Protective Effects and Magnetic Resonance Imaging Temperature Mapping of Systemic and Focal Hypothermia in Cerebral Ischemia

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Background and Purpose—Hypothermia is potentially the most effective protective therapy for brain ischemia; however, its use is limited because of serious side effects. Although focal hypothermia (FH) has a significantly lower stress profile than systemic hypothermia (SH), its efficacy in ischemia has been poorly studied. We aimed to compare the therapeutic effects of each treatment on various short- and long-term clinically relevant end points.

Methods—Sprague–Dawley rats were subjected to transient (45 minutes) occlusion of the middle cerebral artery. One hour after arterial reperfusion, animals were randomly assigned to groups for treatment with SH or FH (target temperature: 32°C) for 4 or 24 hours. Lesion volume, edema, functional recovery, and histological markers of cellular injury were evaluated for 1 month after ischemic injury. Effects of SH and FH on cerebral temperature were also analyzed for the first time by magnetic resonance thermometry, an approach that combines spectroscopy with gradient-echo–based phase mapping.

Results—Both therapeutic approaches reduced ischemic lesion volume ($P<0.001$), although a longer FH treatment (24 hours) was required to achieve similar protective effects to those induced by 4 hours of SH. In addition, magnetic resonance thermometry demonstrated that systemic hypothermia reduced whole-brain temperature, whereas FH primarily reduced the temperature of the ischemic region.

Conclusions—Focal brain hypothermia requires longer cooling periods to achieve the same protective efficacy as SH. However, FH mainly affects the ischemic region, and therefore represents a promising and nonstressful alternative to SH.
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Key Words: focal hypothermia ■ ischemia ■ magnetic resonance imaging ■ middle cerebral artery ■ systemic hypothermia ■ temperature

Hyperthermia is highly correlated with poor outcomes in patients with stroke,[1,2] whereas therapeutic hypothermia is considered a promising therapeutic strategy in patients with ischemic stroke.[3,4] However, systemic (whole body) hypothermia (SH) has not yet been implemented as a routine treatment in patients with ischemic stroke, largely because of side effects including shivering, hypotension, arrhythmia, or infection that complicate the clinical management of these patients.[5] Therefore, new strategies to improve the management of therapeutic hypothermia and reduce its adverse effects are urgently needed. In this regard, brain focal hypothermia (FH) has been proposed as a promising alternative to SH[6] because selective hypothermia induction in the focal ischemic area may provide similar protection while achieving faster cooling and avoiding systemic side effects. In fact, recent studies using novel devices to induce FH have shown promising results for stroke therapy.[7,8] However, no study to date has evaluated the comparative efficacies of SH and FH in patients with ischemic stroke.

Previous experimental studies in this field have mainly focused on the optimization of individual parameters of SH treatment, such as the therapeutic time window or treatment duration.[9–14] The results of these studies, as well as those of a meta-analysis study,[3] indicate that a target temperature of 32 to 34°C should be induced as soon as possible after stroke onset. Thus far, only one previous work has suggested that FH

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requires at least 48 hours to elicit protective effects.[12] However, many of these studies bear some limitations, most notably the use of invasive probes, which sample only a restricted local volume of tissue, to confirm the effects of hypothermia in the cerebral ischemic region. In addition, opening the skull for probe insertion may itself alter temperature dynamics. Thus, an accurate and noninvasive procedure is needed to measure the temperature in the ischemic area during therapeutic hypothermia.

The development of magnetic resonance thermometry (MRT) has shown that brain temperature can be measured noninvasively by means of magnetic resonance spectroscopy, which in combination with magnetic resonance imaging (MRI) provides spatial temperature distribution data across the brain, including ischemic and normal tissue, with sufficient reliability for group comparisons.[15,16] Utilizing the principle that the water frequency shift relative to *N*-acetyl aspartate, creatine, or choline is temperature dependent,[15,16] MRT has been validated as a noninvasive assessment of brain temperature in patients with stroke. MRT provides fast temperature measurement with 3-dimensional imaging coverage, and an accuracy of 0.1/0.2°C, allowing for analysis of either changes in or absolute brain temperature.[17,18]

Therefore, the aim of the present work was to analyze the protective effects of optimized SH and FH protocols in a multimodal comparative fashion, using these clinically relevant MR-based techniques in an animal model of ischemia. We evaluated the long-term progress of animals undergoing each procedure using MRI, in combination with functional tests and histological analysis of molecular processes involved in the pathogenesis of ischemic injury, including neuronal loss, early inflammation, and lesion scar formation. We also determined the effects of each hypothermic treatment on brain temperature using both invasive (temperature probes) and noninvasive strategies (MRT).

Materials and Methods

Animals

All experimental protocols were approved by the local Animal Care Committee according to the guidelines established by the European Union (86/609/CEE, 2003/65/CE, and 2010/63/EU). Male Sprague–Dawley rats weighing between 280 and 330 g were used. Animals were housed individually at an environmental temperature of 23°C, with 40% relative humidity and a 12 hours light–dark cycle, and they were given free access to food and water.

Cerebral Ischemia

Transient focal ischemia (45 minutes) was induced by intraluminal occlusion of the middle cerebral artery, following the method described previously.[19] Cerebral blood flow was monitored during surgery, and a baseline MRI evaluation was performed during the occlusion period, before hypothermic treatment. Details about surgical materials and protocols, as well as MRI, are described in the online-only Data Supplement.

Experimental Design

After arterial reperfusion, animals were randomly assigned to the following experimental groups: systemic control group, 4 hours of treatment duration (SC-4h; n=12); SH group, 4 hours of treatment duration (SH-4h; n=12); focal control group, 24 hours of treatment duration (FC-24h; n=12); and FH group, 24 hours of treatment duration (FH-24h; n=12). Using the same randomized protocol, a second study included the following experimental groups: focal control group, 4 hours of treatment duration (FC-4h; n=6) and FH group, 4 of hours treatment duration (FH-4h; n=6). In addition, 3 FC-24h animals and 3 FH-24h animals were included.

