3

# Timing of Hypothermia (During or After Global Cerebral Ischemia) Differentially Affects Acute Brain Edema and Delayed Neuronal Death

Masaru Doshi[1] and Yutaka Hirashima[2]
[1]*Faculty of Pharmaceutical Sciences, Teikyo University*
[2]*Department of Neurosurgery, Imizu City Hospital*
*Japan*

## 1. Introduction

Hypothermia (HT) is one of the most effective neuroprotective therapies for brain injury caused by cardiac arrest in humans (Benard et al., 2002), although there is as yet no evidence of such an effect of HT on cerebrovascular diseases from a large-scale clinical trial. We recently reported the experimental studies on the importance of timing of HT using global cerebral ischemia in mice (Doshi et al., 2009, 2011). we summarized our findings in this chapter because our results may be relevant to clinical studies.

## 2. Experimental methods

### 2.1 Global cerebral ischemia using C57BL/6J mice

Transient forebrain ischemia (global cerebral ischemia), which is induced by occlusion of the bilateral common carotid arteries (BCCA) in mice, causes delayed neuronal death in the hippocampus and is known as a model of brain injury following transient cardiac arrest (Kawase et al., 1999). C57BL/6J mice are widely used as a background strain for genetic alterations and have been valuable for investigating the molecular mechanism of delayed neuronal death following transient forebrain ischemia (Yang et al., 1997; Tajiri et al., 2004). We recently demonstrated that acute brain edema, one of the most important disorders following cerebral ischemia, occurred in the forebrain in this C57BL/6J mouse model (Doshi et al., 2009).

Forebrain ischemia was induced by BCCA occlusion with clips for 15 min under 1 % halothane anesthesia in air using a face mask. Rectal temperature was monitored using a digital thermometer and maintained at 37±0.5℃ with a heating blanket (normothermia, NT). The control mice underwent a sham operation without BCCA occlusion under halothane anesthesia for 15 min.

### 2.2 Evaluation of delayed neuronal death and acute brain edema

Delayed neuronal death in the hippocampus 7 days after reperfusion was determined by both hematoxylin-eosin (HE) staining and TdT-mediated dUTP-biotin nick end labeling

(TUNEL) assay. To evaluate the acute brain edema, the water content in the forebrain 1 hour after reperfusion was determined by the weight differences between wet and dry samples. The percentage of water in the forebrain was calculated as follows: ((wet weight-dry weight) / wet weight)×100.

### 2.3 Induction of hypothermia

HT during ischemia was spontaneously induced by removing the heating blanket, and the mice were allowed to recover from anesthesia at room temperature (23-25℃) until 1 hr after reperfusion. In contrast, artificial HT after reperfusion (rHT) was induced by placing the mice on a refrigerant, which was maintained at 18-19℃ until 1 hr after reperfusion in a styrofoam box. In NT mice, rectal temperature was maintained at 37±0.5℃ during ischemia. In HT mice, rectal temperature significantly decreased by 28-30℃ during ischemia. On the other hand, the patterns of the change in rectal temperature after reperfusion in rHT mice were significantly lower than that in HT mice (Fig. 1).

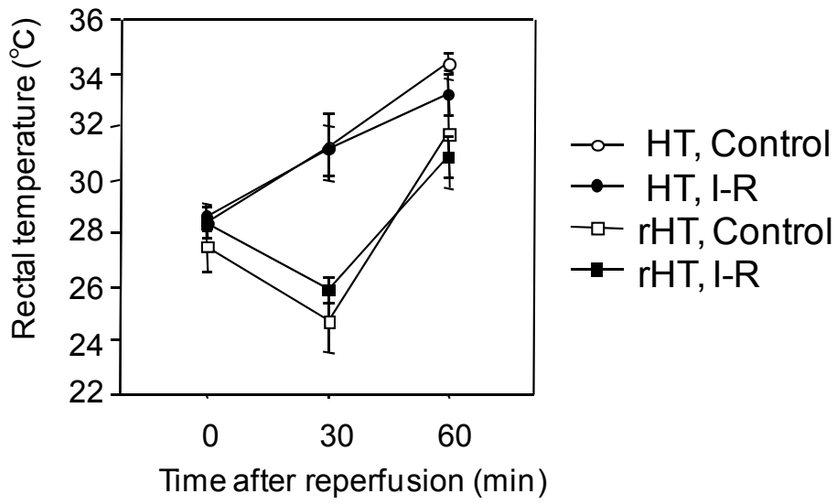



Fig. 1. Changes in rectal temperature after reperfusion in both HT and rHT mice.

