Effects of Delayed Intraischemic and Postischemic Hypothermia on a Focal Model of Transient Cerebral Ischemia in Rats

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Background and Purpose—Intraischemic mild hypothermia has been shown to be neuroprotective in reducing cerebral infarction in transient focal ischemia. As a more clinical relevant issue, we investigated the effect of delayed intraischemic and postischemic hypothermia on cerebral infarction in a rat model of reversible focal ischemia. We also examined the effect of hypothermia on the inflammatory response after ischemia-reperfusion to assess the neuroprotective mechanism of brain hypothermia.

Methods—Rats were subjected to 2 hours of middle cerebral artery occlusion followed by 22 hours of reperfusion under the following protocols: (1) rats were treated with normothermia (37.0°C, 4 hours) and then housed in room temperature (25°C, 18 hours) and (2) rats were treated with hypothermia (33.0°C, 4 hours, brain temperature modulation was started 30 minutes before the reperfusion) and then housed in cold temperature (5°C, 18 hours). Animals were killed 24 hours after the onset of ischemia. The infarct volume was examined with 2,3,5-triphenyl-tetrazolium chloride staining. The accumulation of polymorphonuclear leukocytes (PMNLs) and the expression of intercellular adhesion molecule-1 mRNA were evaluated in both groups.

Results—A significant reduction (P<0.05) in infarct volume was found in the hypothermia group compared with the normothermia group. Compared with the normothermia group, hypothermic treatment also significantly reduced the accumulation of PMNLs (P<0.01) and inhibited the overexpression of intercellular adhesion molecule-1 mRNA at 22 hours of reperfusion after 2 hours of ischemia.

Conclusions—Ischemic brain damage can be reduced with delayed intraischemic and prolonged postischemic hypothermia in a focal model of transient cerebral ischemia in rats. The neuroprotective mechanism of hypothermia may be mediated by suppression of PMNL-mediated inflammatory response after ischemia-reperfusion in this model. (Stroke. 2000;31:1982-1989.)

Key Words: cerebral ischemia, focal ● hypothermia ● intercellular adhesion molecule-1 ● neutrophils ● peroxidase

Ischemic stroke is a leading source of disability in elderly persons, and much emphasis in research is being placed on the early treatment of stroke. The advancement of intravascular techniques and thrombolytic agents, especially recombinant tissue plasminogen activator (rtPA), has been shown to reduce functional deficits within an optimal time window.1,2 The acceleration of recanalization with thrombolytic agents can salvage brain tissue in an ischemic area from irreversible cell death. However, the time window for effective treatment is narrow because longer durations of ischemia and subsequent reperfusion increase the likelihood of brain edema formation and hemorrhagic transformation.3 This phenomenon has been demonstrated in various tissues, especially in the heart and lung, and been termed “reperfusion injury.” The understanding and treatment of reperfusion injury are important in the new era of reperfusion therapy for cerebral stroke.

See Editorial Comment, page 1989

There has been renewed interest in mild hypothermia as a method of cerebral protection in global4,5 and focal4–8 cerebral ischemia. Its neuroprotective effects have been ascribed to a decrease in cerebral blood flow (CBF) and metabolic requirement for oxygen,9 preservation of the blood-brain barrier from breakdown,10 and alteration in neurotransmitter release.11 More recently, there have been reports that hypothermia may attenuate the cell-mediated inflammatory response to cerebral ischemia.12,13 although most studies have shown that mild hypothermia is effective in reducing neuronal injuries when applied intraischemically,4–8 a more clinical relevant issue is whether hypothermia can protect if applied once reperfusion has occurred. Several studies have provided evidence that postischemic hypothermia may protect the brain from brief

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1982
periods of global ischemia. However, few studies have evaluated the effectiveness of postischemic hypothermia in a focal model of transient cerebral ischemia.

