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A Method for Hypothermia-Induction and Maintenance Allows Precise Body and Brain Temperature Control in Mice

Yongshan Mou¹, Brian J. Wilgenburg², Yang-ja Lee¹, and John M. Hallenbeck^{1,*}

¹Clinical Investigations Section, Stroke Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

²Animal Health & Care Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland, USA

Abstract

The benefits as well as mechanisms of hypothermia in brain injuries are actively studied at the bench and in the clinic. However, methods used in controlling hypothermia vary among laboratories, and usually brain temperatures are not monitored directly in animals due to the need for an invasive procedure. Here we show a method, water immersion technique, which we developed recently to regulate body temperature in mice during hypothermia process. This method significantly reduced the temperature variation around target temperature. Importantly, this method demonstrated a parallel and consistent relationship between rectal temperature and brain temperature (the brain temperature was consistently 0.5 C higher than rectal temperature) throughout hypothermia maintenance. This technique may be well-adapted to hypothermia studies in mice and other rodents, especially to the assessment and regulation of brain temperature during studies.

Keywords

Hypothermia; Experimental Method; Animal Model; Mouse; Brain Ischemia; Brain; Body Temperature

1. Introduction

Hypothermia has shown a notable benefit against a variety of brain injuries, and small temperature variations may have a pronounced effect on the outcome of cerebral ischemia (Busto et al., 1989; Ginsberg et al., 1992). However, the relationship between core temperature and brain temperature has not been extensively studied (Bertolizio et al., 2011). The methods to control hypothermia vary in the different laboratories, and brain temperature is usually not monitored directly in survival studies due to the invasiveness of the procedure. Despite clear data that rectal temperature does not always accurately predict brain temperature, many continue to rely solely on rectal temperature measurements during and after ischemia, or fail to measure temperature at all (DeBow et al., 2003). Some authors compared the temperature difference between brain and rectum in rodent models during

*Corresponding Author: John Hallenbeck, hallenbj@ninds.nih.gov.

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hypothermia induced by traditional methods, such as alcohol/water spray plus fan/warm pad, heating infrared lamp, and temperature-controlled chamber; their results, however, showed considerable variation (Finn et al., 1991; Bejanina et al., 1991; Maier et al., 1998; DeBow et al., 2003).

To stabilize the relationship between brain and rectal temperatures, the entire body of the animal needs to be exposed to a medium that has an even temperature. The ice pack/heating pad method, although it is a commonly used surface cooling technique (Cramer et al., 1998; Li et al., 2011; Fujita et al., 2012), suffers from an uneven contact between the ice/heating pad and body surface of animals. In addition, with the ice-pack/heating pad method, it is not easy to keep the animal's body temperature at the target temperature because of a susceptibility to overcooling that is due to the large temperature difference between the ice and the target temperature. Although some authors (DeBow et al., 2003) suggest that the use of a rapid feedback system with lamps, fans, and water spray results in more precise temperature control than other methods, the variation of the temperature with these techniques is still ± 0.5 °C around the desired value. This study presents a water immersion technique that was able to control mice's body temperatures at a very precise level (the temperature variation could be controlled within ± 0.1 °C). Most importantly, it demonstrated a parallel and consistent relationship between rectal temperature and brain temperature. Therefore, it is possible to accurately extrapolate the brain temperature from rectal temperature.

2. Materials and Methods

2.1. Experimental Animals and Target Temperatures

For the hypothermia procedures, a mixed anesthetic gas (5% Isoflurane, 33% O₂, 67% N₂O) was used for induction for both the ice-pack/warming pad and water immersion methods; 1.5% or 1% Isoflurane, plus 33% O₂, 67% N₂O were used to maintain a surgical plane of anesthesia for both methods. To achieve reliable results in a pilot study of hypothermic preconditioning in ischemic stroke, a new technique was designed to cool and regulate brain temperatures in mice. The target temperatures in brains were 32 °C, and 28 °C. According to several reports (Nishio et al., 2000; Yunoki et al. 2003), a 20 min hypothermic treatment had been found optimal for inducing rapid and/or delayed tolerance to ischemia, so this duration of exposure was applied. Sixteen male and female adult mice, age 3–18 months, weighting ≈ 25 –36g, were used in the pilot study: eight mice with the ice/heating pad method at target brain temperatures of 32 °C (Group 1) or 28 °C (Group 2), 4 mice per group; eight mice that underwent the water immersion procedure at a target brain temperature of 32 °C (Group 3) or 28 °C (Group 4), 4 mice per group.

