Acta Neuropathol (2004) 107 : 227–234
DOI 10.1007/s00401-003-0802-2

REGULAR PAPER

Xiaodong Luan · Jie Li · James P. McAllister II
Fernando G. Diaz · Justin C. Clark · Richard D. Fessler
Yuchuan Ding

Regional brain cooling induced by vascular saline infusion into ischemic territory reduces brain inflammation in stroke

Received: 1 August 2003 / Revised: 13 November 2003 / Accepted: 13 November 2003 / Published online: 23 December 2003

Abstract The neuroprotective effect of hypothermia has long been recognized. Use of hypothermia for stroke therapy, which is currently being induced by whole body surface cooling, has been largely limited because of management problems and severe side effects (i.e., pneumonia). Our recent studies have demonstrated the significant therapeutic value of local brain cooling in the ischemic territory prior to reperfusion in stroke. The goal of this study was to determine if cerebral local cooling infusion could reduce stroke-mediated brain injury by inhibiting inflammatory responses. A hollow filament was used to block the middle cerebral artery (MCA) for 3 hours, and then to locally infuse the ischemic territory with 6 ml cold saline (20°C) for 10 min prior to 48-h reperfusion. This cold saline infusion significantly (P<0.01) reduced temperature of the MCA supplied territory (in cerebral cortex from 37.2±0.1°C to 33.4±0.4°C, in striatum from 37.5±0.2°C to 33.9±0.4°C), with the hypothermia remaining for at least 45 min after reperfusion. Consequently, significant (P<0.01) reductions in endothelial expression of intracellular adhesion molecule-1 (ICAM-1), the key step for inflammatory progress, as well as leukocyte infiltration, were evident in both cortex and striatum after reperfusion. As a control, ischemic rats received the same amount of cold saline systemically through a femoral artery. A mild hypothermia was induced in the cerebral cortex (35.3±0.2°C) but not in the striatum (36.8±0.2°C). The reduced cortical temperature returned to normal within 5 min. Brain temperature in ischemic rats perfused locally with saline at 37°C remained normal. Intensive expression of ICAM-1 and accumulation of leukocytes was observed in ischemic control groups without brain cooling infusion. In conclusion, brain hypothermia induced by local pre-reperfusion infusion ameliorated brain inflammation from stroke.

Keywords Cerebral ischemia · Reperfusion injury · Hypothermia · ICAM-1 · Leukocyte infiltration

X. Luan · J. Li · J. P. McAllister II · F. G. Diaz · J. C. Clark
R. D. Fessler · Y. Ding (✉)
Department of Neurological Surgery,
Wayne State University School of Medicine,
Lande Medical Research Building, Room 48,
550 E. Canfield, Detroit, MI 48201, USA
Tel.: +1-313-5771372, Fax: +1-313-5770448,
e-mail: yding@neurosurgery.wayne.edu

J. P. McAllister II
Department of Anatomy and Cell Biology,
Wayne State University School of Medicine,
Detroit, Michigan, USA

Present address:
J. C. Clark
University of Michigan Medical School,
Ann Arbor, Michigan, USA

Introduction

The remarkable benefit of mild (30–34°C) hypothermia in global and focal ischemia has long been recognized, and remains one of the most powerful neuroprotective strategies in cerebral ischemia both experimentally and clinically [2, 6, 9, 20, 27]. Since the majority of stroke patients do not receive treatment until hours after the onset of symptoms, delayed intra-ischemic or postischemic intervention is a more clinically relevant issue in stroke therapy. Some preliminary clinical studies have suggested that the neuroprotective properties of mild or moderate hypothermia in acute ischemic stroke can only be achieved through either more rapid initiation of brain cooling after the onset of stroke or prolonged hypothermic exposure (up to 48–72 h) [26, 38, 39, 40]. Experimentally, several studies have demonstrated the effectiveness of postischemic hypothermia in animal models of global or focal transient cerebral ischemia [8, 9, 21, 28, 35, 44, 45, 49, 51]. Again, a prolonged application of hypothermia is necessary to achieve significant and persistent neuroprotection [7, 9, 23, 46]. It has been demonstrated that mild postischemic hypothermia only delays neuronal damage [12, 23]. Moreover, hypothermia is being currently induced in patients by surface cooling with the use of cooling blankets, "forced" cooling air, alcohol applied to ex-

posed skin, or ice bags placed to the groin, axilla, and neck. This approach requires intense efforts from the medical and nursing staff for induction as well as maintenance of the target temperature, and has induced some severe complications, such as pneumonia in 40% of patients [39]. Therefore, a shorter, more effective mode of postischemic hypothermic intervention appears to be highly desirable.

A few studies have introduced the concept of selective brain cooling in which the brain, rather than the whole body, is selected as a therapeutic end-point for hypothermia [18]. Cold carotid artery perfusion with temperature-decreased blood, localized cerebral ventricular perfusion with a hypothermic solution, and head surface cooling have all been tried clinically and experimentally for selective brain cooling and neuroprotection [3, 22, 30, 37]. Our previous studies have led to the development of a unique hollow filament model to "flush" the microvasculature in the ischemic territory prior to reperfusion (i.e., pre-reperfusion infusion) and demonstrated a significant therapeutic value in stroke [13, 14, 16]. Most recently, by combining the hollow filament infusion technique with therapeutic hypothermia, we have demonstrated that regional brain cooling selectively induced a vascular cold saline infusion before reperfusion significantly decreased neurological deficits and infarct volume (90%) in a severe stroke model (3-h MCA occlusion followed by 48 h of reperfusion) [15]. Importantly, we further indicated that the regional brain cooling induce a long-term neuroprotection by demonstrating functional recovery of motor performance up to 28 days after stroke [32]. However, the protective mechanism of the combined regional hypothermia and vascular infusion remains unknown.

