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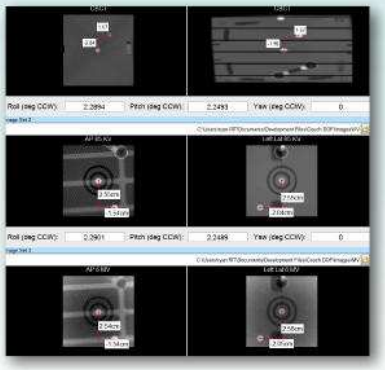

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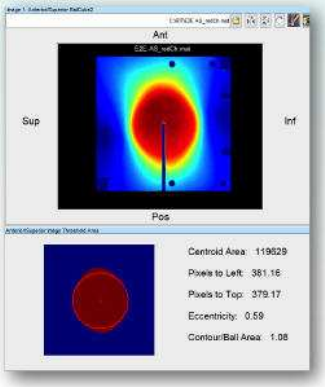

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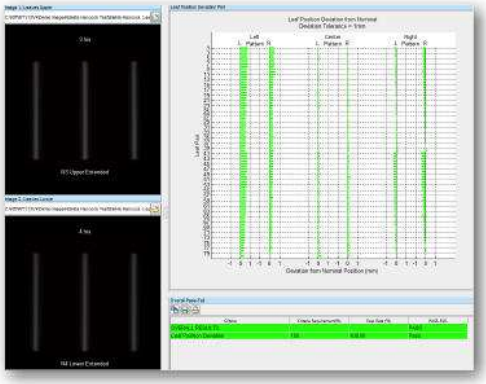

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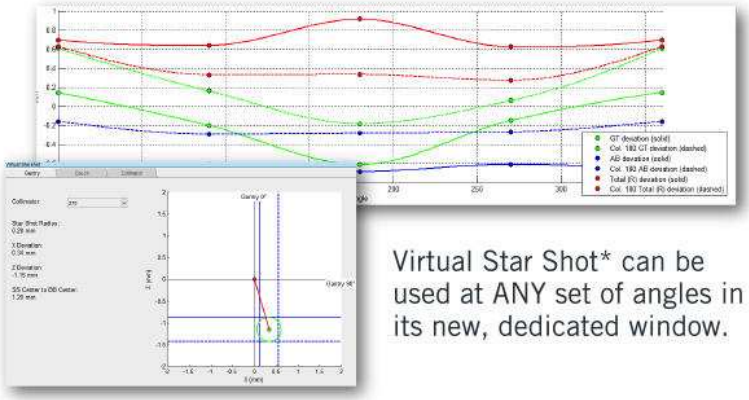

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Temperture distribution and blood perfusion response in rat brain during selective brain cooling

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A rat model was used in this study to examine the transient temperature distribution and blood flow response in the brain during selective brain cooling (SBC) and rewarming. SBC was induced by a head cooling helmet with circulating water of 18 °C or 0 °C. It has been shown that the brain temperature reductions were 1.7±0.2 °C (5 mm beneath the brain surface) and 3.2±1.1 °C (2 mm beneath the brain surface) when the temperature of the water was 18 °C (moderate cooling). The cooling of the brain tissue was more evident when the circulating water was colder (0 °C, deep cooling). The characteristic time that it took for the tissue temperatures to reach a new steady state after the initiation of cooling varied from 5 to more than 35 min and it depended strongly on the blood flow response to the cooling. We used an ultrasound flow meter to measure continuously the blood flow rate in the common carotid artery during the cooling and rewarming. The blood flow rate dropped by up to 22% and 44% during the cooling from its baseline in the moderate cooling group and in the deep cooling group, respectively. Although all brain temperatures recovered to their baseline values 50 min after the helmet was removed, the blood flow rate only recovered to 92% and 77% of its baseline values after the moderate and deep cooling, respectively, implying a possible mismatch between the blood perfusion and metabolism in the brain. The current experimental results can be used to study the feasibility of inducing brain hypothermia by SBC if the blood flow responses in the rat are applicable to humans. The simultaneous recordings of temperature and blood flow rate in the rat brain can be used in the future to validate the theoretical model developed previously.
[DOI: 10.1118/1.2208918]

Key words: bioheat transfer, head injury, ischemia, SBC, hypothermia, CBF

I. INTRODUCTION

In recent years, mild or moderate hypothermia during which the brain temperature is reduced to 30 °C–35 °C has been proposed for clinical use as an adjunct for achieving protection from cerebral ischemia and traumatic brain injury.[1–5] Experimental studies have demonstrated that moderate hypothermia reduces mortality as compared with normothermia or hyperthermia in animal models after severe controlled cortical impact.[6] It has been shown that even a temperature reduction as small as 2 °C in the brain tissue is beneficial to the patients' outcome. The mechanisms of the neuroprotection of hypothermia include the reduction of the metabolic rate by cooling, reduction, or prevention of the release of a neurotransmitter in the brain, reductions in intracranial pressure and cerebral edema formation, and attenuation in the opening of the blood-brain barrier.

The major challenge facing the clinicians is the timely and safe delivery of hypothermia to patients. The inconsistency in the patient outcome in various clinical studies[3,4,7–9] might be due to the delay of hypothermia initiation. Clinical and animal data have suggested a 1–2 h treatment window within which post-ischemic hypothermia must be instituted to confer any significant neuroprotection.[10,11] Based on the clinical and animal studies, it would be ideal to initiate hypothermia treatment in the prehospital settings to maximize the protective effect of hypothermia.