2.2. Hypothermia Procedures

The temperatures of the brain, the rectum and the water were monitored with Thermalert TH-5 digital thermometers (Physitemp, Clifton NJ). The accuracy of all probes is ± 0.1 °C. Temperatures of brain and rectum were recorded at a frequency of one data point per 2-minutes. Rectal temperature was monitored by a RET-3 rectal probe (tip diameter, 0.065 inch/1.66 mm). To monitor brain temperature, a 0.7 mm diameter hole was drilled on the skull 1.0 mm posterior to the bregma, and 1.0 mm lateral to the midsagittal suture. Then an IT-21 flexible implantable probe (diameter 0.016inch/0.41mm, Physitemp, Clifton NJ.) was inserted into brain to a depth of 3–4 mm, and fixed to the scalp with silk suture. After the completion of monitoring, the brain probe was removed, and the animals were euthanized immediately.

2.2.1. Ice/Heating Pad Hypothermia Procedure—After induction of anesthesia, the mouse was transferred onto an ice bag alternating with a warm pad to regulate the body temperature. The mice had to be continuously transferred from the ice bag to the warm pad and back with the anesthesia mask in place to maintain the target temperatures of 28 °C or 32 °C (Fig 1A).

2.2.2. Water Immersion Hypothermia procedure—These experiments were performed in a chemical fume hood. After induction of anesthesia, the mouse was transferred into a (9 × 6 inch) zip-lock plastic bag into which a mixed anesthetic gas was infused (1% Isoflurane, 33% O₂, 67% N₂O) via plastic tubing (inserted through a small opening in the zip-lock bag) at a flow rate of 1.5/L/min to keep the animal anesthetized. The anesthetic gas mixture discharged through the small opening in one corner of the zip-lock plastic bag and exhausted into the chemical fume hood. The plastic bag was inspected for tears or holes prior to use to assure that it was water-tight. The zip-lock bag was then placed into a Styrofoam[®] cooler that was filled with water; the corner of the zip-lock bag through which the anesthetic gases enter and discharge remained above the water level. The mouse was suspended completely below the surface. The temperature of the water was maintained at around 1.0 °C lower than the target brain temperature of the planned study since live animals still generate heat inside of their body although under anesthesia. The temperature of the water was regulated by appropriate additions of hot or cold tap water followed by stirring to maintain the animal's brain temperature at the desired level. Oxygen saturation, heart rate, and respiratory rate were monitored by an Oximeter (STARR Life Sciences Corporation, Oakmont, PA) in survival experiments. The Oximeter was connected to the animal's neck via a CollarClip Sensor (Fig 1B).

3. Statistics

Unpaired Student's t-test was used to compare the means of two groups, and $P < 0.05$ was considered to be statistically significant.

4. Results

We found that the pattern of the temperature-change in rectum and brain during hypothermia was not related to an animal's gender or age. With the ice/heating pad method, the reduction of body temperature occurred very quickly, but it was not easy to control. To keep their temperature close to the target temperature, animals needed to be transferred between the heating pad and ice pack 8–10 times through the 20-minute duration of the maintenance. In addition, the brain temperatures swung with a wide range in each animal compared with the water immersion technique, and that was shown by the scatter graph in Fig. 2. The average of the standard deviations for brain temperatures in ice/heating pad group was 0.288 °C, and in water immersion group was 0.062 °C when target temperature was to 32 °C (Fig. 3A); 0.266 °C and 0.067 °C respectively when target temperature was 28 °C (Fig. 3B). The differences were statistically very significant ($P=0.002$ for 32 °C, $P=0.0007$ for 28 °C) (Fig 3). In addition, the changes of rectal temperature and brain temperature in ice/heating pad method were not tightly coupled during maintaining period (Fig 4A, C).

