

To observe the axonal density, Luxol fast blue (LFB) staining was implemented. The sections were rinsed with distilled water and left in a 1% LFB solution for 12 hours in an oven at 56°C. The excessive stain was then rinsed off using 95% ethyl alcohol and the sections differentiated with a 0.05% lithium carbonate solution. Finally, the sections were rinsed with distilled water and mounted.

Immunohistochemistry

After intracardiac perfusion fixation using 0.9% saline and 4% formaldehyde dissolved in 0.1 M PBS, the brains were removed and immersion-fixed in 4% paraformaldehyde diluted in 0.2 M PBS for 3 days. The fixed brains were then cryoprotected by immersion in 30% sucrose in 0.2 M PBS for 3 days and embedded in an optimal cutting temperature compound. Thereafter, the brains were sectioned at a thickness of 20 μm. For chromogen staining, the sections were rinsed with 0.2 M PBS and incubated in 1% hydrogen peroxide in methanol for 20 minutes at room temperature to quench any endogenous peroxidase activity. Next, the sections were immersed in a blocking solution (0.1% Triton X-100, 1% bovine serum albumin, and 5% normal serum in PBS) for 1 hour, incubated with primary antibodies (mouse anti-neuronal nuclei, 1:500; Santa Cruz Biotechnology, Inc., Dallas, Texas, USA), rabbit anti-ionic calcium binding protein (Iba)-1 (1:500; Wako Pure Chemicals Co; Tokyo, Japan), rabbit anti-glial fibrillary acidic protein (1:500; Dako, Glostrup, Denmark), and mouse anti-endothelial barrier antigen (EBA, 1:500; Covance, Berkeley, California, USA) diluted in a blocking solution at 4°C overnight, rinsed 3 times in PBS containing 10% Tween 20, and incubated with biotinylated secondary antibodies (Vector Laboratories, Burlingame, California, USA) at a dilution of 1:200 for 1 hour at room temperature. The sections were then rinsed 3 times in PBS with Tween-20, incubated with avidin-biotin complex reagents for 1 hour at room temperature (ABC Kit Universal; Vector Laboratories), rinsed 3 times in PBS with Tween-20, and then treated with 3,3-diaminobenzidine tetrahydrochloride as a chromophore. After rinsing with distilled water, the sections were dehydrated using an ethanol series followed by xylene and mounted.

Data Acquisition and Statistical Analysis

Tiled images of each section were captured using a color video camera (Olympus D70 [Tokyo, Japan]) attached to a microscope (Olympus BX51). The stained area and immunohistologic intensity were analyzed using the National Institutes of Health ImageJ program (NIH, Bethesda, Maryland, USA).

The statistical analyses were performed with the aid of commercially available statistics software (SPSS version 19.0; IBM Corp., Armonk, New York, USA). All values are expressed as the mean ± standard deviation. After we conducted a Shapiro–Wilk normality test, a one-way analysis of variance followed by a post-hoc Scheffe test were used to test the between-group differences for normally distributed data. The results were considered significant for probability values less than 0.05. A Kruskal–Wallis test and post-hoc Mann–Whitney U test with a Bonferroni adjustment were performed for multiple comparisons of the remaining data (neurological scores and numbers of hyperactive microglia stained with anti-Iba-1). Associations were considered significant after the Bonferroni correction if $P < 0.0083$ (0.05/6).

RESULTS

Neurologic Performance

The neurologic scores 48 hours after pMCAO are shown in Figure 4. No significant differences in the neurologic scores were found between group A (control, $n = 20$, 4.4 ± 0.5), group B (rats treated with only selective brain hypothermia, $n = 20$, 3.9 ± 0.9), and group C (rats treated with just a craniectomy, $n = 18$, 3.7 ± 0.8). However, given that a Bonferroni-corrected P value <0.0083 was considered statistically significant, group D (rats that received a craniectomy combined with selective brain hypothermia, $n = 18$, 2.8 ± 1.4) showed a significantly better neurologic score than groups A ($P = 0.0003$), B ($P = 0.0072$), and C ($P = 0.0080$).

