Interrupted intracarotid artery cold saline infusion as an alternative method for neuroprotection after ischemic stroke

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Object. Intracarotid artery cold saline infusion (ICSI) is an effective method for protecting brain tissue, but its use is limited because of undesirable secondary effects, such as severe decreases in hematocrit levels, as well as its relatively brief duration. In this study, the authors describe and investigate the effects of a novel ICSI pattern (interrupted ICSI) relative to the traditional method (uninterrupted ICSI).

Methods. Ischemic strokes were induced in 85 male Sprague-Dawley rats by occluding the middle cerebral artery for 3 hours using an intraluminal filament. Uninterrupted infusion groups received an infusion at 15 ml/hour for 30 minutes continuously. The same infusion speed was used in the interrupted infusion groups, but the whole duration was divided into trisections, and there was a 20-minute interval without infusion between sections. Forty-eight hours after reperfusion, H & E and silver nitrate staining were utilized for morphological assessment. Infarct sizes and brain water contents were determined using H & E staining and the dry-wet weight method, respectively. Levels of neuron-specific enolase (NSE), S100β protein, and matrix metalloproteinase 9 (MMP-9) in the serum were determined using enzyme-linked immunosorbent assay. Neurological deficits were also evaluated.

Results. Histology showed that interrupted ICSI did not affect neurons or fibers in rat brains, which suggests that this method is safe for brain tissues with ischemia. The duration of hypothermia induced by interrupted ICSI was longer than that induced via the traditional method, and the decrease in hematocrit levels was less pronounced. There were no differences in infarct size or brain water content between uninterrupted and interrupted ICSI groups, but neuron-specific enolase (NSE), S100β protein, and matrix metalloproteinase 9 (MMP-9) serum levels were more reduced after interrupted ICSI than after the traditional method.

Conclusions. Interrupted ICSI is a safe method. Compared with traditional ICSI, the interrupted method has a longer duration of hypothermia and less effect on hematocrit and offers more potentially improved neuroprotection, thereby making it more attractive as an infusion technique in the clinic.

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Key Words: • brain • injury • neuroprotection • infusion • carotid artery • hypothermia • selective brain cooling • rat

The recent introduction of therapeutic hypothermia has improved acute stroke therapy considerably. It has been shown that mild hypothermia (33°–36°C) is effective for inducing either global or focal ischemia in experimental and clinical studies. Indeed, we have found in our laboratory that there are apparent advantages associated with using ICSI for improving the outcomes of ischemic stroke. Although ICSI is a promising neuroprotective technique, it is difficult to apply it clinically. Trials have suggested that the duration of cooling for mild hypothermia, no less than 48 hours, is one of the most important factors for its neuroprotective properties. However, this duration of treatment is not attainable with ICSI because of an undesirable reduction in hematocrit, which limits its use to no more than 3 hours.

In the present study, we describe a novel method of infusion called “interrupted infusion.” Compared with traditional continuous infusion, the interrupted pattern can effectively prolong hypothermia while using the same amount of liquid. We applied this infusion technique in rats with transient MCAO and observed its tolerance and effectiveness. We postulated that interrupted infusion is a safe cooling method that provides more neuroprotection after ischemic stroke than that provided by the traditional infusion pattern.
Methods

All procedures involving the use of rats were approved by the Animal Research Committee of Nanfang Hospital in Southern Medical University.

Animal Protocol

Eighty-five male Sprague-Dawley rats weighing 250–300 g were used in these experiments. Animals were randomized into the following groups: sham group, which underwent neither MCAO nor infusion, 8 rats; non-infusion group, which had 3 hours of MCAO without infusion, 13 rats; and infusion groups, which underwent different types of infusion after 3 hours of MCAO. The two types of infusion included uninterrupted ICSI and INSI or interrupted ICSI and INSI (13 rats per group, total of 52 rats).

For surgical procedures, anesthesia was induced with 10% chloral hydrate through the peritoneal cavity. Two temperature probes (Physitemp Instruments, Inc.) were each placed in the right brain cortex (3 mm posterior and 3 mm lateral to the bregma and 4 mm below the skull surface) and the rectum for continuous monitoring of brain and core temperatures. A heating pad and bubble wrap were used to maintain the core temperature at 37°C. The left femoral artery of animals was catheterized for monitoring mean arterial pressure, heart rate, arterial gas levels (pH, pO2, and pCO2), and hematocrit.

