Mild Hypothermia Reduces Infarct Size Resulting From Temporary but Not Permanent Focal Ischemia In Rats

Todd R. Ridenour, MD; David S. Warner, MD; Michael M. Todd, MD; and Alice C. McAllister

Background and Purpose: Mild hypothermia (32–35°C) has been repeatedly shown in laboratory models to reduce damage resulting from global cerebral ischemic insults. Little information is available, however, regarding the protective potential of mild hypothermia against focal ischemia. We designed the present study to determine whether mild hypothermia influences outcome from either temporary or permanent middle cerebral artery occlusion in the rat.

Methods: In experiment 1 (permanent occlusion), mechanically ventilated, halothane-anesthetized spontaneously hypertensive rats underwent permanent ligation of the middle cerebral artery. Pericranial temperature was maintained at either 37°C (n=11) or 33°C (n=11) during the first 2 hours of occlusion. In experiment 2 (temporary occlusion), the vessel was occluded for 1 hour only. Pericranial temperature was controlled at either 37°C (n=12) or 33°C (n=14) during ischemia and for 1 hour after reperfusion. In both experiments, the rats were allowed to recover, with neurological function scored at 24 and 96 hours after onset of ischemia. Cerebral infarct volume (as determined by nitro blue tetrazolium staining) was planimetrically evaluated 96 hours after onset of ischemia.

Results: No difference in infarct volume was observed between groups undergoing permanent occlusion (177±53 mm³ for 37°C rats, 167±71 mm³ for 33°C rats [mean±SD]). Although neurologic function correlated with infarct volume at 96 hours (all animals in experiment 1 combined; p<0.01), we were unable to demonstrate an intergroup difference in function. In animals undergoing temporary occlusion, mean±SD infarct volume was 48% less in the hypothermic group (89±54 mm³ for 37°C, 46±31 mm³ for 33°C; p<0.03). Neurological function again correlated with infarct size (p<0.02), but improvement in function approached significance for the hypothermic group (p<0.06) at 24 hours after reperfusion only.

Conclusions: Benefits from mild hypothermia may be obtained under conditions of temporary but not permanent middle cerebral artery occlusion in the rat. (Stroke 1992;23:733-738)

KEY WORDS • cerebral ischemia • hypothermia • rats

Deep hypothermia (i.e., <25°C) unquestionably protects the brain against global ischemia. This therapy is widely used for cardiac surgery and also for some neurosurgical procedures which require cardiac arrest.1-4 However, because deep hypothermia is feasible only with cardiopulmonary bypass, outside of cardiac surgery its application has been extremely limited. In contrast, it recently has become obvious that minor fluctuations in brain temperature (i.e., 2–5°C) also result in major changes in outcome from global ischemia. Mild hypothermia (i.e., 32–35°C) applied either during or immediately after global ischemia reduces brain damage.5-10 Conversely, intra-ischemic hyperthermia increases injury.11-13 Such observations have sparked considerable clinical interest because such small reductions in temperature presumably carry minimal risk and are readily achievable in anesthetized patients undergoing operative procedures where cerebral ischemia may occur.

Surprisingly, despite the data concerning global ischemia, little information is available concerning the impact of mild hypothermia on focal ischemia. Because of persistent collateral circulation, focal ischemia is pathophysiologically unique from global ischemia, and many therapies that work in one situation are of little value in the other. Therefore, the following experiments were designed to explore the potential for mild hypothermia (33°C) to alter infarct volume resulting from either temporary or permanent middle cerebral artery occlusion (MCAO) in the spontaneously hypertensive rat.