SH and FH protocols were selected based on previous studies[9–13] and are described below and in the online-only Data Supplement. Treatments began 1 hour after reperfusion; the target temperature in both methods was 32°C. In all groups, MRI was used to determine the ischemic lesion volume and evaluate edema formation during the occlusion period, before hypothermia treatment (baseline lesion volume), and at 24 hours, 7 days, and 30 days after treatment. Functional outcomes were assessed in the SC-4h, SH-4h, FC-24h, and FH-24h groups using the cylinder test before surgery and 48 hours, 6 days, and 29 days after surgery. Three animals per group were euthanized for histological analyses at 24 hours, 7 days, and 30 days after surgery.

We also analyzed SH- and FH-induced changes in body and brain temperature using implantable probes and MRT in separate groups of animals.

Body rewarming rates after SH were measured using implantable temperature sensors inserted into the peritoneal cavity (n=3 animals). To measure brain temperature during FH or SH, temperature probes were implanted into both hemispheres (n=3 per group). Detailed procedures for the above implantations are described in the online-only Data Supplement.

MRT was used to measure brain temperature in normothermic, SH, and FH groups of animals (n=3 per group) after middle cerebral artery occlusion. Ischemic lesion was confirmed by MRI in all animals 1 day after ischemic injury.

Exclusion Criteria

The following exclusion criteria were used: (1) <70% reduction in relative cerebral blood flow; (2) arterial malformations, as determined by magnetic resonance angiography; (3) baseline lesion volume of <25% or >45% with respect to the ipsilateral hemisphere, as measured using apparent diffusion coefficient maps; (4) absence of reperfusion or prolonged reperfusion (>10 minutes until achievement of at least 50% of the baseline cerebral blood flow) after filament removal; and (5) failure to complete treatment. All excluded or deceased animals were replaced until the total number of animals indicated for each group was attained.

SH Procedure

SH was induced in anesthetized animals 1 hour after reperfusion, for a 4-hour period, using a rectal thermostat-controlled electric pad (NeosBiotec, Pamplona, Spain). After treatment, animals were allowed to rewarm spontaneously. Details about the SH protocol and monitoring are described in the online-only Data Supplement.

FH Procedure

FH was induced 1 hour after arterial reperfusion, for 4 hours or 24 hours of periods, using a previously described cooling device,[20] with some modifications (Figure I in the online-only Data Supplement). Cooling devices were removed immediately after the treatment period. Details about the FH cooling device, protocol, and monitoring are described in supplementary material.

Brain MRT

All MR measurements were performed using a 9.4 T MR system (BrukerBioSpin, Billerica, MA). Our MRT approach consisted of single-voxel magnetic resonance spectroscopy combined with chemical shift imaging[18] and gradient-echo–based phase mapping,[17] all of which rely on temperature-dependent shifts in the water resonance frequency. Details about experimental conditions, sequences, and data processing are described in the online-only Data Supplement and Figures II and III in the online-only Data Supplement.

MRI Assessment and Data Analysis

MRI assessments and analyses were performed according to a previously described protocol.[21] In brief, a baseline MRI evaluation was performed during the occlusion period, including magnetic resonance angiography to confirm middle cerebral artery occlusion, and diffusion tensor imaging with a spin-echo echo-planar imaging sequence to obtain diffusion-weighted images and calculate apparent diffusion coefficient maps for measurement of baseline lesion volume. To analyze lesion volume evolution, T2-weighted images were acquired 24 hours, 7 days, and 30 days after ischemic injury. All image processing was performed with ImageJ (Rasband WS, National Institutes of Health, Bethesda, MD). Additional details about MRI sequences and data analysis are described in the online-only Data Supplement.

Functional Assessment

Functional outcome was evaluated using the cylinder test (online-only Data Supplement).

Brain Histological Analysis

Twenty-four hours after ischemic injury, neurons were immunofluorescently labeled with anti-NeuN antibodies and further stained via terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) to identify dying neurons. Seven days after injury, NeuN labeling was combined with labeling for Iba1, a microglia- and macrophage-expressed inflammatory marker. Finally, 30 days after ischemic injury, NeuN labeling was combined with labeling for the astrocyte marker glial fibrillary acidic protein (GFAP). In addition, lesion scar composition was assessed using glial and fibrotic markers (GFAP and vimentin, respectively). Detailed protocols are described in the online-only Data Supplement.

Statistical Analysis

All data are presented as the mean and SEM (mean±SEM). One-way or 2-way ANOVA followed by the post hoc Bonferroni test was used to identify significant differences in multiple comparisons. The Student *t* test was used to identify significant differences between the 2 groups. Statistical significance was set at $P<0.05$. Statistical analyses were conducted using SPSS Statistics for Macintosh, version 18.0 (IBM, Armonk, NY).

Results

A total of 121 animals were used in this study (Figure IV in the online-only Data Supplement): 100 to compare SH and FH and 21 to assess body and brain temperature during SH and FH procedures. A total of 34 animals were excluded from the comparative study. Twenty-seven animals were excluded after ischemic injury because of nonoptimal baseline lesion volume (17 animals; Figure VA through VC in the online-only Data Supplement), arterial malformations (2 animals; Figure VD in the online-only Data Supplement), or surgical complications (8 animals). Seven animals were excluded during the hypothermia procedures, 4 animals undergoing FH removed the cooling device, and 3 animals undergoing SH died from respiratory complications. No differences in blood glucose levels or weight were observed between the different experimental groups (data not shown).

Invasive Analysis of Body and Brain Temperature During SH

SH was rapidly induced with a decrease in rectal temperature from 37 to 32°C in <15 minutes. The average rectal temperature during SH was 32.3±0.3°C, whereas the average rectal temperature in the systemic control group was 36.9±0.5°C. After the SH procedure, animals were returned to their home cages to spontaneously rewarm. The rewarming rate, measured using an implanted temperature sensor was ≈0.03°C/min (Figure VI in the online-only Data Supplement). Approximately 150 minutes was required for recovery to normothermia. SH caused a reduction in whole-brain temperature that was similar to the observed decrease in body temperature (Figure VIIA in the online-only Data Supplement).

Invasive Analysis of Brain Temperature During FH

An implanted focal cooling device (Figure VIIB in the online-only Data Supplement) did not induce a significant alteration in cerebral temperature in the ipsilateral hemisphere compared with the contralateral hemisphere before cold-water perfusion began. However, when cold water was perfused through the device, a rapid decrease in temperature in the ipsilateral hemisphere was observed (35 to 32°C in 15 minutes). After this initial reduction, the temperature remained stable (31.9±0.4°C). Ipsilateral hemisphere cooling only minimally affected the temperature in the contralateral hemisphere and did not affect body temperature. When cold-water perfusion was stopped, the ipsilateral region spontaneously rewarmed to the baseline temperature in <7 minutes (approximated rewarming rate: 0.5°C/min).