A modified intraluminal filament model was used for inducing MCAO in all animals except those in the sham group, as formerly described by Longa et al.24 Briefly, the right common carotid artery, ICA, and ECA were exposed via a midline incision in the neck, and the ECA was ligated and transected. A piece of nylon filament with a round tip (0.34–0.36 mm) was introduced into the ICA through the recurved ECA and was advanced 18–20 mm beyond the carotid artery bifurcation, until a faint resistance was felt. After 3 hours of MCAO, reperfusion was established by retracting the filament. To verify the success of MCAO surgery and reperfusion, blood flow in the core MCA territory was established by retracting the filament. For surgical procedures, anesthesia was induced with 10% chloral hydrate through the peritoneal cavity. Two temperature probes (Physitemp Instruments, Inc.) were each placed in the right brain cortex (3 mm posterior and 3 mm lateral to the bregma and 4 mm below the skull surface) and the rectum for continuous monitoring of brain and core temperatures. A heating pad and bubble wrap were used to maintain the core temperature at 37°C.

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the infarct area was corrected according to the method described by Swanson et al.35 The infarct area (%) was calculated as follows: [(area of the left hemisphere – non-infarct area of right hemisphere)/area of the left hemisphere] × 100%.

Brain Water Content

The brains minus the cerebellums and brainstems were weighed to assess the wet weight (WW). Thereafter, the brains were dried for 48 hours at 100°C, and the dry weight (DW) was determined. In 5 rats per group brain water content (%) was calculated as [(WW – DW)/WW] × 100%, as previously described.12

Enzyme-Linked Immunosorbent Assay

Serum samples were collected from 8 animals per stroke group after 48 hours of reperfusion and analyzed in duplicate using commercially available ELISA test kits (Cusabio Biotech Co., Ltd.) to measure serum concentrations of NSE, S100β protein, and MMP-9. The assays were performed in accordance with the manufacturer’s specifications. Lower limits of detection for the ELISA were 0.16 ng/ml for NSE, 0.78 × 10⁻⁶ ng/ml for S100β, and 3.9 × 10⁻³ ng/ml for MMP-9. Researchers blinded to the group designations of the specimens collected all data.

Statistical Analysis

Statistical analysis was performed with the SPSS 13.0 for Windows software package (SPSS, Inc.). Data were expressed as means ± standard error. One-way ANOVA was used to determine statistical differences in all observed indicators from different groups. The LSD t-test was used to further analyze differences in post-ANOVA tests. Statistical significance was declared at the p < 0.05 level.

Results

Overall Observations

Twelve animals had to be excluded from the study because of vessel perforation of the filament and brain hemorrhage, premature death, or the absence of laser Doppler flowmetry changes, leaving 73 animals for our analysis. The mean arterial pressure, heart rate, and arterial gas levels were not significantly influenced by infusion and were all measured at close to normal levels. In contrast, hematocrit after uninterrupted infusion was significantly reduced, although it was maintained within a normal physiological range. This significant change in hematocrit levels was not observed in animals that had received interrupted infusions (Table 1). In the sham group, no abnormal behavior was observed and no abnormal changes were found on histological staining. Brain water content was 79.4 ± 0.1%. Serum levels of NSE, S100β, and MMP-9 were 0.19 ± 0.22, 0.14 ± 0.05, and 16.08 ± 2.42 ng/ml, respectively.

Brain Temperature Changes

Baseline brain temperatures of uninterrupted and interrupted ICSI groups before infusion were 37.2 ± 0.1°C and 36.9 ± 0.2°C, respectively, and the core body temperatures of both groups were maintained at approximately 37°C. As shown in Fig. 1, brain temperatures decreased to 36.5°C within approximately 6 minutes of ICSI administered at 15 ml/hour. The average temperatures of spontaneous rewarming in the interrupted ICSI group were 0.5°C in the first interval and 0.6°C in the second interval. The duration of hypothermia induced by interrupted ICSI was approximately 2.7 times more than that induced by uninterrupted ICSI. The final brain temperature in both ICSI groups was approximately 34.6°C.