In the present study, we describe a focal ischemia-reperfusion model in which ischemic brain damage can be reduced by mild hypothermia that is initiated 30 minutes before reperfusion and maintained for a prolonged period. We also examine the effects of mild hypothermia on the activity of myeloperoxidase (MPO) as an indicator of the accumulation of polymorphonuclear leukocytes (PMNLs) and the expression of endothelial adhesion molecule, intercellular adhesion molecule-1 (ICAM-1) in the ischemic tissue to assess the potential neuroprotective mechanisms of hypothermic treatment.

Materials and Methods
The experimental protocols used in this study were approved by the Ethics Committees for Animal Experimentation at Kagawa Medical University. Animals were allowed free access to food and water before the experiments.

Rat MCA Occlusion Model
Adult male Sprague-Dawley rats (n=67; Charles River) weighing 250 to 300 g were used for all experiments. Anesthesia was induced with a nitrous oxide (60%) and oxygen (40%) mixture with 4% halothane in a chamber. After tracheostomy, the animals were ventilated mechanically with a small-animal ventilator. Anesthesia was maintained with a halothane concentration of 1%. A femoral artery was cannulated for the continuous monitoring of arterial blood pressure and for blood sampling. Arterial blood gases were measured a few times to ensure PaO2 and PaCO2 values were within the normal range during the experiments.

The MCA was occluded according to the suture method of Zea Longa et al with minor modifications. Briefly, all branches of the left common carotid artery were isolated and coagulated. The internal carotid artery (ICA) was then isolated, and its extracranial branch, the pterygopalatine artery, was ligated at its origin. A 3-cm length of 3-0 nylon monofilament suture with a rounded tip was introduced into the transected lumen of the external carotid artery and gently advanced into the ICA at an angle of 45° to obstruct the origin of the MCA. For reperfusion, the suture was withdrawn back into the external carotid artery to restore ICA-MCA blood flow.

Experimental Protocols
After the surgery, rats were allowed to recover from the anesthesia. Only rats that showed significant neurological deficits were subjected to the following experiments. They were reanesthetized under halothane, and the brain tissue was modulated through external body heating or cooling started 30 minutes before the reperfusion of the MCA. Rats were subjected to 2 hours of occlusion followed by 22 hours of reperfusion. During the reperfusion, the following protocols were used: (1) Rats were treated with normothermia (37°C) during the first 4 hours of reperfusion and then housed in a room maintained at 25°C for an additional 18 hours (Normo-tMCAO, n=7). (2) Rats were treated with hypothermia (33.0 to 33.5°C) during the first 4 hours of reperfusion and then housed in a room maintained at 25°C for an additional 18 hours (Hypo-tMCAO, n=7). To assess the injury produced after reperfusion, rats were treated with normothermia during 4 hours without reperfusion and then housed in a room maintained at 25°C for an additional 18 hours (P/MCAO, n=7).

For the continuous assessment of CBF, an LDF monitor (model AFL 21; Advance Corp) was used. Blood flow was measured in an ischemic border zone located 3 mm lateral to the midline and 1 mm posterior to the bregma. A 2-mm hole was drilled in the skull, and the dura was left intact to prevent cerebrospinal fluid leakage. Large blood vessels were avoided under microscopic assistance. The LDF probe was held in a micromanipulator and advanced to gently touch the intact dura. Stable baseline LDF readings were obtained for at least 20 minutes before the MCA occlusion. LDF values were recorded continuously during 2 hours of occlusion and the subsequent 4 hours of reperfusion. The CBF values were expressed as a percentage of the baseline value.