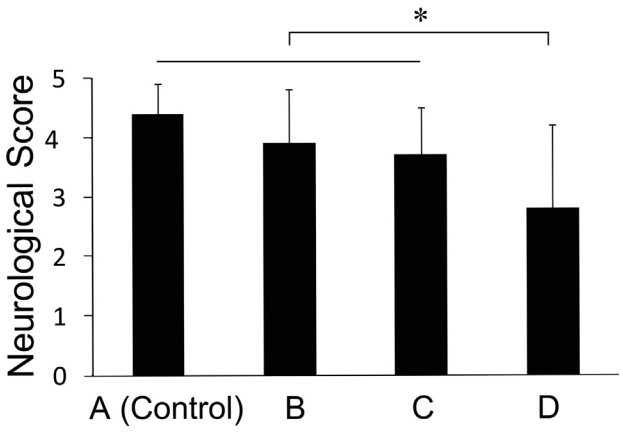

Figure 4. Bar graph showing neurologic scores 48 hours after permanent middle cerebral artery occlusion. Means and standard deviations are presented for all groups. No significant difference in the neurologic scores were found between group A (4.4 ± 0.5), group B (3.9 ± 0.9), and group C (3.7 ± 0.8). However, group D (2.8 ± 1.4) showed a significantly better neurologic score than groups A, B, and C. *Bonferroni-corrected $P < 0.0083$.

Infarct Volume

The evaluation of the infarcted areas and calculations of the RIV for the different treatment groups were performed based on TTC-stained brain sections from 20 (5 initial consecutive rats per group) (Figure 4A). In contrast to the infarcted MCA territory for group A, the other treatment groups exhibited morphologic protection primarily in the cerebral cortex. In the deep brain, the caudoputamen anterior to the coronal level of the anterior commissure was constantly infarcted, regardless of the treatment group, whereas the posterior part of the basal ganglia including the internal capsule was variably affected.

When compared with the RIV for group A ($n = 5$, 56.2 ± 2.1%), the RIV was significantly reduced in group B ($n = 5$, 45.4 ± 7.3%, $P < 0.05$). In addition, when compared with group B, the RIV also was significantly reduced in group C ($n = 5$, 32.1 ± 3.5%, $P < 0.05$). Finally, when compared with group C, a further significant decrease in the RIV was observed for group D ($n = 5$, 19.9 ± 6.7%, $P < 0.05$) (Figure 5B). The RIV data for the 4 groups are presented in Table 1.

Neuronal Cell Death in Cortical Penumbra

The histologic evaluation was focused on the cortical penumbra including the primary motor cortex and primary somatosensory cortex (forelimb region) (Figure 6A). The immunohistochemistry data representing a neuron-specific marker (neuronal nuclei) revealed significant neuronal preservation in group B ($n = 10$, 1736 ± 180/mm², $P < 0.001$) and group C ($n = 10$, 2078 ± 274/mm², $P < 0.001$) when compared with that in the control group (755 ± 260/mm²). Plus, group D ($n = 10$) with the combined treatment exhibited additional preservation of neuronal cells when compared with the group B ($P < 0.001$) and group C ($P < 0.001$) (Figure 6B).

Glial Activation in Cortical Penumbra

The effects of the craniectomy and local hypothermia on the glial activation induced by pMCAO were evaluated in the cortical penumbra including the primary motor cortex and primary somatosensory cortex (Figure 7A). For group A after pMCAO, the immunohistochemical data showed remarkable astrogliosis and microgliosis.

Given that a Bonferroni-corrected P value <0.0083 was considered statistically significant, group B showed a significant reduction of hyperactive Iba-1–positive microglial cells ($n = 10$, 1856 ± 160/mm², $P = 0.0012$) when compared with the control (group A, $n = 10$, 3226 ± 281/mm²). Moreover, when compared with group B, group C also showed a significant reduction of these cells ($n = 10$, 796 ± 59/mm², $P = 0.0012$). Finally, when compared with group C, group D showed a further significant decrease in hyperactive Iba-1–positive microglial cells ($n = 10$, 139 ± 31/mm², $P = 0.0017$) (Figure 7B).

