Use of mild intraischemic hypothermia versus mannitol to reduce infarct size after temporary middle cerebral artery occlusion in rats

Hiroshi Karibe, M.D., Gregory J. Zarow, M.A., and Philip R. Weinstein, M.D.

Department of Neurological Surgery, School of Medicine, University of California, San Francisco, and Neurosurgical Research Laboratory, Department of Veterans Affairs, Veterans Administration Medical Center, San Francisco, California

To determine which of two treatments for reducing ischemic injury after temporal focal ischemia is more effective, the effects of mild (33°C) intraischemic hypothermia were compared with those of mannitol, the most commonly used neuroprotective agent. Four groups of Sprague-Dawley rats underwent 1 hour of endovascular middle cerebral artery occlusion followed by 23 hours of normothermic reperfusion. The four experimental groups were as follows: Group A, saline control; Group B, mannitol (25%, 1 g/kg); Group C, hypothermia; and Group D, hypothermia plus mannitol.

Laser-Doppler estimates of cortical blood flow showed that hypothermia did not affect blood flow during ischemia or reperfusion. Mannitol increased cortical blood flow during ischemia and reperfusion under both normothermic and hypothermic conditions (p < 0.05). Neurological deficit was significantly less severe in treated rats (Group B, p < 0.05; Group C or D, p < 0.01) than in controls (Group A). Infarct volume, measured on semiserial Nissl-stained sections, was significantly smaller in treated rats (p < 0.01) than in controls. Infarct volume was also significantly smaller in rats treated with hypothermia than in those treated with mannitol (Group C vs. Group B, p < 0.05); there was no difference between rats treated with mannitol and those treated with mannitol and hypothermia. All three treatments reduced infarct area in the ischemic penumbra; hypothermia with or without mannitol also reduced infarct area in the ischemic core.

These results demonstrate that both mild intraischemic hypothermia and mannitol reduce infarct size and neurological deficit: hypothermia reduces infarct size more effectively than mannitol, and mannitol adds no significant protection to hypothermia, whereas hypothermia adds significant protection beyond that afforded by mannitol after brief focal ischemia followed by reperfusion in rats. The results suggest that mild intraischemic hypothermia alone, or in combination with mannitol, may be useful in avoiding ischemic injury from temporary vessel occlusion during cerebrovascular surgery.

KEY WORDS • hypothermia • mannitol • focal ischemia • rat

Brief temporary cerebral arterial occlusion for less than 1 hour has been used to prevent intraoperative bleeding and to facilitate dissection during surgery for intracranial aneurysms and removal of arteriovenous malformations. Deep-to-moderate (30°C) hypothermia has been used to minimize ischemic injury induced by temporary vessel occlusion; however, deep hypothermia for purposes other than cardiopulmonary bypass was abandoned because it was complicated by ventricular fibrillation, acidosis, shivering, bleeding dyscrasias, and ischemic sensory neuropathies. Hypothermia was replaced by improved neuroanesthetic techniques and putative neuroprotective agents. In the last few years, mannitol has become the most commonly used neuroprotective agent during temporary intraoperative cerebrovascular occlusion.

Recent studies in small-animal models have shown that even mild (33°C) hypothermia confers striking protection in both global and focal ischemia. Mild hypothermia may carry fewer systemic risks and is easy to achieve. Primates can readily tolerate a 3°C to 4°C reduction of body temperature. These results suggest that mild hypothermia may be useful for brain protection with fewer side effects than deep-to-moderate hypothermia. However, it is not known whether mild hypothermia has a neuroprotective effect during brief focal ischemia. Moreover, the effects of mild hypothermia have not been compared with those of mannitol, the best available neuroprotective agent.