MPO Activity
MPO activity was used as an indicator of tissue PMNL accumulation. The brains were rapidly removed and separated into right and left hemispheres. Each hemisphere was immediately frozen in liquid nitrogen and stored at −70°C for later biochemical analysis. The procedure described by Bradley et al., with minor modifications, was used for the quantification of MPO activity. The brain samples were thawed on ice, and punches were made with a 7-mm-diameter cork borer of the ipsilateral and contralateral cortices.
to obtain tissue samples from the center of the MCA distribution. The wet weight was immediately measured in each sample. Each sample was homogenized in 20 wt/vol of 5 mmol/L potassium phosphate buffer (pH 6.0, 4°C) and centrifuged at 30 000 g (30 minutes, 4°C). The supernatant was discarded, and the pellet was washed again as described earlier. After the supernatant was decanted, the pellet was extracted through suspension in 0.5% hexadecyltrimethylammonium bromide in 50 mmol/L potassium phosphate buffer (pH 6.0, 25°C) for ~2 minutes at an original ratio of tissue wet weight to volume of 1:10. The samples were immediately frozen in liquid nitrogen. Three freeze-thaw cycles were performed with sonication (10 seconds, 25°C) between cycles. After the last sonication, the samples were incubated at 4°C for 20 minutes and centrifuged at 12 500 g (15 minutes, 4°C). Then, 0.1 mL of supernatant was mixed with 2.9 mL of 50 mmol/L potassium phosphate buffer, pH 6.0, containing 0.167 mg/mL o-dianisidine dihydrochloride and 0.005% hydrogen peroxide. The change in absorbance at 460 nm was measured with spectrophotometry. One unit of MPO activity is defined as that which degrades 1 μmol of peroxidase per minute at 25°C, and tissue MPO activities were calculated by using human MPO as a standard.

All chemicals used in this study were supplied by Sigma Chemical Co.

**RT-PCR Analysis**

The brains were rapidly removed and separated into right and left hemispheres. Punch samples were taken with a 7-mm-diameter cork borer from the ipsilateral and contralateral cortices to obtain tissue samples from the center of the MCA distribution. Each sample was divided into 2 specimens: 1 served to determine the ICAM-1 gene expression with RT-PCR and the other was used for MPO activity as described earlier. They were immediately frozen in liquid nitrogen and stored at −70°C for later analysis.

RNA was extracted from the frozen tissue sample with ISOGENTM reagent (Nippon Gene). Briefly, 100 mg of each tissue was homogenized in 1 mL of ISOGENTM. Subsequently, 0.2 mL of chloroform was added, and the mixture was centrifuged for 15 minutes at 15 000 rpm (4°C). This procedure separates the solution into an aqueous phase that contains RNA, an interphase that contains DNA, and an organic phase that contains protein. The aqueous layer was aspirated and added to 0.5 mL of isopropanol for RNA precipitation. The RNA precipitation was then pelleted for 10 minutes at 15 000 rpm (4°C), washed in 100 μL of ice-cold 75% ethanol, and recentrifuged at 15 000 rpm for 10 minutes (4°C). RNA was collected into 50 μL diethylpyrocarbonate-treated water.

RT-PCR was carried out with a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech). One microliter of total RNA (1 μg) was added to 14 μL of RT mixture. After mixing, the samples were incubated at 37°C for 45 minutes, 95°C for 5 minutes, and 4°C for at least 5 minutes. Twenty-five microliters of PCR mixture that contained 10 mmol/L primers (ICAM-1 or β-actin as internal control) and Taq DNA polymerase (Amersham Pharmacia Biotech) was added to RT products. Initial denaturation for 2 minutes at 94°C was followed by 30 cycles of 1 minute at 94°C, 1 minute at 55°C, and 2 minutes at 72°C and a final extension for 6 minutes at 72°C. The PCR products were subjected to 2% agarose gel electrophoresis and visualized by staining with ethidium bromide. The primer sequences were ICAM-1-5′ primer of 5′-AGACACACAAGCAAGAAGAGA-3′ and 3′ primer of 5′-GAGAAGCCCAAACCCGTATG-3′ and β-actin-5′ primer of 5′-ATCACATTGCGAATGAGCG-3′ and 3′ primer of 5′-TTGAAGGTAGAAACGTTGAT-3′.