The water immersion technique, however, allowed the mice to reach the target temperature at a more controlled rate, and to maintain the temperature with very little variations both in the brain and rectum. The changes of rectal temperature and brain temperature were tightly coupled. The brain temperature was consistently about 0.5 °C higher the rectal temperature (Fig. 4B, D). In this study, the range of animals' oxygen saturation rate was 97– 99%; heart rates and respiratory rates were around 400/min, and 50/min. Animals' respiratory rates were constant, with normoxic maintained throughout the procedure.

5. Discussion

The results presented above show distinct effects between those two methods during the hypothermic process. It is clear that ice/heating pad method cannot maintain an animal's body temperature at a target level precisely, nor can it provide a consistent relationship between the temperatures of the brain and those of the rectum. Frequent switching between a cooling medium and a heating medium unavoidably causes swings of body temperature; also, the uneven contact between the animal's body surface and the temperature-regulating medium will lead to differential temperature changes in different parts of body with this method. This especially applies to small animals such as mice.

The ice/heating pad method has obvious flaws, but other more sophisticated techniques also have their limitations. For example, a cooling/heating blanket is one of the more common methods used in brain ischemia studies. This method has some advantages compared with the ice/heating pad method, such as a larger surface with a flexible shape, a mild initial cooling temperature, and the convenience of graduated temperature regulation. However, it is still not easy to wrap a small animal, such as a mouse, evenly while an anesthesia mask is employed. In addition, the self-temperature-regulating speed of a cooling/heating blanket won't be as rapid as temperature adjustment made by mixing cool water with hot water directly. In the water immersion technique, water serves as the ambient temperature control medium that regulates the animal's body temperature. A key advantage of water is that its specific heat is very high compared with that of other mediums. For example, the amount of heat per unit mass required to raise the temperature of water by one degree centigrade is 4.186 Joules or 1 calorie/g/°C by definition. That of ice is 0.51 calorie/g/°C, and of water vapor is 0.48 calorie/g/°C (at constant pressure). The specific heat of human tissue is 0.85 calorie/g/°C and that of air is 0.23 calorie/g/°C. Therefore, the temperature of water once set at the desired temperature remains relatively constant over time scales measured in minutes. In addition, water can conform to any shape thus expanding the contact surface of the hypothermic medium with the animal as compared to an ice bag's more limited surface contact. Finally water temperature can be adjusted to meet any target level, while ice remains at a relatively static temperature. These properties of water make the body-temperature regulation easier and more consistent. Thus, it is possible to control mouse body temperature at a specified level very consistently and accurately by adjusting ambient water temperature.

Accurate monitoring of brain temperature, which is a critical variable for neuroprotection in stroke and other brain injuries, generally requires an invasive recording method that makes it impracticable for survival studies. The water immersion technique, however, makes it possible to extrapolate the brain temperature directly from the rectal temperature without additional instrumentation. The present study shows that during hypothermia induced by means of the water immersion method, the brain temperature is 0.5 °C higher than rectal temperature with very little fluctuation and that the brain and rectal temperature curves remain very parallel at both 32 °C and 28 °C. It has been reported that brain temperature is 0.5–2°C warmer than core temperature in clinical as well as in large animal studies (Hayward et al., 1968; Baker et al., 1972; Rumana et al., 1998; Kurth et al., 2000; McIlvoy et al., 2004). Some authors also find that brain temperature is 0.2 to 0.7°C higher than rectal temperature in their rodent studies (Maier et al., 1998). Our initial data is compatible with the above findings. We also found that with an increase in the anesthetic concentration, the temperature difference between brain and rectum became smaller; moreover, when the ambient temperature (water temperature) was raised to 37°C, there was almost no difference between brain temperature and rectal temperature if animals were still under anesthesia. Once the temperature differential between rectal and brain temperatures for a given level of anesthesia and a given target temperature are established, however, rectal temperatures and

