A magnetic resonance (MR) compatible selective brain temperature manipulation system for preclinical study

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Abstract: There is overwhelming evidence that hypothermia can improve the outcome of an ischemic stroke. However, the most widely used systemic cooling method could lead to multiple side effects, while the incompatibility with magnetic resonance imaging of the present selective cooling methods highly limit their application in preclinical studies. In this study, we developed a magnetic resonance compatible selective brain temperature manipulation system for small animals, which can regulate brain temperature quickly and accurately for a desired period of time, while maintaining the normal body physiological conditions. This device was utilized to examine the relationship between T1 relaxation, cerebral blood flow, and temperature in brain tissue during magnetic resonance imaging of ischemic stroke. The results showed that this device can be an efficient brain temperature manipulation tool for preclinical studies needing local hypothermic or hyperthermic conditions.

Keywords: selective brain cooling, hyperthermia, hypothermia, brain temperature mapping, ischemic stroke

Introduction

Hypothermia has been shown to improve the outcome of acute cerebral ischemia. Its potential has been underlined by randomized clinical trials in patients with global cerebral ischemia after cardiac arrest and in infants with moderate or severe hypoxic-ischemic encephalopathy, in which cooling reduced mortality and disability. In addition, several prospective observational studies have demonstrated that higher body temperatures are associated with poor outcome after stroke.

The mechanism of the effect of hypothermia has been widely studied. Preclinical studies have suggested that hypothermia affects a wide range of cell death mechanisms including reduction of cerebral oxygenation and metabolism, alteration in oxygen free radicals release, decreases in the release of excitotoxic neurotransmitters, reduction or delay in apoptosis, and preservation of the blood–brain barrier. However, there is no final conclusion yet on the exact mechanism behind beneficial hypothermia.

It has been recognized that the protective effect of hypothermia is closely related to the treatment window, duration and degree of the hypothermia. Studies show that the earlier the application of the hypothermia, the better protective effect achieved. It has also been suggested that the brain is highly sensitive to temperature alterations. A small decrease in brain temperature by only a few degrees leads to significant neuroprotective effects in ischemic stroke, while a slightly increased brain temperature exacerbates brain injury.

Thus, for the stroke studies involving applications...
of hypothermia, an accurate, fast, and consistent control of brain temperature for a desired period of time is of crucial importance.

At present, systemic cooling is the most widely used method to induce and maintain mild hypothermia, but the decreasing of body temperature can lead to multiple side effects, such as shivering, pneumonia, arterial hypotension, thrombocytopenia, bradycardia, infection, and myocardial infarctions. Instead, selective brain cooling (SBC) methods can induce hypothermia in the brain, while maintaining normothermic body temperature, which could suppress the side effects. Although several selective cooling devices have been developed, their incompatibility with magnetic resonance (MR) imaging highly limited their applications in preclinical studies.

In this study, we designed a selective brain temperature manipulation system that was able to accurately and consistently regulate the brain temperature of rats. The target temperature can be reached within minutes and maintained for a desired period of time, while keeping the body temperature within normal range. In addition, the system is MR compatible, which highly expands its application in preclinical studies. Utilizing this device, we studied the relationship between the MR parameter T1 (spin-lattice relaxation time), cerebral perfusion, and brain temperature during MR imaging of ischemic stroke.

**Methods**

**Instrument design**

As shown in Figure 1, the MR compatible temperature manipulation device was composed of a brain cooling set, a body heating set, and temperature sensor set, which were placed inside the scanner room, a temperature measurement module and a center control unit (CCU), which were placed outside the scanner room. For MR compatible consideration, in order to avoid radio frequency noise and allow for MR compatibility, optical fibers were used to transfer the signals between the components inside and outside the scanner room.

The brain cooling set was used to control the brain temperature. It was composed of the brain cooling coil, silicone tubing, peristaltic pump (SCI400D/U1; Watson Marlow Inc, Wilmington, MA), cooling water container, and disposal container. The cooling coil was built with a PE 50 polyethylene tube (BD Intramedic™, Franklin Lakes, NJ), which was designed to fit the head of a rat. The cooling water was pushed by the peristaltic pump, flowed through the cooling coil, and went into the disposal container. The flow of cooling water was controlled by adjusting the speed of the pump, which was regulated by an analog voltage signal sent by the CCU.

The body heating set was used to maintain body temperature within the normal physiological range. It was composed of a body heating pad, heating pump (Gaymar, Shakopee, MN), and heating pump control module. The control module was the interface between the pump and CCU, which received the optical signal from the CCU to control the on/off of the heating pump.