Materials and Methods

The present study was approved by the University of Iowa Animal Care and Use Committee. At 13–14 weeks of age, male spontaneously hypertensive rats (Harlan, Indianapolis, Ind.) were denied food for 12–16 hours before the experiment but allowed free access to water. All animals were weighed, then anesthetized with 3–4% halothane in 50% O₂/balance N₂. Each rat was endotracheally intubated and the lungs mechanically venti-
lated to achieve normocapnia. Anesthesia was maintained with 1.2% halothane (inhaled). The tail artery was catheterized for measurement of blood pressure and blood gases. A right subtemporal craniectomy was then performed. Aided by an operating microscope, the dura and arachnoid were opened, and the right middle cerebral artery (MCA) was identified. The MCA was loosely encircled with a 10-0 suture (Ethilon monofilament nylon taper 2870 G3, 8 Circle BV75-3 needle; Ethicon, Inc., Somerville, N.J.) just distal to the lenticulostriate artery. The electroencephalogram was monitored from bilateral needle electrodes percutaneously placed adjacent to the parietal bones, and a reference needle electrode was placed subcutaneously over the right shoulder (Grass model 79E, Quincy, Mass.). During surgical preparation, oropharyngeal temperature was allowed to spontaneously fall to 34-35°C in the hypothermic groups; in the normothermic groups it was maintained at 36-37°C by surface warming with a heat lamp. Pancuronium was given as a 0.4-mg intra-arterial bolus, with subsequent doses of 0.1 mg when necessary for control of ventilation. After preparation, all rats received 50 units heparin. An overall interval of 1 hour was allowed to complete the above tasks.

In experiment 1 (permanent occlusion), rats were assigned randomly to one of two groups based on the pericranial temperature maintained during ischemia. In both groups, the MCA was permanently ligated with the 10–0 suture. A 23 g needle thermistor (YSI Model 524, Yellow Springs, Ohio) was placed immediately adjacent to the craniectomy defect. The wound was loosely closed with suture. Percranial temperature was thereafter servo-regulated with a heat lamp. In the normothermia group (n=11), pericranial temperature was maintained at 37°C. In the hypothermia group (n=11), pericranial temperature was allowed to further decrease spontaneously (over a 10–15-minute interval) to 33°C. These temperatures were maintained for 2 hours. Arterial blood gases were measured immediately before and after 30-minute intervals after MCA ligation. Hematocrit and plasma glucose concentration were measured after 30 minutes of ischemia. At the end of the 2-hour period, the wound was reopened and the thermistor removed. The temporalis and skin overlying the craniectomy were closed in separate layers after hemostasis was assured. During surgical closure and emergence from anesthesia, oropharyngeal temperature was servo-regulated to 37°C in both groups with a heat lamp. Hypothermic rats were rewarmed over a 20–30-minute interval. The tail artery catheter was removed and the halothane discontinued. When spontaneous ventilation resumed, the trachea was extubated. Animals were placed in a room-temperature, oxygen-enriched environment for 1 hour, after which they were returned to their cages for 4 days. Free access to moistened pellet food and water was allowed.

In experiment 2 (temporary occlusion), the protocol was identical to that described above with the following exceptions. A single-pass instrument tie of the 10-0 suture provided occlusion but allowed later release and reperfusion of the MCA. Absence of flow after occlusion was visually verified by a blanching of the vessel distal to the ligature. Again, rats were randomly assigned to either normothermic (37°C; n=12) or hypothermic (33°C; n=14) conditions created by servoregulation of pericranial temperature to the indicated values. In this experiment, the MCA was occluded for 1 hour only. After release of the ligature, the wound was reclosed, and a 1-hour interval of normothermia (37°C) or hypothermia (33°C) was continued in the respective groups. Thereafter, animals were treated as described in experiment 1 with respect to emergence from anesthesia and postoperative recovery.

All animals were neurologically evaluated 24 hours before and 24 and 96 hours after surgery on a scale of 0–3 as follows: 0, no observable deficit; 1, forelimb flexion; 2, decreased resistance to lateral push without circling; and 3, same behavior as 2, with circling. Neurological tests were performed by a single observer who was blinded to the experimental condition of the animal. Evaluations were performed in a dimly lit, quiet room after a brief period of acclimation.

After the 96-hour neurological evaluation, the animals were weighed and anesthetized with 4% halothane in O2. They were then decapitated and the brains removed and frozen at −20°C in 2-methylbutane. Using cryostat, 20- mm thick coronal sections were taken at 360-jm intervals over the rostrocaudal extent of the infarct. The sections were dried and stained for approximations. In this experiment, the MCA was occluded for 1 hour, with the observer blinded to experimental condition, the infarct border was outlined (corpus callosum excluded) using an operator-controlled cursor, and total infarct area within the ipsilateral hemisphere was determined. Infarct volume (cubic millimeters) was computed as the running sum of infarct area multiplied by the 360-μm interval between sections over the extent of the infarct.

