LOCAL SALINE INFUSION INTO ISCHEMIC TERRITORY INDUCES REGIONAL BRAIN COOLING AND NEUROPROTECTION IN RATS WITH TRANSIENT MIDDLE CEREBRAL ARTERY OCCLUSION

OBJECTIVE: 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 limited primarily because of management problems and severe side effects (e.g., pneumonia). The goal of this study was to determine whether local infusion of saline into ischemic territory could induce regional brain cooling and neuroprotection.

METHODS: A novel procedure was used to block the middle cerebral artery of rats for 3 hours with a hollow filament and locally infuse the middle cerebral artery-supplied territory with 6 ml cold saline (20°C) for 10 minutes before reperfusion.

RESULTS: The cold saline infusion rapidly and significantly reduced temperature in cerebral cortex from 37.2 ± 0.1 to 33.4 ± 0.4°C and in striatum from 37.5 ± 0.2 to 33.9 ± 0.4°C. The significant hypothermia remained for up to 60 minutes after reperfusion. Significant (P < 0.01) reductions in infarct volume (approximately 90%) were evident after 48 hours of reperfusion. In ischemic rats that received the same amount of cold saline systemically through a femoral artery, a mild hypothermia was induced only in the cerebral cortex (35.3 ± 0.2°C) and returned to normal within 5 minutes. No significant reductions in infarct volume were observed in this group or in the ischemic group with local warm saline infusion or without infusion. Furthermore, brain-cooling infusion significantly (P < 0.01) improved motor behavior in ischemic rats after 14 days of reperfusion. This improvement continued for up to 28 days after reperfusion.

CONCLUSION: Local prereperfusion infusion effectively induced hypothermia and ameliorated brain injury from stroke. Clinically, this procedure could be used in acute stroke treatment, possibly in combination with intra-arterial thrombolysis or mechanical disruption of clot by means of a microcatheter.

KEY WORDS: Cerebral ischemia, Hypothermia, Infarct, Motor behavior, Prereperfusion infusion, Reperfusion damage

Neurosurgery 54:956-965, 2004 DOI: 10.1227/01.NEU.0000114513.96704.29 www.neurosurgery-online.com

Several studies have demonstrated an effectiveness of postischemic hypothermia in animal models of global or focal transient cerebral ischemia (15, 34, 42, 50, 72, 75, 76). However, a prolonged application of hypothermia has seemed to be necessary to achieve significant and persistent neuroprotection (14, 15, 37, 74). Mild postischemic hypothermia only delays neuronal damage. In a global ischemia model, the therapeutic effect induced by mild postischemic hypothermia (3 h after...
hypothermic intervention seems to be highly desirable. Therefore, a shorter, more effective mode of postischemic complications, such as pneumonia, in 40% of patients (60).

Currently, hypothermia is being induced by surface cooling with the use of cooling blankets, “forced” cooling air, alcohol applied to exposed skin, or ice bags placed on the groin, axilla, and neck. This approach, however, requires intensive efforts from the medical and nursing staff for induction and maintenance of the target temperature and has induced some severe reactions from the medical and nursing staff for induction and maintenance of the target temperature and has induced some severe complications, such as pneumonia, in 40% of patients (60). Therefore, a shorter, more effective mode of postischemic hypothermic intervention seems to be highly desirable.

Our recent studies have used a unique technique to “flush” the microvasculature in the ischemic territory before reperfusion (i.e., prereperfusion infusion) and demonstrated a significant therapeutic value in stroke (20, 22). This procedure may have interfered with a series of injurious events triggered by ischemia and potentiated by reperfusion, preventing the potentially multifactorial interactions between ischemically damaged tissue and reestablished blood flow and oxygenation (23). This technique could provide an alternative approach to induce cerebral hypothermia.