The temperature sensor set (Luxtron Inc, Santa Clara, CA) was composed of two optical temperature sensors, one for the brain temperature and another for the rectal temperature, with the tip size of 0.5 mm. The temperature was derived by the wavelength difference between the emission and reflection lights, which went through the optical fiber between the sensor tip and the temperature measurement module. The temperature measurement module converted the temperature to an analog voltage signal and then transferred it to the CCU.

The CCU was the processing center for the whole system. It was composed of a BASIC Stamp microcontroller (Parallax, Rocklin, CA), analog-to-digital converter (A/D) and digital-to-analog converter (D/A) interface unit, and an optical communication interface circuit. The BASIC Stamp microcontroller, with a small, specialized BASIC interpreter built into the read only memory. It provided twelve digital output ports and input ports, which were used to communicate with other digital devices. A self-developed program was stored in the read only memory to control the processing of the whole system. The A/D and D/A interface circuits were realized by TLV5616C (Texas Instruments Inc, Dallas, TX) and TLC2543 (Texas Instruments Inc) chips. TLV5616 was a 12-bit voltage output digital-to-analog converter with a flexible 4-wire serial interface, while TLC2543 was a 12-bit switched-capacitor, successive-approximation, analog-to-digital converter.
The optical communication interface circuit was composed of an optical-electric converter, comparator, inverter, amplifier and driver, light-emitting diode light, and optical fiber and other components. It provided the conversion between electrical signal and optical signal, and supported the communication between the units inside and outside the scanner room for MR compatibility. The electrical diagrams of the CCU and peripheral circuit, optical communication circuits (transmitter and receiver), and pump control circuit are shown in Figures 2, 3, and 4.

Hypothermia and hyperthermia were induced in the brain by using either cold or warm water respectively. Two fiber optic thermo-probes were used to measure the brain temperature and body temperature. The brain temperature measured with a fiber optic thermo-probe was transferred to the CCU, which then adjusted the flow rate of the peristaltic pump accordingly. The core body temperature was used as the feedback signal to maintain the desired body temperature at 37.0°C ± 0.5°C by switching the heating pump on or off.

Animal procedures

All animal protocols were approved by the Institutional Animal Care and Use Committee at the University of North Carolina at Chapel Hill. A total of 26 Long–Evans rats (n = 26; 300 ± 25 g; Charles River Inc, Wilmington, MA) were used over the course of this study. Among them, nine rats were used to verify temperature manipulation, five rats were used to test the relation between T1 relaxation and temperature, seven rats were used to study the relations between perfusion and temperature in the brain under ischemic stroke, and five rats were used to study the temperature difference between temporal muscle and brain. Anesthesia of the animal was induced (3%) and maintained (1.5%) using mechanical ventilation with isoflurane. Animal body temperature was maintained within 37.0°C ± 0.5°C by a servo-controlled water heating pad during surgery. Each rat was implanted with two fiber optic thermo-probes. A small hole (ϕ = 1.0 mm) was opened in the scalp, 2 mm right of the midline and 2 mm under the bregma on the right side of the head. One probe was inserted at a right angle through the hole, with a depth of 4.0 mm. Another probe was inserted into the rectum, with a depth of 4.0 cm, to measure the core body temperature. The mean arterial blood pressure (MABP) and heart beat rate (HBR) were recorded through a femoral artery catheter with a blood pressure (BP) analyzer (Micro-Med, Louisville, KY).

Experiment to verify temperature manipulation

A total of nine Long–Evans rats (n = 9; 300 ± 25 g) were employed to determine the effectiveness of brain temperature manipulation using the developed system. Five different brain temperature manipulations, hyperthermia (39°C), normothermia (37°C), hypothermia (35°C, 32°C, and 29°C) and a three-phase hypothermia (29°C, 32°C, and 35°C) were conducted. For the different target temperatures, the temperature of the flowing water was preset to 40°C, 25°C, and 15°C respectively. For the three-phase hypothermic condition, the brain temperature was first cooled down to 29°C, maintained for 30 minutes, rewarmed to 32°C, maintained for another 30 minutes, rewarmed to 35°C and maintained for 30 minutes.
sequence50 was utilized to acquire the recovery of the signal for each hypothermia level for 20 minutes, while a Look–Locker method was utilized to study the relation between T1 relaxation and temperature. For each animal, the brain temperature was manipulated as described previously and a 4–0 nylon monofilament with a heat-blunted tip was inserted through an arteriotomy of the common carotid artery. The suture was advanced 20–22 mm into the internal carotid artery to induce middle cerebral artery occlusion. For comparison, a sham operated group (n = 2; 300 ± 25 g) was used, with a similar experimental procedure but without occluding the middle cerebral artery. Three different hypothermic conditions, including 35°C, 33°C, and 31°C were achieved sequentially.