MRT Analysis of Brain Temperature During SH and FH

The effects of normothermic conditions, SH, and FH on brain temperature were determined noninvasively by means of MRT. Similar to the invasive analysis results above, MRT demonstrated that the whole-brain temperature (measured in region of interests [ROIs] 1–3) was similar to the body temperature under normothermic conditions (T_{body}=37.2°C; Figure 1A). However, SH reduced both the whole-brain (32–33°C) and body (T_{body}=32.6°C) temperatures. By contrast, FH reduced the brain temperature from 33 to 32°C in the ischemic region where the cooling device was located (ROI3), but only minimally affected the healthy tissue (ROIs 1–2) or body temperature (T_{body}=35.6°C).

Brain temperature changes because of normothermia, FH, and SH were also accurately determined using gradient-echo–based phase mapping, with a precision of±0.4°C (Figure 1B). In the normothermic group (ΔT_{body}=37.0–37.6°C), minimal temperature changes (ΔT) were observed (ΔT_{ROI1} and ΔT_{ROI2}=0.3°C). In contrast, SH (ΔT_{body}=36.8 to 32°C) resulted in ΔT_{ROI1}=3.9°C and ΔT_{ROI2}=3.7°C. Finally, in the FH group (ΔT_{body}=36–35.7°C), a minimal temperature change was observed in the nonischemic region (ΔT_{ROI1}=1.1°C), in contrast to the larger change in the ischemic region (ΔT_{ROI2}=3.7°C).

Protective Effects of SH and FH Against Ischemic Damage

According to our inclusion criteria, the baseline lesion volume in each animal, determined using apparent diffusion coefficient maps, was between 25% and 45% of the ipsilateral hemisphere: 40.5±1.2% in the SC-4h group, 34.0±1.2% in the SH-4h group, 35.8±1.6% in the FC-24h group, and 34.7±1.8% in the FH-24h group (Figure 2A and 2B; Figure VIII in the online-only Data Supplement). Statistical analysis

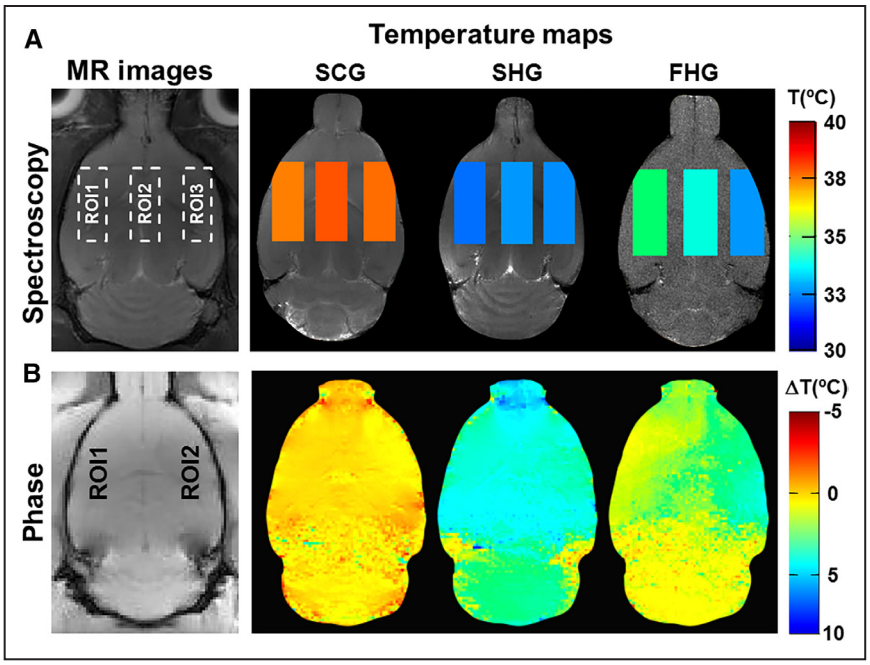

Figure 1. In vivo magnetic resonance (MR) brain temperature mapping in normothermia, systemic hypothermia (SH), and focal hypothermia (FH) in ischemic animals. ROIs indicate where temperature changes were recorded. **A**, RARE T2-weighted image (**left**) and absolute temperature determinations using magnetic resonance spectroscopy (**right**) for each group. **B**, Multiple gradient-echo image (**left**) and brain temperature changes (**right**) determined using gradient-echo–based phase mapping in each group (n=3, per group). ROI indicates region of interest; and SC, systemic control.

of the infarct volumes in both SH- and FH-treated groups compared with their respective control groups showed a significant reduction at the different time points: at 24 hours, $P<0.001$; at 7 days, $P<0.001$, and at 30 days, $P<0.01$); this suggests that hypothermia-mediated protection persisted ≤30 days after ischemic injury. Expressing infarct volume as a percentage of basal lesion volume produced the same results (Figure IX in the online-only Data Supplement).

To determine whether 24 hours of FH were needed to achieve protective effects similar to those induced by 4 hours of SH, an independent group of ischemic animals were treated with 4 hours of FH. Infarct volume analysis showed that a 4 hours of FH treatment did not reduce infarct volume relative to the FC-4h group ($P>0.05$; Figure 2A and 2B; Figure X in the online-only Data Supplement).

Effects of SH and FH on Edema Formation

Edema formation was maximal 24 hours after ischemic injury (Figure 2C), and both SH (4 hours) and FH (24 hours) significantly reduced edema with respect to their control groups ($P<0.05$). However, 4 hours of FH failed to reduce edema formation.

Analysis of Functional Deficits

Ischemic injury was associated with neurological deficits, which were evaluated using the cylinder test 48 hours, 6 days, and 29 days after ischemic injury (Figure XI in the online-only Data Supplement). Although not significantly different, cylinder test scores were asymmetrical, such that the SH-4h and FH-24h groups had better scores (ie, increased recovery) mainly at 29 days after injury relative to their respective control groups.