In this study, we compared the neuroprotective effects of mild intraischemic hypothermia with those of mannitol by measuring infarct volume, brain edema, cortical blood
Materials and Methods

Animal Preparation

Thirty-two male Sprague-Dawley rats weighing 280 to 320 g each were anesthetized with 3% isoflurane, intubated with a 16-gauge angiocatheter, and mechanically ventilated with a rodent ventilator (model 683, Harvard Instruments, South Natick, MA). Anesthesia was maintained with 1.5% isoflurane in a mixture of 70% N₂O and 30% O₂; atropine sulfate (0.04 mg) was injected intraperitoneally to minimize secretions. One femoral artery was cannulated with polyethylene tubing (PE-50) for arterial blood pressure monitoring with a polygraph recorder (model 79D, Grass Instrument Co., Quincy, MA) and for intermittent blood gas analysis (model ABL30, Radiometer, Copenhagen, Denmark). Respiratory adjustments were made to maintain normal arterial blood gases.

Brain temperature was monitored with a 33-gauge thermocouple temperature probe connected to a digital thermometer/thermoregulator (model CN9000, Omega, Stamford, CT) introduced through a small burr hole 3.5 mm lateral to the bregma. The probe was stereotactically lowered 5.0 mm from the skull surface, to place the probe tip in the lateral part of the olfactory bulb, the ischemic core of this model. Rectal temperature was monitored with an analog thermometer (model 43TA, Yellow Springs Instrument Co., Yellow Springs, OH). Brain and rectal temperatures were maintained at 37°C for 30 minutes before the onset of ischemia.

Middle Cerebral Artery Occlusion

All rats underwent 1 hour of focal cerebral ischemia followed by 23 hours of reperfusion. Focal cerebral ischemia was produced by a modification of the endovascular middle cerebral artery (MCA) occlusion technique. Under the operating microscope, the right carotid artery was exposed through a midline cervical incision. The pterygopalatine artery was ligated with a 5-0 silk suture next to its origin. The external carotid artery was dissected and isolated distally by electrocoagulating the terminal superior thyroid, occipital, lingual, and maxillary artery branches. A 3.0 monofilament nylon suture, its tip rounded by heating, was introduced into the lumen of the external carotid artery into the internal carotid artery (ICA) for a length of 22 mm beyond the bifurcation of common carotid artery, occluding the origin of the MCA and anterior cerebral artery. To allow reperfusion, the intraluminal suture was gently advanced 0.5 mm from the skull surface, to place the probe tip in the lateral part of the caudoputamen, the ischemic core of this model.

Rectal temperature was monitored with an analog thermometer (model 43TA, Yellow Springs Instrument Co., Yellow Springs, OH). Brain and rectal temperatures were maintained at 37°C for 30 minutes before the onset of ischemia.

Cortical Blood Flow

Cortical blood flow was monitored by laser-Doppler flowmetry (flowmeter model BPM2 and probe model P433-1, Vasamedics, Inc., St. Paul, MN) during ischemia and reperfusion. A burr hole was made 5 mm lateral and 0.3 mm anterior to the bregma. Under the operating microscope, the laser-Doppler flowmetry probe was stereotactically placed 0.5 mm above the exposed dura matter, avoiding large blood vessels. To prevent dehydration and ensure a clear signal, the space between the dura and the probe tip was filled with saline. The cortical blood flow values were recorded 30 minutes before the onset of ischemia as a baseline and every 10 minutes during ischemia and the first 30 minutes of reperfusion. The cortical blood flow was calculated as a percentage of the baseline.

Experimental Groups

Rats were randomly divided into four groups of eight rats each and subjected to MCA occlusion. During ischemia, control rats (Group A) received saline; treated rats received mannitol (Group B), hypothermia (Group C), or hypothermia and mannitol (Group D). Whole-body hypothermia was induced with an ice pack; brain temperature reached 33°C within 10 minutes before the onset of ischemia, and hypothermia was maintained throughout ischemia.

The rats were rewarmed with a heating pad and a heating lamp; normothermia (37°C) was achieved within 10 minutes after the onset of reperfusion and maintained as monitored during the first 30 minutes of reperfusion.

In normothermic rats (Groups A and B), brain temperature was maintained at 37°C during ischemia and monitored for 30 minutes of reperfusion. In Groups B and D, mannitol (25%, 1 mg/kg) was injected intravenously 10 minutes before the onset of ischemia. In Groups A and C, an identical amount of physiological saline was injected intravenously 10 minutes before the onset of ischemia. After the experiment, the incisions were closed. The rats were returned to their cages to recover from anesthesia and were given free access to food and water.