The aim of this study was to determine whether cold saline infused intra-arterially into the ischemic territory could selectively induce cerebral hypothermia and reduce brain injury from stroke, producing a long-term functional recovery. By using a novel hollow filament developed previously in our laboratory (20, 22, 23), we were able to occlude the middle cerebral artery (MCA) for 3 hours and then infuse cold saline into the MCA-supplied territory in rats. The intraluminal filament MCA occlusion that was used in this study is a well-established stroke model. Clinically, the MCA is the most frequently embolized artery. Reperfusion can occur as a result of spontaneous, surgical, or pharmacologically induced recanalization (36, 53, 58). The therapeutic effects of our novel procedure on severe stroke with 3 hours of MCA occlusion, followed by 48 hours of reperfusion, and long-term neuroprotection up to 28 days were evaluated histologically and functionally. The clinical relevance of this technique is discussed.

MATERIALS AND METHODS

Subjects

Adult male Sprague-Dawley rats (260–300 g; Charles River Breeding Laboratories, Wilmington, MA) were housed in the animal care facility during a 12-hour light/dark cycle through-out the protocol. Animal care and surgical procedures were conducted in accordance with the guidelines approved by the National Institutes of Health and the Wayne State University Animal Investigation Committee. In the stroke group, a 3-hour MCA occlusion was followed by 48 hours of reperfusion (n = 8). In the local cooling infusion group, after a 3-hour MCA occlusion, a 10-minute infusion of cold saline (20°C) was conducted to selectively induce regional brain hypothermia before the onset of reperfusion (n = 8). As a control, infusion of the same amount of cold saline as injected intracerebrally was applied 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-hour MCA occlusion to determine whether a slow local infusion without brain cooling played a predominant role in reducing brain injury from stroke with 3-hour MCA occlusion. To determine whether long-term neuroprotection by brain-cooling infusion had occurred, motor behavior at 14 and 28 days after 3-hour MCA occlusion was tested in three additional groups of animals: 1) the stroke group without infusion (n = 8); 2) the ischemic group with a 10-minute infusion of cold saline (20°C) (n = 7); and 3) normal control animals that were housed for the same time period and examined for motor behavior (n = 8).

Induction of Stroke and Cooling Infusion with an Intraluminal Filament

MCA occlusion was induced by a novel intraluminal hollow filament model that was modified in our laboratory (20, 22) from the technique developed previously by Longa et al. (48). Briefly, animals were anesthetized and maintained with 1 to 3% halothane in 70% N2O and 30% O2 via a facemask. An 18.5- to 19.0-mm length of 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 reanesthetized with halothane, and reperfusion was established by withdrawal of the filament. In the animals with a local infusion, after 3 hours of ischemia, the catheter was withdrawn 1 to 2 mm from the origin of the MCA. During and after the “pullback” of the catheter, 6 ml of cold or warm saline (20 or 37°C) was slowly and constantly injected at the junction of the MCA and ACA, with an infusion pump (Harvard Apparatus, Holliston, MA) used to control the infusion rate at 0.6 ml/min for 10 minutes, approximately 0.25 ml/g brain tissue per minute. A blood collection set was used to connect the filament inserted into the MCA with the syringe held in the infusion pump. The temperature of infused solution was maintained by a water bath along the tube of the blood collection set and the syringe. After infusion, the catheter was withdrawn completely, and reperfusion was established. As
controls, systemic infusion of the same amount of cold saline (20°C) was applied in ischemic animals via the right femoral artery before the onset of reperfusion.

**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 and after infusion while blood flow was reestablished at 1 through 60 minutes until it returned to normal levels. Needle thermistor probes (Hi-Lo Temp Model 8200 thermometer; Mallinckrodt Baker, Inc., Phillipsburg, NJ) were placed into cortex and striatum through holes 3 mm lateral to bregma and 3 mm posterior and 5 mm lateral to bregma, respectively. Body temperature was measured frequently through the rectum before, during, and after saline infusion until it returned to normal levels.