In order to determine how hypothermia affects cerebral perfusion under different ischemic severity, apparent diffusion coefficient (ADC), CBF, and T1 maps were acquired at each hypothermic condition. More specifically, T1 maps were acquired with a Look-Locker method,50 CBF maps with a continuous arterial spin labeling52,53 method, and ADC maps with a three direction diffusion weighted image method. All the data was processed using house made programs, with the procedure the same as in the references.52,53

For the ischemic rats, three ROIs were examined: core, mismatched, and contra. The core region was defined as the region with ADC abnormalities (<mean-3 *SD [standard deviation] of the contralateral hemisphere). The mismatched region was defined as the hypoperfused CBF (<mean-3 *SD
Table 1 MABP and HBR measurements before and after temperature manipulation

<table>
<thead>
<tr>
<th></th>
<th>MABP mmHg</th>
<th>HBR (beats/min)</th>
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</thead>
<tbody>
<tr>
<td>Before</td>
<td>After</td>
<td>Before</td>
</tr>
<tr>
<td>Rat1 (39°C)</td>
<td>76.0</td>
<td>80.0</td>
</tr>
<tr>
<td>Rat2 (37°C)</td>
<td>76.5</td>
<td>72.5</td>
</tr>
<tr>
<td>Rat3 (35°C)</td>
<td>70.0</td>
<td>70.0</td>
</tr>
<tr>
<td>Rat4 (32°C)</td>
<td>77.0</td>
<td>73.0</td>
</tr>
<tr>
<td>Rat5 (29°C)</td>
<td>74.8</td>
<td>71.5</td>
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Abbreviations: MABP, mean arterial blood pressure; HBR heart beat rate.

of the contralateral hemisphere) region without the ADC lesion in the ipsilateral hemisphere. A mirror of the core region in the contralateral hemisphere was chosen as the contra ROI. In contrast, an ROI encompassing both hemispheres, excluding the region at a proximity to the temperature probe, was outlined as sham ROI for rats in the sham group.

To derive the relationship between the change of CBF and change of temperature, a normalized CBF was derived, with the mean value of CBF in the contra ROI at normal temperature (37°C) used as reference.

**Temperature difference between brain and temporal muscle**

Five normal rats (n = 5; 300 ± 25 g) were utilized to study the relationship between the temperature in the temporal muscle and brain. The same procedures outlined above for selective cooling of the brain were used. An additional optical thermal probe was inserted 10 mm into the cleft between the skull and temporal muscle. The temperature of the brain and temporal muscle were recorded simultaneously for 90 minutes after the brain temperature was reduced to 32°C.

**Experimental results**

**Selective brain temperature manipulation**

The results showed the MR compatible selective brain temperature manipulation device achieved the targeted brain temperature within 10 minutes and maintained it for 90 minutes. As demonstrated in Figure 5A, five different levels of targeted temperatures (39°C, 37°C, 35°C, 32°C, and 29°C) were achieved promptly (<10 minutes) and maintained for 90–120 minutes with minimal variation (<0.5°C). The rectal temperature remained in the normal range of 37.0°C ± 0.5°C for all rats. Both MABP and HBR resided within their normal levels under all manipulation (Table 1). There was no significant difference found in MABP (P = 0.40) and HBR (P = 0.19), before and after the manipulation, by two-tailed Student’s t-test.

The three-phase brain temperature manipulation is shown in Figure 5B. First, the 29°C targeted temperature was achieved within 10 minutes and maintained for 20 minutes. It was then rewarmed to 32°C within 10 minutes and maintained for another 20 minutes, and finally rewarmed to 35°C and maintained for the final 20 minutes. The rectal temperature was kept within the range of 37.0°C ± 0.5°C during the entire process of temperature manipulation.

**Correlation between T1 and brain temperature**

Spin-lattice relaxation time T1 was linearly proportional to temperature when it was below 45°C. Our results also demonstrated a similar result. A highly linear correlation (y = 0.011x − 0.0003, R² = 0.9374) was found between the change of T1 and the change of temperature (range: 29°C–37°C) in the gray matter of the brain, as shown in Figure 6.

