Evaluation of Hemodilution and Hypothermia in a Rat Model of Global Cerebral Ischemia

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Cerebrovascular disease and trauma are major causes of death and disability in the United States. Studies have shown that hypothermia can reduce neuronal damage and improve recovery from cerebral ischemia or trauma in animal models, but the optimal use of hypothermia in combination with other treatment modalities has not been established. We studied the combined effect of hemodilution and hypothermia in a rat model of global cerebral ischemia based on the hypothesis that hypothermia may reduce neuronal oxygen requirements, whereas hemodilution may increase cerebral blood flow. Hemodilution was accomplished by intravenous injection of saline. Rats were randomly assigned to three experimental groups: normothermic with a sham injection (sham control), normothermic with a saline injection (normothermic), and hypothermic with a saline injection (hypothermic). Cerebral ischemia was produced by cauterization of the vertebral arteries followed by ligation of the carotid arteries for 10 min. Body and cerebral temperature, cerebral blood flow, and electroencephalographic activity were monitored during ischemia and a 2-h post-ischemic treatment. Cerebral ischemia was followed by a 15-min period of reactive hyperemia, and then a sustained period of hypoperfusion in all treatment groups. Mortality in the hypothermic group (0%) was significantly less than in sham animals (32%). The total number of viable hippocampal CA 1 neurons 21 d after ischemia was greater in the normothermic and hypothermic rats than in the sham group, but the differences were not significant. Motor function improved in all treatment groups between 7 and 21 d post-ischemia. The foot-fault test failed to show significant differences between groups at any time, though there was a trend toward a lower foot-fault rate in hypothermic animals at 7 d. Hypothermia significantly decreased mortality, but this improvement was not reflected by the total number of viable hippocampal neurons or by a motor function test. ©2000 Oklahoma Academy of Science

INTRODUCTION

In Western countries, stroke is the third most common cause of death and the second most common cause of neurologic disability. Stroke, or cerebrovascular accident, results in cerebral ischemia and hypoxia, leading to a cascade of vascular and cellular processes that combine to cause neuronal dysfunction and death. Ischemia induces the

release of excitatory neurotransmitters, glutamate and aspartate, partly by evoking reversed excitatory neurotransmitter transport from glial cells (*1*). The over-activation of glutamate/aspartate receptors causes excessive calcium influx and the generation of free radicals. These events ultimately lead to neuronal swelling and death, partly through activation of the caspase system of enzymes that induce neuronal apoptosis (*2*). The experimental methods that have been found to reduce neuronal damage resulting from cerebral ischemia include interventions that increase cerebral blood flow, reduce oxygen demand, or that prevent neuronal damage mediated by glutamate or ion fluxes (*3*).

Numerous studies have shown that hypothermia may reduce neuronal necrosis resulting from experimental cerebral ischemia (*4-9*). Although brief post-ischemic hypothermia appears to delay neuronal damage rather than provide persistent protection (*8*), hypothermia is protective even when it occurs up to 12 hours after cerebral ischemia (*9*). Due to the significant morbidity and mortality associated with stroke and traumatic brain injury, the clinical use of hypothermia and other neuroprotective modalities to lessen the neurological damage associated with these conditions has received considerable attention (*10-12*).

Because of the multiple mechanisms responsible for ischemic neuronal damage, combination therapy may offer greater protection than hypothermia alone (*13*). Other modalities that have been shown to protect against cerebral ischemia include hemodilution (*14*) and glutamate antagonists (*15*). Hemodilution may increase blood flow during reperfusion following cerebral ischemia by reducing blood viscosity as a result of decreased hematocrit, or by an active vasodilatory process (*16*). Hemodilution in combination with a glutamate antagonist, lubeluzole, provided more complete attenuation of neuronal damage than either intervention alone (*17*).

The interventions used to reduce the brain damage caused by cerebral ischemia have aimed to increase oxygen supply, decrease neuronal oxygen demand, and impede the damaging effects caused by exces-

sive glutamate, calcium, and free radicals. Hemodilution with saline fluids may increase cerebral blood flow and oxygen supply to the brain by two mechanisms. First, hemodilution may decrease blood viscosity by reducing hematocrit, and thereby increase the velocity of blood flow through cerebral vessels via a hemorrheological mechanism (*18*). Second, hemodilution may evoke compensatory vasodilation, and thereby augment cerebral blood flow (*16*).

Because hemodilution may improve blood flow and oxygen delivery, whereas hypothermia appears to reduce the neuronal oxygen requirement, the combined use of these modalities may offer greater protection against brain damage caused by cerebral ischemia. Therefore, we evaluated the effects of hemodilution alone and in combination with hypothermia as interventions to reduce neurological damage in a rat model of global cerebral ischemia.