There are two approaches for achieving a reduction in brain temperature. One is via systemic hypothermia, where the whole body is cooled. This approach may produce deleterious systemic complications and require intensive monitoring. Another approach is called selective brain cooling (SBC) in which the brain is cooled selectively while the rest of the body is kept at the normal temperature. Clinically feasible SBC protocols include a head hood or helmet with water or chemical cooling, head immersion in iced water, nasopharyngeal cooling after tracheal intubation, introcarotid flushing, etc. Simply packing ice or wearing a cooling helmet is easy to implement and may maximize the protection induced by hypothermia in the prehospital settings.[3,8,12,13] A recently published paper[14] introduced a new cooling protocol that used the retrograde perfusion of cold blood into the internal jugular vein in an animal model. In this experiment, the brain temperature was reduced and the rest of the body was kept at the normal temperature. Similar to other SBC protocols, this new method introduced a fast temperature reduction in the brain tissue while minimizing the systemic complications associated with whole body hypothermia.

There are several theoretical studies that have simulated the brain temperature distribution during SBC.[15–19] Most of these theoretical simulations are based on the Pennes bioheat equation,[20] where the blood flow effect is modeled as a heat source proportional to the local blood perfusion rate and the temperature difference between blood and tissue. The accuracy of the theoretical simulations depends on the input of the local blood perfusion rate. Unfortunately, an empirical expression describing how the local blood perfusion rate changes as a function of local tissue temperature and other factors is not available, especially during the simulation of transient heat transfer in the brain. Although cerebral blood flow (CBF) has long been demonstrated to decrease during systemic hypothermia,[21–24] the changes of CBF in response to SBC have not been well studied. The local blood perfusion rate in the brain may be affected, not only by the local tissue temperature, but also by other factors. It is of clinical importance to investigate the blood flow response, since the local blood perfusion rate in the brain during SBC affects the temperature field after steady state, as well as the time duration it takes for the temperatures to reach steady state.[18]

In most of the theoretical simulations, blood perfusion is usually considered either as a constant during the cooling or decreasing only with the local tissue temperature. The temperature dependence of CBF is based on a coupled relationship between CBF and metabolism. Earlier experimental work[25,26] has shown a linear relationship between $1/T$, where T is tissue temperature, and $\log \mathrm{CMRO}_2$ (the cerebral metabolic rate of oxygen consumption). A mathematical expression of this relationship has been given by

$$\mathrm{CMRO}_2 = \mathrm{CMRO}_{2,n} \cdot Q_{10}^{(T_t-37)/10},$$

where $\mathrm{CMRO}_{2,n}$ is the normal cerebral metabolic rate of oxygen consumption, and Q_{10} is a constant factor, which has been reported[26] to vary between 2 and 4.4 based on previous experimental measurements. This law states that the metabolic rate decreases by a factor of Q_{10} with each 10 °C reduction in temperature. Based on a Q_{10} value of 2, it has been calculated that hypothermia decreases the cerebral metabolic rate by an average value of 7% for the first 1 °C reduction in temperature, while the metabolic rate is reduced to 1/2 of the normal value when the temperature reduction is 10 °C. This type of relation is in agreement with the Arrhenius equation. During normal conditions, CBF may follow the same pattern as that of cerebral metabolism.[27] During cerebral ischemia or head injury not only may the Q_{10} value change, but also the CBF may be de-coupled from cerebral metabolism.

A number of studies have been performed to examine the variation of CBF during systemic hypothermia. Using the radioactive microsphere technique, Busija and Leffler[21] measured CBF in anesthetized newborn pigs. They concluded that systemic hypothermia reduced CBF secondarily to the reduction of the cerebral metabolic rate. Verhaegen *et al.*[24] measured the cortical blood flow in anesthetized rats using a laser-Doppler flow meter (LDF), and found that CBF was reduced during moderate hypothermia. Okubo *et al.*[22] examined the effect of systemic cooling on cerebral metabolism and regional CBF variation in newborn piglets. They measured the regional CBF with colored microspheres and demonstrated that the reduction of the cerebral cortex temperature resulted in a decrease in the blood flow in all brain regions. Unlike many experimental studies on CBF response during systemic cooling, there have been only a few studies on the effect of selective brain cooling on CBF, and the results were inconsistent. Laptook *et al.*[13] examined the differences of CBF in newborn swine during SBC versus whole body cooling, and illustrated that the global CBF was reduced during both whole body cooling and selective brain cooling. Ibayashi *et al.*[28] demonstrated that the regional CBF decreased when SBC was implemented on rats. However, a previous study[29] using the LDF technique has shown that the cortical CBF in normal lightly anesthetized rats increased during SBC.