The expected sizes of the PCR products were 234 bp for ICAM-1 and 93 bp for β-actin. The PCR product of ICAM-1 was semiquantitatively analyzed with NIH Image Version 1.61.

**Statistical Analysis**

Differences among groups in cerebral infarct volumes and physiological parameters were analyzed with ANOVA and the Scheffé F test of significance. The laser Doppler CBF values, MPO activities, and relative expressions of ICAM-1 mRNA in the Normo-tMCAO and Hypo-tMCAO groups were compared by unpaired Student’s t test. A 2-tailed probability value of <0.05 was used to indicate a statistically significant difference. All values were expressed as mean±SEM.

**Results**

**Physiological Parameters**

Physiological parameters, including arterial blood gases, blood glucose concentration, hematocrit, and mean arterial blood pressure, were measured at 4 hours of reperfusion after 2 hours of occlusion in each group. Table 1 shows the mean values from the rats used in the infarct volume study; they are all in the normal range. Blood glucose concentration was significantly higher in the Hypo-tMCAO group than in the Normo-tMCAO group (P<0.05).

**Brain Temperature**

Figure 1 shows the time course of brain temperature with a telemetry system in awake rats kept in a room maintained at 25°C and in a cold room maintained at 5°C for 18 hours after 4 hours of controlled brain temperature under general anesthesia. In the hypothermic rats, the brain temperature reduced temporarily to 30°C; then, it recovered gradually to ~35°C and lasted during the observation.

![Figure 1. Time course changes in brain temperature in rats housed in a room maintained at 25°C (n=5) and in a cold room maintained at 5°C (n=5). Measurements were made in awake rats with a telemetry system for 18 hours after 4 hours of controlled brain temperature at 37°C and 35°C under general anesthesia. Values are mean±SEM.](http://stroke.ahajournals.org/doi/fig/10.1161/01.str.84.8.705)
Infarct Volume

As shown in Figure 2, the cortical infarct volume was significantly smaller in the Hypo-tMCAO group (105 ± 639 mm³) than in the Normo-tMCAO group (254 ± 28 mm³, P < 0.05). In the Hypo-tMCAO group, however, the infarct volume was variable. Four rats exhibited clear neuroprotection with hypothermia (0.80 mm³), whereas an additional 3 rats showed a large infarct volume (0.150 mm³). On the other hand, the infarct volume in the subcortical region in the Hypo-tMCAO group (93 ± 18 mm³) was ~30% smaller than that in the Normo-tMCAO group (142 ± 11 mm³) but did not reach statistical significance. Rats with permanent MCA occlusion (pMCAO) exhibited ~30% smaller cortical infarct volumes (173 ± 23 mm³) than the Normo-tMCAO group but again did not reach statistical significance.

Laser Doppler Blood Flow

Introduction of the suture to block the blood flow to the MCA territory produced a similar fall in the relative surface blood flow in the ischemic border zone (medial cortex) in the Normo-tMCAO and Hypo-tMCAO groups (Figure 3). During the MCA occlusion, the values of CBF were reduced to 50% of the baseline in both groups. During the 4 hours of reperfusion, the values of CBF returned to 90% to 120% of the baseline, and they were indistinguishable between the 2 groups.

MPO Activity

Figure 4 illustrates the MPO activities in the MCA area in the ipsilateral and contralateral hemispheres in the Normo-tMCAO and Hypo-tMCAO rats in response to the MCA occlusion at 2 different time points. At 4 hours of reperfusion after 2 hours of occlusion, MPO activities in the ipsilateral hemisphere were higher in the Normo-tMCAO group (0.017 ± 0.006 U/g wet tissue) than in the Hypo-tMCAO group (0.006 ± 0.001 U/g wet tissue), but this was not statistically significant. At 22 hours of reperfusion after 2 hours of occlusion, a dramatic increase in MPO activity was shown in the ipsilateral MCA area (0.142 ± 0.047 U/g wet tissue) and a small increase was observed in the contralateral hemisphere (0.035 ± 0.018 U/g wet tissue) in the Normo-tMCAO rats.