The specific aim of this study was to determine if cold saline infused intra-arterially into the ischemic territory could reduce brain inflammation after stroke. To accomplish this task, we used a hollow filament procedure, developed in our laboratory [13, 14], to first occlude the middle cerebral artery (MCA) for 3 h, and to then infuse cold saline into the MCA supplied territory in rats. The synergistic effects of our combined infusion and cooling procedure on the inflammatory response in ischemic brain were evaluated by immunocytochemistry.

Materials and methods

Subjects

Throughout the protocol, adult male Sprague Dawley rats (260–300 g, Charles River) were housed in the animal care facility with a 12-h light/dark cycle. Animal care and surgical procedures were conducted in accordance with the guidelines approved by NIH and the Wayne State University Animal Investigation Committee. In the stroke without treatment group, a 3-h MCA occlusion was induced and followed by 48 h of reperfusion (n=8). In the local cooling infusion group, after a 3-h MCA occlusion, a 10-min infusion of cold saline (20°C) was performed to selectively induce regional brain hypothermia before the onset of reperfusion for 48 h (n=8). As a control, cold saline equal to the volume injected intracerebrally was delivered through a femoral artery in ischemic animals (n=8) to determine whether systemically administered cold saline (20°C) could induce neuroprotection. As an additional control, local saline infusion at body temperature (37°C) (n=7) was performed after a 3-h MCA occlusion to determine if a slow (0.6 ml/min vs 3 ml/min in our previous studies [13, 14, 16]) local infusion without brain cooling played a predominant role in reducing brain injury from stroke with 3-h MCA occlusion.

Induction of stroke and cooling infusion with an intraluminal filament

MCA occlusion was induced by a novel intraluminal filament model that was modified in our laboratory [13, 14, 15] from the technique developed previously by Zea Longa et al. [47]. Briefly, animals were anesthetized and maintained with 1–3% halothane in 70% N_2O and 30% O_2 using a facemask. A length of 18.5–19.0 mm modified PE-50 catheter (with 0.2-mm outer diameter and 0.1-mm inner diameter) was inserted into the right external carotid artery via an arteriotomy, and passed up the lumen of the internal carotid artery into the intracranial circulation. The filament was lodged in the narrow proximal anterior cerebral artery (ACA) and blocked the MCA at its origin. Three hours after MCA occlusion, animals were re-anesthetized, and reperfusion was established by withdrawal of the filament. In the animals with a local infusion, the catheter was withdrawn 1 mm from the origin of the MCA (after 3 h of ischemia). During and after the "pull back" of the catheter, 6 ml of cold or warm saline (20° or 37°C) was slowly and constantly injected at the junction of MCA and ACA, using an infusion pump (Harvard Apparatus) to maintain an infusion rate of 0.6 ml/min for 10 min (approximately 0.25 ml/g brain tissue per min). After infusion, the catheter was completely withdrawn and reperfusion established.

To verify MCA occlusion, neurological deficits in rats were examined during ischemia with a modified scoring system developed by Zea Longa et al. [47] as follows: 0, no deficits; 1, unable to extend the contralateral forelimb; 2, flexion of contralateral forelimb; 3, mild circling to the contralateral side; 4, severe circling, and 5, falling to the contralateral side. Animals that did not have severe neurological deficits (score less than 4) during MCA occlusion were excluded from further analysis.

Physiological variables, such as blood pressure, blood gases (pH, pO_2, PCO_2) and hematocrits were monitored before and after surgical procedure.

Brain and body temperature monitoring

Brain temperature was monitored ipsilaterally in the area supplied by the MCA both before and during the local infusion of saline, as well as after infusion while blood flow was re-established over 1–60 min until it returned to normal levels. Needle thermistor probes (Hi-Lo Temp Model 8200 thermometer, Mallinckrodt, New Jersey) were placed into cortex and striatum through holes at 3 mm lateral to bregma, and 3 mm posterior and 5 mm lateral to bregma, respectively. Body temperature was frequently measured through the rectum before, during and after saline infusion until it returned to normal levels. One-way (infusion conditions: none, local cooling, local warming, systemic cooling) ANOVAs with repeated measures on different time points were employed to assess brain and body temperature, with a significance level at $P<0.05$.

Animals from each group were anesthetized and placed on a water circulating heating pad under a heating lamp throughout the surgical procedure. When cerebral and rectal temperature both returned to normal levels, animals were allowed to awaken and were placed in a warm environment for additional 3 h.

Histological analysis for ICAM-1 expression and leukocytes infiltration

At 48 h after MCA occlusion and reperfusion, ischemic animals were deeply anesthetized with Nembutal (50 mg/kg, i.p.) and killed by cardiac perfusion of saline followed by 4% paraformaldehyde in

