Remote Ischemic Per-Conditioning
A Novel Therapy for Acute Stroke?

Cecil D. Hahn, MD, MPH; Cedric Manlhiot, BSc; Michael R. Schmidt, MD; Torsten T. Nielsen, MD; Andrew N. Redington, MD

Background and Purpose—Remote ischemic preconditioning is a phenomenon by which a short period of sublethal ischemia to an organ protects against subsequent ischemia in another organ. We have recently demonstrated that remote ischemic conditioning by transient hind limb ischemia delivered during ischemia and before reperfusion can provide potent cardioprotection, a phenomenon we termed per-conditioning. This study evaluated whether remote ischemic per-conditioning may provide neuroprotection in a clinically relevant rat model of acute ischemic stroke.

Methods—Remote ischemic conditioning by transient limb ischemia was used in a rat transient middle cerebral artery occlusion model of acute stroke. A total of 39 P60 rats were randomly allocated to receive preconditioning, per-conditioning, or sham conditioning. Cerebral ischemia was maintained for 120 minutes followed by reperfusion. The resulting infarct size at 24 hours was quantified using computerized image analysis of 2–3-5-triphenyl tetrazolium chloride-stained brain sections.

Results—Compared with control, both pre- and per-conditioning significantly reduced brain infarct size with the more clinically relevant per-conditioning stimulus being superior to preconditioning.

Conclusions—Remote per-conditioning by transient limb ischemia is a facile, clinically relevant stimulus that provides potent neuroprotection in a model of regional brain ischemia–reperfusion injury. Further studies are required to better understand the mechanisms and biology of this response before translation to randomized controlled trials of remote per-conditioning for acute ischemic stroke. (Stroke. 2011;42:00-00.)

Key Words: acute ischemic stroke ▪ neuroprotection ▪ remote ischemic conditioning

Acute ischemic stroke is a leading cause of morbidity and mortality worldwide. Because the timing of stroke is usually unpredictable, clinically relevant neuroprotective strategies must be effective when delivered after the onset of ischemia, thus targeting reperfusion injury. Local ischemic preconditioning is one of the most potent innate mechanisms of cellular protection against ischemia–reperfusion injury, but its use in the treatment of acute stroke is necessarily limited by the need to induce it directly in the area at risk before the onset of ischemia. Remote ischemic preconditioning is a phenomenon by which a short period of sublethal ischemia to an organ protects against subsequent ischemia in another organ. Although the remote activation of protection overcomes some of the limitations of local preconditioning, it also must be delivered before the onset of ischemia.

We have recently demonstrated that a remote ischemic conditioning stimulus, induced by transient ischemia of the limb, can provide potent cardioprotection when delivered during ischemia and before reperfusion, a phenomenon we termed as per-conditioning. The objective of this proof-of-principle study was to evaluate the neuroprotective efficacy of remote ischemic per-conditioning in a clinically relevant ischemia–reperfusion model of acute ischemic stroke.

Methods
A total of 39 male P60 Sprague-Dawley rats (Harlan Laboratories, Inc, Indianapolis, IN) were randomly allocated to 3 groups: a control group, which received no remote pre- or per-conditioning, a preconditioning group, and a per-conditioning group (Figure 1). Remote preconditioning was initiated during the 40 minutes before surgery, whereas per-conditioning was initiated during the 40 minutes before reperfusion. The remote conditioning stimulus consisted of 4 cycles of 5 minutes of left hind limb ischemia (using an atraumatic tourniquet tightened to achieve limb pallor) followed by 5 minutes of reperfusion. Focal cerebral ischemia was achieved using transient right middle cerebral artery occlusion, performed surgically under isoflurane anesthesia, according to previously published methods. After surgical exposure of the right carotid bifurcation, a monofilament was advanced to the internal carotid artery/middle cerebral artery junction to achieve a drop in cerebral blood flow of =75% measured by a laser Doppler probe over the ipsilateral cerebral cortex. After 120 minutes of ischemia, blood flow to the internal carotid artery and middle cerebral artery was restored by removal of the monofilament occluder. At 24 hours post-transient right middle cerebral artery occlusion surgery, animals were euthanized with...
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against global cerebral ischemia or focal cerebral ischemia demonstrated that remote ischemic preconditioning can protect brain barrier penetration. Indeed, prior studies have demonstrated that transient limb ischemia liberates a low-molecular-weight (<15 kDa) hydrophobic dialyzable humoral factor(s) with ischemic preconditioning as a neuroprotective strategy. We have recently shown that remote ischemic preconditioning can protect against global cerebral ischemia7 or focal cerebral ischemia using permanent occlusion8 and ischemia–reperfusion9 models. However, because the timing of acute ischemic stroke is unpredictable, preconditioning has limited clinical applicability. Local interruption of cerebral reperfusion (postconditioning) also appears to be neuroprotective when applied within 30 seconds or 3 to 6 hours after ischemia.10,11 However, postconditioning also has limited clinical applicability, because current therapies (such as thrombolysis with intravenous tissue-type plasminogen activator) provide little control over the timing and extent of reperfusion.