Brain Histological Analysis After SH and FH

A histological analysis performed 24 hours after ischemic injury revealed areas of TUNEL-positive (dead or dying) cells in the striatum and cortex in both control groups, whereas TUNEL-positive cells were mainly found in the striatum in both hypothermia-treated groups (Figure 3). The majority of TUNEL-positive cells colocalized with NeuN immunoreactivity, indicating neuronal death in the ischemic region. This distribution was consistent with the hyperintense areas observed in T2 maps and T2-weighted MR images recorded 24 hours after injury.

Seven days after ischemic injury, NeuN immunoreactivity had disappeared from the injured area (Figure 4), where dead cells had previously been observed (Figure 3). The distribution of Iba1 immunoreactivity corresponded to these NeuN-negative areas.

At 30 days after injury, the lesion cores in the control groups were negative for GFAP and NeuN immunoreactivity; however, the lesion cores in both hypothermic groups included GFAP-positive cells (Figure 5).

Lesion scars were also studied 30 days after injury (Figure 6). Markers of the 2 main components of the lesion scar were studied: GFAP as a marker of the glial component, and vimentin, an intermediate filament protein expressed in fibroblast-like cells, as a marker of the fibrotic component. Control animals showed a clear distinction between the vimentin-positive lesion core and the uninjured areas, where only GFAP immunoreactivity was observed. The peri-infarct region was characterized by the colocalization of these markers, indicating reactive astrogliosis. In contrast, the lesion cores in hypothermia-treated animals did not show this stratification. The area identified as the lesion core was positive for both markers, similar to the peri-infarct areas of untreated animals. Thus, the primarily fibrotic phenotype observed in the lesion cores of control animals was not observed in hypothermia-treated animals.

Discussion

SH is considered the most promising neuroprotective therapy for cerebral ischemia. However, clinical success in patients

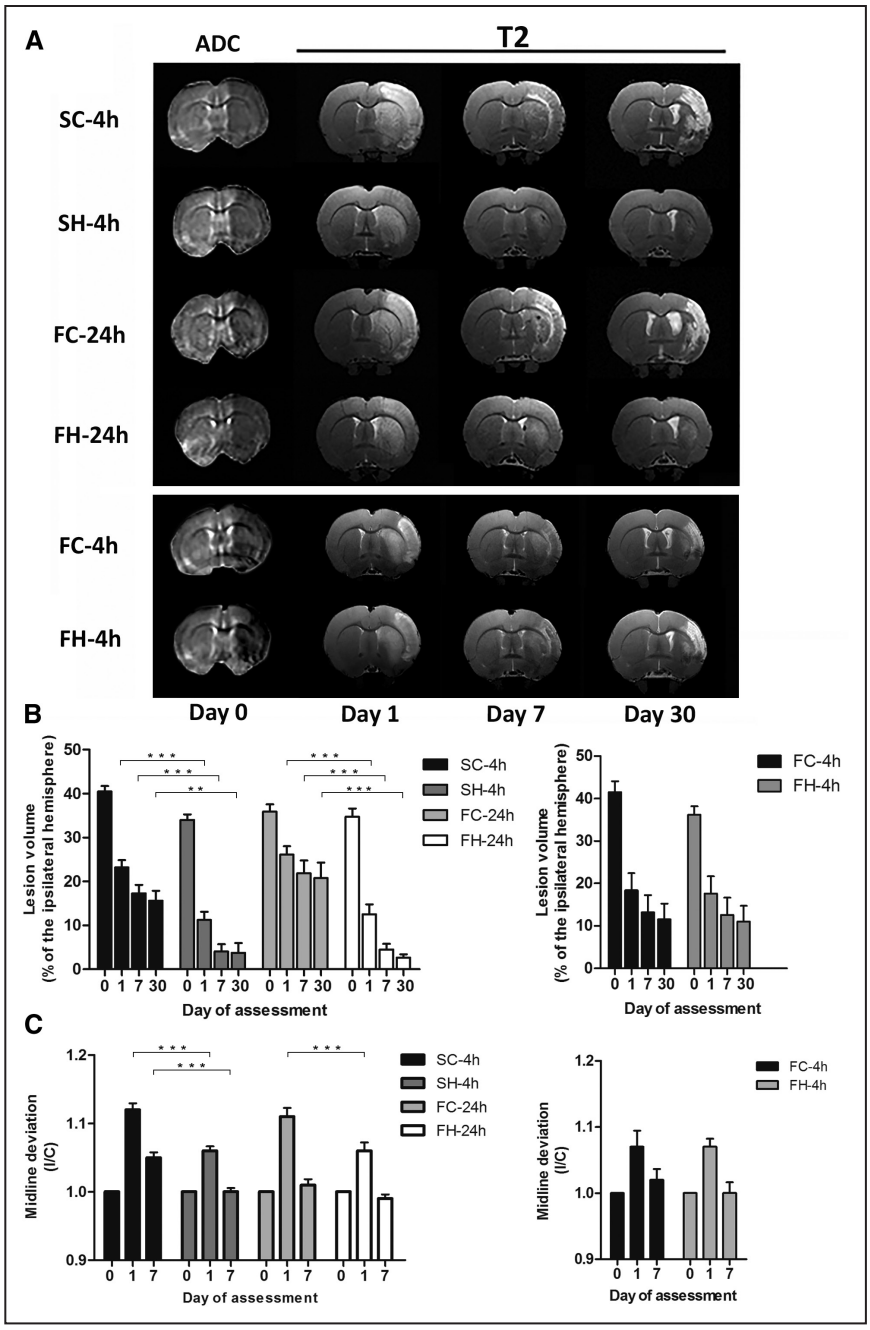

Figure 2. Magnetic resonance imaging (MRI) assessments of ischemic injury evolution. apparent diffusion coefficient (ADC) maps were recorded before treatment to ensure that all animals included in the study were subjected to similar levels ischemic damage. Lesion volume evolution was assessed using T2-weighted images recorded 24 hours, 7 days, and 30 days after ischemia induction. **A**, MRI assessment of animals subjected to 4 hours of systemic hypothermia (SH) or 24 and 4 hours of focal hypothermia (FH). **B**, Quantitative analysis of lesion volumes of animals subjected to 4 hours of SH or 24 and 4 hours of FH. **C**, Edema evolution in animals subjected to 4 hours of SH or 24 and 4 hours of FH. Data are shown as mean±SEM.*P<0.05, **P<0.01, ***P<0.001; using 2-way ANOVA followed by the post hoc Bonferroni test (n=12 for baseline and 24-h assessments, n=9 for 7 d assessment, and n=6 for 30 d assessment in the systemic control (SC)-4h,SH-4h, focal control (FC)-24h, and FH-24h groups; n=6 in the FC-4h and FH-4h groups).