LDF has been used to monitor blood perfusion in a superficial area. Although the output from LDF is measured not in easily interpretable units of flow but rather in hertz, it offers the potential for measuring continuously the relative change of blood flow in a small regional volume and has repetitive accuracy. To measure the blood perfusion rate of brain tissue, the LDF probe has to be inserted into the brain to have access to the measured region. Another limitation of LDF is that it may not be suitable for measuring global blood flow change in brain. Microspheres, a popular technique for measuring the local blood perfusion rate in the tissue, are injected into the blood and allowed to circulate freely until they impact in the capillaries. Using different colored or different sized microspheres, it is possible to determine the blood perfusion at different time instants. However, the microspheres are not injected frequently and it results in a low temporal resolution. In pervious studies,[2,22,30] CBF was measured only once or twice during hypothermia in the animal models. In recent years, the MRI has been used to scan the cerebral perfusion in animal models during brain hypothermia.[31] Due to the relatively long acquiring time for the 3-D MRI scan, the MRI may not be suitable for studying the response of blood perfusion during cooling. Considering the complicated and fast varying behavior of the blood flow response during cooling and rewarming, it is of clinical importance to develop an experimental approach for measuring continuously the global CBF while simultaneously monitoring the temperatures of the brain tissue. It is estimated that more than 90% of the blood supplied to the brain is from the common carotid arteries. If the blood flow rate of the common carotid artery can be measured continuously, it can be used in an *in vivo* setting for studying the transient behavior of temperature and blood flow responses during selective brain cooling and rewarming.

In this study, we performed *in vivo* experiments to investigate the transient temperature distribution in a rat brain during head surface cooling and rewarming. An ultrasound flow meter was used to measure continuously the blood flow rate in the common carotid artery (CCA) to investigate its response to cooling. Temperature distribution in the brain tissue, the scalp temperature, the rectal temperature, as well

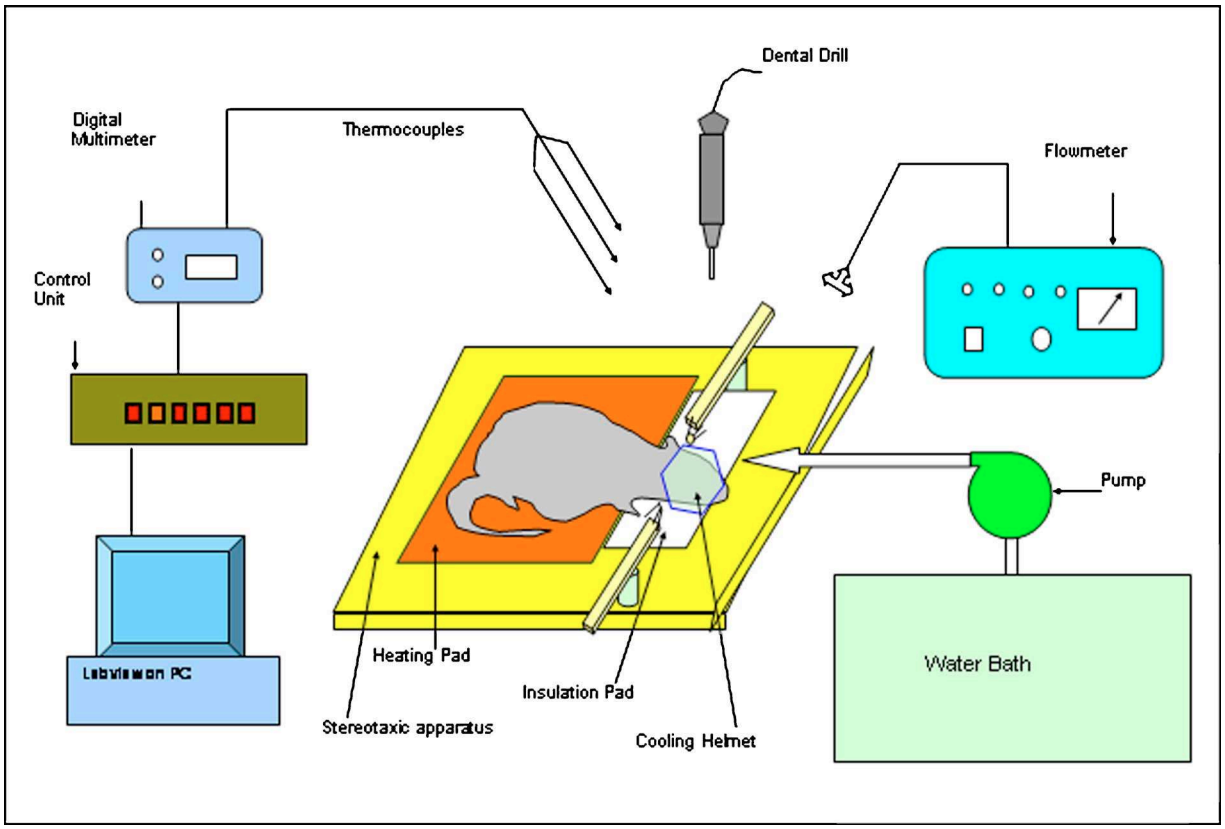

FIG. 1. Schematic diagram of the experimental setup for the measurements of the brain temperatures and blood flow.

as the arterial blood temperature of the CCA, were recorded and monitored continuously during the experiments. We analyzed the simultaneous measurements of both the tissue temperature and blood flow rate to better understand the role played by the local blood perfusion rate on the brain temperature.