**Relationship between CBF and temperature**

The relationship between CBF and brain temperature at different ischemic severity is shown in Figure 7. Cooling the
brain always resulted in decreases in CBF, while the rate of decrease varied between the different regions. Highly linear relationships were observed between CBF and brain temperature independent of the brain perfusion status. However, the rate of CBF reduction in response to hypothermia differed among different ROIs. The sham group had the greatest slope ($y = 0.08x - 2.1$), followed by the contralateral region ($y = 0.07x - 1.6$), the mismatch region ($y = 0.05x - 1.2$), and the core lesion region ($y = 0.02x - 0.2$).

**Relation of temperature between brain and temple muscle**

As shown in Figure 8, a stable difference between the brain and temporal muscle temperature was found on each animal (only the result from one animal was shown) through the entire 90 minutes after the brain temperature reaches the target temperature of 32°C. Although the magnitude of the temperature difference was different for each animal, from 2°C to 5°C respectively (not shown), the difference was stable throughout the whole cooling period for each animal.

**Conclusion and discussion**

We have designed a MR compatible selective brain temperature manipulation system that can manipulate the brain temperature of rats quickly and accurately while maintaining normal body temperature and physiology. The results showed that the targeted temperatures were reached within 10 minutes and were maintained for a long period of time (>90 minutes), with minimal variation (<0.5°C). The system was also very flexible and could produce the desired temperature pattern.

In our study, the BP seems low compared to other studies. The BP was measured from the femoral artery, with the sensor on the same level as the animal. One possible reason for the measured low BP is that there is a systemic bias of the device that we used. Because the device was connected to the femoral artery during the whole procedure of temperature manipulation for all the animals, the conclusion that there is no significant difference in BP during brain temperature manipulation should still be valid.

To ensure MR compatibility, two issues were addressed in the design. First, any metallic or ferromagnetic part was not allowed inside the scanner room due to safety considerations. Second, any radio frequencies generated by electronic components could affect the quality of the MR image. Thus, for our design, all the digital circuits were placed outside the scanner room. Optical fibers were utilized for the signal communication to eliminate the radio frequency interference. The MR compatibility of the system expands its application in preclinical studies.

The efficiency of cooling was closely related to the temperature of the cooling water and the rate of water flow. For this device, the temperature of the water was found to be the major factor affecting the cooling efficiency, while adjustment of the flow rate was utilized to enhance the initial cooling and as a fine adjustment once the target temperature was reached. For each targeted temperature, the temperature of the cooled water was manually preset (4°C for 29°C target temperature, 10°C for 32°C, and 20°C for 35°C). At the start of cooling, a high flow rate was used to expedite the cooling process. After the temperature was within 2°C of the target temperature, the rate of water flow was reduced slowly, until the target temperature was reached. The brain temperature could be maintained within 0.5°C of the targeted temperature by the adjustment of water flow during the stable period.

It has been suggested that brain metabolism is sensitive to temperature alterations;26–28 therefore in order to evaluate how temperature alters brain metabolism, an accurate measurement of brain temperature is important. In many studies,
the temperature at the temporal muscle has been used as an indicator of brain temperature. However, it has been reported that the temperature in the temporal muscle does not accurately reflect the brain temperature. In our study, surface cooling was used to produce hypothermia. A large temperature gradient was built across the brain from the warmer central structures to the cooler periphery (as big as from 10°C on the surface to 30°C inside the brain). The temperature at the temporal muscle was much lower than the brain, because it is closer to the surface cooling coil. In addition, under certain circumstances, direct measurement of the brain temperature with a temperature probe is not applicable due to its invasive nature, and its inability to provide a spatial temperature distribution. Thus, for many applications, a pixel-by-pixel noninvasive brain temperature map is preferred. Five noninvasive brain temperature mapping MR techniques have been proposed: the longitudinal relaxation time T1, the ADC, proton spectroscopic imaging, and temperature-sensitive contrast agents. We chose the T1 based method for brain temperature mapping because the other four methods have drawbacks that make them unsuitable for an ischemic stroke study. These limitations include: (1) For the diffusion coefficient method, the ADC value can change within 10 minutes after ischemic occlusion, resulting in an inaccurate temperature measurement; (2) Proton resonance frequency of water can be changed when cerebral hemodynamics are altered; (3) The temperature sensitive contrast agents usually have a nonlinear response to temperature making their relationship hard to define; (4) The spatial resolution of proton spectroscopy is usually very poor and not suitable for temperature mapping. Alternatively, for T1 mapping methods, a highly linear relationship between the change of temperature and T1 has been reported, and there is no T1 change immediately after ischemic occlusion at magnetic fields < 3T.