Histopathological Analysis

The impact of interrupted ICSI on brain tissues was illustrated by photomicrographs of H & E and silver staining sections. The H & E staining revealed swollen brain cells; fractured, pyknotic, or apparently missing cells; and disordered neurons that were loosely arranged in infarct regions as well as adjacent normal regions. There were no obvious differences in the various regions examined—that is, cortex, striatum, and hippocampus—between uninterrupted and interrupted ICSI (5 rats per group; Fig. 2). Evaluation of the nerve fibers in brains revealed no significant changes in the fibers of the normal brain regions adjacent to infarct regions—that is, corpus callosum, cortex, and striatum—between the 2 infusion patterns (5 rats per group; Fig. 3). Overall, there was no difference in the injuries to the neurons and fibers in the interrupted ICSI group, as compared with injuries in the uninterrupted ICSI group.

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### TABLE 1: Physiological variables in animals before and after infusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Uninterrupted Infusion</th>
<th>Interrupted Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>before 96.3 ± 8.5</td>
<td>110.5 ± 11.8</td>
</tr>
<tr>
<td></td>
<td>after 93.0 ± 10.2</td>
<td>95.7 ± 7.3</td>
</tr>
<tr>
<td>heart rate (min⁻¹)</td>
<td>before 366.2 ± 40.3</td>
<td>378.2 ± 23.5</td>
</tr>
<tr>
<td></td>
<td>after 354.7 ± 28.4</td>
<td>365.9 ± 47.1</td>
</tr>
<tr>
<td>pO₂ (kPa)</td>
<td>before 17.3 ± 3.8</td>
<td>17.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>after 14.5 ± 4.0</td>
<td>15.4 ± 2.1</td>
</tr>
<tr>
<td>pCO₂ (kPa)</td>
<td>before 5.2 ± 0.1</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>after 5.4 ± 0.3</td>
<td>5.5 ± 0.2</td>
</tr>
<tr>
<td>pH</td>
<td>before 7.33 ± 0.02</td>
<td>7.36 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>after 7.32 ± 0.01</td>
<td>7.34 ± 0.03</td>
</tr>
<tr>
<td>hematocrit (%)</td>
<td>before 46.5 ± 2.3</td>
<td>47.1 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>after 41.7 ± 1.6†</td>
<td>45.5 ± 2.1</td>
</tr>
</tbody>
</table>

* Values represent the mean ± SE. MAP = mean arterial pressure. † p < 0.05, compared with before the infusion (LSD t-test).
Infarct Size, Brain Water Content, and Functional Outcome

The infarct size in ischemic brains with 3 hours of MCAO followed by 48 hours of reperfusion was measured in the 5 stroke groups (8 rats per group; Fig. 4). The H & E staining showed infarction within the MCAO territory, including the cortex and the neostriatum. Although significant differences were revealed between the noninfusion group (50.5 ± 5.3%) and the 2 ICSI groups (uninterrupted ICSI, 33.8 ± 3.8%; interrupted ICSI, 34.2 ± 4.6%), no differences were detected between the 2 ICSI groups. Additionally, no differences were detected between the noninfusion group and the 2 INSI groups. Thus, these data indicated that infarct size did not differ significantly between interrupted and uninterrupted ICSI brains.

In comparisons among groups, brain water content in the uninterrupted (81.6 ± 0.2%) and interrupted (81.0 ± 0.3%) ICSI groups was significantly lower than in the noninfusion group (82.7 ± 0.3%). As with infarct size, no differences in brain water content were detected between the 2 ICSI groups. Additionally, although there was a slight trend toward a reduction of water content in the 2 INSI groups, the trend did not reach statistical significance (5 rats per group; Fig. 5). Analysis of variance revealed no significant differences between groups in the improvement of neurological deficits after 48 hours, which indicated that neither interrupted nor uninterrupted ICSI could improve neurological deficits in early MCAO-treated rats (13 rats per group; Fig. 6).