II. MATERIALS AND METHODS

The IACUC at University of Maryland Baltimore County has reviewed and approved the experimental protocols. This study used 16 Sparague-Dawley rats (mean±SD, 482±18 g) provided by the Charles River Laboratory (Boston, MA). The rats were anesthetized with intraperitoneal injections of sodium pentobarbital (40 mg/kg) and were put on a heating pad to keep a normal body core temperature. The rectal temperature was monitored throughout the experiment by a thermocouple inserted into the rectum. Additional anesthetic was administrated as needed.

Through a 20–30 mm midline neck incision from the caudal end of the larynx to the suprasternal notch, the common carotid arteries on both sides were dissected free from the surrounding nerves and fascia. Preliminary experimental data have suggested that there was no significant difference between the blood flow rate and the temperature between the left and right common carotid arteries. A T106/T206 Animal Research Flowmeter (Transonic® System Inc., New York) with a 0.7 mm V flow probe was used to measure the blood flow rate in the common carotid artery, Q. We placed the flow probe around the common carotid artery so that the artery was nestled within the deepest angle of the V reflector and the flow rate of the left CCA was recorded. The flow probe was left inside the tissue for continuous measurements of the blood flow rate during the experiment. One thermocouple was attached to the surface of the left CCA using tissue glue[32] to monitor the arterial temperature during the experiment, since the rectal temperature measurement by the rectal probe may be different from the arterial temperature due to the heating pad and cooling helmet. The skin was then closed.

The experimental setup is shown in Fig. 1. The rat head was fixed in a stereotactic device (Stoelting Inc., Wood Dale, IL). After retraction of the scalp, a craniotomy was made over the left and right parietal cortex with a dental drill, using the coronal and interparietal sutures as margins. With a small drill bit (0.4 mm diameter), a small burr hole was made 3 mm lateral and 2 mm posterior to the bregma. Drilling was done with a continuous drip of 0.9% saline solution to prevent heat injury of the underlying cerebral cortex. The schematic diagram of the SBC setting and the sites of the thermocouples are shown in Fig. 2. Three fine thermocouples (50 μm diam wire) 3 mm apart were bound together and inserted through the burr hole to various depths in the brain tissue. The first one was always placed between the skull and scalp. The other two were 2 and 5 mm underneath the brain surface. The locations of the thermocouples were verified later from the dissection of the brain tissue after the experi-

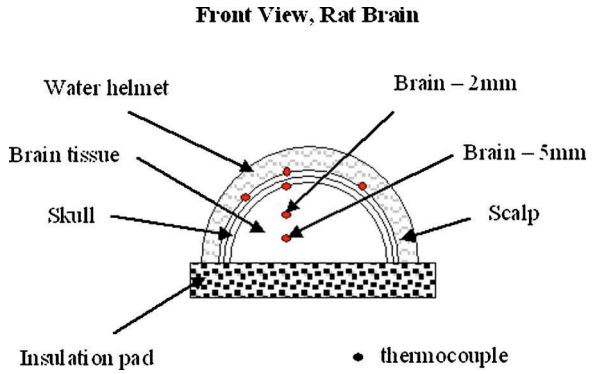

FIG. 2. Thermocouple locations inside the rat brain and on the head surface.

ment. The calvarium was then sealed with small amount of bone wax (Stoelting Inc., Wood Dale, IL). The overlaying muscles were approximated and sutured. The skin was closed. The rat was placed on its abdomen position with its head resting on an insulation pad. Several copper-constantan thermocouples were placed on various locations of the head surface for monitoring the skin temperature. A helmet with circulating water was used to induce selective brain cooling in rats. Preliminary experiments were performed to minimize the contact resistance between the helmet and the scalp by applying a highly thermal conductive paste (OMEGATHERM® 201, Omega Engineering, INC, CT), although the contact resistance could not be eliminated completely. In this experiment the contact resistance was controlled to ensure that it did not vary significantly from one rat to another. The heat transfer characteristics of the cooling helmet include the inlet and outlet temperatures, and the coolant flow rate. In this experiment, the inlet temperatures were controlled as $18.4\pm0.2\ ^\circ\mathrm{C}$ (or $0.0\pm0.2\ ^\circ\mathrm{C}$ for the deep cooling group) while the coolant flow rate was 1250 mm^3/s by setting a high-resolution peristaltic pump (World Precision Instruments, Inc., Sarasota, Florida) at 56 rpm.

The experimental protocol is as follows. After the surgery the rat head was exposed to the surrounding air and was allowed to recover for 20 min. The baseline temperatures and blood flow rate in the left CCA were recorded for 10 min. Then, for the first rat group (eight rats), a 35 min moderate cooling was induced by the cold surface of the helmet placed on the rat head. During the cooling, all the temperatures and blood flow rate were recorded. The helmet was then removed from the head and a 50 min recovery was implemented for the rat to reestablish its baseline temperature distribution. The above procedures were repeated for the second rat group (eight rats). All the data were acquired and recorded with a LabView® program running on a personal computer. The temperatures were recorded every ten seconds while the blood flow rate was measured every minute.

After the experiment, the rat was sacrificed with a sodium pentobarbital overdose I.P. (150 mg/kg). The entire brain was taken from the skull and weighed (2.06 ± 0.05 g, $n=16$). The brain was sectioned to confirm that the temperature sensors were properly positioned and no bleeding was caused by the insertion. The thicknesses of different layers (scalp, bone, and brain tissue) of the head were measured.