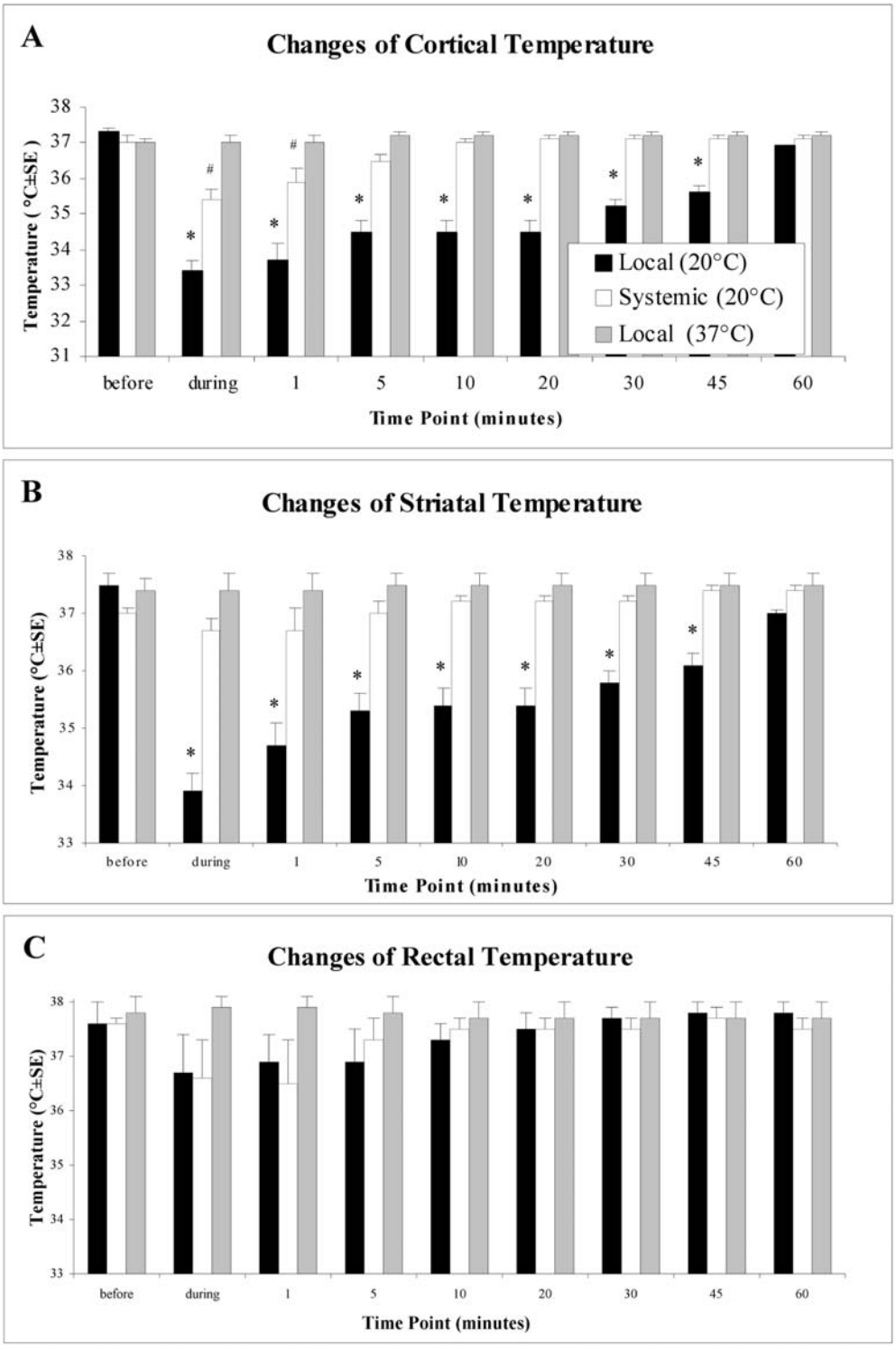

Fig. 1 Graphs depicting changes of brain temperature in cortex (**A**) and striatum (**B**), as well as body temperature (**C**) before, during and after saline infusion via either the MCA or femoral artery in different ischemic groups, including local infusion at 20°C, systemic infusion at 20°C and local infusion at 37°C. In the cortex (**A**), the cold saline (20°C) infusion via MCA rapidly and significantly ($P<0.01$, indicated by *) reduced temperature of the MCA supplied ischemic territory as compared to all groups, with the hypothermia remaining for at least 45 min after reperfusion. A mild hypothermia was induced (35.3± 0.2°C) in the ischemic rats that received the same amount of cold saline (20°C) systemically through a femoral artery. This significantly ($P<0.01$, indicated by #) reduced cortical temperature recovered to normal within 5 min. Brain temperature remained normal during ischemia and reperfusion in the third group of ischemic rats that was perfused locally with body temperature saline (37°C). In the striatum (**B**), the cold local saline infusion reduced temperature significantly ($P<0.01$, indicated by *), with the hypothermia remaining for at least 45 min. However, the cold systemic infusion did not reduce the temperature significantly in the striatum (36.8± 0.2°C). Brain temperature remained normal during ischemia and reperfusion in the ischemic rats perfused locally with warm saline (37°C). In ischemic groups with either local or systemic cold saline (**C**), the body temperature measured from the rectum was slightly reduced but remained above 36°C, and soon returned to normal levels (37.5°C). Values are means ± SE (*MCA* middle cerebral artery)

0.1 M phosphate buffer (PB), pH 7.4. Coronal brain sections, cut at a thickness of 30 µm and taken from +2.0 mm to –4.0 mm bregma, were processed for immunohistochemistry to determine endothelial expression of intracellular adhesion molecule-1 (ICAM-1), the key step for inflammatory progress, and leukocyte infiltration. All histological analyses were performed in a blind fashion.

Immunocytochemistry procedure

Two series of frozen sections were incubated at 4°C for 48 h either with a monoclonal anti-rat ICAM-1 antibody (1:3,000; 1A29, Seikagaku) [36, 43] or with a polyclonal rabbit anti-human myeloperoxidase antibody (1:1,500; A0398, DAKO). Myeloperoxidase, a heme lysosomal enzyme present in azurophilic granules of neutrophils and in smaller quantities in monocytes, has been commonly used as a marker of polymorphonuclear (PMN) leukocytes [1, 4]. Ex-

pression of ICAM-1 or myeloperoxidase was visualized using routine immunoperoxidase techniques.