In our design, a thermo-probe is always required as the real time feedback signal for the system. It has been reported that the temperature in the temporal muscle cannot accurately reflect the brain temperature. In our study, the temperature difference between the temporal muscle and brain ranged from 2°C to 5°C across different animals. There are two possible reasons for these variations. First, the temperature gradient under surface cooling makes the temperature measurement very sensitive to the position of the probe. Second, the fit of the cooling coil to the animal head could vary for different animals, resulting in variations in heat conductance. Nevertheless, although the temperature difference between the temporal muscle and brain varies from animal to animal, it remains stable over a long period of time for each individual animal. This stable relationship allows us to use the temporal muscle temperature as the feedback control signal to keep the brain temperature stable for the desired period of time. Since the T1 map could be used to derive the brain temperature after data acquisition, we could classify the animals into different groups during the later data analysis stage.

Hypothermic conditions induce the reduction of CBF in a normal brain. This finding was consistent with findings previously reported by Cheng et al and Laptook et al. They reported that a decrease of temperature of about 4°C (systemic cooling) resulted in a reduction of 40% of CBF. In our study, a comparable result was found, with a 40% reduction of CBF when the brain temperature dropped from 37°C to 33°C. There are several physiological factors that may account for the observed reduction of CBF, such as changes of BP or changes of arterial blood gases. In our study, there was no significant change of MABP before and after the cooling, and blood gas was also well controlled by mechanical ventilation. It is highly possible that the status of auto-regulation plays a critical role on how CBF will respond to brain temperature manipulation. If the auto-regulation is dysfunctional in the core area it may be only slightly impaired in the mismatched region, leading to different CBF characteristics in response to temperature manipulation. More studies are needed to further investigate whether or not brain metabolism under hypothermia also responds in a similar manner as that of CBF.

For active animals, the brain temperature is tightly related to body temperature because of the fast exchange of heat between the brain and body through blood flow. When the surface cooling was used to reduce the brain temperature, the efficiency of cooling depends on multiple factors, including the temperature of the cooling water, the flow of the cooling water, local blood flow, the temperature of the perfused blood, the heat production of the brain, and the thermal conductivity of brain tissue and muscle. Zhu and Diao's simulation shows the cooling efficiency is largely dependent on the depth of tissue. For small animals, the small diameter of the brain and the large contact surface make it much easier to reduce the brain temperature. Therefore, for larger animals or humans where the diameter of the brain is larger, it would be much harder to regulate brain temperature. Although our device works well on small animals such as rats, its efficiency of cooling on larger animals or humans needs further investigation. In addition, besides the external cooling, the brain temperature is affected by several other factors. Kiyatkin et al have shown that anesthesia or stress will also have an effect on
hypothermia. So careful experiment design in these cases is critical before application of SBC devices.

Some similar SBC devices have been developed and utilized clinically and preclinically, eg, cool cap studies,\textsuperscript{33,34} the ChillerPad\textsuperscript{TM} system (Seacoast Technologies Inc, Portsmouth, NH),\textsuperscript{48} intravascular infusion of cold solution,\textsuperscript{49} local metal coil cooling,\textsuperscript{47} and SBC from the pharyngeal surface.\textsuperscript{73} Compared to those methods, the device we designed in this study has the following advantages. First, it is an effective way to induce local hypothermia condition in small animals in preclinical studies. It is very fast (<10 min) with a wide temperature range (as low as 29°C), accurate, and maintains temperature (<0.5°C) in a controllable way. Second, it is MR compatible, which extends its application for MR imaging studies, while most other methods have no proven record on working under a MR environment. Third, the setting up of the device is simple and flexible, and doesn’t need a stereotactic holder or specially designed frame. This gives it the flexibility to be used in a variety of experimental conditions. Fourth, in our model, the method of deriving the temperature with T1 mapping has been validated, which not only makes the temperature measurement noninvasive, but also provides the spatial distribution of temperature. Fifth, with our system, a stable relationship of temperature between the temporal muscle and brain under hypothermia has been found, which could have potential applications in other studies needing temperature control.

This system has been proven to maintain brain hypothermia under anesthesia for as long as 90 minutes successfully. For studies needing longer durations, an improved design will be needed to allow the animal to wake up and move free in the cage. Improvements could possibly be done by using stretchable tubes and limiting the animal activity space, etc.

In conclusion, in our study, a selective brain temperature manipulation device has been designed and verified in a MR environment. This device has potential to be a valuable tool to further understand the mechanisms of local hypothermic and hyperthermic conditions in preclinical studies of the brain.

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Disclosure
The authors report no conflicts of interest in this work.

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