Hypothermic treatment (Hypo-tMCAO) significantly suppressed the increase in MPO activity in the ipsilateral MCA area (0.032 ± 0.012 U/g wet tissue; P < 0.01) compared with the Normo-tMCAO rats. These results indicate that immediate and prolonged posts ischemic hypothermia inhibits the accumulation of PMNLs in the ischemic area at 22 hours of reperfusion after 2 hours of occlusion.

ICAM-1 mRNA Expression: RT-PCR Analysis

The expression of mRNA for ICAM-1 was determined with RT-PCR in the Normo-tMCAO (n = 3) and Hypo-tMCAO (n = 3) animals. The PCR products were visualized with ethidium bromide staining and are given in Figure 5, which clearly shows the band of interest (234 bp). The expression of ICAM-1 mRNA was clearly seen in 3 of 3 in the Normo-tMCAO group and 1 of 3 in the Hypo-tMCAO group. However, a weak expression was detected in the others in the...
Figure 5. RT-PCR analysis of ICAM-1 and β-actin (internal control) mRNA. The PCR products were electrophoresed on agarose gel and visualized with ethidium bromide. Samples were taken from the MCA territory after 2 hours of occlusion and 22 hours of reperfusion. Relative intensities of the PCR products (ICAM-1/β-actin) were determined with an image-analyzing computer. MPO activities also were determined with the samples used for RT-PCR analysis.

Hypo-tMCAO group with an image analyzing system. The expression was semiquantitatively determined with β-actin mRNA (93 bp) as internal control. The values were higher in the Normo-tMCAO group (1.16 to 1.93) compared with the Hypo-tMCAO group (0.35 to 0.97). The MPO activities obtained from the same samples were also higher in the Normo-tMCAO group (0.039 to 0.301 U/g wet tissue) compared with the Hypo-tMCAO group (0.009 to 0.086 U/g wet tissue). There were, however, some discrepancies in correlation between the ICAM-1 mRNA and the MPO activity in each sample.

Discussion

Small decreases in the intraischemic brain temperature have been shown to confer a significant degree of neuroprotection in global4,5 and focal6–8 cerebral ischemia in animals. The more clinical relevant issue of hypothermia in a practical use depends on the effectiveness when applied during the delayed intraischemic or posts ischemic period. Delayed intraischemic hypothermia is usually effective when it is initiated relatively soon after the onset of a focal ischemia.31 In addition, hypothermia initiated after brief global ischemia appears to be neuroprotective when it is achieved during the early posts ischemic period.14–16 For instance, a neuroprotective effect has been demonstrated in the CA1 sector of the hippocampus even when hypothermia was initiated 1 hour after a brief global ischemic event in the gerbil.14 These promising results obtained for global ischemia raised the possibility that mild hypothermia may also be of therapeutic use when applied after transient focal ischemia. The window of therapeutic opportunity for focal ischemia, however, appears to be shorter than that for brief global ischemia because of more severe intraischemic insults. In a model of transient focal cerebral ischemia, a significant reduction (32%) in the volume of infarction was obtained when mild hypothermia was established immediately after reperfusion and was maintained for a prolonged period, but a delay in hypothermia until 30 minutes postreperfusion failed to achieve statistical significance in the reduction of infarct volume.18 The observation that a reduction in temperature immediately after reperfusion, but not after a 30-minute delay, resulted in cerebroprotection suggests that mild hypothermia affects some of the injury mechanisms that occur early in the reperfusion process. In our experiment, a cooling process was begun during the final 30 minutes of ischemia to achieve the hypothermic condition at the time of reperfusion. Although this method involves the effect of hypothermia on brain injury during the late intraischemic period, it is essential to establish hypothermic conditions at reperfusion to assess the effect of posts ischemic hypothermia on brain injury. Our present study demonstrated that delayed intraischemic and prolonged posts ischemic hypothermia could indeed be beneficial in reducing the size of cortical infarction induced by transient focal ischemia. Two important features in the protective effect were observed in this study. First, although mild hypothermia significantly reduced the volume of cortical infarction by 60%, the effect was variable in individual animals. Four of 7 rats exhibited clear neuroprotection with hypothermia (<80 mm³), whereas the others showed a pretty large infarct volume (>150 mm³). Second, the protective effect with hypothermia was obtained only in the cortical regions, not in the subcortical regions.