Physiological values and infarct volumes were compared between groups at each measurement interval for each experiment by one-way analysis of variance. Non-parametric neurological scores were compared between groups using the Mann-Whitney U test. The association between infarct size versus neurological deficit score was analyzed with the Spearman rank correlation coefficient. Parametric values are represented as mean±SD. Significance was assumed at the value p<0.05.

Results

Physiologic values for experiments 1 and 2 are presented in Table 1. Arterial blood gases and pH, mean arterial pressure, plasma glucose, and hematocrit were similar between groups at each measurement interval. There were no differences for preischemic or postschismic body weights between groups in either experiment.
TABLE 1. Physiological Values for Experiments 1 (Permanent MCA Occlusion) and 2 (Temporary [1-Hour] MCA Occlusion)

<table>
<thead>
<tr>
<th>Value</th>
<th>Before ligation</th>
<th>30 Minutes after ligation</th>
<th>96 hours after ischemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>149±8</td>
<td>145±12</td>
<td>144±7</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.42±0.05</td>
<td>7.43±0.05</td>
<td>7.44±0.03</td>
</tr>
<tr>
<td>Pacr (mm Hg)</td>
<td>37.6±3.8</td>
<td>37.5±2.5</td>
<td>36.8±3.7</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>162±32</td>
<td>170±25</td>
<td>171±32</td>
</tr>
<tr>
<td>Oropharyngeal temperature (°C)</td>
<td>36.3±0.6*</td>
<td>35.4±0.6*</td>
<td>36.5±0.4</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>309±12</td>
<td>295±20</td>
<td>291±21</td>
</tr>
</tbody>
</table>

Before ligation:
- Mean arterial pressure (mm Hg)
- Arterial pH
- Pacr (mm Hg)
- PaO2 (mm Hg)
- Oropharyngeal temperature (°C)
- Body weight (g)

30 Minutes after ligation:
- Mean arterial pressure (mm Hg)
- Arterial pH
- Pacr (mm Hg)
- PaO2 (mm Hg)
- Cranial temperature (°C)
- Plasma glucose (mg/dl)
- Hematocrit (%)

Values are mean±SD. MCA, middle cerebral artery; normothermia, 37°C; hypothermia, 33°C.

* p<0.05, † p<0.001 by one-way analysis of variance.

Before MCAO, oropharyngeal temperature was modestly reduced in the hypothermic versus normothermic groups in both experiments (p<0.05). As intended, intraischemic pericranial temperature was reduced in the hypothermic group in both experiments (experiment 1: 33.2±0.3°C versus 37.0±0.1°C, p<0.001; experiment 2: 33.2±0.4°C versus 37.0±0.1°C, p<0.001).

Twenty-four hours before ischemia, all rats were neurologically normal. In experiment 1 (permanent occlusion), one rat in the hypothermia group died during the recovery interval. In experiment 2, two rats in the hypothermia and one in the normothermia group died. There were no differences in mortality rates between groups in either experiment (χ² analysis).

Infarct volumes (mean±SD) for both experiments are presented in Figure 1. In experiment 1, infarcted tissue extended over the rostrocaudal aspect of the neocortex. Infarcted tissue was also present in the more rostral and lateral aspects of the basal ganglia. Pericranial temperature did not affect infarct volume resulting from permanent occlusion (177±53 mm³ for 37°C rats; 167±71 mm³ for 33°C rats). In contrast, in experiment 2 (temporary occlusion), infarct was almost exclusively restricted to cortical tissue. Mean infarct volume was 48% smaller in the hypothermic group (89±54 mm³ for 37°C, 46±31 mm³ for 33°C; p<0.03).