The blood flow rate and temperature at each trial were analyzed and expressed as mean±SD. Differences among the mean values were determined by one-way repeated measures ANOVA. The post hoc comparisons between the baseline measurement and cooling or rewarming were performed by Dunnett's method.[33] Significance was evaluated at the 5% confidence level.

III. RESULTS

The experimental data have shown consistency between the temperature of the common carotid artery ($T_a=36.5\pm0.4\ ^\circ\mathrm{C}$) and the rectal temperature ($T_{\mathrm{rec}}=37.0\pm0.5\ ^\circ\mathrm{C}$) during both the baseline and cooling. It implies a minor effect of the cooling helmet on the temperatures of the common carotid arteries. The cooling duration is 35 ± 3 min. The recorded tissue temperatures at the end of each trial are given in Fig. 3. As compared with the baseline values, the moderate cooling resulted in a temperature drop

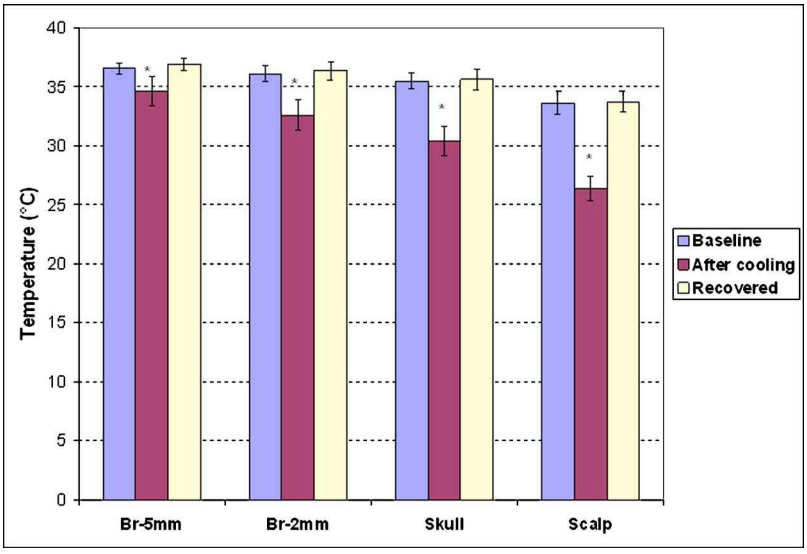

FIG. 3. The tissue temperatures at different locations for the baseline, 35 min after the moderate cooling, and 50 min after the helmet removal. The temperature values are expressed as means±SD($n=8$). $^*p<0.05$.

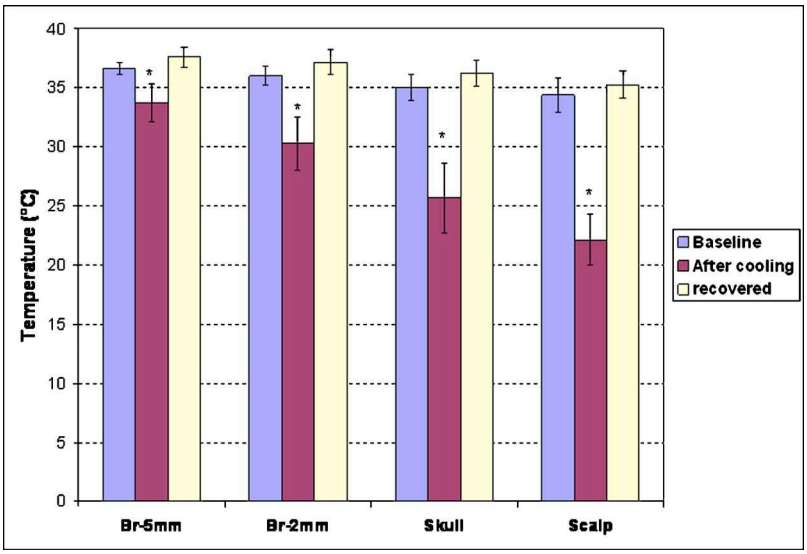

FIG. 4. The tissue temperatures at different locations for the baseline, 35 min after the deep cooling, and 50 min after the helmet removal. The temperature values are expressed as means±SD(n=8). $^{*}p<0.05$.

of 1.7±0.2 °C, 3.2±1.1 °C, 4.9±1.4 °C, and 7.5±1.1 °C at the sites of brain-5 mm, brain-2 mm, skull, and scalp, respectively. The temperature reductions further increased to 2.9±1.6 °C, 5.8±2.1 °C, 9.4±2.7 °C, and 12.2±2 °C, 35 min after the cooling in the deep cooling group (Fig. 4). Note that the cooling on the head surface resulted in a significant reduction in the temperature at all recording sites, while all temperatures recovered to their baseline values 50 min after the helmet was removed. No significant difference was found between the recovered and the baseline temperatures.

Table I illustrates the mean blood flow rates at the end of each trial and their standard deviations. Blood flow rates at the baseline were similar for both rat groups (group 1: 4.6±0.46 ml/min versus group 2: 4.5±0.42 ml/min). It decreased by 22% and 44% from its baseline during the moderate and deep cooling, respectively. After the helmet was removed, the blood flow rate almost recovered (92%) to its baseline level in the moderate cooling group. However, the blood flow rate after the rewarming was only 77% of its baseline value in the deep cooling group. Since blood flow and metabolism act as heat sources to the local tissue temperature, a recovered tissue temperature after rewarming in the deep cooling group suggested a possible increase in the metabolic heat generation rate or other forms of heat generation in the tissue during the rewarming.