In this study, we used 120 minutes of MCA occlusion followed by 22 hours of reperfusion. It is believed that there is a limited beneficial effect in reperfusion after 120 minutes of focal cerebral ischemia in rats.22,23 In transient focal ischemia produced by unilateral MCA–common carotid artery occlusion in Long-Evans rats, an ischemic duration of <90 minutes was well tolerated, showing very small infarct volumes; however, prolongation of the ischemic duration to 120 minutes resulted in a dramatic increase in infarct volume.23 In a suture MCA occlusion model, temporary occlusion that lasted 120 minutes resulted in an extensive cerebral infarction after 24 hours that was indistinguishable from the damage produced by permanent ischemia in the rat.22 Our studies showed that hypothermia that was initiated after 90 minutes of ischemia and that persisted after reperfusion significantly reduced the cortical infarct volume in the same suture model. These findings led us to the concept that focal ischemic injury is an ongoing process that involves deleterious mechanisms, is triggered during ischemia, and persists into the posts ischemic period; posts ischemic hypothermia can suppress the deleterious processes. In these animals, there was a certain variation in the size of cortical infarct; more severe or dense focal cerebral ischemic insults could not be associated with beneficial effects by hypothermia. We may postulate that 120 minutes of occlusion is a “border” time in this model, between spread of the cortical infarction and salvage from the neuronal death with hypothermia. A similar phenomenon was observed in the subcortical regions; the infarct volumes in the subcortical regions were not affected by intervention with hypothermia, which may be due to poor collateral blood supply and dense ischemia in these areas.

During reperfusion after ischemia, whereas the restoration of oxygen and glucose supply reinstates the oxidative phos-
phorylation that helps to normalize energy demands, a deleterious cascade can be triggered that may antagonize the beneficial effect of reperfusion. The cause of an early reperfusion injury is multifactorial. The possible mechanisms of cerebral reperfusion injury include a secondary elevation of excitatory amino acid, Ca\(^{2+}\) influx that produces further elevation of cytosolic free Ca\(^{2+}\) concentration, free radical formation, blood-brain barrier injury, and increased expression of endothelial adhesion molecules. Recently, there have been various studies on the involvement of glutamate accumulation in transient focal ischemia, but published studies have not shown effective neuroprotection by delayed treatment with MK-801. These studies suggest that the secondary amino acid release during reperfusion may play only a limited role in the evolution of cerebral damage after transient ischemia.

In this study, LDF measurements were used to continuously monitor the changes of local blood flow during 2 hours of occlusion and 4 hours of reperfusion with or without hypothermic intervention after reperfusion. Although it has been reported that systemic hypothermia significantly lowered the CBF in experimental work, the present results showed changes in blood flow after reperfusion that were indistinguishable between the 2 groups. In regard to ischemic protection, preservation in blood flow after reperfusion in hypothermic rats would likely attenuate ischemic damage combined with reduced brain metabolism. The results show only relative rates of cortical blood flow; it is essential to verify quantitatively both blood flow and metabolic changes when studying temperature modulation on ischemic brain injuries. We only assessed the changes in blood flow during 2 hours of occlusion and 4 hours of reperfusion in anesthetized rats. One may speculate that ischemic brain damage is an ongoing process even if reperfusion is established and that microcirculatory changes, including the plugging phenomenon, will occur after our observation.