Assessment of Motor Performance and Paralysis

Twenty-four hours after the onset of ischemia, motor performance was measured with an inclined plane test, a balance beam test, and a prehensile test. In the inclined plane test, the rat was placed in head-up position at the top of a 60-cm × 30-cm board, which was covered with a thin rubber pad and fixed at an angle of 60° from the horizontal. In the balance beam test, the rat was placed at the center of a wooden rod (70 cm long, 3.2 cm in diameter). In the prehensile test, the forepaws of the rat were hung on a horizontally stretched nylon rope (70 cm long, 4 mm in diameter). All motor performance tests were conducted approximately 70 cm above a thick sponge pad. Each test was scored based on how long the rats stayed on each hurdle (0 = < 1 second; 1 = 1 to 10 seconds; 2 = 11 to 20 seconds; 3 = 21 to 30 seconds; and 4 = > 30 seconds). Two trials were given in each test, and the better score was recorded. To avoid fatiguing the animals, the trials were conducted 5 minutes apart. Paralysis was also measured as previously described: 0 = dead; 1 = coma; 2 = falls to the left; 3 = circling; 4 = flexion of left forepaw; 5 = no deficit. The scores on each test were summed to give a total neurofunctional deficit score.

Histological Assessment

The rats were reanesthetized with chloral hydrate (4%, 5 ml, intraperitoneally) and decapitated. The brains were removed within 1 minute, frozen in powdered dry ice, cut with a cryostat into 20-μm thick sections, and stained with cresyl violet. The infarct areas of these 20-μm sections, obtained at 1-mm intervals, were analyzed by a videodigitizer image-analysis system (Imaging Research, Inc., St. Catharines, Ontario, Canada). To compensate for brain swelling in the ischemic hemisphere, the infarct area on each slide was calculated by subtracting ipsilateral normal tissue area from contralateral normal tissue area. Infarct volume was calculated by summing the infarct areas from each slide and multiplying by the interval width (1 mm).

Infarct areas in cortex (ischemic penumbra) and caudoputamen (ischemic core) were measured from slides obtained at 3, 4, and 5 mm from the frontal pole. The infarct area of each region was expressed as a percentage of the contralateral normal tissue area, and the average of these three slides was used for comparison. Brain edema volume was estimated by subtracting the volume of the left (nonischemic) hemisphere volume from that of the right (ischemic) hemisphere.

Statistical Analysis

Analysis of variance was used to determine the statistical significance of differences between groups. Fisher’s protected least significant difference was used to compare differences in brain edema volume, infarct volume, infarct area, and physiological variables between groups. Multivariate analysis of variance was used to compare differences in cortical blood flow between groups. All parametric values were expressed as means ± standard deviation. The Mann–Whitney U-test was used for comparison of neurofunctional deficit between groups. Bonferroni’s correction for multiple groups comparisons was used so that overall experimental differences could be considered significant at p < 0.05.
Hypothermia versus mannitol for neuroprotection

**Physiological variables before ischemia and during ischemia and reperfusion**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Ischemia</th>
<th>During Ischemia</th>
<th>During Reperfusion</th>
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<tr>
<td>PaCO₂ (mm Hg)</td>
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<tr>
<td>Group A</td>
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<td>36.3 ± 2.0</td>
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<td>35.7 ± 1.5</td>
<td>37.2 ± 2.3</td>
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<tr>
<td>PaO₂ (mm Hg)</td>
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<td></td>
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<tr>
<td>Group A</td>
<td>128.1 ± 12.9</td>
<td>126.7 ± 10.9</td>
<td>127.8 ± 14.9</td>
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<tr>
<td>Group B</td>
<td>122.3 ± 12.6</td>
<td>122.9 ± 10.0</td>
<td>123.1 ± 12.3</td>
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<tr>
<td>Group C</td>
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<td>129.6 ± 10.2</td>
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<td>103.6 ± 3.9</td>
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<td>Group D</td>
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<td>brain temperature (˚C)</td>
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<tr>
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<td>32.4 ± 0.4†</td>
<td>37.6 ± 0.4</td>
</tr>
<tr>
<td>Group D</td>
<td>37.0 ± 0.3</td>
<td>32.6 ± 0.6†</td>
<td>36.7 ± 0.3</td>
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</table>