Immunolabeling quantification

ICAM-1-positive vessels and leukocytes immunostained with myeloperoxidase were counted randomly throughout 24 non-overlapping cortical and striatal regions (0.25 mm^2 each). The regions were selected in the ipsilateral (ischemic) hemisphere supplied by the MCA from three sections along frontoparietal cortex and dorsolateral striatum under a light microscope at ×400 [13, 17, 50]. To determine the frequency of ICAM-1-positive vessels, the number of immunoreactive vessels less than 100 μm in diameter (estimated by width of vessel segment) was counted and regarded as the total number of microvessels [36]. In each microscopic field, all labeled leukocytes (neutrophils and monocytes) that were visible in the vascular lumen, the intramural wall or the brain parenchyma, were counted. Statistical differences in the number of labeled vessels or cells in the different ischemia groups were analyzed by ANOVA and Duncan's multiple range tests, with a significance level of $P<0.05$.

Results

Regional brain hypothermia

The cold saline (20°C) infusion via MCA rapidly reduced the temperature of the MCA supplied ischemic territory, in cortex from 37.2±0.1°C to 33.4±0.4°C (Fig. 1A), and in striatum from 37.5±0.2°C to 33.9±0.4°C (Fig. 1B). ANOVA indicated that the temperature differences were significant in the cortex [$F_{(8, 48)}=35.88$, $P<0.01$], and striatum [$F_{(8, 48)}=26.66$, $P<0.01$]. The Duncan's multiple range test revealed that the temperatures were significantly lowered during local infusion, and then gradually recovered after the reperfusion in both cortex and striatum. This significant degree of hypothermia remained for at least 45 min after reperfusion. A mild hypothermia was induced in cortex (35.3±0.2°C) [$F_{(8, 48)}=13.21$, $P<0.01$], but not in striatum (36.8±0.2°C) [$F_{(8, 48)}=1.48$, $P>0.05$] in the ischemic rats that received the same amount of cold saline systemically through a femoral artery (Fig. 1A, B). Further, the Duncan's multiple range test indicated that temperatures were significantly decreased during systemic infusion, and the reduced cortical temperature recovered to normal within 5 min. Brain and body temperature remained normal during ischemia and reperfusion in the third group of ischemic rats that was perfused locally with saline at body temperature (37°C) (Fig. 1A–C). In ischemic groups with either local or systemic cold saline, the body temperature measured from the rectum was slightly reduced but remained above 36°C, and soon returned to normal levels (Fig. 1C).

There were no significant differences in arterial blood pressure, blood pH, hematocrits and blood gases among the ischemic animal groups before or after saline infusion (data not shown).

Inflammatory endothelial ICAM-1 expression and leukocyte infiltration

Microvessels in the ischemic lesion strongly expressed ICAM-1 immunoactivity in ischemic rats after 48 h of reperfusion without brain cooling, in contrast to that in ischemic rats with local brain cooling (20°C) infusion. ICAM-1 immunoreactivity was also strongly expressed in microvessels from ischemic brain that received systemic cold (20°C) saline infusion or with local warm (37°C) saline infusion. Immunoreactivity was rarely found in brain tissue beyond infarct regions. Quantitative analysis demonstrated a significant ($P<0.01$) reduction in number of ICAM-1-positive vessels both from cortex and striatum in ischemic group with brain cooling infusion. In cortex, 1.8±0.5 ICAM-1-positive vessels was found per 0.25-mm^2 region, com-

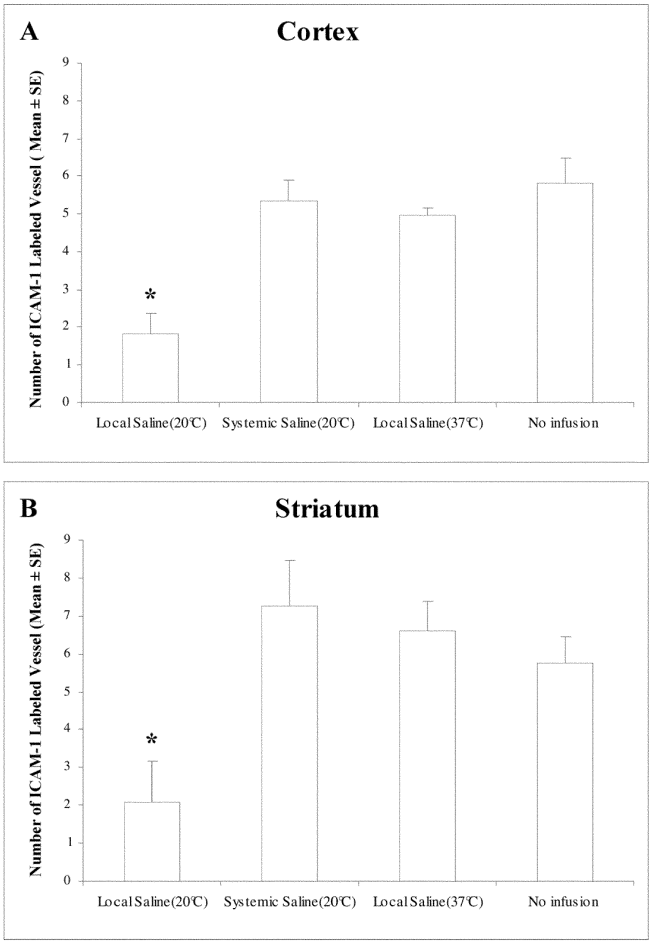

Fig. 2 Graph of the number of ICAM-1-immunolabeled blood vessels, which indicates magnitude of the inflammatory injury after stroke (3-h MCA occlusion followed by 48-h reperfusion) in four different stroke treatment groups (local cold infusion, systemic cold infusion, local warm infusion, and no infusion). Significant ($P<0.001$, indicated by *) reductions in numbers of ICAM-1-positive vessels per 0.25 mm^2 within cortical (**A**) and striatal (**B**) regions were found in rats with local brain cooling, in contrast to control ischemic rats with other infusion procedures or without infusion. There was no significance seen among those control groups. Values are means ± SE

pared to 5.8±0.7 in ischemic rats without saline infusion, 5.0±0.2 in rats with local warm infusion and 5.4±0.6 in rats with systemic cold saline infusion (Fig. 2A). In striatum, there were only 2.1±1.1 ICAM-1-immunolabeled vessels seen after local brain cooling infusion, in contrast to 5.8± 0.7 in ischemic rats without saline infusion, 6.6±0.8 in rats with local warm infusion and 7.3±1.2 in rats with systemic cold saline infusion (Fig. 2B).