PMNLs have been considered to be directly involved in the pathogenesis and development of ischemia-reperfusion injuries in various tissues. Postischemic influx of leukocytes may contribute to an ischemia-reperfusion injury by plugging the microvasculature resulting in the “no-reflow” phenomenon during reperfusion and by releasing cytotoxic products. Leukocyte infiltration in the area of cerebral infarction is a well-documented histopathological finding in the laboratory studies. Zhang et al has used a rat model of focal ischemia to study the time course of PMNL infiltration of the ischemic zone. In animals with transient MCA occlusion, PMNLs were noted in the intraparenchymal lesion at 6 hours and significant increases in numbers of neutrophils were present by 24 hours after the onset of ischemia. The period of maximal infarct expansion is well correlated with the time course of PMNL infiltration of the ischemic lesion, suggesting that the progression of ischemic damage results from the accumulation of PMNLs. The adhesion of leukocytes to the endothelium is the requisite first step to acute inflammation during an ischemia-reperfusion injury. Leukocyte sequestration into ischemic tissue is facilitated by adhesion molecules on the surfaces of both PMNLs and endothelial cells. Among adhesion molecule receptors found on PMNLs, the CD11/CD18 glycoprotein complex (β\(_2\)-integrins) appears to play a determinant role in the PMNL–endothelium interaction. ICAM-1 is an endothelial ligand for the β\(_2\)-integrins on PMNLs. ICAM-1 is constitutively expressed at low levels on vascular endothelium and can be upregulated by ischemia-reperfusion in brain microvessels. In a rat model of MCA occlusion and reperfusion, Zhang et al found that ICAM-1 mRNA was detected 1 hour after ischemia and peaked at 10 hours of reperfusion. Factors that directly antagonize adhesion molecules have been shown to reduce reperfusion injury. For instance, anti–ICAM-1 monoclonal antibody reduced PMNL infiltration and brain damage after reperfusion. The protective effect of this therapy was insignificant in permanent ischemia, indicating that PMNL adhesion and inflammatory tissue damage are more important when reperfusion is established. Evidence indicates that the temporal profile of the expression of ICAM-1 mRNA after transient MCA occlusion differs from the expression of ICAM-1 protein and the accumulation of PMNLs in the ischemic brain. Therefore, it is quite reasonable that there are some discrepancies in correlation between the expression of ICAM-1 mRNA and the accumulation of PMNLs as assessed by MPO activity in the ischemic brain observed in our experiment.

Emerging evidence suggests that hypothermia attenuates the cell-mediated inflammatory response to cerebral ischemia. A recent study has provided quantitative results with MPO activity that indicate the accumulation of PMNLs in the ischemic brain after ischemia-reperfusion of the MCA is attenuated by intraischemic hypothermia. In addition, intraischemic hypothermia reduced the volume of infarction by 59% compared with the normothermic animals. This is an important observation because it suggests that a reduction in the inflammatory response after ischemia-reperfusion contributes to the neuroprotective effects of hypothermia. Our present study also reveals quantitative evidence that demonstrates delayed intraischemic and postischemic hypothermia significantly attenuates the accumulation of PMNLs in the ischemic brain and the volume of infarction after ischemia-reperfusion. It is believed that PMNL adhesion and inflammatory tissue damage are more important when reperfusion is established; the therapeutic intervention of postischemic hypothermia to reduce ischemic cell damage is reasonable to apply. Furthermore, ICAM-1 mRNA expression is suppressed in the ischemic brain treated with postischemic hypothermia after ischemia-reperfusion compared with the normothermic animals. To our knowledge, there has been no report of the effect of hypothermia on the expression of ICAM-1 mRNA in ischemic brain. Because hypothermia may also influence the energy-dependent transcription of adhesion molecule, it will be important in future studies to determine whether these hypothermia-induced reductions in expression of ICAM-1 mRNA correlate with reductions in ICAM-1 protein levels.