Animals from each group were placed on a water-circulating heating pad under a heating lamp throughout the surgical procedure under anesthesia. When cerebral and rectal temperature both returned to normal levels, animals were allowed to awake and were placed under warm conditions for an additional 3 hours.

**Physiological Variable Monitoring**

Physiological variables were monitored to determine whether saline infusion may cause physiological changes. Blood pressure was measured through the right femoral artery before ischemia and 30 minutes after local or systemic saline infusion after reperfusion in ischemic/reperfused rats. Blood gases (pH, PO₂, PCO₂) and hematocrits were also examined by a blood gas analyzer (ABL 700 Series; Radiometer, Copenhagen, Denmark) at the same two time points via a femoral artery.

**Neurological Examination**

To verify MCA occlusion, neurological deficits in rats were examined during ischemia. The deficits were scored on a modified scoring system developed by Longa et al. (48), 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 < 4) during MCA occlusion were excluded from further analysis.

**Histological Analysis for Infarct Volume**

Forty-eight hours after the onset of reperfusion, animals were deeply anesthetized with pentobarbital sodium (Nembutal; Abbott Laboratories, North Chicago, IL; 50 mg/kg intraperitoneally) and killed by cardiac perfusion of saline followed by 4% paraformaldehyde in 0.1 mol/L phosphate buffer, pH 7.4. Brains were sectioned serially through the MCA territory in the frontoparietal sensorimotor cortex and the dorsolateral neostriatum from +2.0 to −4.0 mm from bregma. Infarct volume was determined by a histological technique with hematoxylin and eosin staining that has been widely used in previous studies (17, 20, 22, 28, 66, 77).

The histological analyses were performed in a blinded manner. The infarct region, defined as the area with reduced staining or containing eosinophilic-necrotic cell bodies, was traced with a Tri Simplex projector (×15; Bausch & Lomb, Rochester, NY). By use of a scanner and an image analysis system (MetaMorph Imaging System; Universal Imaging Corp., West Chester, PA), the areas of noninfarcted tissue ipsilateral and contralateral to the occluded side were measured. To minimize the error introduced by edema, an indirect method for calculating infarct volume was used (66). The noninfarcted region in the ipsilateral hemisphere was subtracted from that in the contralateral hemisphere. The infarct volume is presented as a percentage of the volume of the contralateral hemisphere.

**Motor Behavioral Evaluation**

Animals from the two ischemic groups with or without brain-cooling infusion and the normal control group were examined with four different motor testing paradigms (foot fault placing, parallel-bar crossing, and ladder and rope climbing) at 14 and 28 days after the surgical procedure in ischemic rats or after housing in normal control animals. All rats were tested three times on each trial day. Behavior analyses were performed in a blinded manner.

A modified forelimb foot-fault-placing test was used to examine forelimb function. The foot-placing apparatus consisted of an elevated (100 cm) grid surface (10 × 110 cm², with a square opening of 9 cm² and grid wire diameter of 1.0 mm) connected to platforms at each end. In each trial, the animal was encouraged by noise or prod to traverse the grid surface for 1 minute. Occasionally, animals placed a forelimb inaccurately, which fell through one of the openings in the grid. These mistakes were considered foot faults. The rate of contralateral forelimb foot faults made per meter in 1 minute was calculated. Parallel-bar testing was used to test hindlimb coordination. This apparatus consisted of two parallel wooden rods (each 1.0 cm in diameter and 115 cm long), with an inter-rod distance of 2.5 cm, connected to platforms at each end. The number of times that the subject placed two hind paws on one rod, dropped a hind paw below the rod, or fell or swung under the rods was recorded. The number of errors made per meter in 1 minute was recorded. The rope-climbing test was used to examine coordination of both forelimbs and hindlimbs. A rope (thick braided twine) 1.5 cm in diameter was suspended vertically from a platform 1 m above the base. On the first testing day, the rats were pretrained by gentle encouragement to climb the upper one-third of the rope before testing. Animals were also tested with a ladder-climbing task that required motor skills similar to those in rope climbing. A single wooden rod with crossbars at 3-cm intervals was suspended vertically from a platform 1 m above the base. Rats were tested after the short pretraining procedure.
Statistical Analysis