PMN leukocytes infiltrated into ischemic areas were labeled with myeloperoxidase immunocytochemistry. When the numbers of leukocytes per 0.25 mm^2 of striatal and cortical regions were compared in ischemic rats, it was found that a significant (P<0.001) reduction was associated with pre-reperfusion brain cooling infusion (20°C). In cortex (Fig. 3A), the average number of infiltrated leukocytes was reduced from 50.2±2.3 in ischemic rats without saline infusion to 2.2±1.5 in ischemic rats with local brain cooling. However, there were no significant reductions in infiltrating leukocytes from ischemic rats with local warm infusion (38.9±2.5) and in rats with systemic cold saline infusion (46.5±3.1). In striatum, only 2.0±1.4 infiltrating leukocytes were seen after local brain cooling infusion, in contrast to 41.8±5.9 in ischemic rats without saline infusion. In addition, the slightly reduced numbers of infiltrating leukocytes in rats with local warm infusion (28.1±3.9) and in rats with systemic cold saline infusion (31.5±1.4) did not reach significant levels (Fig. 3B).

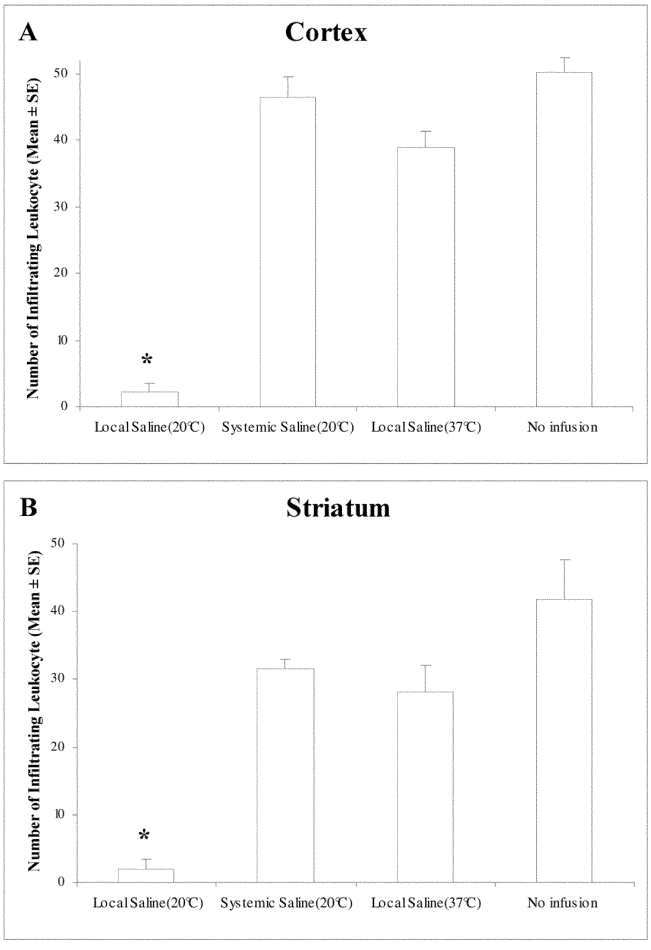

Fig. 3 Increased numbers of infiltrating leukocytes indicate an increased inflammatory injury of ischemic animals with 3-h MCA occlusion. After 48 h of reperfusion, a significant decrease in leukocyte infiltration was revealed in ischemic rats from the local brain cooling group, showing a largely reduced number of infiltrating leukocytes in ischemic cortex (**A**) and striatum (**B**) (P<0.01, indicated by *). However, no significant reduction in the number of infiltrating leukocytes was found from other ischemic animal groups without local brain cooling. Values are means ± SE

Discussion

Our previous studies have demonstrated a significant decrease in neurological deficits and infarct volume (90%) in severe stroke (3-h MCA occlusion) after 48 h of reperfusion, as well as a significant improvement in motor function 28 days after stroke, following brain cooling infusion treatment [15, 32]. By elucidating causal mechanisms responsible for inflammation-related brain injury, this study continues towards the goal of our previous studies [15, 32], which are to further the development of a therapeutic procedure that combines both saline infusion and regional cerebral hypothermia of ischemic territory. In ischemic rats infused intracerebrally with cold saline, we demonstrated a significant reduction in inflammatory leukocyte infiltration and endothelial expression of ICAM-1. This reduced brain inflammation could have led to reduced brain damage and improved long-term functional outcome.

The complex effects of redundancy and overlap generated by inflammatory cells and their mediators [10] are highlighted in stroke. Polymorphonuclear (PMN) leukocyte infiltration in areas of cerebral infarct and cytokine-mediated inflammatory reactions play a pivotal role during reperfusion. The present study demonstrated, for the first time, that brain cooling infusion treatment effectively inhibits stroke-mediated inflammation. The association of reduced inflammatory responses and decreased brain damage due to MCA occlusion provides insight into the potential mechanisms of the protection afforded by hypothermia and local saline infusion.