*Values are mean ± standard deviation. Group A: control (eight rats), Group B: mannitol (eight rats), Group C: hypothermia (seven rats), Group D: hypothermia and mannitol (eight rats). MABP = mean arterial blood pressure. † p < 0.05 versus Group A (control) or Group B (mannitol).

**RESULTS**

Physiological Variables

Except for artificially controlled temperature, all physiological variables were within the normal range throughout the experiments (Table 1). There were no statistically significant differences between groups except for mean arterial blood pressure, which was lower, although still in the normal range, in hypothermic rats (Groups C and D) during ischemia (p < 0.05). One rat in Group C was excluded from the analysis because of inadequate blood gas control.

Measurement of Cortical Blood Flow

Immediately after the onset of MCA occlusion, cortical blood flow decreased sharply. During ischemia, cortical blood flow was significantly higher in rats treated with mannitol (Groups B and D) than in control (Group A) (p < 0.05); however, cortical blood flow decreased sharply after the onset of ischemia in all rats (Table 2). No significant difference was detected between rats treated with hypothermia alone (Group C) and controls or between rats treated with mannitol alone (Group B) and rats treated with hypothermia and hypothermia (Group D).

After the onset of reperfusion, cortical blood flow recovered rapidly in all rats. During the first 30 minutes of reperfusion, cortical blood flow was significantly higher in rats treated with mannitol (Groups B and D) than in control animals (Group A) (p < 0.05). No significant difference was detected between rats treated with hypothermia alone (Group C) and controls or between rats treated with mannitol only (Group B) and those treated with both mannitol and hypothermia (Group D). Postischemic hyperemia was not observed in controls.

Motor Performance and Paralysis

Neurofunctional deficits were significantly less severe in treatment groups than in controls. Although hypothermia plus mannitol produced the greatest protection against parasitic, there were no significant differences between treatment groups (Table 3). No rat died within 24 hours after the onset of ischemia.

Brain Edema Volume

No significant difference between the groups was detected in the volume of the nonischemic hemisphere. Brain edema volume was significantly smaller in treated rats than in controls (p < 0.05) (Fig. 1 left). There were no significant differences between treatment groups.

Infarct Volume

Infarct volume was significantly smaller in treated rats than in controls (p < 0.05). This volume was significantly smaller in rats treated with hypothermia alone (Group C) than in those treated with mannitol alone (Group B).
There was no significant difference between rats treated with hypothermia alone (Group C) and those treated with hypothermia and mannitol (Group D) (Fig. 1 right).

The infarct area in cortex (ischemic penumbra) was significantly smaller in treated rats than in controls (p < 0.05). There were no significant differences between treatment groups (Fig. 2 left). The infarct area in caudoputamen was significantly smaller in rats treated with hypothermia (Groups C and D) than in controls (Group A) (p < 0.05). There was no significant difference between rats treated with mannitol alone (Group B) and controls (Fig. 2 right).

**Discussion**

This study shows that mild intraschismic hypothermia reduces brain edema, neurofunctional deficit, and infarct volume in rats after 1 hour of temporary MCA occlusion followed by 23 hours of reperfusion. Mild hypothermia was more effective than mannitol in reducing infarct size and conferred additional protection, especially in caudoputamen, compared with mannitol. These results suggest that, although it may be difficult to reproduce in clinical settings, mild intraschismic hypothermia alone or in combination with mannitol may be useful for brain protection during brief temporary focal ischemia.