Biochemical Markers of Neuronal Injury

Serum levels of NSE, S100β, and MMP-9 were compared among the 5 stroke groups (8 rats per group; Fig. 7). The ELISA of all 3 markers showed significant differences for only NSE and MMP-9 between the noninfusion group (NSE, 2.2 ± 0.3 ng/ml; MMP-9, 52.3 ± 5.2 ng/ml) and the interrupted ICSI group (NSE, 1.2 ± 0.2 ng/ml; MMP-9, 32.4 ± 4.0 ng/ml). There were no significant group differences for serum levels of S100β, but all infu-
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Discussion

Hypothermia has long been recognized as a neuroprotective remedy; however, a variety of adverse side effects limits the usefulness and possible efficacy of systemic cooling. Many SBC methods have been developed to avoid the side effects of systemic cooling. Cold liquid infusion from the carotid artery is one of the most promising SBC methods because of its high efficiency for inducing hypothermia. However, crystalloid solution infusion into either the vertebral artery, as studied by Nishihara et al. and Ohta et al. or the carotid or jugular veins, as studied by Ding and colleagues and Wen et al. were all performed using simple continuous infusion. Although hypothermia induced by the traditional infusion pattern improved the outcomes of brain ischemia in almost all of these studies, this pattern is technically difficult because of an inevitable drop in hematocrit levels and limitations on the duration of treatment. To decrease these negative effects and provide a better method, we present here a novel infusion pattern, called “interrupted infusion,” to achieve SBC. Key features of interrupted ICSI are as follows: 1) longer treatment duration, 2) less effect on hematocrit levels, and 3) more potentially improved neuroprotection, as compared with the traditional infusion pattern.
First, interrupted infusions can prolong treatment, which is an advantage given that its limited duration is the main shortcoming of ICSI. Many clinical trials and laboratory studies have suggested that a longer duration of cooling is needed to obtain the full neuroprotective effects against ischemia, possibly because of the diverse pathophysiological processes at work over an extended time course. In the present study, traditional uninterrupted ICSI was maintained for approximately 24 minutes with the brain temperature maintained under 36.5°C (it takes about 6 minutes to decrease the brain temperature from 37°C to 36.5°C). But with the same amount of liquid as that used in uninterrupted ICSI, the duration of interrupted ICSI could be maintained for up to approximately 64 minutes, which is 2.7 times longer than the traditional method. This prolonged duration provided a relatively long time for the body to absorb or metabolize the redundant water that was infused. In fact, urination was seen in most rats receiving interrupted infusions but was rarely observed in the traditional infusion groups.

The second advantage of interrupted infusions was the less affected hematocrit levels. A theoretical model of ICSI in the human brain showed that a continuously falling hematocrit level was the main time-limiting factor in the traditional method. In the combination model of ICSI and an ice cap, the amount of infused volume was reduced by 55% over a 6-hour period and better hematocrit values were obtained, as compared with the ICSI method alone. In this combined method, however, core body temperature was severely decreased because of the ice cap. In the present study, interrupted ICSI induced a less drastic decrease in hematocrit levels than traditional ICSI, and the core body temperature was not significantly changed. Thus, this advantage improves the safety of the ICSI method.

Third, compared with traditional infusion, interrupted ICSI has more positive molecular biological outcomes for ischemic stroke. We used standard biochemical markers to assess neuronal injury. The first marker, NSE, is localized mainly within neurons and endocrine cells. High serum NSE was associated with a poor outcome in ischemic stroke, and NSE has been reported as a useful biochemical marker for neuronal injury from various causes. The second marker, S100β, is found primarily in glial and Schwann cells and can be used to detect glial cell injury. As with NSE, previous studies have shown that S100β correlates with stroke severity and outcomes. Matrix metalloproteinase 9 is a good marker for blood-brain barrier breakdown after cerebral ischemia, and a clinical study has shown that MMP-9 has a significant correlation with brain edema. It has been shown by several groups to play an important role in ischemic stroke. In the present study, we found that although there were no significant differences in infarct size, brain water content, or neurological deficits between interrupted and uninterrupted ICSI groups, interrupted ICSI decreased the NSE and MMP-9 serum levels.

![Fig. 6. Bar graph showing neurological scores in noninfusion and infusion groups. No significant differences were detected by ANOVA.](image)

![Fig. 7. Bar graph showing NSE, S100β, and MMP-9 serum levels in noninfusion and infusion groups. *p < 0.05 (ANOVA followed by LSD t-test), infusion groups versus noninfusion group (8 rats per group).](image)
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These molecular biological changes suggested that there might be more neuroprotection in interrupted infusion than in uninterrupted infusion. Additionally, the dry-wet method showed lower water retention in brains from rats treated with interrupted ICSI, coincident with a change in MMP-9 serum levels, thus suggesting the effect of interrupted ICSI on brain edema may be better than traditional infusion.