In the present experiment, we killed the animals 24 hours after ischemia and did not attempt longer survival
periods; this relatively short survival period may not allow an assessment of the final infarct volume. Therefore, it could be argued that we simply retarded the inevitable development of ischemic brain damage with postischemic hypothermia. Indeed, postischemic hypothermia (3 hours) after global ischemia has shown to be effective in reducing neuronal damage 3 days after ischemia, but the protective effect was less evident at 7 days and no protection was documented at 2 months. In this setting, postischemic hypothermia may be most advantageous in extending the therapeutic window for delayed pharmacological treatment. When questions regarding therapeutic strategies are addressed, whether chronic protection can be documented with postischemic hypothermia in our model must be determined.

In conclusion, we describe a focal ischemia-reperfusion model in which ischemic brain damage can be ameliorated with mild hypothermia initiated 30 minutes before reperfusion and persisting after reperfusion. The results of the present study demonstrate that both the expression of ICAM-1 mRNA and the accumulation of PMNLs in the ischemic brain are inhibited by delayed intraschismic and postischemic hypothermia. An active response by PMNLs is believed to participate in cerebral injuries; it is possible that the potential neuroprotective mechanisms of hypothermia are mediated in part through the suppression of PMNL-mediated inflammation. Although further work must be performed to evaluate the long-term effects, postischemic hypothermia may provide an approach to potentially reduce ongoing damage during reperfusion in patients with ischemic stroke, and the immediate application of mild hypothermia at thrombolytic therapy for acute stroke may also be feasible.

References

This study by Kawai and colleagues examines the effects of mild hypothermia (33°C) on infarct volume, polymorphonuclear leukocyte (PMNL) accumulation, and the expression of intercellular adhesion molecule-1 (ICAM-1) messenger RNA following 2 hours of transient middle cerebral artery (MCA) occlusion in rats. Hypothermia was initiated 30 minutes before reperfusion of the MCA and continued in a cold environment (5°C) for an additional 18 hours. Hypothermia significantly reduced the accumulation of PMNLs, as assessed by myeloperoxidase activity, and inhibited the overexpression of ICAM-1 mRNA compared with that in normothermic animals. In addition, infarct volume was significantly reduced by the hypothermic treatment. The authors conclude that prolonged mild hypothermia is neuroprotective in a model of transient focal ischemia and that this neuroprotective consequence may be mediated by leukocyte-mediated inflammatory processes.

As discussed by the authors, various laboratories have reported the neuroprotective properties of mild-to-moderate hypothermia when introduced in models of transient global and focal ischemia. The present study emphasizes the role of temperature-sensitive inflammatory processes in therapeutic hypothermia. Previous investigators have demonstrated that hypothermia attenuates neutrophil infiltration after CNS injury. For example, Toyoda and colleagues reported that intraschismic hypothermia reduced neutrophil accumulation in a rat model of transient focal ischemia. More recently, similar results have been reported in models of brain and spinal cord trauma, in which hypothermia and hyperthermia have been reported to affect the acute and more chronic inflammatory consequences of brain injury. Thus, temperature manipulations after various CNS injuries significantly affect the inflammatory response to injury. Taken together, these data are consistent with an acute detrimental inflammatory component of CNS injury and suggest an association between hypothermic protection and inflammatory processes.

In the present study, infarct volume was assessed at 24 hours after ischemia. This is a relatively short survival period and may not assess final infarct volume. Previous ischemia and trauma studies have indicated that longer survival periods may be necessary to adequately assess the effects of therapeutic interventions, including hypothermia. As with any acute injury study, chronic histopathological and behavioral outcome measures are required to critically evaluate the consequences of therapeutic interventions in terms of clinical relevance.

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