One-way (infusion conditions: none, local cooling, local 37°C, systemic cooling) analyses of variance (ANOVAs) with repeated measures on different time points were used to assess brain and body temperature and physiological variables. ANOVA was also used to analyze statistical differences in infarct volume from different ischemia groups. Functional performance on motor tasks was evaluated by the number of faults or duration of performance in each trial. Separate one-way ANOVAs of different groups (ischemia, ischemia and cooling, and nonischemia) with repeated measures indicated that the temperature differences were significant [in the cortex, \( F_{(8, 48)} = 35.88, P < 0.01 \), and striatum, \( F_{(8, 48)} = 26.66, P < 0.01 \)]. Duncan’s multiple-range test revealed that the temperatures were lowered significantly during local infusion and then gradually recovered after reperfusion in both cortex and striatum. A significant degree of hypothermia remained for up to 60 minutes after reperfusion. A mild hypothermia was induced in the cortex (35.3 ± 0.2°C) \( F_{(8, 48)} = 13.21, P < 0.01 \) but not in the 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 and B). Furthermore, Duncan’s multiple-range test indicated that temperatures were decreased significantly during systemic infusion, and the reduced cortical temperature recovered to normal within 5 minutes. In the third group of ischemic rats that was perfused locally with saline at body temperature (37°C), brain temperature remained normal during ischemia and reperfusion (Fig. 1A and B). ANOVA failed to detect any significant difference in temperature in the cortex \( F_{(8, 32)} = 1.99, P > 0.05 \) and the striatum \( F_{(8, 32)} = 0.31, P > 0.05 \). 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).

RESULTS

Regional Brain Hypothermia

The cold saline (20°C) infusion via the MCA rapidly reduced the temperature of the MCA-supplied ischemic territory in cortex from 37.2 ± 0.1 to 33.4 ± 0.4°C (Fig. 1A) and in striatum from 37.5 ± 0.2 to 33.9 ± 0.4°C (Fig. 1B). ANOVA with repeated measures indicated that the temperature differences were significant [in the cortex, \( F_{(8, 48)} = 35.88, P < 0.01 \), and striatum, \( F_{(8, 48)} = 26.66, P < 0.01 \)]. Duncan’s multiple-range test was used to further analyze differences in post-ANOVA tests. A significance level of \( P < 0.05 \) was used for all tests.

Physiological Variables after Saline Infusion

ANOVA indicated that there were no significant differences in arterial blood pressure, blood pH, and blood gases among the ischemic animal groups before or after saline infusion (systemically or locally). Hematocrits after either systemic or local saline infusions were slightly reduced, but the differences did not reach significant levels.

Infarct Volume

Effective occlusion of the MCA is critical to this study. In all ischemic animals, the MCA occlusions were verified by a severe neurological deficit (score 4 or 5) during ischemia. All ischemic rats showed a consistently high score of 4 or 5, compared with 0 in nonischemic rats in the present study and in our previous studies (21, 22, 24, 25). In addition, the intraobserver and interobserver variability was minimal. Therefore, it is highly likely that the same degree of ischemia was present in all groups.