Previous studies have begun to investigate the mechanisms through which hypothermia or brain vascular infusion provide protection against stroke-induced inflammatory responses. Chen et al. [5] demonstrated that hypothermia could produce a reduction in ischemic damage with minimal inflammatory injury. Their finding that intra-ischemic hypothermia attenuates neutrophil infiltration in rat neocortex after focal ischemia/reperfusion injury has also been confirmed by others [23, 24, 34, 41]. In addition, the reduction of injury and leukocyte infiltration has been correlated with a diminished over-expression of ICAM-1 mRNA [28, 42]. A more recent study further indicated that mild hypothermia decreases inflammatory reposes in both brain inflammation induced by lipopolysaccharide and stroke, implicating a direct anti-inflammatory effect of cooling [11]. The benefits of vascular infusion include removal of biochemical by-products and toxic mediators, such as cytokines and adhesion molecules ac-

cumulating in the ischemic territory [16], leading to significantly reduced vascular-parenchymal infiltration of inflammatory leukocyte and endothelial expression of adhesion molecule with improving the cerebral microcirculation [13].

This study has emphasized a synergistic effect of combined vascular infusion and brain cooling on reducing brain inflammatory injury. In our previous studies [13, 14, 16], 7–10 ml of warm saline infusion (37°C) administered over 3–4 min significantly reduced brain injury in rats with 2-h MCA occlusion. A further reduction of infarct volume, however, was not induced by infusion of saline at 23°C for the same short duration (3–4 min), while a mild hypothermia was produced for up to 3 min, suggesting that removal of deleterious compounds rather than the brief period of postischemic hypothermia plays a crucial role in the neuroprotection. In contrast, 6 ml of warm saline infusion for 10 min in this study did not reduce brain inflammation. This negative result could be due to a more severe stroke model (3- vs 2-h MCA occlusion), or less infusion volume per minute (0.6 vs 2.5–3 ml) and less hypothermic duration (3 vs 45 min). Moreover, no significant reduction in inflammation was found in ischemic rats with cold saline administered systemically while only a mild and brief (5 min) hypothermia was induced in cortex (but not in the striatum). These findings suggest that a slow infusion with a small volume might not be able to remove accumulated toxins and biochemical byproducts as effectively as a fast infusion with larger volume, and that the combination of local infusion and brain cooling synergistically produce more profound neuroprotection in a severe stroke model with 3-h MCA occlusion. In addition, by combining hypothermia (with 20°C saline) with the local "flushing", the total volume of infusion could be reduced by up to 30% (from 10 to 6 ml), and infusion speed could be decreased by up to 75% (2.5 or 3 ml to 0.6 ml/min), which may make this procedure safer and more feasible in a clinical setting.

Since stroke is a complex and progressive process involving deleterious mechanisms that are active during the entire ischemic and postischemic period, beyond inflammation, our model for simultaneously infusing the microvasculature of the ischemic territory with saline and cooling the brain region prior to reperfusion could be active through other therapeutic mechanisms. A large number of basic studies have shown that the mechanisms of neuroprotection conferred by hypothermia are multifactorial [3, 9, 19, 27]. There is extensive evidence linking hypothermia to reductions in free radical formation and the associated lipid peroxidation [20]. Mild hypothermia reduces the rate of metabolism of arachidonic acid following postischemic reperfusion [29]. It has been reported that mild brain hypothermia beginning immediately after ischemia (within 30 or 60 min, but not 90 min) delays consumption of endogenous antioxidant enzymes and energy supplies with decreased accumulation of lactate and lipid peroxidation [48]. Mild hypothermia may also convey neuroprotection by virtue of improving vascular circulation. Brain cooling to 31°C was found to increase cerebral blood flow more than twofold [31]. Upon reperfusion of the MCA, a more rapid return of cerebral blood flow was obtained with mild hypothermia than with normothermia [25]. The therapeutic effect of the technique to "flush" the microvasculature in the ischemic territory prior to reperfusion in our previous studies may involve removal of free fatty acids from the ischemic territory, attenuating oxygen free radical reactions (unpublished data). In addition, erythrocytes trapped in the capillary bed of the ischemic penumbra region could be washed away [33]. Pre-reperfusion infusion and brain cooling, therefore, may provide the ultimate neuroprotective "cocktail" that could limit multiple injurious events during reperfusion.

Conceivably, a therapeutic procedure, which combines pre-reperfusion infusion into an ischemic region with coincident cerebral hypothermia and perhaps subsequent recanalization of an occluded intracranial vessel, may improve the outcome by minimizing secondary brain injury and lengthening the therapeutic window for stroke patients.

Acknowledgements This work was supported by the American Heart Association Midwest Affiliate (0255930Z) and a Wayne State University Research Stimulation Grant to Yuchuan Ding. We are grateful to Mr. Yandong Zhou for his help with manuscript preparation, and Dr. Hun Park for his help in blood pressure measurements.