with acute ischemic stroke has not yet been attained, most likely because of the considerable side effects associated with systemic cooling. Thermoregulation is a highly efficient physiological mechanism that remains intact even during severe pathophysiological processes, such as ischemic stroke. In consequence, therapeutic hypothermia is useful in patients who are anesthetized or in a deep coma, but it is complicated in most patients with ischemic stroke, mainly by the side effects of vasoconstriction and shivering, which may interfere with the beneficial effects of the treatment. In fact, induction of therapeutic hypothermia requires the use of additional pharmacological tools to inhibit thermoregulatory mechanisms.[22] These clinical limitations could be observed in our study, since animals subjected to SH had to be anesthetized to induce SH treatment. Previous studies have also probed the effect of SH in awake animals using fans and fine water misters for cooling, and an overhead infrared lamp for warming, with beneficial results in ischemic animal models.[11,12] However, like humans, adult rats have an efficient thermoregulation mechanism. In our hands, inducing SH in awake animals is technically demanding, and the environmental conditions are extremely stressful for the animals. Therefore, we decided to test SH under anesthetized conditions that allowed us to accurately control body temperature, and that reduced the hypothermic stress on the animals. In fact, this setup is more clinically relevant, and thus more easily translated, than awake-animal protocols.

Novel techniques mainly based on surface and endovascular cooling procedures have been developed and tested in

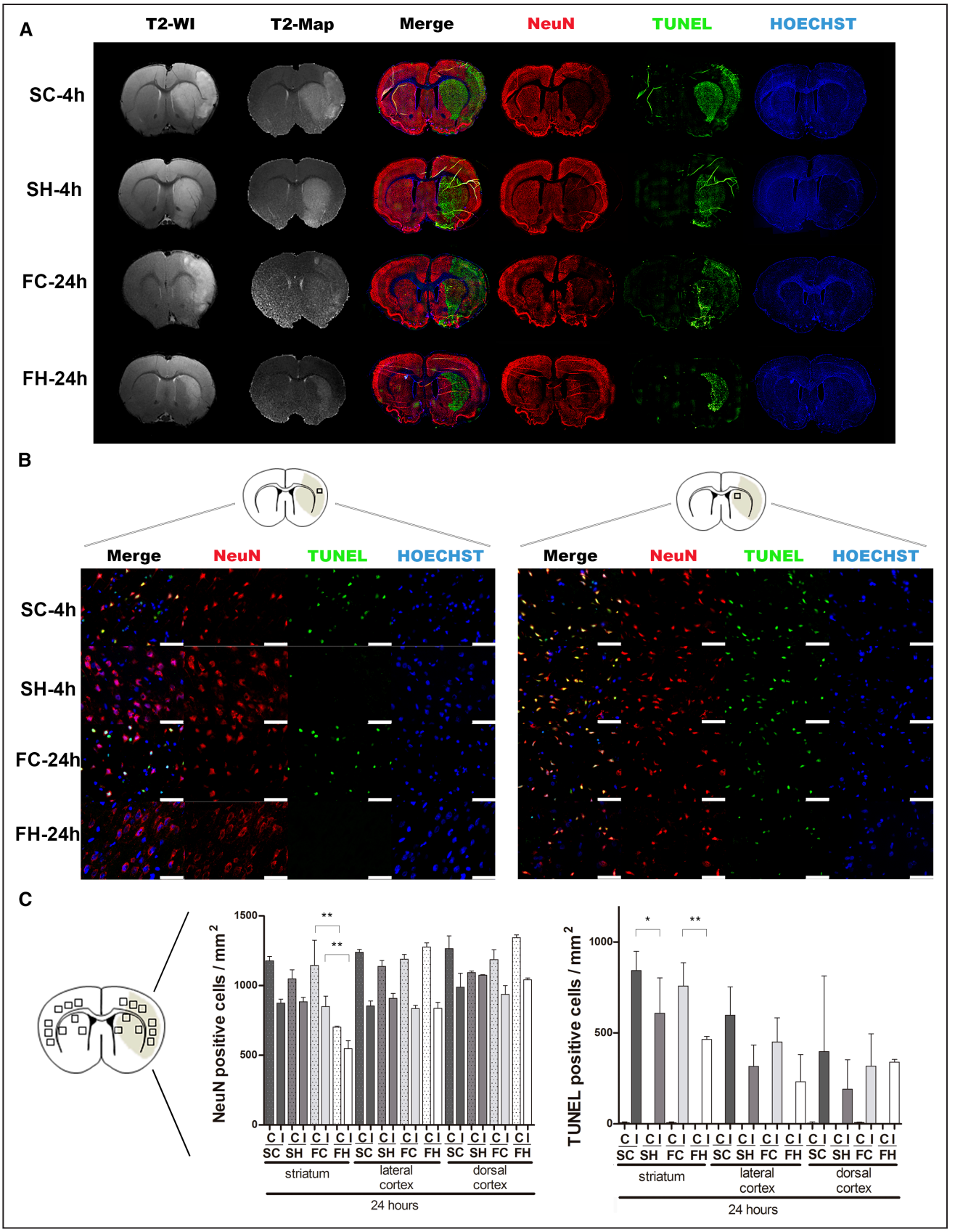

Figure 3. Histological marker expression 24 hours after ischemia. Immunofluorescent labeling of neurons (NeuN, red), dead cells (TUNEL, green), and nuclei (Hoechst, blue) 24 hours after ischemia in the systemic control (SC)-4 hours, systemic hypothermia (SH)-4 hours, focal control (FC)-24 hours, and focal hypothermia (FH)-24 hours groups. **A**, Whole-brain reconstructions from multiple (*Continued*)

Figure 3 Continued. photomicrographs showing the general distribution of each marker, merged images, and correspondence with T2-weighted magnetic resonance images and maps. **B**, High-magnification (×400) photomicrographs showing representative cortical and striatal immunoreactivity patterns in the 4 principal treatment groups. **C**, Neuron density and apoptotic cell density in different brain regions. Scale bars, 50 μm. C indicates contralateral hemisphere; I, ipsilateral hemisphere; and TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling. Data are shown as mean±SEM. **P*<0.05, ***P*<0.01, ****P*<0.001; using 2-way ANOVA followed by the post hoc Bonferroni test (n=3, per group).