In contrast, remote ischemic per-conditioning, delivered to a nonvital organ after the onset of cerebral ischemia and before cerebral reperfusion, has great potential for clinical translation to a randomized controlled trial in adults with evolving myocardial infarction. Ambulance personnel applied eight 5-minute cycles of intermittent upper arm ischemia using a blood pressure cuff during transportation to hospital for emergency coronary angioplasty. This per-conditioning stimulus resulted in a significant reduction in final infarct size in patients with anterior myocardial infarcts compared with controls.12

It is interesting that the neuroprotective efficacy of per-conditioning was greater than preconditioning in our model despite an identical limb ischemia stimulus. Although the biology of response to pre- and per-conditioning might vary, and mechanistic studies were beyond the scope of this proof-of-principle study, we suggest that this difference more likely reflects the kinetics of the humoral factor within the bloodstream. The duration of ischemia was 120 minutes, much longer than the ischemic period (40 to 60 minutes) in most models of cardioprotection. Although speculative, we suggest that the humoral factor(s) may have a temporal profile of decay that reduces its effectiveness proportionate to the interval between stimulus and reperfusion, arguing for performing remote ischemic per-conditioning as close to the onset of reperfusion time as possible.

<table>
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<tr>
<th>Group 1: Control</th>
<th>Group 2: Pre-conditioning</th>
<th>Group 3: Per-conditioning</th>
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<tr>
<td>n=12</td>
<td>n=14</td>
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<td>120 min ischemia</td>
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<td>reperfusion</td>
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Figure 1. Surgical protocols: 39 male P60 Sprague-Dawley rats (270 to 330 g) were randomly allocated to 3 groups. The control group (n=12) received only sham pre- and per-conditioning, consisting of restraint and application of a tourniquet to the contralateral hind limb without tightening. The pre-conditioning group (n=14) received 4 cycles of hind limb ischemia applied and released at 5-minute intervals immediately before transient middle cerebral artery occlusion surgery and sham per-conditioning immediately before reperfusion. The per-conditioning group (n=13) received sham preconditioning and 4 cycles of hind limb ischemia applied and released at 5-minute intervals immediately before reperfusion. Animals were housed in controlled conditions of heating, lighting, and humidity and were allowed free access to feed and water.

carbon dioxide and the brains immediately removed, sectioned at 2-mm intervals, stained with 2–3-5-triphenyl tetrazolium chloride, and fixed in formalin. Sections were photographed and the resulting infarct size was measured using a computerized image analysis system. The protocol was approved by the institutional animal care committee (Ricerca Biosciences), and all experiments were conducted in accordance with the US Department of Agriculture animal welfare act.

Statistical Analysis
To account for the wide distribution in infarct sizes, all comparisons were performed in log-linear (natural log transformation of the dependent variable) regression models with maximum likelihood methodology for parameter estimation. Data from the control group were used as the reference category and between-group contrasts were created for all pairwise comparisons based on a single regression model for each outcome. All statistical analyses were performed using SAS Version 9.2 (SAS Institute, Cary NC) with the GENMOD procedure.

Results
Both preconditioning and per-conditioning produced potent neuroprotection, evidenced by significant reductions in total, cortical, and subcortical infarct volumes compared with controls (Figure 2). Furthermore, the more clinically relevant stimulus of per-conditioning, delivered after the onset of ischemia and before reperfusion, resulted in neuroprotection that was superior to that achieved by preconditioning.

Discussion
This is the first study showing the effectiveness of per-conditioning as a neuroprotective strategy. We have recently demonstrated that transient limb ischemia liberates a low-molecular-weight (<15 kDa) hydrophobic dialyzable humoral factor(s) that induces cardioprotection in animals and humans, properties that are likely associated with blood–brain barrier penetration. Indeed, prior studies have demonstrated that remote ischemic preconditioning can protect against global cerebral ischemia or focal cerebral ischemia using permanent occlusion8 and ischemia–reperfusion9 models. However, because the timing of acute ischemic stroke is
In summary, remote per-conditioning by transient limb ischemia is a facile, clinically relevant stimulus that provides potent neuroprotection in a model of regional brain ischemia–reperfusion injury. Further studies are required to better understand the mechanisms and biology of this response and any effect on important end points of clinical relevance (such as behavioral outcomes) before translation to randomized controlled trials of remote per-conditioning for acute ischemic stroke.

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References
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