References

1. Barone FC, Hillegass LM, Price WJ, White RF, Lee EV, Feuerstein GZ, Sarau HM, Clark RK, Griswold DE (1991) Polymorphonuclear leukocyte infiltration into cerebral focal ischemic tissue: myeloperoxidase activity assay and histologic verification. J Neurosci Res 29:336–345
2. Barone FC, Feuerstein GZ, White RF (1997) Brain cooling during transient focal ischemia provides complete neuroprotection. Neurosci Biobehav Rev 21:31–44
3. Busto R, Ginsberg MD (1998) The influence of altered brain temperature in cerebral ischemia. In: Ginsberg MD, Bogousslavsky J (eds) Cerebrovascular disease pathophysiology, diagnosis, and management. Blackwell Science, Malden, pp 287–307
4. Chatzipanteli K, Alonso OF, Kraydieh S, Dietrich WD (2000) Importance of posttraumatic hypothermia and hyperthermia on the inflammatory response after fluid percussion brain injury: biochemical and immunocytochemical studies. J Cereb Blood Flow Metab 20:531–542
5. Chen H, Chopp M, Vande LA, Dereski MO, Garcia JH, Welch KM (1992) The effects of post-ischemic hypothermia on the neuronal injury and brain metabolism after forebrain ischemia in the rat. J Neurol Sci 107:191–198
6. Chopp M, Knight R, Tidwell CD, Helpern JA, Brown E, Welch KM (1989) The metabolic effects of mild hypothermia on global cerebral ischemia and recirculation in the cat: comparison to normothermia and hyperthermia. J Cereb Blood Flow Metab 9:141–148
7. Colbourne F, Corbett D (1995) Delayed postischemic hypothermia: a six month survival study using behavioral and histological assessments of neuroprotection. J Neurosci 15:7250–7260
8. Colbourne F, Sutherland GR, Auer RN (1996) An automated system for regulating brain temperature in awake and freely moving rodents. J Neurosci Methods 67:185–190
9. Colbourne F, Sutherland G, Corbett D (1997) Postischemic hypothermia. A critical appraisal with implications for clinical treatment. Mol Neurobiol 14:171–201

10. Del Zoppo GJ, Becker KJ, Hallenbeck JM (2001) Inflammation after stroke: is it harmful? Arch Neurol 58:669–672
11. Deng H, Han HS, Cheng D, Sun GH, Yenari MA (2003) Mild hypothermia inhibits inflammation after experimental stroke and brain inflammation. Stroke 34:2495–2501
12. Dietrich WD, Busto R, Alonso O, Globus MY, Ginsberg MD (1993) Intraischemic but not postischemic brain hypothermia protects chronically following global forebrain ischemia in rats. J Cereb Blood Flow Metab 13:541–549
13. Ding Y, Li J, Rafols JA, Phillis JW, Diaz FG (2002) Prereperfusion saline infusion into ischemic territory reduces inflammatory injury after transient middle cerebral artery occlusion in rats. Stroke 33:2492–2498
14. Ding Y, Yao B, Zhou Y, Park H, McAllister JP II, Diaz FG (2002) Pre-reperfusion flushing of ischemic territory: a therapeutic study in which histological and behavioral assessments were used to measure ischemia-reperfusion Injury in rats with stroke. J Neurosurg 96:310–319
15. Ding Y, Li J, Luan X, Lai Q, McAllister JP II, Phillis JW, Zhong J, Park H, Clark J, Guthikonda M, Diaz FG (2003) Neuroprotection by regional brain cooling and local saline infusion in ischemic territory of rats with transients middle cerebral artery. Neurosurgery (in press)
16. Ding Y, Young C, Li C, Luan X, McAllister JP II, Clark J, Diaz FG (2003) Reduced inflammatory mediator expression by pre-reperfusion infusion into ischemic territory: a real-time polymerase chain reaction analysis. Neurosci Lett 353:173–176
17. Garcia JH, Liu KF, Yoshida Y, Lian J, Chen S, Zoppo GJ del (1994) Influx of leukocytes and platelets in an evolving brain infarct (Wistar rat). Am J Pathol 144:188–199
18. Ginsberg MD, Busto R (1998) Combating hyperthermia in acute stroke: a significant clinical concern. Stroke 29:529–534
19. Ginsberg MD, Busto R (1998) Small animal models of global and focal cerebral ischemia. In: Ginsberg MD, Bogousslavsky J (eds) Cerebrovascular disease: pathophysiology, diagnosis, and management. Blackwell Science, Malden, pp 14–35
20. Ginsberg MD, Sternau LL, Globus MY, Dietrich WD, Busto R (1992) Therapeutic modulation of brain temperature: relevance to ischemic brain injury. Cerebrovasc Brain Metab Rev 4:189–225
21. Huang FP, Zhou LF, Yang GY (1998) The effect of extending mild hypothermia on focal cerebral ischemia and reperfusion in the rat. Neurol Res 20:57–62
22. Huh PW, Belayev L, Zhao W, Koch S, Busto R, Ginsberg MD (2000) Comparative neuroprotective efficacy of prolonged moderate intraischemic and postischemic hypothermia in focal cerebral ischemia. J Neurosurg 92:91–99
23. Inamasu J, Suga S, Sato S, Horiguchi T, Akaji K, Mayanagi K, Kawase T (2000) Post-ischemic hypothermia delayed neutrophil accumulation and microglial activation following transient focal ischemia in rats. J Neuroimmunol 109:66–74
24. Ishikawa M, Sekizuka E, Sato S, Yamaguchi N, Inamasu J, Bertalanffy H, Kawase T, Iadecola C (1999) Effects of moderate hypothermia on leukocyte-endothelium interaction in the rat pial microvasculature after transient middle cerebral artery occlusion. Stroke 30:1679–1686
25. Jiang Q, Chopp M, Zhang ZG, Helpern JA, Ordidge RJ, Ewing J, Jiang P, Marchese BA (1994) The effect of hypothermia on transient focal ischemia in rat brain evaluated by diffusion- and perfusion-weighted NMR imaging. J Cereb Blood Flow Metab 14:732–741
26. Kammersgaard LP, Rasmussen BH, Jorgensen HS, Reith J, Weber U, Olsen TS (2000) Feasibility and safety of inducing modest hypothermia in awake patients with acute stroke through surface cooling: a case-control study: the Copenhagen Stroke Study. Stroke 31:2251–2256
27. Kataoka K, Yanase H (1998) Mild hypothermia – a revived countermeasure against ischemic neuronal damages. Neurosci Res 32:103–117
28. Kawai N, Okauchi M, Morisaki K, Nagao S (2000) Effects of delayed intraischemic and postischemic hypothermia on a focal model of transient cerebral ischemia in rats. Stroke 31:1982–1989
29. Kubota M, Nakane M, Narita K, Nakagomi T, Tamura A, Hisaki H, Shimasaki H, Ueta N (1998) Mild hypothermia reduces the rate of metabolism of arachidonic acid following postischemic reperfusion. Brain Res 779:297–300
30. Kuluz JW, Gregory GA, Yu AC, Chang Y (1992) Selective brain cooling during and after prolonged global ischemia reduces cortical damage in rats. Stroke 23:1792–1796
31. Kuluz JW, Prado R, Chang J, Ginsberg MD, Schleien CL, Busto R (1993) Selective brain cooling increases cortical cerebral blood flow in rats. Am J Physiol 265:H824–H827
32. Li J, Luan X, Lai Q, Clark J, McAllister JP II, Fessler RD, Diaz FG, Ding Y (2003) Long-term neuroprotection in ischemic rats induced by regional brain cooling with saline infusion into ischemic territory: a behavioral analysis. Neurol Res (in press)
33. Liu S, Connor J, Peterson S, Shuttleworth CW, Liu KJ (2002) Direct visualization of trapped erythrocytes in rat brain after focal ischemia and reperfusion. J Cereb Blood Flow Metab 22: 1222–1230
34. Maier CM, Ahern K, Cheng ML, Lee JE, Yenari MA, Steinberg GK (1998) Optimal depth and duration of mild hypothermia in a focal model of transient cerebral ischemia: effects on neurologic outcome, infarct size, apoptosis, and inflammation. Stroke 29:2171–2180
35. Maier CM, Sun GH, Kunis D, Yenari MA, Steinberg GK (2001) Delayed induction and long-term effects of mild hypothermia in a focal model of transient cerebral ischemia: neurological outcome and infarct size. J Neurosurg 94:90–96
36. Okada Y, Copeland BR, Mori E, Tung MM, Thomas WS, Zoppo GJ del (1994) P-selectin and intercellular adhesion molecule-1 expression after focal brain ischemia and reperfusion. Stroke 25:202–211
37. Ooboshi H, Ibayashi S, Takano K, Sadoshima S, Kondo A, Uchimura H, Fujishima M (2000) Hypothermia inhibits ischemia-induced efflux of amino acids and neuronal damage in the hippocampus of aged rats. Brain Res 884:23–30
38. Schwab S, Schwarz S, Aschoff A, Keller E, Hacke W (1998) Moderate hypothermia and brain temperature in patients with severe middle cerebral artery infarction. Acta Neurochir Suppl (Wien) 71:131–134
39. Schwab S, Schwarz S, Spranger M, Keller E, Bertram M, Hacke W (1998) Moderate hypothermia in the treatment of patients with severe middle cerebral artery infarction. Stroke 29:2461–2466
40. Steiner T, Friede T, Aschoff A, Schellinger PD, Schwab S, Hacke W (2001) Effect and feasibility of controlled rewarming after moderate hypothermia in stroke patients with malignant infarction of the middle cerebral artery. Stroke 32:2833–2835
41. Toyoda T, Suzuki S, Kassell NF, Lee KS (1996) Intraischemic hypothermia attenuates neutrophil infiltration in the rat neocortex after focal ischemia-reperfusion injury. Neurosurgery 39: 1200–1205
42. Wang G, Deng H, Maier C, Sun G, Yenari M (2002) Mild hypothermia reduces ICAM-1 expression, neutrophil infiltration and microglia/monocyte accumulation following experimental stroke. Neuroscience 114:1081
43. Wang X, Siren AL, Liu Y, Yue TL, Barone FC, Feuerstein GZ (1994) Upregulation of intercellular adhesion molecule 1 (ICAM-1) on brain microvascular endothelial cells in rat ischemic cortex. Brain Res Mol Brain Res 26:61–68
44. Xue D, Huang ZG, Smith KE, Buchan AM (1992) Immediate or delayed mild hypothermia prevents focal cerebral infarction. Brain Res 587:66–72
45. Yanamoto H, Hong SC, Soleau S, Kassell NF, Lee KS (1996) Mild postischemic hypothermia limits cerebral injury following transient focal ischemia in rat neocortex. Brain Res 718: 207–211