The infarct volumes in brains with a 3-hour MCA occlusion followed by 48 hours of reperfusion were reconstructed from the four ischemic groups, with or without different saline infusions, including local infusion at 20 or 37°C and systemic infusion at 20°C (Fig. 2). Infarction within the territory of the occluded MCA included the sensorimotor frontoparietal cortex and the dorsolateral neostriatum. The ANOVA detected a significant difference in infarct volume among the different experimental groups \( F_{(3, 18)} = 39.64, P < 0.001 \). Duncan’s multiple-range test showed that infarct areas from ischemic animals without local cooling infusions or with control infusion (local at 37°C or systemic at 20°C) were clearly larger than infarct areas in rats with a local cooling infusion. The average infarct volume was 54.2% (standard error [SE], ±1.9%) of the volume of the contralateral hemisphere in ischemic rats, compared with an infarct volume of only 3.8% (SE, ±2.6%) in rats with the local cooling infusion. This 90% reduction in infarct volume was statistically significant \( P < 0.001 \). Our ANOVA also indicates that there were no significant differences in infarct sizes between the control stroke group (54.2 ± 1.9%), the local warm (37°C) saline infarction (54.4 ± 4.0%), and the systemic cooling (20°C) infarction group (43.4 ± 9.0%). Although a slight reduction in infarct volume was found in the systemic cooling infusion group, the difference did not reach a significant level. The reduced lesion was restricted to the ischemic core in the striatum of ischemic rats with brain-cooling infusion, in contrast to the extensively infarcted area, including both the dorsolateral portion of the neostriatum and frontoparietal cortex in the rats without brain-cooling infusion.

Long-term Motor Functional Recovery

Animal performances on all four motor tasks showed differences among the three animal groups (stroke, stroke with cold infusion, and normal control) at Days 14 and 28 after reperfusion (Fig. 3). At first, we analyzed the effect of the brain-cooling infusion on ischemic animals. Statistically significant effects were detected among the three groups for all the tasks, including forelimb foot fault placing \( F_{(2, 23)} = 6.08, P < 0.01 \), parallel-bar traversing \( F_{(2, 23)} = 14.75, P < 0.001 \), rope climbing \( F_{(2, 23)} = 22.70, P < 0.001 \), and ladder climbing \( F_{(2, 23)} = 10.82, P < 0.001 \). The post hoc test (Duncan's
Regional Brain Cooling: Synergistic Neuroprotection of Local Infusion and Regional Brain Cooling

The focus of this study was on developing a combined therapeutic procedure that used regional infusion to induce cerebral hypothermia in ischemic territory, with a novel hollow filament being inserted into the internal carotid artery. Although the results are based on a fairly small number of rats in each group (7, 8), the highly significant reductions in brain damage and motor deficits suggest a therapeutic effect of the brain-cooling infusion procedure. A statistically significant (P < 0.001) reduction (90%) of infarct volume was achieved 48 hours after reperfusion in rats. Importantly, this brain-cooling infusion significantly (P < 0.01) improved motor behavior in ischemic rats at Days 14 through 28 after reperfusion.

DISCUSSION

The focus of this study was on developing a combined therapeutic procedure that used regional infusion to induce cerebral hypothermia in ischemic territory, with a novel hollow filament being inserted into the internal carotid artery. Although the results are based on a fairly small number of rats in each group (7, 8), the highly significant reductions in brain damage and motor deficits suggest a therapeutic effect of the brain-cooling infusion procedure. A statistically significant (P < 0.001) reduction (90%) of infarct volume was achieved 48 hours after reperfusion in rats. Importantly, this brain-cooling infusion significantly (P < 0.01) improved motor behavior in ischemic rats at Days 14 through 28 after reperfusion.
study, 6 ml of warm saline infusion for 10 minutes did not reduce brain infarction. The negative result in contrast to our previous study could be because of a more severe stroke model (3 versus 2 h of MCA occlusion) or less infusion volume per minute (0.6 versus 2.5–3 ml) and shorter duration of hypothermia (3 versus 60 min). In addition, no significant reduction in infarct volume was found in ischemic rats with cold saline administered systemically, because only a mild and brief (5 min) hypothermia was induced in the cortex (but not in the striatum). Taken together, these findings support the notion that the combination of local infusion and brain cooling synergistically produce more profound neuroprotection in a severe stroke model with 3-hour MCA occlusion. In addition, by combining hypothermia with the local "flushing," the total volume of infusion could be reduced by 30% (from 10 to 6 ml), and infusion speed could be decreased by 75% (2.5 or 3 to 0.6 ml), which may make this procedure more feasible and safe in a clinical setting.