Two typical temperature transients during the experiment are shown in Figs. 5(a) and 5(b). In Fig. 5(a), once the cooling helmet was applied, temperatures at all measuring sites decreased fast and established their steady state during the cooling. The other trend was observed where the temperatures kept decreasing during the cooling and no steady state was established at the end of the cooling, as shown in Fig. 5(b). Comparing the transient temperature profiles with the

TABLE I. Average blood flow rates and their standard deviations at the end of each trial.

	Baseline Q (ml/min)	After 35 min cooling Q (ml/min)	After 50 min rewarming Q (ml/min)
Group 1 (n=8)	4.6±0.46	3.6±0.61[a]	4.3±0.67
Group 2 (n=8)	4.5±0.42	2.5±0.46[a]	3.5±0.84[a]

[a] $p<0.05$.

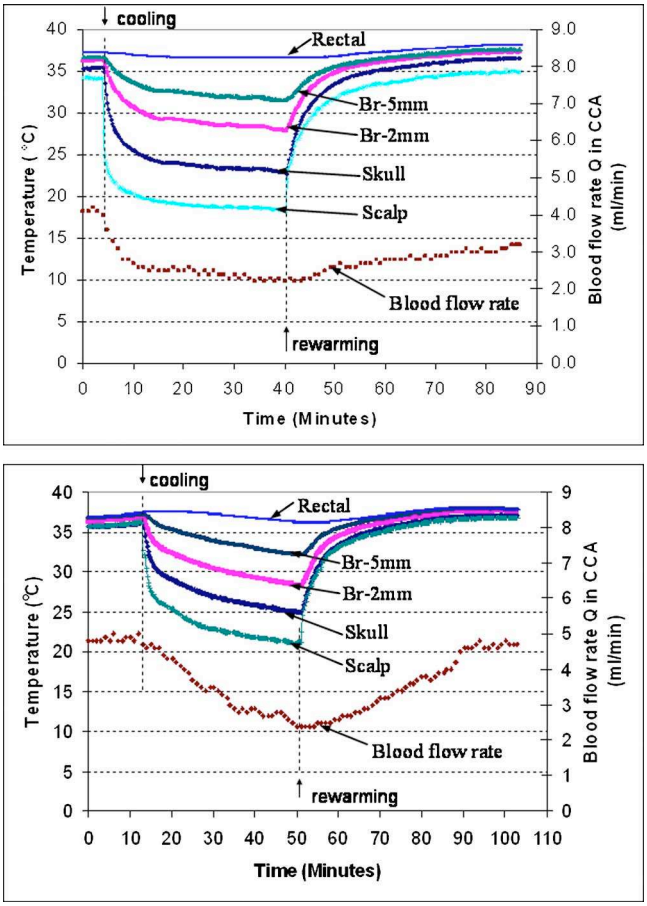

FIG. 5. Typical temperature responses (left y axis) to the helmet cooling and rewarming at various brain locations and transient blood flow rate of CCA (right y axis) during the trials. (a) Fast response; and (b) slow response.

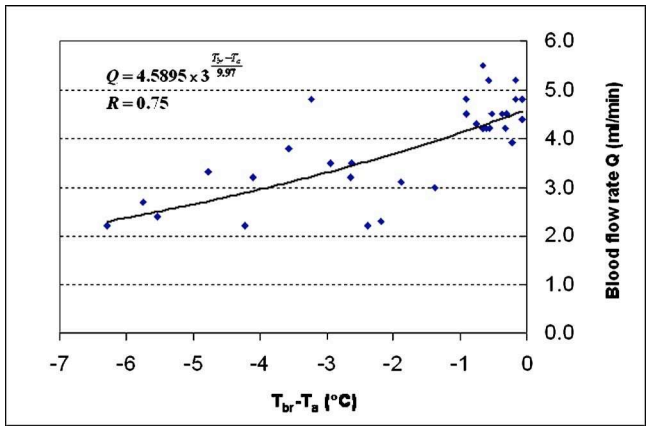

FIG. 6. The relationship between the blood flow of CCA and the average brain temperature after 35 min cooling. Experimental results are represented by scattered data. The line represents a fitted curve.

blood flow rate variation in either Fig. 5(a) or Fig. 5(b), one notices a very similar transient behavior between the tissue temperature and blood flow rate in the CCA. The fast response of blood flow shown in Fig. 5(a) is evident in approximately 1/3 of the rats studied (3 out of 8 rats in the moderate cooling group, and 2 out of 8 rats in the deep cooling group), while the rest of them possess the slow response [see Fig. 5(b)]. It seems that the variability of the response was not induced by the degree of the cooling. In transient heat transfer analysis, a characteristic time is usually defined to measure how fast the temperature field approaches its steady state. The larger the characteristic time, the longer it takes for the temperature field to establish a steady state. As shown in Fig. 5(a), the steady state has been established within the cooling process and the characteristic time varies between 5 to 35 min. However, in some experiments the steady state was never established during the 35 min cooling. One may state that the characteristic time then must be larger than 35 min. The very large characteristic time in those experiments may be the direct result of the unstable blood perfusion rate in the brain. As shown in Figs. 5(a) and 5(b), the varying pattern of the blood flow rate in the CCA during the cooling is very similar to that of the brain temperatures.