46. Yanamoto H, Nagata I, Nakahara I, Tohnai N, Zhang Z, Kikuchi H (1999) Combination of intraischemic and postischemic hypothermia provides potent and persistent neuroprotection against temporary focal ischemia in rats. Stroke 30: 2720–2726
47. Zea Longa E, Weinstein PR, Carlson S, Cummins R (1989) Reversible middle cerebral artery occlusion without craniectomy in rats. Stroke 20:84–91
48. Zhang H, Tong ET (2002) Neuroprotection of mild hypothermia beginning at different time intervals on cerebral ischemia/reperfusion injury. J Neurochem 81 (Suppl 1):108–111
49. Zhang RL, Chopp M, Chen H, Garcia JH, Zhang ZG (1993) Postischemic (1 hour) hypothermia significantly reduces ischemic cell damage in rats subjected to 2 hours of middle cerebral artery occlusion. Stroke 24:1235–1240
50. Zhang RL, Chopp M, Chen H, Garcia JH (1994) Temporal profile of ischemic tissue damage, neutrophil response, and vascular plugging following permanent and transient (2H) middle cerebral artery occlusion in the rat. J Neurol Sci 125:3–10
51. Zhang ZG, Chopp M, Chen H (1993) Duration dependent postischemic hypothermia alleviates cortical damage after transient middle cerebral artery occlusion in the rat. J Neurol Sci 117: 240–244