To determine long-term neuroprotective effects resulting from the local cerebral hypothermia, all animals were tested with different motor activity paradigms, including forelimb foot-fault placing, parallel-bar crossing, and rope and ladder climbing. These motor paradigms have consistently and accurately measured motor functions in our previous studies on normal, traumatically injured, and stroked rats by examining the functions of forelimbs and hindlimbs and coordination of all four limbs (17–19, 21, 24, 25).

A modified forelimb foot-fault-placing paradigm, which has also been studied in other stroke or brain injury models (39, 51, 57, 62, 63), was used to examine sensorimotor function in forelimb responses to visual, tactile, and proprioceptive stimuli. The parallel-bar-crossing test, which depends on the acquisition of motor skills, is sensitive to hindlimb coordination impairment (52). The rope-climbing test, which has been used to evaluate balance and coordination of forelimb and hindlimb movements in normal, alcohol-affected animals (43) and traumatically injured animals (21), was used to evaluate motor ability in our stroke model. The ladder-climbing test also evaluates the ability to balance and coordinate forelimb and hindlimb movements. The combination of these four different motor tasks provided an overview of improved motor performance after brain-cooling infusion in focally ischemic rats.

**Clinical Relevance**

The inherent practical difficulties and expense associated with surface cooling, the current technique for achieving generalized hypothermia, with poor control of body temperature and the occurrence of medical complications, have tended to...
discourage their continued use and prompted consideration of other options, such as selective brain cooling. A few studies have introduced the concept of selective brain cooling in which brain rather than whole-body temperature is selectively reduced as a therapeutic end point for hypothermia (7). Cold carotid artery perfusion with temperature-reduced blood, localized cerebral ventricular perfusion with hypothemic solution, and head-surface cooling have all been conducted clinically and experimentally for brain cooling (3, 7, 35, 45, 46, 49, 54, 71). Most recently, endovascular cooling with an indwelling catheter in the inferior vena cava was used for moderate hypothermia in patients with acute stroke (29, 44). Our study provides a potentially effective technique to reduce brain temperature regionally, particularly in the ischemically affected area, coupled with intravascular infusion.

Although our model is not yet applied in a clinical setting, its clinical potential is realistic. Clinically, intra-arterial thrombolytic therapy by means of a microcatheter has been used successfully to reopen acutely occluded cerebral vessels, such as the MCA, either by mechanically disrupting or suctioning out the clot. This technique also delivers thrombolytic drugs during human stroke (1, 2, 5, 6, 27, 47, 55, 67, 69). Most recently, mechanical thrombolysis, defined as the mechanical dissolution of clot with a microcatheter coincident with infusion of thrombolytics, has been performed in patients (56). An infusion microcatheter was advanced distally to the site of occlusion and gradually pulled back through the clot to permit infusion of thrombolytics drugs distal to, within, and proximal to the occluded segment. Saline is known to be safe and is already used on a regular basis for intra-arterial infusion during human endovascular procedures. Microcatheters placed in the MCA are continuously flushed with heparinized saline to prevent clot propagation on the distal catheter tip. These studies using intra-arterial catheterization support our hypothesis that local cooling infusion of saline into the ischemic territory before reperfusion is feasible in clinical practice. The clinical application of saline perfusion distal to an occlusion in humans will be simpler than performing similar procedures in animals with smaller-diameter vessels. Finally, this procedure represents a normal part of performing endovascular interventions for many neuroendovascular surgeons.