It is difficult to study all the factors that may affect the response of the blood flow rate to the head surface cooling, since the blood flow rate may be a function of the local temperature, time, and other unknown factors. We would like to isolate the factors and to examine how the local temperature alone affects the blood flow rate. In this study, the brain temperature T_{br} is represented by the average value of the tissue temperatures of two locations (brain-2 mm, and brain-5 mm) at the end of each trial. The relationship between the brain temperature T_{br} and the blood flow rate is then plotted in Fig. 6. A curve fit of the scattered data ($R=0.75$) has been performed and the relationship is expressed as

$$Q = 4.59 \cdot 3^{(T_{br}-T_a)/9.97},$$

where T_a is the arterial temperature at each trial. It is noticed that the first coefficient 4.59 is very close to the blood flow rate at the baseline, and the second coefficient 9.97 is close to 10. Therefore, the relationship between the local tissue temperature and the blood flow rate is very similar to the Q_{10} law with $Q_{10}=3$. Although the local metabolism was not directly measured in this experiment, it is possible that a coupled relationship between the blood flow and metabolism existed at the end of the 35 min cooling in this experiment.

IV. DISCUSSIONS AND CONCLUSIONS

Although blood supply to the brain is via the common carotid arteries and the vertebrate artery, it is estimated that more than 90% is from the common carotid arteries. The common carotid artery further bifurcates into the internal and external carotid arteries and the blood flow in the internal carotid artery supplies the brain tissue. Unfortunately, the ratio of the blood flow in the internal carotid artery to that in the external carotid artery has not been determined in this study and in the literature. Therefore, it is hard for us to estimate the average CBF in the rat brain from the current experimental procedures. In addition, whether this ratio is unchanged or not during cooling and rewarming needs to be investigated. Considering that there is a very minor change in the weight of the brain tissue in the rat group, it may be possible to use the blood flow rate as an index of the global CBF and to study how the global CBF changes during head surface cooling and rewarming. In the current experiment, the brain tissue temperature and blood flow rate in the CCA shared a similar transient profile. According to the Pennes bioheat equation, the effect of blood perfusion is modeled as a perfusion source term that is proportional to the local blood perfusion rate. If the blood perfusion rate keeps decreasing, tissue temperature will decrease as well due to the decrease in the strength of the perfusion source term. It is reasonable to expect a very similar transient profiles between the local blood perfusion rate and tissue temperature. Our results, therefore, can be interpreted as that the ratio of the blood flow in the internal carotid artery to that in the external carotid artery did not change significantly during the cooling in the current experiment.

Most of the previous studies have shown that systemic hypothermia is associated with a decrease in CBF. Unfortunately, the effect of SBC on CBF has not been extensively investigated. In the current experiment the blood flow rate in the common carotid artery in rats was measured directly and continuously using a Transonic® flow meter. Unlike other flow measurement techniques, the high temporal resolution of the flow meter used in this study allows continuous measurement of the blood flow rate. We not only observed a reduction in the blood flow rate of the CCA in all the rats studied, but also monitored how the blood flow reduction evolved during the cooling and rewarming.

The actual mechanisms of the blood flow response to cooling are not fully understood. It has been suggested that cooling may have a direct effect on the smooth muscle cell of the arterial wall to cause constriction and alter vascular responses. Cooling may also increase blood viscosity. All those factors have led to an increase in flow resistance in

brain vasculature.[21] The blood flow response, on the other hand, may be much more complicated and may not be explained by the local tissue temperature alone.

Blood flow plays an important role in determining both the steady state and transient temperature behavior in tissue. Based on the Pennes bioheat equation,[20] the effect of blood flow can be modeled as a heat source during head surface cooling. In this experiment, we have observed similar transient profiles between the tissue temperature and the blood flow rate. During a transient heat transfer process such as changing the boundary condition due to cooling, the temperature should decrease continuously until a new steady state is established. The blood flow decrease would slow down the process of establishing a new steady state. Our previous theoretical study[18] has predicted a much smaller characteristic time (less than 5 min) for the small size of the rat head when the blood flow rate is unchanged during the cooling. However, our experimental results have demonstrated a wide range of the characteristic time that is much longer than 5 min. It implies that it is the change of the blood perfusion rate in tissue that contributes significantly to the transient characteristic time. Other factors, such as the thermal diffusivity of the tissue and the boundary condition, play a minor role in the transient processes of heat transfer in SBC. In the current experiment, the cooling time is selected as 35 min. The cooling duration may have an effect on the blood flow response. If the cooling were allowed to continue, it would be interesting to see how long it would take to establish the steady state. However, a varying cooling time might affect the rewarming and it would introduce uncertainty to the rewarming process. If rewarming is not a concern, it will be worthwhile to perform experiments in the future to test the cooling tolerance in rats.