patients with stroke to optimize SH protocols for clinical use. Several trials are ongoing, although no clear evidence has been obtained thus far.[6] However, current research efforts aim to develop safer and more effective SH therapies, the alternative use of FH to rapidly induce hypothermia in the ischemic region had been poorly studied. In this study, we have compared SH and FH protocols featuring commonly used target temperature, therapeutic window, and duration parameters. We demonstrate that, when initiated 1 hour after arterial reperfusion, FH in awake animals induces similar protective effects to those of SH against ischemic damage. Efficacy is dependent on treatment duration; FH needs to be maintained for at least 24 hours to achieve protective effects similar to those of 4 hours of SH. Edema, which frequently occurs during the acute phase of stroke, hampers the effects of hypothermia and is closely related to patient outcome; however, it too was reduced by both methods.

Other beneficial effects of therapeutic hypothermia include reduction of neuronal death and attenuation of the inflammatory response.[23] Thus, we analyzed neuronal loss and microglia and macrophage activation during the follow-up period in control and hypothermia-treated animals. Histological assessment of the brain samples confirmed similar protective efficacies for SH and FH. In addition, analysis of the residual ischemic lesion in the control groups at 30 days after the intervention showed that the strong fibrotic phenotype observed in the control animals, indicated by high vimentin immunoreactivity, was attenuated in both hypothermia-treated groups. Lesion scar development is a critical factor that influences the regenerative potential in the central nervous system. Scar formation seems to play dual roles in isolating the injured area and limiting inflammation; however, this isolation may also impede the regenerative process.[24] In this regard, the hypothermia-induced decrease in fibrotic lesion scar formation may contribute to regeneration of the damaged tissue, as has been described by other similar studies.[23]

In a separate comparison of hypothermia-induced temperature changes measured using MRT and invasive temperature probes, we showed that SH reduced both body and brain temperatures to 32°C, whereas FH induced a similar temperature reduction mainly in the ischemic region. As mentioned, FH required 6-fold longer treatment than SH to achieve a similar effect. Comparing local and SH is difficult because it is hard to perfectly match treatment protocols (same temperature profile in the brain, same cooling and rewarming rate, etc.). However, this disparity suggests that the protective effects of SH may include noncerebral physiological mechanisms, whereas the

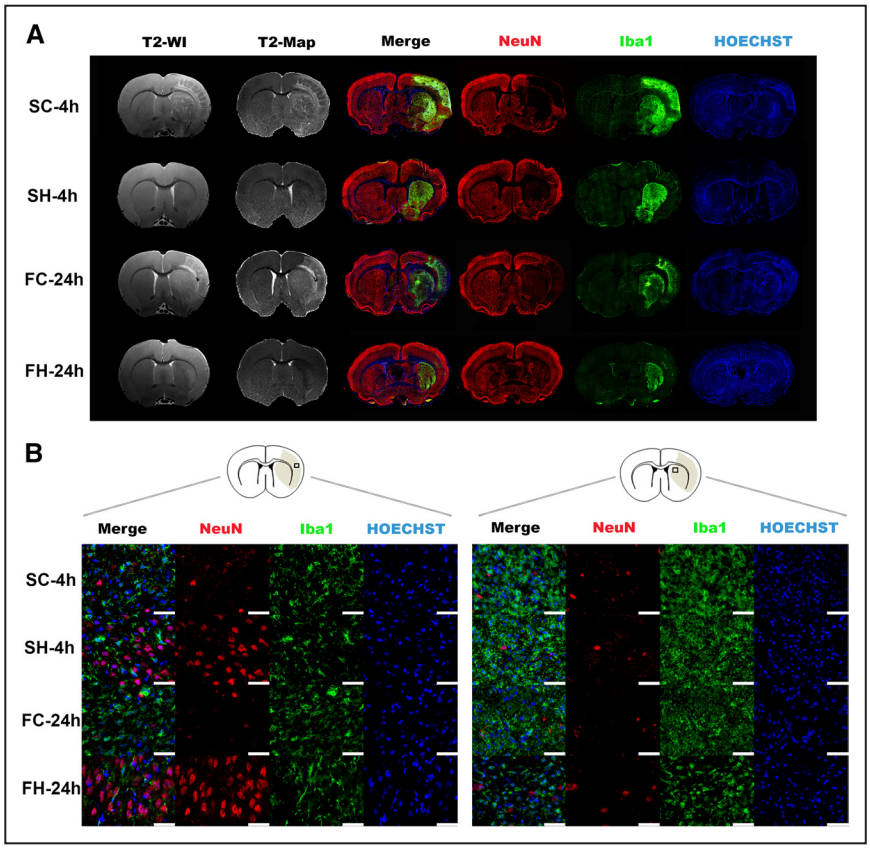

Figure 4. Histological marker expression 7 days after ischemia. Distribution of neurons (NeuN, red), Iba1-labeled microglia and macrophages (green), and nuclei (Hoechst, blue) 7 days after ischemia in systemic control (SC)-4 hours, systemic hypothermia (SH)-4 hours, focal control (FC)-24 hours, and focal hypothermia (FH)-24 hours groups. **A**, Whole-brain reconstructions from multiple photomicrographs showing the general distribution of each marker, merged images, and correspondence with T2-weighted magnetic resonance images and maps. **B**, High-magnification (×400) photomicrographs showing representative immunoreactivity patterns in the cortex and striatum. Scale bars, 50 μm (n=3, per group).