Meanwhile, the number of hypertrophic astrocytes was significantly reduced in group B ($n = 10$, 558 ± 90/mm², $P < 0.001$) and

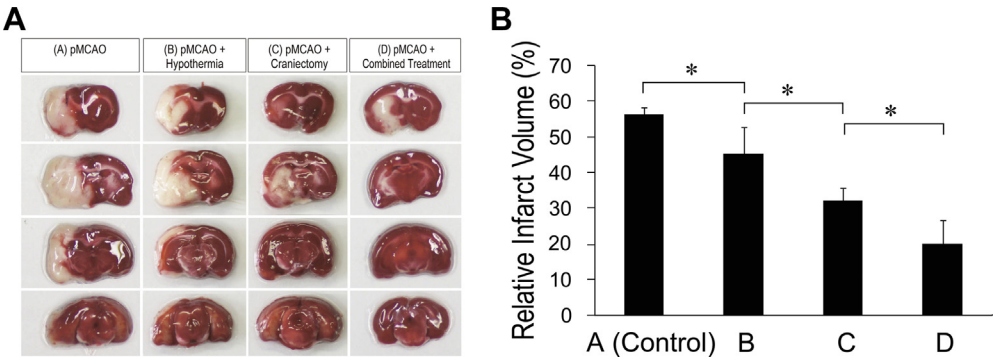

Figure 5. Infarct volume 48 hours after permanent middle cerebral artery occlusion (pMCAO). (**A**) Representative 2,3,5-triphenyl-tetrazolium chloride (TTC)-stained brain slices from rats after pMCAO showing the effect of craniectomy and brain hypothermic treatment. (**B**) Bar graph showing TTC-derived relative infarct volume (RIV). Means and standard deviations are presented for all groups. The RIV differed significantly between the groups (group A > group B > group C > group D). *$P < 0.05$.

Table 1. RIV Based on TTC-Stained Brain Sections after pMCAO

	Group A	Group B*	Group C†	Group D‡
Mean ± SD	56.2 ± 2.1	45.4 ± 7.3	32.1 ± 3.5	19.9 ± 6.7
Median (IQR)	56.1 (0.6)	46.8 (9.7)	32.5 (1.2)	22.5 (6.7)
Range	53.5–59.3	34.9–52.6	26.4–35.7	8.9–18.2

RIV, relative infarct volume; TTC, 2,3,5-triphenyl-tetrazolium chloride; pMCAO, permanent middle cerebral artery occlusion; SD, standard deviation; IQR, interquartile range.
*Significant difference ($P < 0.05$) between Group A and Group B.
†Significant difference ($P < 0.05$) between Group B and Group C.
‡Significant difference ($P < 0.05$) between Group C and Group D.

group D ($n = 10$, 925 ± 230/mm², $P < 0.001$) when compared with the control group ($n = 10$, 5560 ± 857/mm²), whereas the number was not significantly reduced in group C ($n = 10$, 5312 ± 835/mm², $P = 0.950$) (Figure 7C).

Axonal and Vascular Structures in Core Infarct Region

To evaluate the beneficial effects of the combined treatment of selective brain hypothermia and a craniectomy as regards to altering the axonal structures in the core of the infarction, the specimens were stained with LFB. The histologic evaluation focused on the infarcted caudoputamen (Figure 8, top panel). The data showed completely destroyed axonal bundles in group A compared with the normal specimens (group N). In contrast, the combined treatment (group D) showed more preserved axonal bundles with a normal structure and higher myelin stain intensity when compared with groups A, B, and C. However, none of the treatment groups showed any normal-appearing cellular structures in the core of the infarction.

To examine the effect of the combined treatment on the blood–brain barrier (BBB) in the core of the infarction, the endothelial cell morphology was immunohistochemically assessed using an antibody to EBA (Figure 8, bottom panel). In the infarcted caudoputamen of group A, no EBA-positive vessels were observed, indicating complete BBB disruption. However, group D exhibited