Forebrain ischemia was induced by BCCA occlusion for 15 min under 1 % halothane anesthesia in both HT and rHT mice, as described in the Materials and Methods. The control mice (Control) underwent a sham operation without BCCA occlusion under halothane anesthesia for 15 min. The rectal temperature was monitored 0, 30, and 60 min after reperfusion. Data are expressed as mean ± SE (control : n=3, I-R : n=7). Statistical analysis was performed by analysis of variance (ANOVA) of repeated measures for the comparison of the changes in rectal temperature between the HT and rHT groups.

## 3. Effect of hypothermia during ischemia on occurrence of delayed neuronal death

HT during ischemia has been shown to protect against delayed neuronal death in several animal models of cerebral ischemia including the global cerebral ischemia in C57BL/6J mice (Yang et al., 1997). We confirmed the protective effect of HT during ischemia against

delayed neuronal death in the hippocampus under our experimental conditions. As shown in Table 1, there were no histological changes in the hippocampus 7 d after reperfusion in all HT mice, whereas delayed neuronal death occurred in three of the four NT mice. In this model, controlled NT during cerebral ischemia is important for the induction of neuronal death following cerebral ischemia (Ohtaki et al., 2006), supported by data revealing that HT during ischemia protects against neuronal death in the BCCA occlusion C57BL/6J mouse model (Yang et al., 1997). Our data in this study were consistent with previous data. However, the neuroprotective effect of HT after ischemia-reperfusion still remains to be solved in NT mice.

| Mice | The number of delayed neuronal death-positive mice / total mice |
|---|---|
| NT (ischemia 15 min) | 3 / 4 |
| HT (ischemia 15 min) | 0 / 5* |
| HT (ischemia 45 min) | 0 / 5* |

*p<0.05 vs NT (Fisher's exact test)

Table 1. Effect of HT during ischemia on occurrence of delayed neuronal death in hippocampus of C57BL/6J mice.

## 4. Effect of timing of hypothermia on occurrence of acute brain edema

Brain edema, defined as an increase in brain water content, is one of the major reperfusion pathologies following cerebral ischemia along with delayed neuronal death and infarction. The increase in brain water content results in serious pathologic situations such as elevation of intracranial pressure and reduction of cerebral blood flow, and subsequently causes cerebral herniation and death (Kempski, 2001). Even now, the treatment options for brain edema are limited to the use of hyperosmotic agents, such as glycerol or mannitol, and surgical decompression. That is, despite the clinical significance of brain edema, the mechanisms of brain water transport and edema formation in ischemic injuries remain unclear.

Several studies have shown that HT, which was monitored by measuring rectal temperature, protects against delayed neuronal death and infarction (Maier et al., 1998; Tsuchiya et al., 2002), but few studies have investigated the effects of HT on brain edema in mouse models of cerebral ischemia. Therefore, we investigated the effect of HT on the occurrence of acute brain edema following the global cerebral ischemia in C57BL/6J mice.

### 4.1 Effect of hypothermia during ischemia on occurrence of acute brain edema

We first investigated the effect of HT during global cerebral ischemia on the occurrence of acute brain edema in the C57BL/6J mouse model. In both NT and HT mice, the water content 1 h after reperfusion was significantly higher than that of the control mice, but no significant differences were detected between the NT and HT mice (Fig. 2). This data indicated the ineffectiveness of HT during ischemia against acute brain edema, unlike its effect on neuronal death, suggesting that HT during ischemia may not necessarily be an effective therapy for all reperfusion pathologies following cerebral ischemia. However, we found that the rectal temperature of the HT mice recovers from HT during ischemia to NT mice levels within 1 hr after reperfusion. Therefore, we speculated that the ineffectiveness of

HT during ischemia against acute brain edema is due to the immediate recovery of rectal temperature after reperfusion at room temperature in the C57BL/6J mouse model.