brain temperatures remained parallel during the hypothermic exposure with the water immersion technique. That property makes the brain temperature predictable by simply monitoring rectal temperature. More experiments will be needed to permit gauging brain temperatures under other defined conditions, but brain temperatures can be accurately predicted by monitoring rectal temperatures during hypothermia studies in murine models with the water immersion technique for the ranges of temperature and surgical plane of anesthesia we have employed.

Some issues associated with application of the water immersion technique, however, need to be addressed. In order to avoid respiratory distress, an appropriate oxygen supply should be confirmed no matter what kind of anesthetic technique is employed. In addition, a piece of tissue paper put around animal's nose in the plastic bag is helpful. If there is a small leak in the bag, it can be easily noted, and the water will be absorbed by the paper. The duration of hypothermia depends on the purpose of individual experiment. In mechanism studies, short periods of hypothermia are usually employed (Nishio et al., 2000; Yunoki et al. 2003); in clinical trials, however, longer durations are considered more effective (Liping, et al. 2009; Van der Worp HB, et al 2010).

In summary, the water immersion technique described above offers precise temperature control without repeated animal movement and with continuous physiological monitoring in order to more accurately model the physiological properties and efficacy of therapeutic hypothermia. The most important feature is that this method allows us to predict brain temperature accurately by extrapolating from rectal temperature and without resorting to invasive instrumentation. This improvement in the technique for hypothermia induction and maintenance may be adaptable to experiments with larger rodents.

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References

1. Baker MA, Stocking RA, Meehan JP. Thermal relationship between tympanic membrane and hypothalamus in conscious cat and monkey. *J Appl Physiol.* 1972; 32:739–742. [PubMed: 5034336]
2. Bejanian M, Jones BL, Syapin PJ, Finn DA, Alkana RL. Brain Temperature and Ethanol Sensitivity in C57 Mice: A Radiotelemetric Study. *Pharmacology Biochemisto' & Behavior.* 1991; 39:457–463.
3. Bertolizio G, Mason L, Bissonnette B. Brain Temperature: heat production, elimination and clinical relevance. *Paediatr Anaesth.* 2011; 4:347–358. [PubMed: 21371165]
4. Busto R, Dietrich WD, Globus MY-T, Ginsberg MD. The Importance of Brain Temperature in Cerebral Ischemic Injury. *Stroke.* 1989; 20:1113–1114. [PubMed: 2756546]
5. Cramer WC, Toorop GP. Focal cerebral ischemia in the mouse: Hypothermia and rapid screening of drugs. *Gen Pharmacol.* 1998; 30:195–200. [PubMed: 9502174]
6. DeBow S, Colbourne F. Brain temperature measurement and regulation in awake and freely moving rodents. *Methods.* 2003; 30:167–171. [PubMed: 12725783]
7. Finn DA, Bejanian M, Jones BL, Babbini M, Syapin PJ, Alkana RL. The Relationship between Brain Temperature during Intoxication and Ethanol Sensitivity in LS and SS Mice. *Alcoholism: Clinical and Experimentals Reseach.* 1991; 4:717–724.
8. Fujita M, Wei EP, Povlishock JT. Effects of Hypothermia on Cerebral Autoregulatory Vascular Responses in Two Rodent Models of Traumatic Brain Injury. *J Neurotrauma.* 2012; 29(7):1491–8. [PubMed: 22364620]