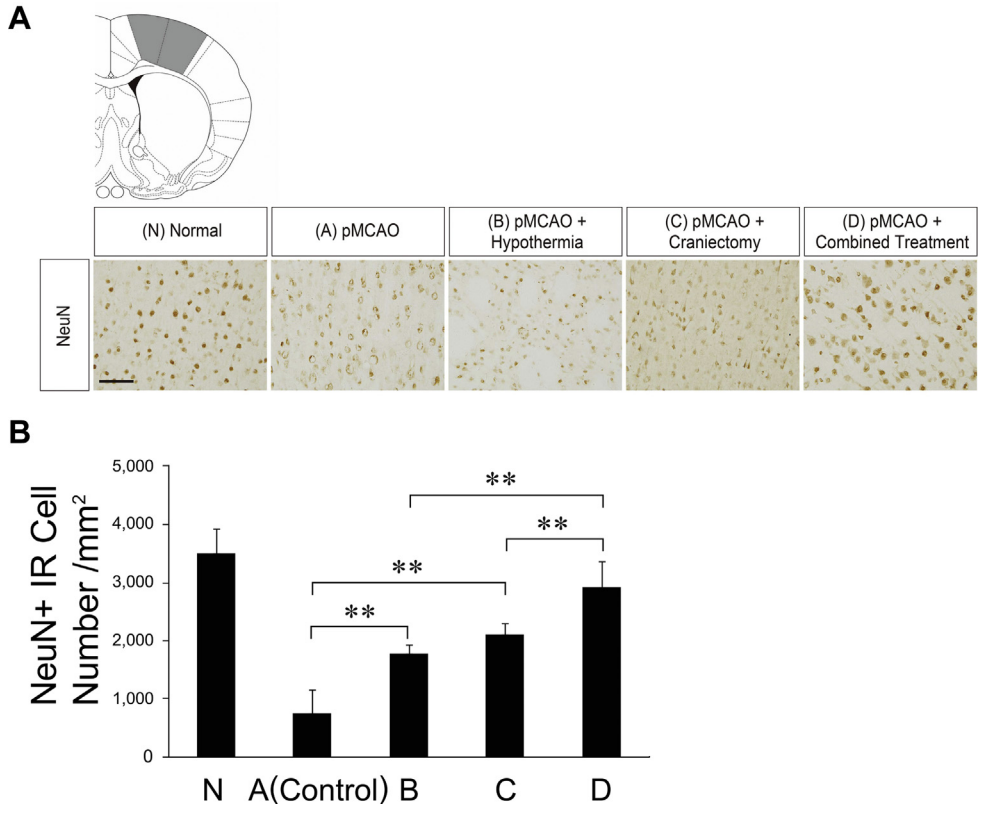

Figure 6. Neuronal preservation in ischemic penumbra 48 hours after permanent middle cerebral artery occlusion (pMCAO). (**A**) Neuronal nuclei immunohistochemistry (NeuN, scale bar 100 μm). The microscopic observation of the primary motor cortex and primary somatosensory cortex was based on brain sections (20 μm) from each group. (**B**) Quantitative analysis of NeuN-immunohistochemistry. Means and standard deviations are presented for all groups. NeuN-immunohistochemistry revealed significant neuronal preservation in group B (1736 ± 180/mm², $P < 0.001$) and group C (2078 ± 274/mm², $P < 0.001$) when compared with that in the control group (755 ± 260/mm²). Furthermore, group D exhibited additional preservation of neuronal cells when compared with group B ($P < 0.001$) and group C ($P < 0.001$). **$P < 0.01$. IR, immunoreactivity to neuronal nuclear protein.