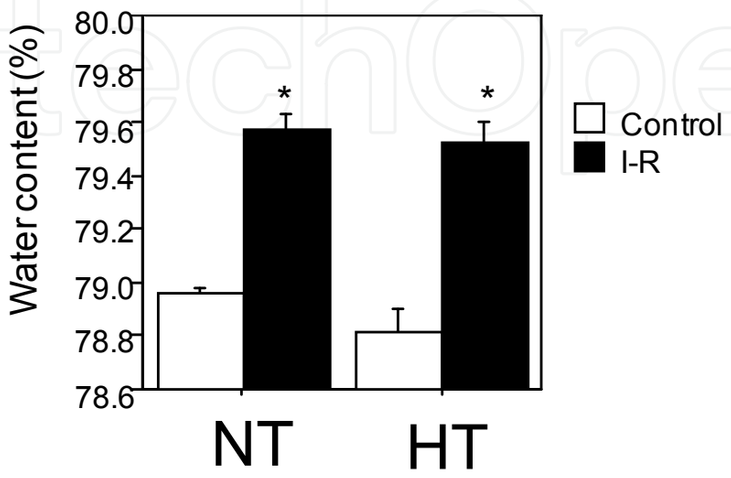



Fig. 2. Effect of HT during ischemia on occurrence of brain edema in C57BL/6J mice.

Forebrain ischemia was induced by BCCA occlusion for 15 min under 1 % halothane anesthesia in both normothermia (NT) and hypothermia (HT). The control mice (Control) underwent a sham operation without BCCA occlusion under halothane anesthesia for 15 min. The water content in the forebrain treated with BCCA occlusion for 15 min and 1 h after reperfusion (I-R) was measured. Data are expressed as the mean ± SE (control : n=3, I-R : n=5). Statistical analysis was performed by two-way ANOVA and an unpaired Student's *t*-test for comparison between control and I-R mice groups (*p<0.01 vs control).

### 4.2 Effect of hypothermia after reperfusion on occurrence of acute brain edema

We next investigated the effect of rHT on acute brain edema in the C57BL/6J mouse model. The water content 1 hr after reperfusion was significantly higher in both the HT and rHT mice than in the control mice ($p$<0.05). However, the water content 1 hr after reperfusion was significantly lower in the rHT mice than in the HT mice (Fig. 3). Already, we have shown an increase in brain water content during BCCA occlusion in a previous study (Doshi et al., 2009). Therefore, these results indicated that rHT suppresses the additional increase in brain water content after reperfusion, but not the increase in brain water content during BCCA occlusion.

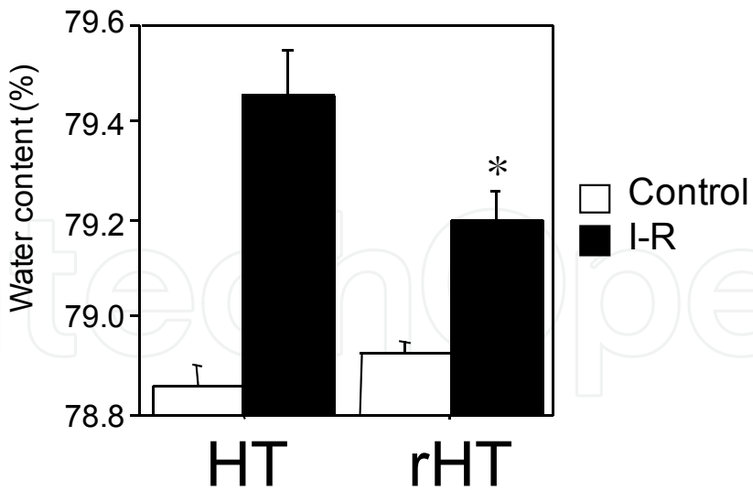



Fig. 3. Effect of HT after reperfusion on occurrence of brain edema in the BCCA occlusion C57BL/6J mouse model.
Forebrain ischemia was induced by BCCA occlusion for 15 min under 1 % halothane anesthesia in both HT and rHT mice. The control mice (Control) underwent a sham operation without BCCA occlusion under halothane anesthesia for 15 min. The water content in the forebrain treated with BCCA occlusion for 15 min and 1 hr after reperfusion (I-R) was measured. Data are expressed as mean ± SE (control : n=3, I-R : n=7).
Statistical analysis was performed by two-way ANOVA and the unpaired Student's *t*-test (*$p$<0.05 vs HT).

## 5. Conclusion

HT during ischemia protects against delayed neuronal death in the hippocampus. In addition, rHT suppresses aggravation of acute brain edema following global cerebral ischemia in mice. Therefore, these findings indicate that the timing of therapeutic HT differs depending on the pathology following global cerebral ischemia. Further experimental studies should be performed to establish a standard method for the clinical application of therapeutic HT because there are still several controversial issues in the development of cooling techniques and in the determination of optimal duration and temperature.