Neurological scores assigned at the 96-hour recovery interval as a function of infarct volume are given in Figure 2. In experiment 1, no differences in neurological score were observed between hypothermic and normothermic groups at either 24 or 96 hours after MCAO. However, at both intervals, a significant correlation between infarct volume and neurological score was present (24 hours: r=0.53, Z=2.37, p<0.02; 96 hours: r=0.61, Z=2.48, p<0.01). In experiment 2 (temporary occlusion), an improvement in the neurological score in the hypothermic versus normothermic rats approached significance at 24 hours after ischemia (Z=1.86, p<0.06), but this effect was absent at 96 hours after MCAO (Z=1.05, p<0.3). A correlation between neurological score and infarct volume was nearly significant at 24 hours after ischemia (r=0.49, Z=1.87, p<0.06) and...
function of pericranial temperature (37°C versus 33°C) during
function was correlated with infarct volume in both experiments,
rats evaluated 96 hours after onset of ischemia in experiments 1
3, decreased resistance to lateral push with circling) for individual
FIGURE 2. Neurological deficit scores (0, no observable deficit;
Infarct Volume (mm³)
D Pfnumnt  MCAO - 37°C
•OB O»
• 1hrHCAO-33°C
O 1hrMCAO-37°C
Permanent MCAO - 37°C
Permanent MCAO - 33°C
was significant at 96 hours after MCAO ($r=0.59,
Discussion
As early as 1957, it was speculated that hypothermia
might protect the brain against focal ischemic insults. Rosomoff19 found a reduction in infarct size after partial
excision of the MCA in dogs if body temperature was
reduced to 24°C within 15 minutes after interruption of
flow. However, later work performed under more care-
fully controlled conditions failed to support that finding.
In fact, when primates underwent permanent MCAO
with a reduction of body temperature to 29°C for 48
hours, outcome was worse than that observed for normothermic counterparts.20,21 This worsened outcome as-
associated with hypothermia was speculated to result from
increased blood viscosity at reduced body temperatures
perhaps impeding collateral circulation. The failure to
document improved outcome with moderate hypother-
mia, along with the numerous complications associated
with surface cooling to 28–30°C,21–23 effectively elimi-
nated the clinical use of nonbypass hypothermic therapy
for focal cerebral ischemia. A more recent report on the
influence of hypothermia in focal ischemia is that of
Onesti et al.24 In that report, rats undergoing coagulation
of the MCA were cooled to 24°C during the first hour of
ischemia. While the success of that study in finding
protective efficacy from hypothermia against permanent
focal ischemia was not observed in our study, the degree
of hypothermia needed to achieve a protective effect
again holds little clinical relevance outside the domain of
cardiopulmonary bypass.
Interest in mild hypothermia as a cerebral protectant
has emerged almost serendipitously. The development of
rodent recovery models has allowed considerable
progress in the understanding of the pathophysiology
and pharmacology of cerebral ischemia.25–27 In the
course of such model development, it has become clear
that brain temperature must be controlled because
minor fluctuations are associated with substantial dif-
fences in histopathologic outcome from global isch-
emia.3 Besides constituting a methodological advance,
such observations have been welcomed because mild
hypothermia appears to offer at least as much protective
potential as most pharmacological agents. Such findings
may have the greatest relevance to the anesthetized
patient, in whom 2–5°C reductions of body temperature
are readily achievable and tolerated in most cases. In
fact, anesthetized patients spontaneously cool to 34–
35°C if not actively warmed.
Accordingly, the current study was performed to
determine whether mild hypothermia similarly offers
protection against either permanent or temporary focal
ischemic insults. We observed that mild hypothermia
influenced neither pathological nor neurological out-
come in the spontaneously hypertensive rat undergoing
permanent MCAO. This makes intuitive sense because
the ligature was still in place after the animals were
rewarmed. It would be difficult to conjecture how the
beneficial effects of mild hypothermia would persist
beyond the rewarming interval. In contrast, in experi-
ment 2, a 4°C reduction of pericranial temperature
persisting throughout 1 hour of MCAO and the first
hour of recirculation was associated with a 48% reduc-
tion in infarct volume. There was also a nearly signifi-
cant improvement in neurological function at 24 hours
after ischemia. Finally, the size of the infarct was
substantially less in either temporary occlusion group
than was observed with permanent occlusion (statistics
not performed due to incomplete concurrency between
experiments). This finding is consistent with previous
observations regarding the effects of duration of isch-
emia on infarct size28 and suggests that duration of
ischemia remains a critical determinant of outcome,
regardless of the pericranial temperature.
The mechanism of the observed protection was not
addressed by this outcome study, and thus we can only
speculate. Because there is a log-linear reduction of
metabolic rate associated with progressive hypothermia
(untill at least 27°C),29,30 hypothermic brain protection
has traditionally been attributed to a reduction of
cerebral metabolic rate (CMR). This might explain why
the beneficial effects of mild hypothermia on outcome
from focal ischemia have been overlooked. If CMR
reduction was the mechanism for hypothermic brain
protection, there should be some correlation between
temperature and protective efficacies, and one would
expect little benefit from a mild reduction of tempera-
ture. According to this reasoning, more severe hypo-
thermia should be pursued so as to achieve optimal benefit.
Severe hypothermia (i.e., <30°C), however, carries con-
siderable risk for complications that include myocardial
ischemia, arrhythmias, coagulopathies, and altered dis-
position of pharmacological agents.22 Many of these
risks could be circumvented by cardiopulmonary bypass.
However, cardiopulmonary bypass involves anticoagu-
ation (complicating neurosurgical procedures signifi-
cantly) as well as its own risk of neurological injury, such
as embolic events.
Although a favorable balance between metabolic
supply and demand during ischemia would seem to
favor an improved outcome, some experimental evidence argues against a reduction of CMR as being a sufficient criterion for brain protection. Because CMR falls by approximately 50% when brain temperature is reduced to 27°C, we would predict that at 33°C, a 20% reduction in CMR was achieved in our experiment. Previous work in our laboratory failed to demonstrate a reduction in infarct size when isoflurane, a volatile anesthetic, was administered in doses sufficient to result in a 50% reduction in CMRglu.16 Thus, other protective mechanisms of mild hypothermia must be present. Attention has recently been focused on the roles of excitatory neurotransmitters in ischemic brain damage. Extracellular concentrations of these neurotransmitters are increased during and after a reversible global insult. Several laboratories have specifically documented a profound attenuation of the increase in glutamate concentration when brain temperature is reduced to as little as 33−35°C.7,13,22 Such observations have not yet been made in focal ischemia models, but given the results of this experiment, such investigations would seem indicated.