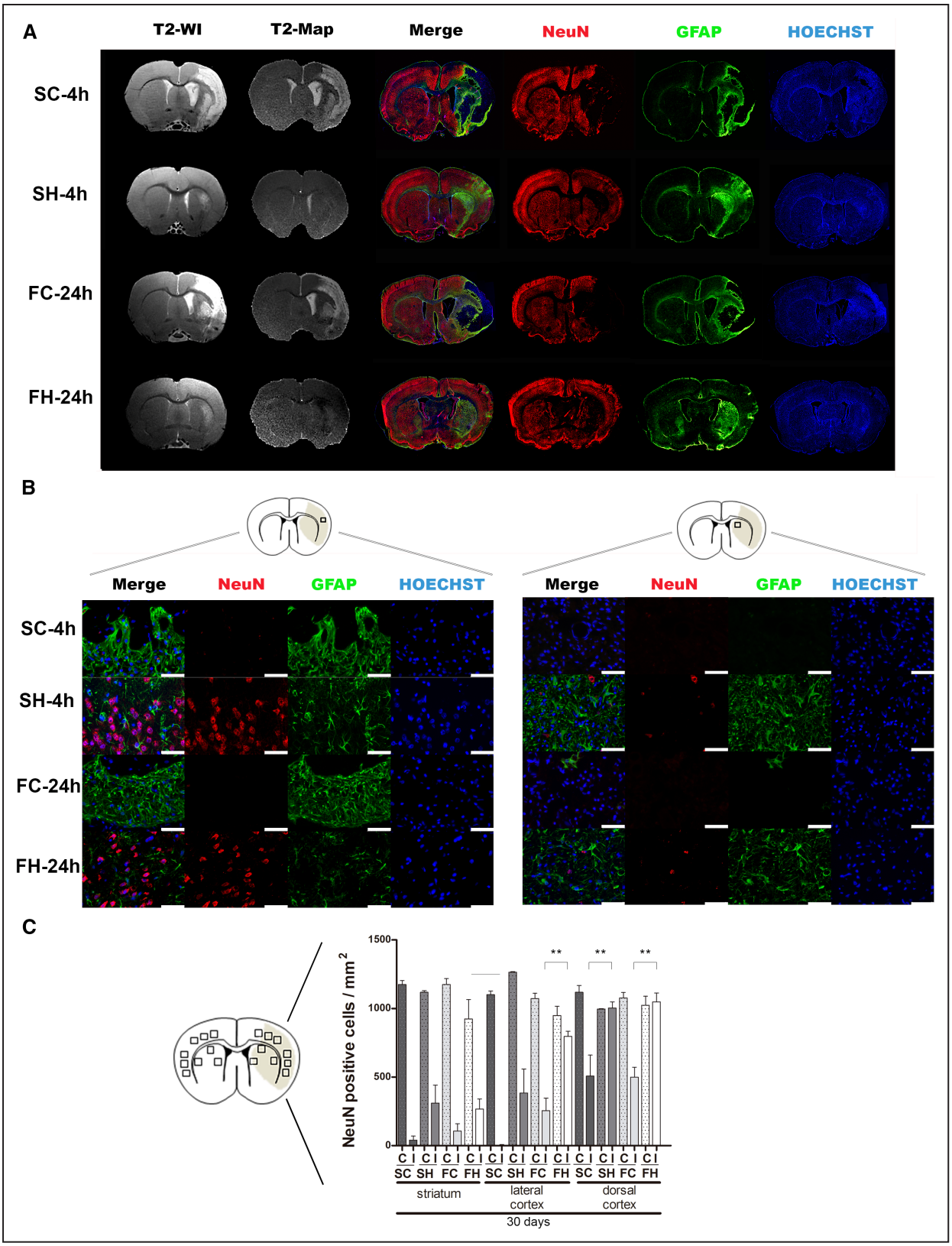

Figure 5. Histological marker expression 30 days after ischemia. Distribution of neurons (NeuN, red), astrocytes (GFAP, green), and nuclei (Hoechst, blue) 30 days after ischemia in the systemic control (SC)-4 hours, systemic hypothermia (SH)-4 hours, focal control (FC)-24 hours, and focal hypothermia (FH)-24 hours groups. **A**, Whole-brain reconstructions from multiple photomicrographs showing the general distribution of each marker, merged images, and correspondence with T2-weighted magnetic resonance images and maps. **B**, High magnification (×400) photomicrographs showing representative immunoreactivity patterns in the cortex and striatum. **C**, Neuron density in different brain regions. Scale bars, 50 μm. Data are shown as mean±SEM. GFAP indicates glial fibrillary acidic protein. ***P*<0.01; using 2-way ANOVA followed by the post hoc Bonferroni test (n=3, per group).