The volume of physiological saline infused with this procedure is a critical issue that may affect the feasibility and safety in clinical settings. Unlike in humans, the microcatheter used in experimental rats cannot be inserted directly into the MCA because of the small size of the vessel. It is likely that only 3 ml, half of the total (6 ml) solution, injected posterior to the junction of the MCA and ACA flows into the MCA-distributed territory, whereas the rest flows to the ACA territory. This volume is 10 to 15% of total blood volume in rats with a body weight of approximately 260 g (as used in this study) (4). In rats, blood flow is approximately 0.75 ml/min for the entire brain (brain weight, 1.8–2.0 g for 260–300 g body weight), or 15% of cardiac output (4). In humans, the blood flow through the brain tissue averages 750 ml/min in normal adults (brain weight, 1500 g) (32) or 540 ml/min in elderly stroke patients (68). These figures suggest that an infusion volume of approximately 500 to 750 ml can be applicable in adult patients with an average blood volume of approximately 5000 ml (31). Therefore, it is feasible that 50 to 70 ml/min of infusion volume can be safely administered to patients. Furthermore, rapid crystalloid fluid (Ringer’s solution) infusion with a similar volume (600–1400 ml) over a 10-minute period has been used in fluid replacement therapy for traumatically injured patients or normal adults (26, 64, 65). Conceivably, a therapeutic procedure that combines a prerereperfusion infusion into an ischemic region with coincident cerebral hypothermia and perhaps subsequent mechanical recanalization of an occluded intracranial vessel or thrombolytic therapy may improve outcome beyond the level achieved by current therapies.

REFERENCES


Ding et al. provide an interesting investigation evaluating the use of 20°C saline infusion to ameliorate focal cerebral ischemia. This article describes an interesting new approach to a long-realized avenue of potential stroke therapy. The importance of temperature of stroke has been demonstrated time and again; yet, to date, efforts have remained futile with regard to the fully fledged use of this potential therapeutic modality. The idea of intra-arterial infusion of chilled saline is both novel and relevant to the ongoing effort to treat cerebral ischemia. Especially important is the exploration of the effect on long-term outcomes. As the authors continue to develop this therapy for use in humans, it will be interesting to see whether saline is the optimal agent or whether another chilled colloid or crystalloid is even more efficacious. Whatever the result, the authors describe a simple, yet ingenious, approach to stroke therapy, and we look forward to additional preclinical data examining both the mechanism and the safety of this treatment in different species.

J. Max Findlay
Edmonton, Alberta, Canada

Hypothermia is neuroprotective under a variety of ischemic conditions in animal models, as the authors mention. Demonstration of this in humans has been more difficult (2, 4). For example, meta-analysis of head injury studies revealed no evidence for a beneficial effect of hypothermia in human head injury (3). Side effects include an increased risk of infection. Other toxicity may occur, including platelet dysfunction and an increased risk of bleeding, among others (5). Conversely, there may be improvement in outcome when hypothermia is induced after cardiac arrest (1). For patients with brain injury, including ischemic stroke and subarachnoid hemorrhage, methods of locally cooling the brain may be advantageous in that systemic side effects might be avoided, the difficulties with shivering in the awake patient might be ameliorated, and induction might be achieved more rapidly. The work of Ding et al. reported here supports this concept. It seems to me that the detrimental effects of perfusing the brain with saline rather than oxygenated blood may be substantial. The main problems in the methods of this

Acknowledgments

This work was supported by the American Heart Association Midwest Affiliate Grant-in-Aid Fund and a Wayne State University Research Stimulation Fund (to YD). We are grateful to Dr. Hun Park for help with blood pressure measurements and to Yandong Zhou for help with manuscript preparation.

COMMENTS

Ding et al. provide an interesting investigation evaluating the use of 20°C saline infusion to ameliorate focal cerebral ischemia. This article describes an interesting new approach to a long-realized avenue of potential stroke therapy. The importance of temperature of stroke has been demonstrated time and again; yet, to date, efforts have remained futile with regard to the fully fledged use of this potential therapeutic modality. The idea of intra-arterial infusion of chilled saline is both novel and relevant to the ongoing effort to treat cerebral ischemia. Especially important is the exploration of the effect on long-term outcomes. As the authors continue to develop this therapy for use in humans, it will be interesting to see whether saline is the optimal agent or whether another chilled colloid or crystalloid is even more efficacious. Whatever the result, the authors describe a simple, yet ingenious, approach to stroke therapy, and we look forward to additional preclinical data examining both the mechanism and the safety of this treatment in different species.