MATERIALS and METHODS

Animals: The experimental studies, which were approved by the Institutional Animal Care and Use Committee (IACUC), used 80 male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 310-345 g. Animals were randomly assigned to one of three treatment groups and were identified only by a serial code number to blind investigators to the treatment group. The treatment groups consisted of a normothermic group with a sham injection (sham), a normothermic hemodilution group (normothermic), and a hypothermic hemodilution group (hypothermic). Animals were housed individually in the laboratory at 24±2°C with lights on between 0600 and 1800 h. Food (laboratory chow) and tap water were provided ad libitum under the supervision of a certified animal care technician.

Global Cerebral Ischemia: A four-vessel occlusion procedure was used to induce global cerebral ischemia (*19*). The vertebral arteries were cauterized on Day 1, and the carotid arteries were occluded for 10 min on Day 2. On Day 1, rats were anesthetized with 1.5% isoflurane in a mixture of 70% nitrous

oxide and 30% oxygen by face mask. The dorsal neck area was opened by using a midline incision, and the alar foramina were exposed by dissecting the deep musculature from the posterior arches of the first cervical vertebra (20). The vertebral arteries were accessed through the foramina and permanently occluded with a high temperature cautery unit (Model RS-209, Raboz Surgical Instruments, Rockville, MD). The wounds were closed, and the animals were placed on their backs so that incisions could be made in the midline ventral side of the neck. The common carotid arteries were exposed, and a ligature was loosely placed around each artery. The neck was bandaged to protect the ligature, and 1% lidocaine was infiltrated for postoperative pain control. Animals also received an intramuscular injection of cefazolin (20 mg/kg) before they were returned to their cages.

Twenty-four hours later, the rats were anesthetized as before, placed in a stereotactic apparatus, and the cranium exposed. We drilled a hole through the cranium, and a thermistor probe was inserted through the dura to the level of the hippocampus. The thermistor probe was connected to a temperature monitoring system (Model TM-12, Physitemp Instruments, Clifton, NJ) to record temperature changes in the hippocampus. A second hole was drilled to the level of the dura, and a laser-Doppler probe (Model BLF21, Transonic Systems Inc., Ithaca, NY) was placed on the dura to measure blood flow. Two stainless steel screws were inserted into the occiput for recording electroencephalographic (EEG) activity. Following the placement of probes for monitoring cerebral blood flow, temperature, and EEG, the carotid ligatures were tightened for 10 min to produce global cerebral ischemia. The ligatures were then released to enable reperfusion of the brain. Temperature changes, blood flow, and EEG activity were monitored and recorded throughout the experimental protocol.

Interventions: Interventions were performed immediately after 10 min of global cerebral ischemia if the animals met two criteria. The criteria were a flat EEG commencing within 30 s after carotid artery occlusion and lasting throughout the remainder of the ischemic period and a cerebral blood flow of less than 0.5 tissue perfusion units at the end of the 10-min ischemic period, as determined by laser-Doppler flowmetry. Animals meeting these requirements were assigned to treatment interventions. Fifty-six animals (70%) survived surgical and ischemic procedures and met the criteria for treatment interventions. Pulsinelli et al. (19) reported that a similar percentage of rats survived and met the criteria for achieving global cerebral ischemia in their study.

After global cerebral ischemia, cerebral temperatures were rapidly adjusted to normothermic (37±0.5°C) or hypothermic (33±0.5°C) levels in the designated treatment groups and were maintained at these levels for 2 h post-ischemia. The desired cerebral temperatures were achieved by adjusting core body temperature and by external heating or cooling of the head with a heat lamp or fan, respectively. Core body temperature was controlled by changing the temperature of water that was continuously circulated through a rubber pad placed under the animals.

Normothermic animals and hypothermic animals were also subjected to hemodilution. Normothermic rats received a tail vein injection of 3.6 mL/kg body weight of 0.9% saline at 37°C, whereas hypothermic animals received a tail vein injection of the same quantity of cold saline (4°C). Animals in the sham group received a tail-vein needle stick but did not receive intravenous fluid administration. This volume of saline was estimated to initially increase blood volume approximately 7% based on data on the body fluid composition of the rat (21). This level of hemodilution was selected because moderate hemodilution has been shown to ameliorate neurological signs and reduce infarct volume after cerebral ischemia, whereas profound hemodilution may impair oxygen transport and be detrimental in cerebral ischemia (18).

Evaluation: The effectiveness of interventions was evaluated in terms of mortality, motor function, and hippocampal histopa-

thology. Mortality was expressed as the percent of animals that did not survive 21 d post-ischemia. Motor function was evaluated in 30 randomly selected animals by using a foot-fault test (4). After global cerebral ischemia, the test was performed at weekly intervals by an investigator who was blinded to treatment interventions. In this test, animals were placed on an elevated 30 x 30 cm platform of heavy wire screen (1.5-cm squares) attached to a wood frame. As the animals moved about the platform, the investigator counted the number of times either hind foot missed the wire screen (foot fault). The data were expressed as the ratio of foot faults to total steps during a 2-min observation period.

Histopathology: The animals were brought to the histology laboratory at 21 d post-ischemia and were anesthetized with sodium pentobarbital (50 mg/kg body weight, intraperitoneal). A thoracotomy was performed followed by transcardial perfusion of heparinized saline for 30 s, and formaldehyde:glacial acetic acid:methanol (FAM; 1:1:8) for 25 min (22,23). The animals were decapitated, and the heads were immersed in FAM for 4 h. Next, the calvaria were removed to expose the brains and fixation continued overnight. The brains were then removed from the skulls and positioned in a rodent brain matrix (ASI Instruments, Warren, MI) for coronal sectioning. A 4-mm tissue block was removed from the brain immediately posterior to the optic chiasm, which contains most of the hippocampus. The blocks were processed and embedded in paraffin in the usual manner.

Histological sections were cut at a thickness of 6μm. The first 60 sections were discarded and the next section was saved for evaluation (Level A). This process was repeated to obtain subserial sections (B-H) from several levels within the hippocampus (24). The sections were stained with celestin blue B and acid fuchsin (25) to selectively demonstrate damaged and viable neurons within the CA 1 region of the hippocampus.

Because of a slight variability in the microscopic morphology of the brains, it was necessary to identify a common starting point for counting viable hippocampal neurons. The level selected was the region in which the neurons of the CA 1 sector of the hippocampus, subiculum, and the dentate gyrus are in juxtaposition (Fig. 1). The viable neurons were counted and totaled for each animal in the entire CA 1 sector at the starting level and three following levels.

Statistics: The mortality data were compared by using the chi-square test. The histopathology data (neuron counts) were analyzed by using a one-way ANOVA, and the foot-fault data were analyzed by using a two-way, nonparametric ANOVA (Friedman) followed by Dunn's multiple comparison test. The level of statistical significance was set at $P < 0.05$.

RESULTS

Blood Flow and Temperature: Cerebral blood flow (CBF) was measured before, during, and after bilateral carotid artery occlusion (BCAO) in each animal to verify cerebral ischemia. As shown in Fig. 2, BCAO immediately reduced CBF to less than 20% of baseline blood flow in all treatment groups. At the end of the 10-min period of BCAO, CBF had declined to less than 15% of baseline. Immediately after terminating BCAO, a period of reactive hyperemia was observed, and CBF rebounded to 120-150% of baseline for about 10 min. The CBF then declined to less than baseline values. After 60 min, CBF was 57% of baseline in the groups receiving hemodilution (normothermic and hypothermic) and 76% of baseline in the sham group. Blood flow did not change significantly during the remainder of the 2-hour post-ischemic period.

The mean body temperature in all treatment groups was 36-37° C during ischemia, whereas the mean cerebral temperature fell to 35-36° C in all groups during ischemia (Fig. 3). After ischemia, the mean body temperature gradually increased to 37-38° C in the sham and normothermic groups, and fell to 35-36° C in the hypothermic group. The mean cerebral temperature after ischemia was 36-37° C in the sham and normothermic groups, but declined to 33-34° C in the hypothermic group.
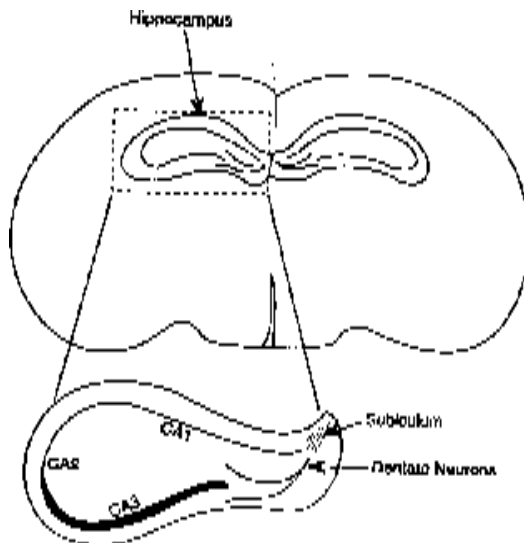
Figure 1. Starting point for counting viable neurons at the juxtaposition of the hippocampus, subiculum, and dentate gyrus.

The bipolar EEG was recorded before, during, and after the global ischemic event. Representative recordings of the pre-ischemic, ischemic, and post-ischemic EEG are shown in Fig. 4. During complete or global ischemia, spontaneous electrical activity disappeared in 1 to 2 min and was isoelectric during the remainder of the ischemic period. Following reperfusion through the carotid arteries, bursts of EEG activity began to appear in 5 to 10 min, and the EEG pattern returned to normal in about 90 min.
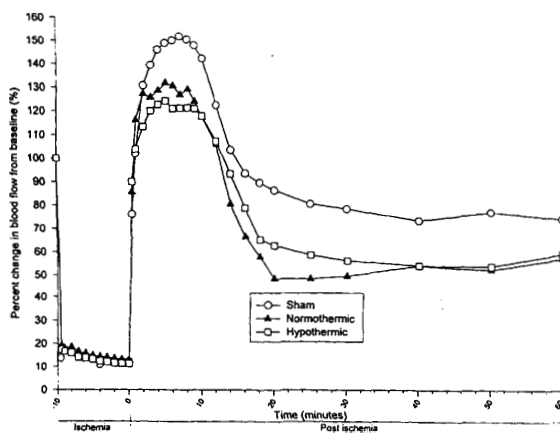

Figure 2. Mean percent change in cerebral blood flow from baseline in sham, normothermic, and hypothermic animals during ischemia and a 60-min post-ischemic period.

Mortality: The cumulative mortality 3 wk after ischemia and interventions is shown in Fig. 5. None of the animals in the hypothermic group died during the 3-wk post-ischemic interval, whereas the mortality rates in the sham control group and the normothermic group were 31.8% and 15.8%, respectively. The mortality rate of hypothermic animals was significantly less than that of the sham animals, whereas the mortality rate of normothermic animals was not significantly different from the sham controls.

Motor Function: Motor function was assessed with the foot-fault test in randomly selected animals from each treatment group. The test was performed at weekly intervals after cerebral ischemia. Sham controls had a higher foot-fault rate than did normothermic and hypothermic animals during each observation period, but the differences between groups were not significantly different (Table 1). However, the foot-fault rates decreased in all groups from Day 7 to Day 14, and the foot-fault rates were significantly less in all groups on Day 21 than on Day 7.

Hippocampal Histopathology: Four levels of the CA 1 area of the hippocampus were histologically evaluated for viable neurons in each animal that survived 21 d post-ischemia. Figure 6 shows the mean number of viable neurons in each treatment group. A total of 2,656 neurons were counted in four levels of the hippocampus in the untreated external control. The sham treatment group demonstrated the greatest ischemic neuronal damage, with a mean of 763 viable neurons (range 161 to 1,872). The normothermic group had less ischemic damage with a mean of 976 viable neurons (range 252 to 1,885), whereas the hypothermic group demonstrated the least ischemic damage with a mean of 1,199 viable neurons (range 662 to 2,555). There was wide variability in the number of viable neurons in animals within each treatment group, and the total number of viable neurons in treatment groups was not significantly different. However, there was a trend toward more viable neurons in the groups that received hemodilution (normothermic) or hemodilution and hypother-
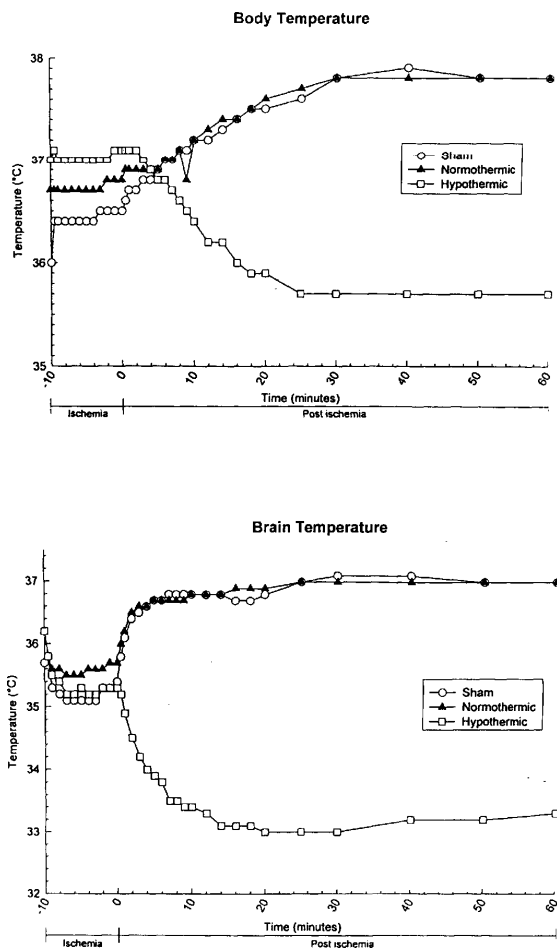
Figure 3. Mean body and brain temperatures in sham, normothermic, and hypothermic animals during ischemia and a 60-min post-ischemic period.

mia (hypothermic) when compared to the sham group (Fig. 6).

DISCUSSION

In the present study, we used an established model of global cerebral ischemia and incorporated direct measurement of cerebral blood flow, cerebral temperature, and body temperature. These parameters were monitored during and after ischemia that was produced by four-vessel occlusion, and enabled verification of cerebral ischemia, reperfusion, and post-ischemic cerebral and body temperature. We used this model to evaluate the effect of hemodilution compared to hemodilution with hypothermia on the outcome of global cerebral ischemia, as indicated by mortality, motor function, and his-

topathology. Hypothermia may decrease neuronal metabolism and oxygen consumption, whereas hemodilution may increase cerebral blood flow and oxygen supply during the reperfusion period. Hence, combined hemodilution and hypothermia interventions may be more effective than either treatment alone. The benefits of hemodilution have been partly attributed to hemorheologic improvement in the microcirculation (18), but other investigators found evidence that hemodilution may evoke an active vasodilatory process that may improve cerebral blood flow (16). The latter finding may indicate that hemodilution can increase cerebral blood flow without inducing a significant reduction in hematocrit and blood viscosity.

We found that 2 h of post-ischemic hemodilution and hypothermia completely prevented animal death during the 3-wk post-ischemic observation period, whereas mortality was not significantly reduced by hemodilution alone compared to a sham control group. Ohtaki and Tranmer reported that hemodilution with hetastarch infusion was more effective than hemodilution with saline in protecting against neuronal damage caused by cerebral ischemia (14). Furthermore, the degree of hemodilution employed in our study may have not been sufficient to reduce hematocrit and blood viscosity sufficiently to cause a significant increase in blood flow.

Other assessment parameters were not significantly affected by hemodilution and hypothermia in this study, although there was a trend toward improved motor function and neuronal survival in the group that received hemodilution and hypothermia compared to sham controls. Our study confirms that animals can survive short periods of global cerebral ischemia while sustaining marked neuropathologic damage in the CA 1 region of the hippocampus.

We found that the total number of viable neurons counted in four levels of hippocampal CA 1 tissue was higher in the hypothermic group than in the normothermic and sham control groups (Fig. 6), but the differences were not significant (P = 0.27). This finding may partly result from the higher

EEG: Pre-ischemia

During 4 vessel occlusion

Recovery EEG: Post-ischemia
(flow initiated for 16 min)

Recovery EEG: Post-ischemia
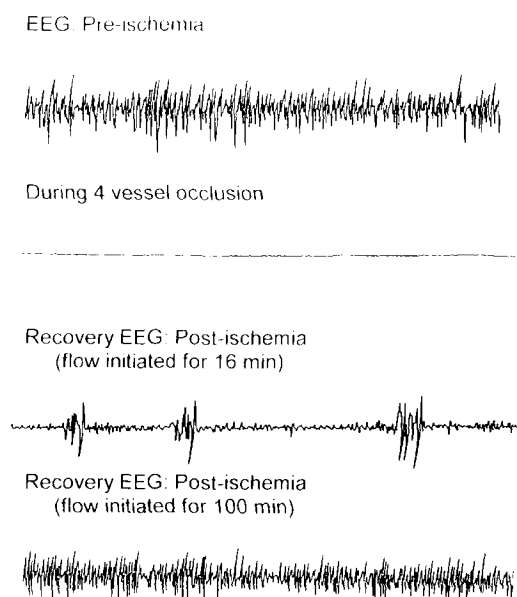(flow initiated for 100 min)

Figure 4. Representative bipolar EEG from normothermic animals before ischemia (Pre-ischemia EEG), during global cerebral ischemia (four-vessel occlusion), and during cerebral reperfusion for 16 and 100 min (Recovery EEG: Post-ischemia).

mortality rate in the sham group (31.8%) compared to the hypothermic group (0%), because the sham animals that died before histological evaluation may have had fewer viable neurons than did the surviving animals. Other investigators have reported a wide variance in the degree of CA 1 injury in rat models of cerebral ischemia (26). Furthermore, there appears to be a poor correlation between histological preservation and functional endpoints in some studies. For this reason, we also evaluated motor function by using a foot-fault test. Although the foot-fault rate was marginally lower in the

hypothermic group compared to the sham group at 7 and 14 d post-ischemia, the differences between groups were not significant. This measure of motor function improved significantly ($P < 0.05$) in all groups from Day 7 to Day 21 (Table 1).

Other investigators have reported that cerebral ischemia is often followed by a brief period of cerebral hyperperfusion and a protracted period of hypoperfusion (27,28). We also observed a post-ischemic period of cerebral hyperperfusion lasting 10-15 min, followed by a period of hypoperfusion that persisted throughout the 2-h post-ischemic observation period in all treatment groups (Fig. 2). These perturbations during ischemia and reperfusion may contribute to blood-brain barrier disruption and edema formation and thereby exacerbate neuronal damage (27,28). Huang et al. (28) found that intra-ischemic hypothermia significantly reduced post-ischemic hyperperfusion, but had no effect on delayed hypoperfusion, whereas combined intra-ischemic and post-ischemia hypothermia prevented post-ischemic hyperperfusion and delayed post-ischemic hypoperfusion.

A large number of studies have shown that mild hypothermia protects against neuronal damage and functional disability caused by brief periods of cerebral ischemia (3-9,25,29). Also, studies have indicated that brief periods of post-ischemic hypothermia may only delay neuronal damage, whereas protracted hypothermia may provide sustained histopathologic and behavioral neuroprotection (30,31). For example, 3 h of post-ischemic hypothermia at 30°C in the rat provided marked neuroprotection at 7 d but

TABLE 1. Foot-fault rate of rats at 1, 2, and 3 wk after global cerebral ischemia.

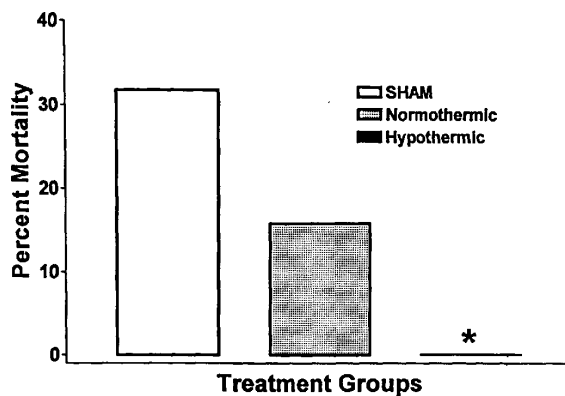|	Treatment		
	Sham $n = 9$	Normothermic $n = 10$	Hypothermic $n = 11$
Week	Foot-Fault Rate x10^2		
1	1.12 ± 0.46	1.11 ± 0.29	0.68 ± 0.32
2	0.90 ± 0.37	0.28 ± 0.19	0.33 ± 0.25
3	0.43 ± 0.25	0.23 ± 0.15	0.17 ± 0.11

Values are the mean ± SEM for each treatment group.

Figure 5. Cumulative mortality in sham (n = 22), normothermic (n = 19), and hypothermic (n = 15) groups 3 wk after global cerebral ischemia and interventions. *Significantly different from sham group (P < 0.05).
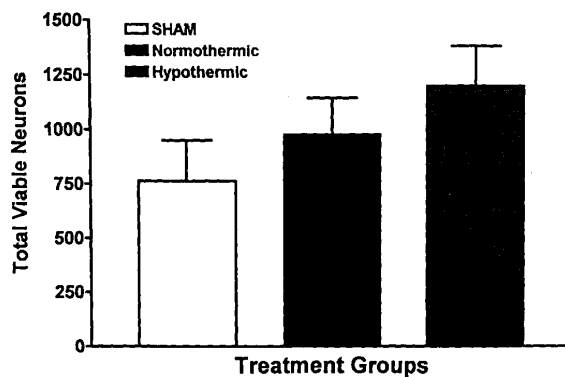

Figure 6. Number of viable neurons counted in four levels of rat hippocampal CA 1 tissue 3 wk after global cerebral ischemia. The bars represents the mean ±SEM of viable neurons in sham (n = 9), normothermic (n = 13), and hypothermic (n = 11) animals. Differences between treatment groups were not significantly different.

not at 2 months of recovery (8). In contrast, mild post-ischemic hypothermia for 1 to 3 d appears to produce sustained neuroprotection for up to 6 months (29,32).

Other investigations suggest that the combined use of hypothermia and other treatment modalities may have a beneficial effect on survival following cerebral ischemia. These interventions are partly based on the hypothesis that neuronal degeneration may be ongoing for several months after a transient ischemic insult and that prolonged neuroprotection may reduce neurologic deficits and improve survival. Coimbra et al. (13) found that combining 7 h of post-

ischemic hypothermia with an anti-inflammatory/antipyretic drug administered from 14 to 72 h reduced neuronal damage at both 7 d and 2 months of recovery compared to hypothermia alone. The drug benefits were partly attributed to a reduction in post-ischemic hyperthermia that occurred during the first 3 d of recovery. Other interventions that may potentiate the benefits of hypothermia include hemodilution and glutamate antagonists. However, the neuroprotective benefit of the glutamate antagonist MK-801 was partly attributed to drug-induced hypothermia (33).

Although sustained post-ischemic hypothermia appears to provide significant cerebral neuroprotection, hypothermia also appears to have a graded effect on neurologic outcome (34). The precise mechanism by which reductions in brain temperature provide neuroprotection is not clear. Hypothermia appears to exert several beneficial effects that attenuate neuronal loss, including reduced energy utilization (35) and inhibition of ischemia-induced release of excitotoxic neurotransmitters such as glutamate and aspartate (36). These excitatory neurotransmitters have been shown to activate calcium influx and the generation of free radicals that activate the caspase system, leading to neuronal DNA damage and apoptosis (2).

There is growing interest in the clinical use of neuroprotective drugs and procedures to prevent neurological damage caused by acute stroke and brain trauma (10-12). Further studies are needed to assess the efficacy of different levels of post-ischemic hypothermia administered alone and in combination with other neuroprotective treatments for the prevention of neurologic damage caused by brain trauma and ischemia.

ACKNOWLEDGMENTS

REFERENCES

1. Phillis JW, Ren J, O'Regan O. Transporter reversal as a mechanism of glutamate release from the ischemic rat cerebral cortex: studies with DL-threo-β-benzyl-oxyaspartate. Brain Res 2000; 868:105-112.

2. Krupinski J, Lopez E, Marti E, Ferrer I. Expression of caspases and their substrates in the rat model of focal cerebral ischemia. Neurobiol Dis 2000;7:332-342.

3. Nagahiro S, Uno M, Sato K, Goto S, Morioka M, Ushio Y. Pathophysiology and treatment of cerebral ischemia. J Med Invest 1998;45:57-70.

4. Green EJ, Dietrich WD, van Dijk F, Busto R, Markgraf CG, McCabe PM, Ginsberg MD, Schneiderman N. Protective effects of brain hypothermia on behavior and histopathology following global cerebral ischemia in rats. Brain Res 1992;580:197-204.

5. Busto R, Dietrich WD, Globus MY-T, Valdes I, Scheinberg P, Ginsberg MD. Small differences in intraischemic brain temperature critically determine the extent of ischemic neuronal injury. J Cerebral Blood Flow Metab 1987;7:729-738.

6. Colbourne F, Corbett D. Delayed and prolonged post-ischemic hypothermia is neuroprotective in the gerbil. Brain Res 1994;654:265-272.

7. Baker CJ, Onesti ST, Solomon RA. Reduction by delayed hypothermia of cerebral infarction following middle cerebral artery occlusion in the rat: a time-course study. J Neurosurg 1992;77:438-444.

8. Dietrich WD, Busto R, Alonso O, Globus MY, Ginsberg MD. Intraischemic but not post-ischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J Cereb Blood Flow Metab 1993;13:541-549.

9. Lipton P. Ischemic cell death in brain neurons. Physiol Rev 1999; 79:1431-1568.

10. Marion DW, Penrod LE, Kelsey SF, Obrist WD, Kochanek PM, Palmer AM, Wisniewski SR, DeKosky ST. Treatment of traumatic brain injury with moderate hypothermia. N Engl J Med 1997;336:540-546.

11. Biagas KV, Gaeta ML. Treatment of traumatic brain injury with hypothermia. Curr Opin Pediat 1998;10:271-277.

12. Devuyst G, Bogousslavsky J. Clinical trial update: neuroprotection against acute ischemic stroke. Curr Opin Neurol 1999;12:73-79.

13. Coimbra C, Drake M, Boris-Moller F, Wieloch T. Long-lasting neuroprotective effect of post-ischemic hypothermia and treatment with an anti-inflammatory/antipyretic drug. Stroke 1996;27:1578-1585.

14. Ohtaki M, Tranmer BI. Role of hypervolemic hemodilution in focal cerebral ischemia of rats. Surg Neurol 1993;40:196-206.

15. Lazarewicz JW, Gadamski R, Parsons CG, Danysz W. Protection against post-ischaemic neuronal loss in gerbil hippocampal CA 1 by $glycine_β$ and AMPA antagonists. J Neural Transm 1997; 104:1249-1254.

16. Todd MM, Wu B, Warner DS. The hemispheric cerebrovascular response to hemodilution is attenuated by a focal cryogenic brain injury. J Neurotrauma 1994; 11: 149-160.

17. Aronowski J, Strong R, Grotta JC. Combined neuroprotection and reperfusion therapy for stroke. Effect of lubeluzole and diaspirin cross-linked hemoglobin in experimental focal ischemia. Stroke 1996;27:1571-1577.

18. Tu YK, Kuo MF, Liu HM. Cerebral oxygen transport and metabolism during graded isovolemic hemodilution in experimental global ischemia. J Neurol Sci 1997;150:115-122.

19. Pulsinelli WA, Brierley JB. A new model of bilateral hemispheric ischemia in the unanesthetized rat. Stroke 1979;10:267-272.

20. Pulsinelli WA, Buchan AM. The four-vessel occlusion rat model: method for complete occlusion of vertebral arteries and control of collateral circulation. Stroke 1988;19:913-914.

21. Altman PL, Dittmer DS. Biology Data Book, volume III, 2nd ed., Federation of American Societies for Experimental Biology, Bethesda (MD): 1974. 1847 p.

22. Agardh CD, Kalimo H, Olsson Y, Siesjo BK. Hypoglycemic brain injury. I. Metabolic and light microscopic findings in rat cerebral cortex during profound insulin-induced hypoglycemia and in the recovery period following glucose administration. Acta Neuropathol (Berlin) 1980;50:31-41.

23. David GB. The effect of eliminating shrinkage artifacts on degenerative changes seen in CNS material. Excerpta Medica 1955;8:777-778.

24. Auer RN, Olsson Y, Siesjo BK. Hypoglycemic brain injury in the rat. Correlation of density of brain damage with the EEG isoelectric time: a quantitative study. Diabetes 1984;33:1090-1098.

25. Minamisawa H, Nordstrom C, Smith M, Siesjo B. The influence of mild body and brain hypothermia on ischemic brain damage. J Cerebral Blood Flow Metabol 1990;10:365-374.

26. Corbett D, Nurse S. The problem of assessing effective neuroprotection in experimental cerebral ischemia. Prog Neurobiol 1998;54:531-548.

27. Kagstrom E, Smith ML, Siesjo BK. Local cerebral blood flow in the recovery period following complete cerebral ischemia in the rat. J Cereb Blood Flow Metab 1983;3:170-182.

28. Huang FP, Zhou LF, Yang GY. The effect of extending mild hypothermia on focal cerebral ischemia and reperfusion in the rat. Neurol Res 1998;20:57-62.

29. Kataoka K, Yanase H. Mild hypothermia-a revised countermeasure against ischemic neuronal damages. Neurosci Res 1998;32:103-117.

30. Colbourne F, Sutherland G, Corbett D. Post-ischemic hypothermia. A critical appraisal with implications for clinical treatment. Molec Neurobiol 1997;14:171-201.

31. Laptook AR, Corbett RJT, Sterett R, Burns DK, Garcia D, Tollefsbol G. Modest hypothermia provides partial neuroprotection when used for immediate resuscitation after brain ischemia. Pediatr Res 1997;42:17-23.

32. Colbourne F, Corbett D. Delayed post-ischemic hypothermia: a six-month survival study using behavioral and histologic assessments of neuroprotection. J Neurosci 1995;15:7250-7260.

33. Zhang L, Mitani A, Yanase H, Kataoka K. Continuous monitoring and regulating of brain temperature in the conscious and freely moving ischemic gerbil: effect of MK-801 on delayed neuronal death in hippocampal CA 1. J Neurosci Res 1997;47:440-448.

34. Hoffman WE, Thomas C. Effects of graded hypothermia on outcome from brain ischemia. Neurol Res 1996;18:185-189.

35. Laptook AR, Corbett RJT, Sterett R, Garcia D, Tollefsbol G. Quantitative relationship between brain temperature and energy utilization rate measured in vivo using ^{31}P and ^{1}H magnetic resonance spectroscopy. Pediatr Res 1995;38:919-925.

36. Busto R, Globus MY-T, Dietrich D, Martinez E, Valdes I, Ginsberg MD. Effect of mild hypothermia on ischemia-induced release of neurotransmitters and free fatty acids in rat brain. Stroke 1989;20:904-910.