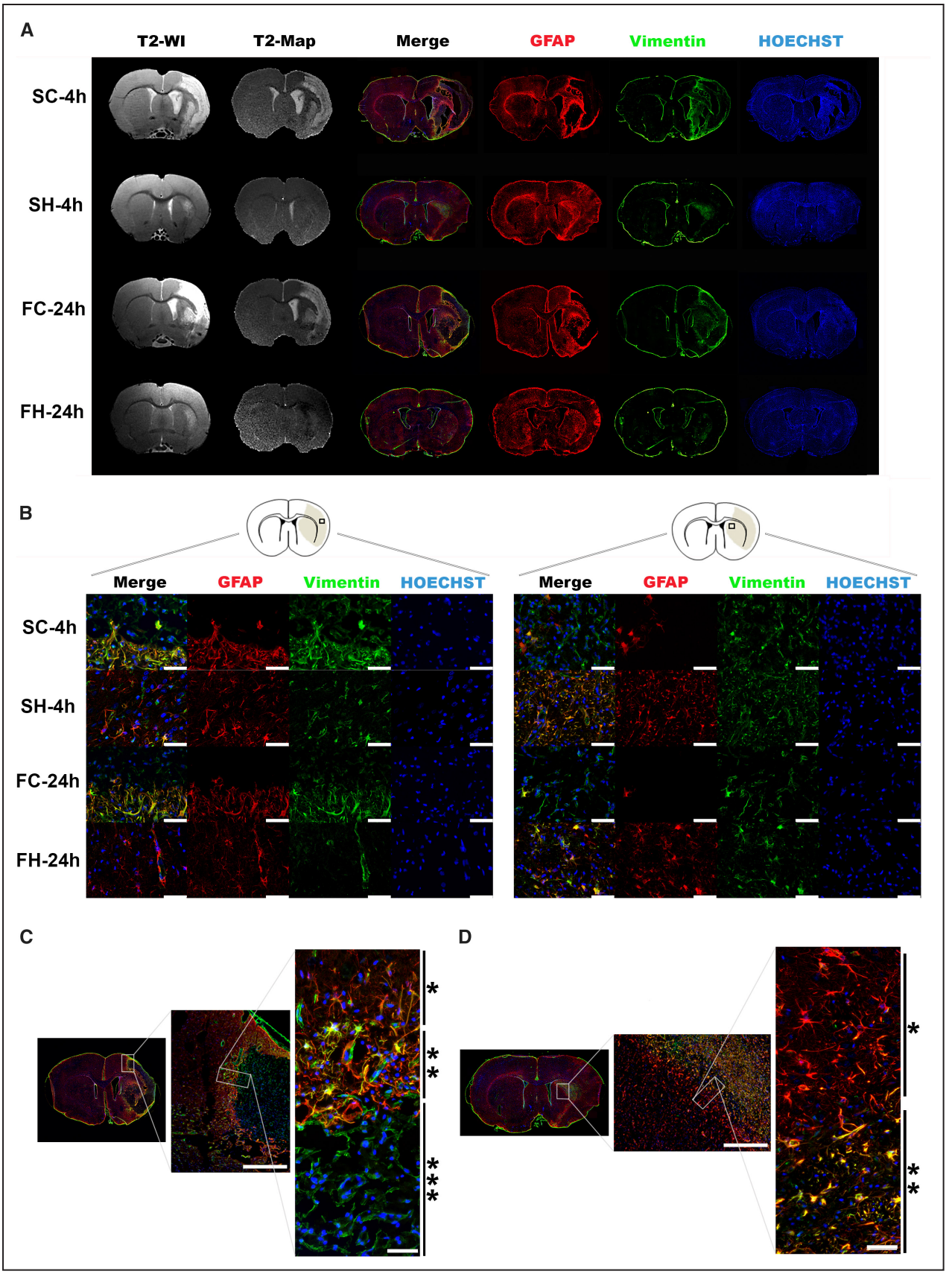

Figure 6. Histological examination of lesion scars 30 days after ischemia. Distribution of astrocytes (GFAP, red), fibroblast-like cells (vimentin, green), and nuclei (Hoechst, blue) 30 days after ischemia in the systemic control (SC)-4 hours, systemic hypothermia (SH)-4 hours, focal control (FC)-24 hours, and focal hypothermia (FH)-24 hours groups. **A**, Whole-brain reconstructions from (*Continued*)

Figure 6 Continued. multiple photomicrographs showing the general distribution of each marker, merged images, and correspondence with T2-weighted magnetic resonance images and maps. **B**, High-magnification (×400) photomicrographs showing representative striatal and cortical immunoreactivity patterns in the 4 principal treatment groups. **C**, Characteristic stratified lesion scar observed in control animals. **D**, Characteristic lesion scar observed in hypothermia-treated animals. GFAP indicates glial fibrillary acidic protein. *GFAP$^+$/vimentin$^-$ areas (nonfibrotic phenotype), **GFAP$^+$/vimentin$^+$ (reactive gliosis, mild-fibrotic phenotype), ***GFAP$^-$/vimentin$^+$ areas (fibroblast-like cells, highly fibrotic phenotype). Scale bars, 50 and 500 μm (n=3, per group).

effects of FH are mediated only through local effects in the ischemic region. Consistent with this, previous evidence has indicated that SH affects both the brain and peripheral organs.[25] This difference may explain why FH requires a longer treatment period to achieve a protective effect. It is also possible that the protective effect of 4 hours of SH could be partly mediated by the use of anesthesia during the hypothermic treatment period because anesthesia is well known to protect against cerebral ischemia.[26] However, a previous study in awake animals subjected to cerebral ischemia showed that 48 hours of focal cooling was needed to mitigate the ischemic injury, whereas 12 hours of SH was enough.[12] These data indicate that anesthesia does not mediate the effect of SH. However, rewarming was faster in the local cooling group, an effect that is harmful for the ischemic tissues, and potentially could explain why longer cooling was needed. In fact, 1 recent study suggests that edema may be worsened by rewarming from focal cooling.[27] In our study, systemic rewarming was slow, and focal rewarming was found to be difficult to control, as a minimal increase in the bath temperature (adjusted to 4°C) induced an immediate increase in brain temperature. Thus, one of the limitations of this study was the inability to accurately control this parameter. However, as 4 hours of FH did not increase the size of the ischemic region, we feel faster focal rewarming cannot explain the differences observed between 4 hours of SH and 24 hours of FH in our ischemic model.

Overall, hypothermia seems to be a complex therapy affecting multiple molecular components of the ischemic cascade, and which may be active throughout the body or in the lesioned area alone. In recent years, the pathways mediating hypothermia-mediated neuroprotection have attracted increasing interest.[28] Understanding these mechanisms may facilitate the design of pharmacological alternatives with similar protective efficacy and without side effects.[29]

A limitation of this study is that FH application to the surface of the head is not a translational approach because the thickness of the skull would necessitate the use of skin-damaging low temperatures and long treatment periods to achieve the target temperature within the brain. In fact, the beneficial effects of selective brain cooling using a cooling helmet are more evident in infants because of their smaller head size and reduced skull thickness. However, the recent introduction of new approaches to cerebral FH induction, including the use of a cooling neck collar and intracarotid infusion of cold saline, has shown great promise.[6,8,14] It should be noted that, in line with our results, hypothermia seems to be an effective strategy mainly in models of transient ischemia, suggesting that the combination of hypothermia and reperfusion therapies may be the most promising strategy in humans.[30] Although some data suggest that FH could be beneficial in permanent ischemia,[12] further studies are necessary to validate its use as a monotherapy.

Finally, magnetic resonance spectroscopy has been tested as a noninvasive and reliable tool for the measurement of brain temperature in both animal and human studies.[31] This strategy provides temperature distribution data across the brain, including the ischemic and normal tissue, allowing us to determine the effects of FH on the ischemic regions. This study represents the first use of MR as a thermometric tool to determine and compare the effects of SH and FH on cerebral temperature. Using brain temperature mapping, we have demonstrated that SH affects the temperature throughout brain (healthy and ischemic regions), whereas FH reduces the temperature mainly in the ischemic region. These findings were verified by gradient-echo–based phase mapping, a novel but validated MR technique.

Conclusions

Focal brain hypothermia is an effective therapeutic alternative to SH that circumvents the side effects associated with systemic cooling, enabling better patient management, and allowing the design of personalized treatments according to the characteristics of the ischemic lesion.

Disclosures

None.

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