J Mocco
E. Sander Connolly, Jr.
New York, New York

In this novel experimental design, the middle cerebral artery of rats was occluded with a hollow filament for 180 minutes, after which cold saline (6 ml, 20°C) was flushed through the filament into the ischemic region for 10 minutes, then the filament was removed, allowing for reperfusion. With proper controls for comparison, it was found that the preceding cold saline infusion resulted in significant and prolonged brain cooling of both cortex and striatum and a significant reduction in infarct volume measured at 48 hours. The authors point out that this type of regional brain cooling might be feasible in humans during intra-arterial thrombolysis or thrombectomy, despite the larger-caliber vessels and greater brain mass being dealt with. If technically possible, something as intuitive as this might move directly to clinical testing rather than be delayed for confirmatory experimental results in large animals.
study are its lack of randomization, questionable blinding, and failure to confirm ischemia with a measurement of cerebral blood flow. Another experiment to be performed might be to infuse the rat’s own blood or an oxygen-carrying blood substitute cooled to various temperatures. In addition, allosteric scaling laws may need to be applied to correct for differences in time factors between rats and humans (6). From a practical perspective, the thermodynamics of cooling a 25-g rat brain need to be scaled up to the human brain.

R. Loch Macdonald  
Chicago, Illinois


The authors present the results of a novel therapeutic approach in experimentally induced ischemia of the middle cerebral arterial territory in rats. The stroke model they used is a well-established one that has been known for years. This approach consists of local infusion with 6 ml cold saline (20°C) for 10 minutes, followed by reperfusion, which cools the ischemic territory of the cortex and the striatum to approximately 33°C for 60 minutes. The body temperature remains almost unchanged. A significant reduction of the infarct volume was achieved, averaging approximately 90%. Also, motor behavior was found to be significantly improved 14 days after reperfusion. A control group in which 20°C saline was systemically infused through the femoral artery demonstrated only mild hypothermia lasting for approximately 5 minutes, whereas the striatum remained unaffected. No significant reduction in infarct volume was observed in this group. In a second control group in which a 37°C saline was locally infused, the brain temperature remained normal. Also in this control group, the size of the infarct volume was not significantly reduced.

The authors describe an interesting approach for the treatment of stroke. Further investigations and prospective, randomized trials need to be performed in patients. If the results achieved in this animal model hold true in humans and can be combined with local intra-arterial thrombolysis or mechanical disruption of a clot, as the authors mention in the Discussion, the treatment modality presented might be a valuable advance for stroke patients that could potentially improve outcomes.

Gabriele Schackert  
Dresden, Germany

In this article, Ding et al. present evidence that the perfusion of cooled normal saline into the middle cerebral arterial territory after transient arterial occlusion and immediately before arterial reperfusion induces significant regional cooling of brain tissue, reduces the neurological deficit after the ischemic injury, and reduces the size of the brain tissue infarct 48 hours after reperfusion in the rat.

The article appropriately addresses the importance of the clinical management of ischemic stroke and the potential for improving outcome with the use of regional brain cooling during cerebral arterial catheterization, although its relevance is partially limited to the rat model. The testing of neuroprotection by selective arterial perfusion of cooled normal saline in rats is a novel design, and it contributes to the evolution in understanding the effects and possible applications of this approach to stroke treatment. Even though the applicability to human disease is uncertain, this study is a necessary first step in evaluating this creative approach.

Murat Gunel  
New Haven, Connecticut

Neurosurgeons’ Library

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