Initiating the cooling as soon as possible after brain ischemia or traumatic head injury is critical to maximize the protective effect of hypothermia. In most of the theoretical and animal studies, the focus is on the required time to establish a new steady state after the initiation of the cooling. It has been shown that several hours may be needed for the systemic cooling when the entire body is cooled. A theoretical study by the authors has suggested a time duration between 20 and 30 min for an adult's head via head surface cooling in humans.[18] An animal study on piglets demonstrated that it took more than 40 min to establish steady state after head surface cooling.[13] The discrepancy between the theoretical prediction and animal experimental data may be explained by the complicated blood flow response to cooling observed in the current experiment. Steady state can be established within a very short time duration (less than 10 min) if the blood flow reaches its steady state within 5 min. On the contrary, in the majority (two thirds) of the rats studied, the steady state temperature field was never established during the 35 min cooling. It suggests that during future simulation of the transient processes, the relationship between the CBF and tissue temperature cannot be simply described by the Q_{10} law. Other factors may also contribute to the complicated blood flow response to cooling. Our experimental data suggest that caution may be needed when one interprets results of theoretical simulation, since the complicated blood flow response is usually overlooked in the theoretical model.

Rapid rewarming may dangerously result in rebound intracranial pressure elevation and cerebral perfusion pressure reduction. The importance of gradual rewarming has been emphasized in multiple clinical and animal studies.[34–37] Using a rabbit model, Enomoto *et al.*[30] demonstrated that rapid rewarming caused an increase in the cerebral metabolic rate for oxygen that was temporarily greater than the increase in cerebral blood flow. This "mismatch" indicated a transient abnormality in flow-metabolism coupling, which resulted in an adverse effect on cerebral neuroprotection of hypothermia. By analyzing the CBF and cerebral oxygen consumption in anesthetized goats during brain hypothermia and subsequent rewarming, Hoffman *et al.*[26] concluded that the inability of brain function to recover following hypothermia was not due to the direct effects of cold on brain tissue but to an inadequate recovery of the cardiovascular system. The current study showed that the blood flow in the common carotid artery failed to recover to its baseline value 50 min after the cooling helmet was removed in the deep cooling group, although the tissue temperatures were recovered. Although the global metabolism in the brain was not measured directly, it suggested that after the rewarming the metabolic heat generation should be higher than its baseline value to compensate for the decreased strength of the blood perfusion source term. Thus, the metabolism outpaced the increase in the blood flow rate in the rat brain during the rewarming. Considering that the rewarming rate in the deep cooling group was higher than that in the moderate group, one may suggest that the observed mismatch was caused by the rapid rewarming rate, as demonstrated by other studies.[30,35]

Another limitation of this research is that no analgesia was provided and EEG[27,38] was not used to quantify the effective dose of pentobarbital during the experiments. The blood flow response to cooling is a very complicated process. The local pH value, PaO_2, and $PaCO_2$, as well as the coupling relationship between the CBF and metabolism, may directly or indirectly influence by the local temperature. Nonphysiological factors such as an anesthetic have been demonstrated to have systemic effects on blood pressure and hormones. Pentobarbital has been suggested to have a protective mechanism on cerebral circulation.[39–41] At this stage we do not know whether the anesthetic affected directly the blood flow response to cooling. Since the rats were under general anesthesia, it is also possible that the response to cooling in an unanesthetized rat may be different from that in ananesthetized rat, Furthermore, in an unanesthetized rat, additional shivering and stress may be induced and, thus, to cause changes in cerebral blood flow and cerebral glucose utilization. Unfortunately, current available techniques would not allow the direct measurement of the CBF in an unanesthetized rat. Future studies are needed to understand the mechanisms of anesthetic effects during head surface cooling and thus a better controlled experimental protocol can be designed to investigate the blood flow response to selective brain cooling.

The current experiment on rats will be useful for validating the previously developed theoretical models based on the Pennes bioheat equation.[18,19] Previous experimental and theoretical studies have suggested that the Pennes equation overestimates the blood effect on tissue temperature via neglecting the heat recaptured by the countercurrent veins and the temperature variation along the arteries. Temperature variation along the arteries and arterioles in the brain tissue can be more evident, especially during hypothermia, when the temperature of the brain tissue is lower than 37 °C. An overestimation of the blood flow effect in tissue can result in a small penetration of cooling to the deep brain tissue during head surface cooling and a short time duration to establish a steady state. Once the actual ratio of blood flow in internal to external carotid arteries is determined, the average blood perfusion rate in brain tissue can be determined from the measured blood flow rate of the common carotid artery and the weight of the brain tissue of the rats. The measured blood perfusion rate, as well as the thicknesses of brain tissue, skull, and scalp, the temperature of the carotid arterial blood supplied the brain, and the temperature of the circulating water can then be used as input to the Pennes bioheat equation to predict theoretically the radial temperature distribution in the brain tissue. The comparison between the theoretical predictions and experimental measurements will be used to evaluate whether the Pennes bioheat equation is accurate in predicting the temperature distribution in brain tissue during head surface cooling.

In summary, the current *in vivo* experimental study on rats has demonstrated that head surface cooling via a cooling helmet is a feasible way to achieve brain hypothermia. Different blood flow responses to cooling have been observed during the experiment. The local blood flow rate not only depends strongly on the local tissue temperature during steady state, but also varies as a function of time during the transient process. The results have also shown a possible mismatch between the global blood perfusion and metabolism during rewarming. We believe that the current study of the blood flow response to cooling and rewarming may be applicable to humans. It will further help to understand thermoregulation during clinical treatments for patients suffering brain ischemia or traumatic head injury, and therefore, provide better treatment protocols.

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