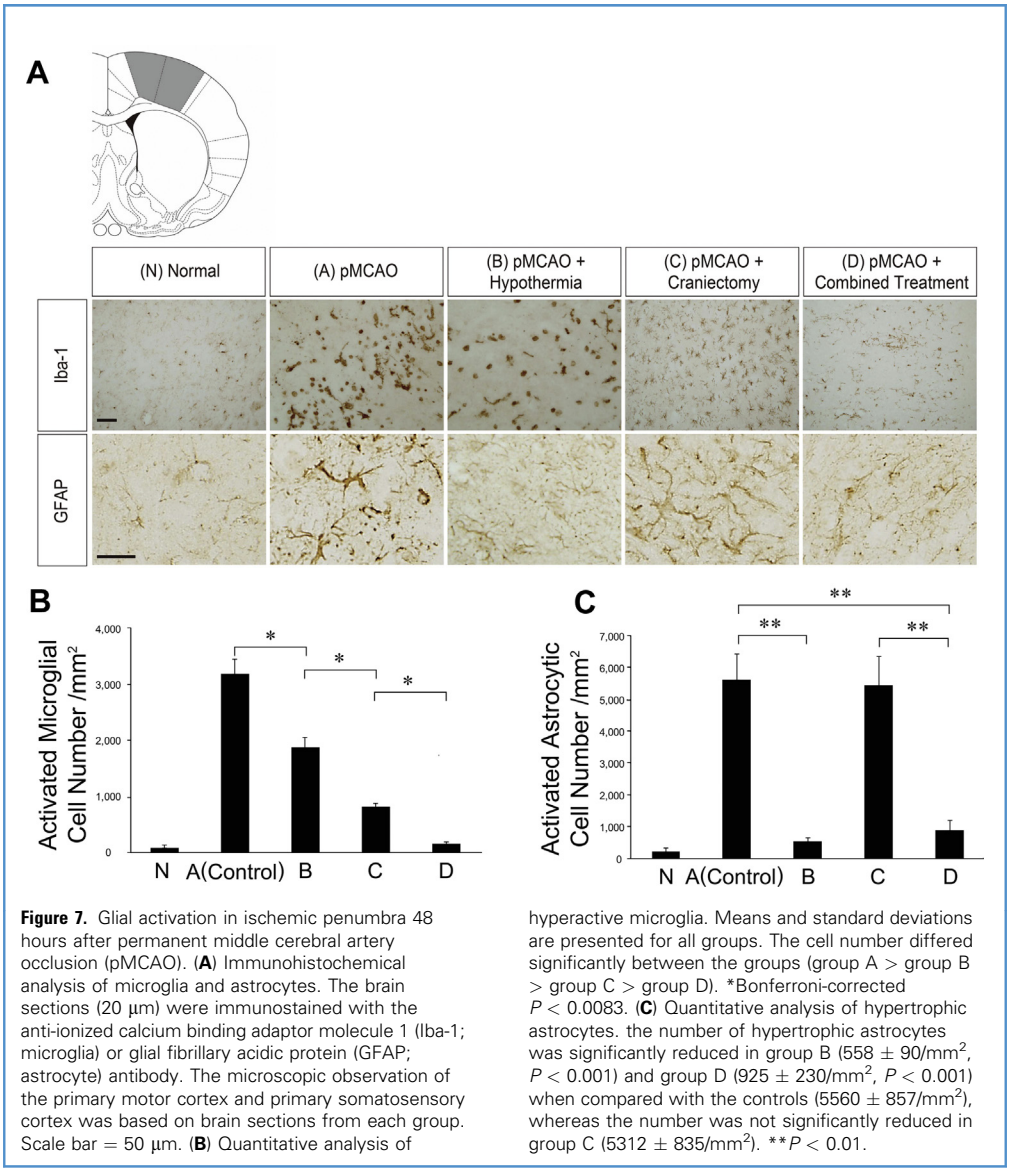

Figure 7. Glial activation in ischemic penumbra 48 hours after permanent middle cerebral artery occlusion (pMCAO). (**A**) Immunohistochemical analysis of microglia and astrocytes. The brain sections (20 μm) were immunostained with the anti-ionized calcium binding adaptor molecule 1 (Iba-1; microglia) or glial fibrillary acidic protein (GFAP; astrocyte) antibody. The microscopic observation of the primary motor cortex and primary somatosensory cortex was based on brain sections from each group. Scale bar = 50 μm. (**B**) Quantitative analysis of hyperactive microglia. Means and standard deviations are presented for all groups. The cell number differed significantly between the groups (group A > group B > group C > group D). *Bonferroni-corrected $P < 0.0083$. (**C**) Quantitative analysis of hypertrophic astrocytes. the number of hypertrophic astrocytes was significantly reduced in group B (558 ± 90/mm^2, $P < 0.001$) and group D (925 ± 230/mm^2, $P < 0.001$) when compared with the controls (5560 ± 857/mm^2), whereas the number was not significantly reduced in group C (5312 ± 835/mm^2). **$P < 0.01$.

more preservation of EBA expression than group A, although the other treatment groups failed to conserve the BBB in the core of the infarction.