Further laboratory work is indicated by the results of these experiments. The temperature of 33°C was chosen because it is feasible to achieve that temperature with minimal difficulty in humans. However, a dose−response study with respect to severity of hypothermia versus outcome from temporary focal ischemia might indicate whether even a temperature as low as 33°C was necessary to achieve the protection observed. Indeed, if the primary mechanism of action of hypothermia is inhibition of ischemia-induced increases in extracellular excitatory neurotransmitter concentrations, such a mechanism might be induced by a less-severe reduction in brain temperature.32 Finally, to make experiments 1 and 2 more directly comparable, rats in both experiments were maintained hypothermic for a duration of 2 hours. Now that a specific protective effect has been identified for hypothermia during temporary MCAO, it would be of further interest to determine whether the maintenance of hypothermia throughout the first hour of reperfusion offers additional advantage as opposed to rewarming immediately after reperfusion.

It is tempting to extrapolate the results of this and other related laboratory studies to the clinical arena. Given the consistency of improved outcome across numerous laboratories and rodent ischemia models as well as the apparently modest deleterious aspect of mild hypothermia, it would seem reasonable to recommend application of this modality routinely in patients undergoing nonbypass procedures that carry a high risk for cerebral ischemic insults. However, other considerations are relevant. Validation of the above observations in higher species would seem important. If it can be replicated that mild hypothermia is protective against temporary but not permanent focal ischemia, the information would be relevant to selection of patients for whom this modality could be used. For example, temporary occlusion of a major intracranial vessel during cerebral aneurysm clipping would be physiologically most similar to the experimental model described herein. In contrast, other procedures that might generate permanent focal insults by release of calcific plaques into the circulation (e.g., carotid endarterectomy) may be less responsive to hypothermic protection. Finally, patients at risk for intraoperative cerebral ischemia typically have diffuse vascular disease. Mild hypothermia, while unlikely to produce coagulopathies or arrhythmias, still has substantial hemodynamic and respiratory effects (e.g., total body oxygen consumption is increased by as much as 48% in shivering, mildly hypothermic patients during immediate postoperative recovery).33 The efficacy of mild hypothermia for cerebral protection must still be weighed against potential myocardial stresses associated with this therapy in patients with significant atherosclerosis.

In conclusion, a reduction of pericranial temperature to 33°C was demonstrated to be protective against temporary, but not permanent, focal cerebral ischemia in this rat model. In the case of temporary ischemia, mean infarct size was reduced by 48% and a modest (but transient) improvement in neurological outcome from the MCAO was observed. This study and those demonstrating brain protection against global ischemia with mild hypothermia should encourage debate concerning the utility of this therapeutic modality in the neurosurgical patient.

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