Focal ischemia due to MCA occlusion severely decreases cortical blood flow in the ischemic core; ischemia is less severe in the penumbra. Therapies that decrease infarct size usually prevent recruitment of the penumbra into the infarction process. Even in the ischemic penumbra, reperfusion after prolonged ischemia induces so-called reperfusion injury, which is characterized by postschismic hyperemia, oxygen–free radical production, vasogenic edema caused by blood–brain barrier disruption, and delayed postschismic hypoperfusion. Infarct volume after hypothermic ischemia correlates positively with the degree of postschismic hyperemia.

Mild hypothermia reduces free-radical production, postschismic hyperemia, delayed postschismic hypoperfusion, brain edema, and blood-brain barrier disruption after 3 hours of focal ischemia followed by reperfusion in rats. Hypothermia during prolonged temporary focal ischemia reduces postschismic cell injury. In this study, hypothermia reduced penumbra injury even though there was no difference in postschismic cortical blood flow compared with controls. Furthermore, no postschismic hyperemia was observed, even in control animals. This observation suggests that hypothermia during brief focal ischemia may not modify postschismic hemodynamic events. However, hypothermia can protect against ischemic injury by mechanisms other than reducing reperfusion injury.

One of the important toxic metabolic events in the penumbra is excitotoxicity induced by glutamate. Glutamate activates excitatory amino acid receptors during ischemia, resulting in increased intracellular calcium, which is neurotoxic and activates a variety of reactions mediated by phospholipases, proteases, and protein kinases. Hypothermia reduces glutamate release, intracellular mediator activation, and free fatty acid accumulation in global ischemia. Preliminary studies in our laboratory have shown that mild hypothermia reduces glutamate release in ischemic penumbra in the same focal ischemia model. Thus, mild intraschismic hypothermia may also exert protection by reducing excitotoxic reactions.

A unique finding of this study is that hypothermia reduced infarct size in the ischemic core. In this area, neuronal cell damage occurs within 15 to 30 minutes because of severe cortical blood flow reduction, energy failure, and subsequent destruction of ion homeostasis under nor-
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...thermic conditions.39 Although no other treatment has been shown to reduce striatal necrosis after temporary MCA occlusion in rats, similar protection was observed during permanent MCA occlusion after pretreatment with dextrophan, a noncompetitive glutamate receptor antagonist.13 In global ischemia, the beneficial effects of hypothermia have been attributed to reduced energy demand27,35 and prevention of adenosine triphosphate depletion.19,20 Mild hypothermia may extend the time threshold of cell survival during ischemia by maintaining energy metabolism. This study shows that the protective effects of hypothermia previously demonstrated during brief global ischemia can be extended to focal ischemia.

One potential adverse effect of hypothermia is reduction of collateral circulation because of increased blood viscosity.28,41 This study demonstrates that during ischemia there is no difference in cortical blood flow between hypothermic rats and controls, which suggests that mild intraschismic hypothermia may carry less risk due to increased blood viscosity than might have been anticipated. Because mannitol increases cortical blood flow by reducing blood viscosity,2 we also examined the combination of mannitol and hypothermia. Even though mannitol increased cortical blood flow, it conferred no additional protection compared with hypothermia alone. Although we did not measure cortical blood flow in the caudoputamen, previous studies demonstrated that mannitol increases cortical blood flow only in the ischemic penumbra and not in the ischemic core.26,36 Mannitol also exerts a protective effect by scavenging oxygen free radicals.30 Hypothermia also reduces oxygen-free radical production.18 The lack of additional effect suggests that the protective mechanisms of hypothermia at least duplicate those of mannitol.

Conclusions

We conclude that mild intraschismic hypothermia or mannitol reduces infarct volume and neurofunctional deficit; that hypothermia reduces infarct size more effectively than mannitol, especially in the ischemic core; and that hypothermia significantly increases protection compared to mannitol alone. In addition, the neurofunctional deficit scores suggest that hypothermia in combination with mannitol may potentially have the greatest protective effect. Mild hypothermia had no adverse effects after 1 hour of MCA occlusion followed by 23 hours of reperfusion in rats. Although hypothermia may be difficult to reproduce in clinical settings, these observations suggest that mild intraschismic hypothermia may be useful during temporary vessel occlusion for cerebrovascular surgery.

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References