## 6. References

Benard, SA., Gray, TW., Buist, MD., Jones, BM., Silvester, W., Gutteridge, G., & Smith, K. (2002). Treatment of comatose survivors of out-of-hospital cardiac arrest with induced hypothermia, *N Engl J Med*, 346, 557-563.

Doshi, M., Kuwatori, Y., Ishii, Y., Sasahara, M., & Hirashima, Y. (2009). Hypothermia during ischemia protects against neuronal death but not acute brain edema following transient forebrain ischemia in mice, *Biol Pharm Bull*, 32, 1957-1961.

Doshi, M., Higuchi, E., & Hirashima, Y. (2011). Hypothermia after reperfusion suppresses aggravation of acute brain edema following transient forebrain ischemia in mice, *J Health Sci*, 57, 82-85.

Kawase, M., Murakami, K., Fujimura, M., Morita-Fujimura, Y., Gasche, Y., Kondo, T., Scott, RW., & Chan, PH. (1999). Exacerbation of delayed cell injury after transient global ischemia in mutant mice with CuZn superoxide dismutase deficiency, *Stroke*, 30, 1962-1968.

Kempski, O. (2001). Cerebral edema, *Seminars in Nephrology*, 21, 303-307.

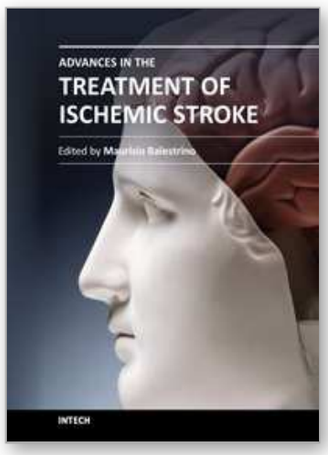
Maier, CM., Ahern, K., Cheng, ML., Lee, JE., Yenari, MA., & Steinberg, GK. (1998). Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia, *Stroke*, 29, 2171-2180.

Ohtaki, H., Nakamachi, T., Dohi, K., Yofu, S., Hodoyama, K., Matsunaga, M., Aruga, T., & Shioda, S. (2006). Controlled normothermia during ischemia is important for the induction of neuronal cell death after global ischemia in mouse, *Acta Neurochir*, 96, 249-253.

Tajiri, S., Oyadomari, S., Yano, S., Morioka, M., Gotoh, T., Hamada, J-I., Ushio, Y., & Mori, M. (2004). Ischemia-induced neuronal cell death is mediated by the endoplasmic reticulum stress pathway involving CHOP, *Cell Death Differ*, 11, 403-415.

Tsuchiya, D., Hong, S., Suh, SW., Kayama, T., Panter, SS., & Weinstein, PR. (2002). Mild hypothermia reduces zinc translocation, neuronal cell death, and mortality after transient global ischemia in mice, *J Cereb Blood Flow Metab*, 22, 1231-1238.

Yang, G., Kitagawa, K., Matsushita, K., Mabuchi, T., Yagita, Y., Yanagihara, T., & Matsumoto, M. (1997). C57BL/6 strain is most susceptible to cerebral ischemia following bilateral common carotid occlusion among seven mouse strains: selective neuronal death in the murine transient forebrain ischemia, *Brain Res*, 752, 209-218.




**Advances in the Treatment of Ischemic Stroke**
Edited by Dr. Maurizio Balestrino

ISBN 978-953-51-0136-9
Hard cover, 246 pages
**Publisher** InTech
**Published online** 02, March, 2012
**Published in print edition** March, 2012

In recent years research on ischemic stroke has developed powerful therapeutic tools. The novel frontiers of stem cells therapy and of hypothermia have been explored, and novel brain repair mechanisms have been discovered. Limits to intravenous thrombolysis have been advanced and powerful endovascular tools have been put at the clinicians' disposal. Surgical decompression in malignant stroke has significantly improved the prognosis of this often fatal condition. This book includes contributions from scientists active in this innovative research. Stroke physicians, students, nurses and technicians will hopefully use it as a tool of continuing medical education to update their knowledge in this rapidly changing field.



**InTech Europe**
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

**InTech China**
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen