Postischemic (1 Hour) Hypothermia Significantly Reduces Ischemic Cell Damage in Rats Subjected to 2 Hours of Middle Cerebral Artery Occlusion

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Background and Purpose: We investigated the effect of hypothermia induced 1 hour after transient (2-hour) middle cerebral artery occlusion on the extent of ischemic cell damage in the rat.

Methods: Middle cerebral artery occlusion was induced extracranially by insertion of a nylon filament into the right internal carotid artery. Two groups of rats were investigated: (1) rats (n=10) subjected to normothermic (37°C) ischemia and normothermic reperfusion; and (2) rats (n=10) subjected to normothermic ischemia and 1 hour of normothermic reperfusion followed by 3 hours of hypothermia (30°C). All rats were killed 1 week after the experiment, and brain sections were stained with hematoxylin and eosin for evaluation of ischemic cell damage.

Results: Infarct volume in normothermic rats involved 20.9±4.6% of the hemisphere, whereas hypothermic rats exhibited a significantly smaller (P<.001) infarct volume of 11.1±2.7%. The numbers of surviving (or structurally intact) neurons within large sections of the cortex and striatum were significantly greater for hypothermic compared with normothermic rats (P<.01).

Conclusions: Our data suggest that postischemic induction of hypothermia significantly reduces ischemic cell damage after 2 hours of middle cerebral artery occlusion in the rat, and that an interval of time of at least 1 hour after ischemia exists in which hypothermic intervention is effective in either salvaging or postponing irreversible neuronal injury. (Stroke 1993;24:1235-1240)

KEY WORDS • cerebral arteries • hypothermia • neuronal damage • rats

There have been numerous reports on the effects of hypothermic interventions on ischemic cell damage in an array of experimental models of either global or focal cerebral ischemia.1-10 The preponderance of data supports a protective role of hypothermia in salvaging neurons from ischemic cell damage.

Of great interest and potential clinical significance is whether hypothermia induced after an ischemic insult has beneficial effects on the ischemic brain. Hypothermia induced after transient forebrain ischemia in the rat reduces ischemic cell damage in the hippocampus when instituted within 30 minutes of reperfusion.5,11,12 The effectiveness of this therapy depends on the duration of ischemia.8,11,12

To our knowledge, there have not been studies on the effects that hypothermia may have on ischemic cell damage when hypothermia is induced after transient focal ischemia. Whether moderate hypothermia (30°C) might also protect against ischemic injury after transient middle cerebral artery (MCA) occlusion remains an important but as yet uninvestigated question. The objective of the present experiment was to ascertain whether hypothermia induced 1 hour after transient (2-hour) occlusion of the MCA in the rat reduces ischemic cell damage.

Materials and Methods

Twenty-four male Wistar rats (weighing 270 to 300 g) were used in the experiments. Animals were allowed free access to food and water before and after all procedures. Rats were anesthetized with 0.5% to 1.5% halothane in 70% N₂O and 30% O₂ by use of a face mask. A PE-50 catheter was introduced into the right femoral artery to measure blood gases before ischemia and during MCA occlusion and reperfusion. Arterial blood pressure was continuously monitored before MCA occlusion and for 15 minutes after MCA occlusion in all animals. Arterial blood pressure was also measured after 3 hours of reperfusion in four animals in each group.

Middle cerebral artery occlusion was induced by advancing a 4-0 surgical nylon filament into the internal carotid artery (ICA) to block the origin of the MCA.13-15 Under the surgical microscope, the right common ca-
rotid artery (CCA), external carotid artery (ECA), and ICA were isolated via a midline incision at the neck. The distal end of the ECA was ligated, and the origin of the ECA was loosely tied with 5-0 silk suture. The CCA and ICA were temporarily clamped using microsurgical clips. A 4-0 surgical nylon filament with its tip rounded by heating near a flame was introduced into the lumen of the ECA through a puncture of the ECA. The silk suture around the ECA stump was tightened around the intraluminal nylon filament to prevent bleeding, and the microsurgical clips were removed. A length of 18.5 to 19.5 mm of nylon filament, determined according to the animal’s body weight, was gently advanced from the ECA into the lumen of the ICA until the suture blocked the origin of the MCA. The incision was temporarily closed using skin clips. Anesthesia was terminated after 15 minutes of MCA occlusion in both groups, and animals awoke 5 to 10 minutes thereafter. After 2 hours of ischemia, anesthesia was reinstalled, and reperfusion was instituted by withdrawing the intravascular filament until the distal tip became visible at the origin of the ECA. In both groups, halothane anesthesia was maintained throughout 4 hours of reperfusion time to allow accurate temperature control.

Rectal temperature was controlled with a feedback-regulated water heating system. Rats were randomly divided into two groups: (1) a normothermic group (n=10), in which 37°C body temperature was maintained during MCA occlusion and for an additional 4 hours of reperfusion; and (2) a hypothermic group (n=10), in which 37°C body temperature was maintained during MCA occlusion and 1 hour of reperfusion and hypothermia (30°C) was then induced and maintained for 3 hours. Whole-body hypothermia was instituted by spraying alcohol on the animal’s skin and fanning room air (22°C) toward the animal’s body. The heating system was set to maintain the rat’s body temperature at 30°C. After 3 hours of hypothermia, the animals were rewarmed to 37°C using a heating lamp and pad.

Additional rats were used to measure changes in rectal, striatal, and cortical temperatures during 2 hours of MCA occlusion and 4 hours of recirculation. Two animals were normothermic during the entire experimental procedure, and the other two animals were subjected to 3 hours of hypothermia after 2 hours of ischemia and 1 hour of reperfusion. To measure brain temperature, four 1-mm burr holes were drilled into the animal’s skull. Brain temperature was simultaneously measured in the ipsilateral parietal cortex (5.5 mm lateral, 1.0 mm posterior to the bregma, and 4 mm below the dura) and striatum (4 mm lateral, 1.0 mm posterior to the bregma, and 6 mm below the dura) as well as in the contralateral cortex and striatum. Before MCA occlusion, microthermocouples (100 μm) were placed into 27-gauge needles and were then inserted into the brain. Brain and rectal temperatures were recorded every 5 minutes.

Seven days after MCA occlusion, animals were anesthetized intramuscularly with ketamine (44 mg/kg) and xylazine (13 mg/kg) and were perfused with heparinized saline and 10% neutral buffered formalin. Brains were cut, using a rodent matrix, into seven coronal slabs of 2-mm thickness each. The slabs were labeled A (frontal) through G (occipital). Histological sections (approximately 6 μm thick) were obtained from each 2-mm-thick paraffin block and stained with hematoxylin and cosin. The areas of the infarct and the right hemisphere in each section (slices A through G) were measured with an Imagist-2 Image Analysis System (PGT, Princeton, NJ). Each section stained with hematoxylin and cosin was evaluated at ×2.5 magnification. Infarct and hemisphere volumes (in cubic millimeters) were determined by multiplying the appropriate area by the thickness of the section interval. The size of the lesion is presented as percentage of infarct volume to volume of ipsilateral hemisphere.

Counting of histologically intact neurons was performed on a standard section corresponding to a coronal section interaural 8.2 mm, bregma 0.8 mm. This section is centered in the ischemic lesion. Each hemisphere was divided into eight anatomically distinct regions (Fig 1). The numbers of histologically intact neurons were counted in homotopic areas in all eight regions of both cerebral hemispheres. The features of the abnormal neurons (red, swollen, ghosts) have been defined in a companion article. Data on the numbers of surviving neurons are presented as the ratio of numbers of morphologically intact neurons present in the ischemic region to numbers of morphologically intact neurons present in the homotopic contralateral nonischemic region. The histological observations were carried out in a blinded fashion.

**Statistical Analysis**

A one-way analysis of variance was performed to compare the percent infarct volumes between the normothermic and hypothermic MCA occlusion groups. The ratios of intact neurons in the ipsilateral and contralateral hemispheres between the two groups were analyzed by a Mann-Whitney test. Values of P≤.01 were considered significant. All data are presented as mean±SD.

**Results**

All rats exhibited a similar type of focal neurological deficit with failure to fully extend the left forepaw when anesthesia was terminated after 15 minutes of MCA occlusion. No significant differences in blood gas values and arterial pressures were detected between normothermic and hypothermic groups at any time points, and all values were within normal physiological ranges (data

![FIG 1. Diagram of coronal section of rat brain illustrating division of right hemisphere into eight regions for pathological evaluation. Regions are as follows: (1) piriform cortex, (2) insular cortex, (3) parietal cortex, (4) hind limb and forelimb area of cortex, (5) frontal and cingulate cortex, (6) caudate putamen, (7) globus putamen, and (8) preoptic area.](http://stroke.ahajournals.org/DownloadedFromStrokeVol24No8August1993)
not shown). One normothermic rat died at 72 hours after reperfusion. Autopsy revealed a large ipsilateral hemispheric infarct and extensive brain edema. Measurements derived from this animal’s brain are not included in the data reported in the present study.

Infarct volume in normothermic rats involved 20.9±4.6% of the hemisphere, and hypothermic rats exhibited a significantly smaller (P<.001) infarct volume of 11.1±2.7%.

Fig 2 illustrates the brain (right side) and rectal temperature changes during the experimental procedure in a representative hypothermic MCA occlusion animal. Rectal temperature of the experimental animal (n=4) was 37.0±0.2°C during the ischemic period and remained at 37.0±0.2°C in the normothermic reperfusion period. In animals subjected to delayed postischemic hypothermia, body temperature was reduced to 30.0±0.2°C during the hypothermic reperfusion period (n=2). No significant differences were detected between temperatures in the cortex and striatum at any time during the experiment. Before ischemia, brain temperature was elevated above the rectal temperature by 0.7°C; it declined transiently by approximately 0.6°C after the onset of MCA occlusion and returned to preischemic levels within 15 minutes. Brain temperature was 37.3±0.4°C during the course of ischemia and remained at 37.3±0.4°C in the normothermic reperfusion period (n=4). Brain temperature fell to 30.3±0.5°C in the hypothermic reperfusion period (n=2). The temporal profile of brain temperatures in the nonlesioned left side was nearly identical to that in the lesioned right hemisphere (data not shown).

Coronal sections of brain from a representative normothermic rat are shown in Fig 3, A and B. All the normothermic rats exhibited a sharply demarcated infarct within regions 1, 2, 3, 4, 6, and 8 (Fig 3, A). Macrophages, microvessels, and cavitation were present within the infarct (Fig 3, B). Fig 3, C and D, shows a coronal section of brain from a representative hypothermic rat exhibiting a smaller infarct within regions 1, 2, 6, and 8 (Fig 3, C) than observed in normothermic rats. Selective neuronal damage was present in the areas peripheral to the site of necrosis (Fig 3, D). Four of the hypothermic rats exhibited selective neuronal damage and incomplete necrosis in regions 3 and 7, without pan necrosis. None of the hypothermic rats exhibited necrosis in region 4. Region 5, which is supplied by the anterior cerebral artery, did not exhibit neuronal changes in either normothermic or hypothermic rats.

The Table summarizes percentages of morphologically intact neurons in both experimental groups. Hypothermic rats had a significantly higher percentage of surviving neurons than normothermic rats at multiple brain sites. Significant differences in the numbers of surviving neurons were found in regions 1, 2, and 6 and were especially marked in regions 3 and 4.

Discussion

We have confirmed that in all normothermic animals a brain lesion of comparable size develops within the territory of the occluded artery. Moreover, whole-body hypothermia (30°C) induced 1 hour after reperfusion following 2 hours of MCA occlusion significantly ameliorates the extent of ischemic brain damage. To our knowledge, this is the first demonstration that a postischemic intervention (delayed by 1 hour) significantly reduces ischemic cell damage in rats with transient MCA occlusion. Our data also suggest that a time interval of at least 1 hour is present after transient (2-hour) focal ischemia in the rat that allows for an effective therapeutic intervention to be instituted. In the intraluminal filament model of MCA occlusion, both permanent and transient (2-hour or more) MCA occlusion evoke a similar volume of brain infarct; therefore, in this model, 2 hours of transient focal ischemia is equivalent to permanent focal ischemia with respect to the final histological damage. However, recent studies indicate that hypothermia induced after the onset of permanent focal ischemia produced by coagulation of the MCA has little benefit in reducing ischemic cell damage; this is in sharp contrast to the beneficial effects of hypothermia in ameliorating cell damage after transient focal ischemia induced by the intraluminal filament occlusion of the MCA. Thus, although the final lesion may be the same after permanent or transient focal ischemia, transient focal ischemia may allow for successful hypothermic intervention after ischemia, but permanent focal ischemia may not. These data imply that reperfusion alone after an ischemic event may not
FIG 3. Coronal sections of rat brains subjected to normothermic (A, B) and hypothermic (C, D) middle cerebral artery occlusion. A, A sharply demarcated brain lesion or infarct is noted in the right hemisphere, involving regions 1, 2, 3, 4, 6, 7, and 8 (original magnification ×2.5). B, An enlargement (original magnification ×200) of the box in A shows macrophages (arrows), cavitation (arrowhead), and microvessels (open arrow). C, A small infarct is noted in the right hemisphere, involving regions 1, 2, 6, and 8 (original magnification ×2.5). D, An enlargement (original magnification ×200) of the box in C shows scattered red neurons (arrows) and intact neurons (open arrows) at the periphery of the brain lesion.
always improve the biological outcome; however, reperfusion may prolong the time during which therapy may be instituted. The ability of hypothermia to improve both neurological and histopathological outcome after transient global cerebral ischemia has been recognized.\textsuperscript{2,7,8,12} The mechanisms causing this protective effect are less clear. The protective effect of hypothermia against ischemic cell damage in the brain has been attributed to reduction in brain energy demand.\textsuperscript{20,21} However, it has become evident that metabolic perturbation by hypothermia is insufficient to protect brain from ischemic cell damage.\textsuperscript{22,23} Ischemic cell damage has also been attributed to the neurotoxic effects of glutamate released after onset of ischemia. Glutamate functions as a fast excitatory transmitter and is a powerful neurotoxin, and glutamate may play a key role in ischemic brain damage.\textsuperscript{24–28} Hypothermia induced during ischemia reduces the release of excitatory neurotransmitters, including glutamate.\textsuperscript{2} However, the possible postischemic hypothermic modulation of excitatory amino acid release in this model is unknown.

Comparison of the ischemic lesion in animals in which hypothermia is induced concurrent with ischemia and reperfusion with that in animals in which hypothermia is induced 1 hour after ischemia reveals different degrees of protection. Hypothermia during ischemia and reperfusion has a more beneficial effect on tissue survival than delayed postischemic hypothermia. Animals with hypothermia concurrent with MCA occlusion and reperfusion exhibit minor ischemic damage with only selective neuronal injury in the cortex and small focal areas of infarct within the basal ganglia.\textsuperscript{10} In contrast, as shown in the present study, animals in which hypothermia is induced 1 hour after MCA occlusion develop pannecrosis both in the cortex and in the basal ganglia, although the volume of the infarct was significantly smaller than in normothermic MCA occlusion animals.

We and others have mapped brain and rectal temperature during and after ischemia in normothermic and hypothermic animals.\textsuperscript{3,10,29} Our present data indicate that brain temperature under both normothermic and hypothermic conditions is 0.7°C greater than rectal temperature. These data are consistent with previous findings.\textsuperscript{10}

In summary, we report that inducing whole-body hypothermia (30°C) after 1 hour of reperfusion and 2 hours of MCA occlusion is an effective way to reduce the size of a focal ischemic brain lesion in rats. These data imply that reperfusing a region of cerebral tissue subjected to a focal ischemic insult of short duration, coupled with a therapeutic intervention (such as hypothermia), may reduce the biological impact of an ischemic stroke.

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**References**

It has been shown that stroke damage can be reduced by hypothermia in several animal models. Most of these studies were conducted in global ischemia models or hypothermia was induced at the time ischemia was produced. Such a strategy might be useful for neuroprotection during surgical procedures but would not be helpful for most stroke patients. Previous investigations have not addressed the question of whether it is possible to reduce damage if hypothermia is induced after the onset of transient focal ischemia. Focal strokes are the most frequent type. Zhang and colleagues have now shown that hypothermia is protective when begun 1 hour after transient ischemia in a focal stroke model. They cite a previous article by Ridenour et al1 in which hypothermia failed to modify permanent focal ischemia.

There are several important implications of the work of Zhang et al. There is still no proof that any form of therapy is effective for treatment of patients after the onset of ischemic stroke. This article provides further support for the concept of a "window of opportunity" for specific stroke therapy. The investigators demonstrated that hypothermia reduced the lesion volumes and increased the fractions of morphologically intact neurons compared with untreated stroke subjects. This suggests a robust effect that may also be present in patients. The possibility that such treatment will only be useful in transient ischemia is less encouraging because vascular occlusion often does not resolve for many hours or days, if ever. However, if thrombolytic therapy becomes practical, transient ischemia may become more common.

This article was not intended to describe a clinically useful therapy but simply to show the potential for a method of treatment. An unresolved issue is how to induce hypothermia rapidly in stroke patients. It is relatively easy to quickly induce hypothermia in small anesthetized animals with large ratios of surface area to volume. Several techniques for producing hypothermia in adult humans have been proposed, but many are quite time consuming, and such delays will reduce efficacy. Systemic hypothermia may also be dangerous for elderly stroke victims who are likely to have cardiac disease. Mechanical methods for local cooling of the head are unlikely to reduce the temperature of the deep brain structures. Consequently, the practical utility of hypothermia for focal stroke therapy has yet to be established.

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Reference
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