DISCUSSION

This experimental study investigated the additional neuroprotective effects on permanent hemispheric infarction when applying selective brain hypothermia as a supplementary treatment to a decompressive craniectomy. The combined treatments were found to reduce the infarct volume and improve the neurologic outcomes. The decrease in the infarct volume was associated with the survival of at-risk neurons and reduced neuroinflammation in the ischemic penumbra.

To date, clinical studies on the use of hypothermia for acute ischemic stroke have been limited to feasibility and remain inconclusive in terms of effectiveness, whereas hypothermia has been shown to be neuroprotective in experimental models of focal ischemic stroke.[7-9] In the case of malignant hemispheric infarction, previous clinical series of systemic hypothermic treatment reported a decrease in the intracranial pressure (ICP).[6,11] Yet, the overall results for systemic hypothermia were worse than those for decompressive surgery due to a higher mortality and higher complication rates. Thus, although prolonged (>10 days) hypothermia can reduce the ICP due to a malignant brain infarct,[11] the high incidence of systemic complications related to whole-body cooling and deficient clinical evidence supporting the beneficial effects of hypothermia explain the low popularity of systemic hypothermia in the clinical field.

Several experimental studies already have explored combining systemic hypothermia and a decompressive craniectomy. When Doerfler et al.[16] and Jieyong et al.[19] applied this combination

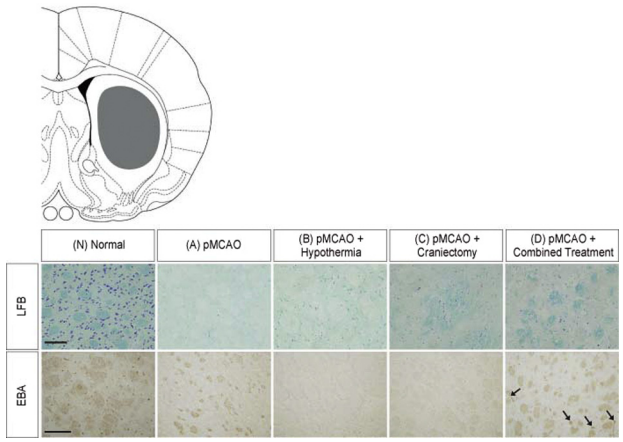

Figure 8. Axonal and vascular structures in infarcted caudoputamen 48 hours after permanent middle cerebral artery occlusion (pMCAO). *Top*: Luxol fast blue (LFB) staining for histologic assessment of axonal density (scale bar 100 μm). *Bottom*: Immunohistochemical assessment of endothelial barrier antigen (EBA)-positive endothelial cells (scale bar = 100 μm). Note the EBA-positive blood vessels (*arrows*) in group D.

treatment with a rat pMCAO model, they reported a reduced infarct volume and improvement of the neurologic outcomes when compared with only a craniectomy.

However, selective brain hypothermia combined with a craniectomy rarely has been investigated. Szczygielski et al.[20] combined a craniectomy and selective brain hypothermia for closed head injuries in mice and reported a decrease in posttraumatic brain edema when compared with only a craniectomy. Plus, Allahtavakoli et al.[21] used a method of local brain cooling for pMCAO by pouring cold saline (2 drops per second) on the exposed rat brain and reported a reduced infarct size and improved neurologic performance. In contrast, the present study used a homeothermic system adjusting the temperature of the cooling coil and a closed system comprising a metal coil, silicone tube, overhead swivel, and peristaltic pump to allow the rats with pMCAO to move freely for 2 days. The present prolonged combined treatment also showed a reduced infarct size and better neurologic scores when compared with only a craniectomy. Moreover, the results revealed more neuronal preservation and reduced neuroinflammation in the ischemic penumbra, along with a greater preservation of axonal bundles and the BBB in the ischemic core.