The effect of ICSI is a synergistic neuroprotection of hypothermia and flushing. The mechanisms of action of hypothermia are varied, so benefits could be attributed to blocking the cascade of ischemia on many physiological levels. With regard to flushing, previous studies have found that ischemic rats who received INSI at 120–180 ml/hour for 3–4 minutes had a significantly smaller infarct size and fewer neurological deficits in the acute phase of ischemic stroke, which was attributed to adequate reperfusion and ameliorated inflammation. Similarly, we assessed the kinetics of hypothermia and flushing in a 2-hour MCAO rat model and found that the flushing effect in ICSI (10–25 ml/hour) decreased over a delayed start time, whereas the hypothermia effect was preserved for relatively longer times (data not shown). In the present study, the INSI parameter (at 15 ml/hour) for the uninterrupted or interrupted infusion for 30 minutes did not induce obvious neuroprotection in a 3-hour MCAO rat model, and the most probable explanation for this finding is that these animals experienced a more severe stroke. Even so, this evidence does not suggest that flushing does not contribute to ICSI neuroprotection.

The depth of cooling is another important factor for therapeutic hypothermia. Cerebral metabolism is temperature dependent. Hypothermia reduces brain O2 consumption by approximately 5% per degree Celsius of body temperature between the range of 22°C–37°C. A study of systematic hypothermia in ischemic rats showed that 33°C and 34°C were the optimal temperatures for hypothermia, but another study found that mild hypothermia was as protective as more severe hypothermia. Regardless, 36.5°C is considered to be the temperature at which hypothermia has neuroprotective effects.

No previous studies have documented the rate of temperature fluctuations in brain tissue, especially ischemic brain tissue. In the present study, we observed the status of neurons and fibers in ischemic brain, which would likely be affected by fluctuating temperatures induced by ICSI. We observed no adverse histopathological changes to normal, ischemic, or transition zone neurons or fibers by interrupted ICSI, which indicates that quick changes in this range of temperatures from hypothermia are safe for brain tissue after ischemic stroke.

A clinical trial revealed that the rewarming period seems to be crucial for the outcomes of ischemic stroke. Authors found that a shorter duration of rewarming (<16 hours) was associated with a more pronounced increase in intracranial pressure. In the present study, we adapted this method of rapid rewarming, because there are individual differences in spontaneous rewarming rates in rats that can affect the total duration of hypothermia. We observed that rapid rewarming returned the brain temperature to baseline within 15–20 minutes in all animals studied.

Theoretically, traditional ICSI for the human brain cannot be maintained for over 3 hours, and its potential role in the management of ischemic stroke is as the "igniter" for rapidly induced hypothermia so as not to miss the therapeutic window. However, data in this study indicated that interrupted ICSI could maintain a longer duration of hypothermia, which not only improves the availability of ICSIs but also sets the stage for clinical combination therapy. Next, it will be interesting to investigate the neuroprotective effects obtained with the combination of interrupted ICSI and neuroprotective agents. Clinically, it is possible to adapt ICSI or an ICSI combined with neuroprotectants through an intervention technique.

Conclusions

Intracarotid artery cold saline infusion with an interrupted pattern not only is a safe method, but can also significantly prolong the whole duration of hypothermia with fewer side effects and noninferior neuroprotection for ischemic stroke, which would ultimately enhance the clinical availability of the hypothermic infusion technique.

Disclosure

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Author contributions to the study and manuscript preparation include the following. Conception and design: Pan, Y Ji, Wu, Z Ji. Acquisition of data: Y Ji, Song, Xu, Wang. Analysis and interpretation of data: Y Ji, Song, Xu, Wang. Drafting the article: Y Ji. Critically revising the article: Pan, Y Ji, Wu. Reviewed submitted version of manuscript: Pan, Y Ji, Wu, Z Ji. Approved the final version of the manuscript on behalf of all authors: Pan. Statistical analysis: Y Ji. Administrative/technical/material support: Pan. Study supervision: Pan.

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References

6. Colbourne F, Sutherland G, Corbett D: Postischemic hypo-

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