9. Ginsberg MD, Sternau LL, Globus MY-T, Dietrich WD, Busto R. Therapeutic Modulation of Brain Temperature: relevance to Ischemic Brain Injury. *Cerebrovascular and Brain Metabolism Reviews*. 1992; 4:189–225. [PubMed: 1389956]
10. Hayward JN, Baker MA. Role of cerebral arterial blood in the regulation of brain temperature in the monkey. *Am J Physiol*. 1968; 215:389–403. [PubMed: 4969787]
11. Kurth CD, Steven JM. Keeping a cool head. *Anesthesiology*. 2000; 93:598–600. [PubMed: 10969290]
12. Li J, Benashski S, McCullough L. Post-Stroke Hypothermia Provides Neuroprotection through Inhibition of AMP-Activated Protein Kinase. *Journal of Neurotrauma*. 2011; 28:1281–1288. [PubMed: 21446786]
13. Liu, Liping; Midori, A. Yenari. Clinical application of therapeutic hypothermia in Stroke. *Neurol Res*. 2009; 31:331–335. [PubMed: 19508813]
14. Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK. Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. *Stroke*. 1998; 29:2171–2180. [PubMed: 9756600]
15. McIlvoy L. Comparison of brain temperature to core temperature: a review of the literature. *J Neurosci Nurs*. 2004; 36:23–31. [PubMed: 14998103]
16. Nishio S, Yunoki M, Chen ZF, Anzivino MJ, Lee KS. Ischemic tolerance in the rat neocortex following hypothermic preconditioning. *J Neurosurg*. 93:845–851. [PubMed: 11059667]
17. Rumana CS, Gopinath SP, Uzura M, Valadka AB, Robertson CS. Brain temperature exceeds systemic temperature in head-injured patients. *Crit Care Med*. 1998; 26:562–567. [PubMed: 9504587]
18. Van der Worp HB, Macleod MR, Kollmar R. Therapeutic hypothermia for acute ischemic stroke: ready to start large randomized trials? *J Cereb Blood Flow Metab*. 2010; 30:1079–93. [PubMed: 20354545]
19. Yunoki M, Nishio S, Ukita N, Anzivino MJ, Lee KS. Hypothermic preconditioning induces rapid tolerance to focal ischemic injury in the rat. *Exp Neurol*. 2003; 181:291–300. [PubMed: 12782001]

1. A precise method to control body temperature in mice by using water immersion technique.
2. This method allows us to predict brain temperature accurately by extrapolating from rectal temperatures.
3. This improved technique for induction and maintenance of hypothermia may be useful for experiments with larger rodents.

Figure 1.

Different methods used to regulate animal's body temperature in hypothermic studies. (A) The conventional ice pack/heating pad procedure. To keep body temperature close to the target level, animal need to be transferred between the heating pad and ice pack alternatively. (B) The water immersion technique. Animal was kept immobile during the procedure, and the body temperature was regulated just by adding appropriate amount of cold or hot waters into the box. In practice, a weight was placed on the bag (along the bag opening) to keep the animal in the bag completely immersed in the water (styrofoam box not shown).

Figure 2.

Scatter graphs of brain temperature of the individual animal during the hypothermic procedure with two different methods targeted to two different temperatures (32 °C and 28 °C). Four animals were used in each group. (A): Ice/heating pad method, 32 °C as a targeted temperature, (B) Water immersion method, 32 °C as a targeted temperature, (C) Ice/heating pad method, 28 °C as a targeted temperature, (D) Water immersion method, 28 °C as a targeted temperature.

Figure 3. Comparison of standard deviations of brain temperature from four animals in each group between these two methods (ice/heating pad, water immersion) targeted to two different temperatures. (A) Targeted temperature: 32 °C. ** $P < 0.01$ compared to ice/heating pad method. (B) Targeted temperature: 28 °C. *** $P < 0.001$ compared to ice/heating pad method.

Figure 4.

Comparison of temperature curves of brain and rectum during hypothermic procedures in two different methods and at two different target temperatures. (A) Ice/heating pad method, target temperature at 32 °C, (B) water immersion method, target temperature at 32 °C, (C) Ice/heating pad method, target temperature at 28 °C, (D) water immersion method, target temperature at 28 °C.