The therapeutic time window for hypothermic treatment of cerebral ischemia (the optimal duration for hypothermia and maximal postischemic delay for hypothermic application) has not yet been precisely established. This issue depends on the hypothermic method, treatment object, treatment duration, and related complications, which clearly distinguish systemic hypothermia and selective brain hypothermia. Systemic hypothermia for ischemic stroke is commonly started a few hours after stroke onset and applied for 24–48 hours. It reduces the metabolic rate and release of excitatory amino acids and influences the enzyme activity in the ischemic nervous tissue.[22]

In contrast, selective brain hypothermia via a craniectomy site can be started as an adjuvant treatment after a craniotomy 1–3 days after stroke onset and applied for a more prolonged period.[23] The effects of such selective hypothermia are still not elucidated in clinical settings. However, it could reduce secondary brain injuries occurring in the subacute phase of a stroke lasting one to several days postischemia. The generation of reactive oxygen species by injured cells, activated inflammatory responses, apoptosis, BBB disruption, and edema formation could be reduced by hypothermia during this period.[24] In particular, neuroinflammation is a crucial element in the ischemic cascade, leading to cell damage in the subacute phase of a stroke. The activation of microglia and astrocytes with the subsequent production of inflammatory mediators, including reactive oxygen, nitrogen species, adhesion molecules, proinflammatory cytokines, and chemokines attracting extravasated leukocytes, results in cell damage and death.[25,26] Thus, combining selective brain hypothermia and a craniectomy may help to increase the survival of at-risk neurons in the ischemic penumbra in addition to decreasing the ICP.[27]

In the present study, the size of the ischemic core decreased in the order of group A > group B > group C > group D. The number of activated microglia also was reduced in the order of group A > group B > group C > group D. This is explained by the gradual migration and accumulation of microglial cells and macrophages into the core region after a stroke. Meanwhile, activated astrocytes are known to accumulate in the peri-infarct region surrounding the core region, yet not inside the ischemic core.[28] This different behavior of activated astrocytes can partially explain the different number of activated astrocytes in groups B and C when compared with the number of activated microglial cells.

The effect of selective brain cooling on the cortical cerebral blood flow is of critical concern. In the present study, the temperature of the cooling coil was adjusted to 20°C to achieve the target hypothermic brain temperature (~33°C). The cooling coil temperature, 20°C, seems to have a beneficial effect on the cortical cerebral blood flow. In a previous study by Ogura et al.,[29] hypothermia at 20–35°C was found to dilate the intracerebral arterioles in rats in a temperature-dependent manner. In addition, Kuluz et al.[30] reported a 2-fold baseline increase in cortical cerebral blood flow at a cortical brain temperature of 30.9°C in a study using rats.

For systemic hypothermic treatment, the benefit to humans has not yet been established despite well-documented beneficial effects in rodent models.[31] However, the human application of systemic hypothermic treatment involves several clinical issues not represented in animal models.[32] Systemic cooling takes longer in humans, and typical stroke patients may not be able to tolerate systemic hypothermia due to old age and comorbidity. Systemic hypothermia in humans also can induce such unfavorable effects as shivering, immune suppression, pneumonia, and cardiovascular events.

It is also questionable whether the beneficial results of selective brain hypothermia in a rodent model can have clinical implications and benefits for humans. Therefore, the current study has several limitations that should be considered before extrapolating and applying the results to the case of a human stroke. First, the volume of the cerebral hemisphere is very small in rats and the whole MCA territory was cooled by cerebral surface cooling. In the

case of a human brain, the cooling depth is limited and can be affected by local cooling methods. Second, the time course of human selective brain hypothermia combined with a decompressive craniectomy would differ considerably from that used in the current study. As most decompressive hemicraniectomies are performed 1–3 days after pMCAO,[23] selective brain hypothermia in humans would be more delayed and the duration even prolonged to more than a week.

Thus, efficient clinical application of a decompressive craniectomy with selective brain hypothermia requires further studies to devise appropriate surgical techniques and hypothermic technology. For example, a decompressive craniectomy including resection of the temporalis muscle, originally devised to maximize external herniation of an infarcted swollen brain and achieve greater decompression, could facilitate external cooling of the brain via the operative wound.[33]

CONCLUSIONS

A craniectomy reduced the infarct volume and improved the neurologic outcomes in a rat model of permanent MCA infarction. Furthermore, when selective brain hypothermia was combined as a supplementary treatment for a prolonged duration, significant additional neuroprotection was observed. This included a reduced infarct volume and improved neurologic outcomes, which were related to more neuronal preservation, reduced neuroinflammation, and a greater preservation of axonal bundles and the BBB.

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