Remote Limb Perconditioning and Postconditioning
Will It Translate Into a Promising Treatment for Acute Stroke?

David C. Hess, MD; Md Nasrul Hoda, PhD; Kanchan Bhatia, PhD

Preconditioning is an adaptive process of endogenous protection in which small, sublethal doses of a harmful agent protect the organism against a later lethal dose of the same agent. The basic principle is evident in human thought and literature, encapsulated in Nietzsche’s statement, “what does not kill me makes me stronger.” Ischemic preconditioning, the use of short episodes of sublethal ischemia to protect against a later episode of lethal ischemia, is one of the most effective cardioprotectants known. Moreover, ischemic conditioning is protective in organ ischemia of multiple animal species and effective even when applied after the onset of ischemia.

Ischemic conditioning targets ischemia-reperfusion injury. Depending on the temporal relation of the sublethal ischemia to the lethal ischemia, the ischemic stimulus can be applied before ischemia and vessel occlusion (preconditioning), during the ischemia and before reperfusion (perconditioning), or after the lethal ischemic episode, during reperfusion (postconditioning; Figure 1). Preconditioning has limited clinical application and is best suited to clinical situations of predictable ischemia, such as coronary artery bypass grafting or carotid endarterectomy; perconditioning and postconditioning are ideally suited to treatment of acute ischemic stroke because they can be applied during or after the ischemic period. Conventional preconditioning and postconditioning require occluding and releasing the artery supplying the target organ. Although percutaneous interventions are commonplace in the treatment of myocardial infarctions and more stroke patients are receiving intra-arterial interventions, accessing a coronary or cerebral artery is not always feasible, practical, nor safe.

One of the most significant breakthroughs in the ischemic conditioning field came with the discovery that ischemic conditioning can also be performed at a distance or remote from the ischemic target organ. Transient, sublethal mesenteric, renal, and limb ischemia all protect against lethal myocardial ischemia. This review focuses on the development of limb remote per-and posts ischemic conditioning because this emerging therapy has the most clinical relevance and translational potential for the acute treatment of ischemic stroke. We searched Pubmed with the search terms, “remote conditioning,” “limb preconditioning,” “remote perconditioning,” “remote postconditioning” “perconditioning and stroke/cerebral ischemia,” and “postconditioning and stroke/cerebral ischemia.”

Historical Development of Remote Limb Conditioning

Ischemic preconditioning was first reported in 1986 from the laboratory of Keith Reimer, where 4 cycles of a 5-minute period of occlusion followed by a 5-minute period of reperfusion of the circumflex artery in the dog reduced infarct size in a subsequent 40-minute period of circumflex artery occlusion but not in a 3-hour occlusion.

In 1993, Przyklenk et al. showed that transient occlusion of the circumflex artery protected against subsequent left anterior descending artery occlusion, indicating that preconditioning could be applied at a distance in the same organ. The phenomenon of preconditioning at a distance was extended to another remote organ, when Gho et al. demonstrated that 15 minutes of mesenteric sublethal ischemia protected rat heart from ischemia. If the mesenteric ischemia was permanent, there was no protection, demonstrating the need for reperfusion of the distant organ. Fifteen minutes of renal ischemia was also cardioprotective but only when combined with hypothermia. Hexamethonium, a nicotinic receptor and ganglion blocker, abolished the cardioprotective effect of distant mesenteric occlusion but not preceding nonremote, coronary occlusion. This was the first suggestion that remote conditioning had an underlying neural mechanism.

Remote preconditioning was extended to limb ischemia when Birnbaum and Kloner demonstrated that rabbit hindlimb ischemia, produced by a combination by subtotal occlusion of the femoral artery with electric stimulation of the hindlimb ischemia, produced by a combination by subtotal occlusion of the femoral artery with electric stimulation of the hindlimb muscle, protected the heart against later ischemia. In 2005, Kerendi et al. moved the remote ischemic stimulus in time, from before ischemia to later during the period of organ ischemia, and showed that renal ischemia immediately before cardiac reperfusion (perconditioning) was cardioprotective; this effect was blocked by an adenosine antagonist. The first demonstration of remote limb preconditioning was reported by Schmidt et al. in 2007 in a pig model, in which they showed that 4 cycles of 5 minutes of ischemic condition applied with a tourniquet to the hindlimb reduced...
myocardial infarct size, improved functional indices, and reduced malignant arrhythmias after 40 minutes of left anterior descending occlusion. This protection was abrogated by blockade of the mitochondrial potassium ATP channel (mito-K\textsubscript{ATP}) with glibenclamide.

These preclinical findings launched a number of randomized clinical trials of remote limb preconditioning to protect the heart. In a meta-analysis of 23 randomized clinical trials of remote conditioning, regardless of timing, most (15) involving patients undergoing cardiac surgery, limb conditioning did not reduce mortality or major adverse cardiovascular events compared with no conditioning but did reduce the incidence of myocardial infarction and troponin release.\textsuperscript{9} Most of these trials were small and underpowered to detect differences in clinical outcomes. A relevant perconditioning clinical trial for stroke clinical trials was the Danish prehospital use of blood pressure cuff inflation/deflation for 4 cycles of 5-minute occlusion in patients with ST-elevation myocardial infarction before primary percutaneous coronary intervention.\textsuperscript{10} The primary end point was myocardial salvage index at 30 days after percutaneous coronary intervention estimated by gated single photon emission computed tomography. The intervention group (N=73) had higher mean and median salvage indices, suggesting myocardial salvage in the per-protocol analysis than the control group (N=69). The benefit was greatest in the subset of patients with coronary vessel occlusion on admission angiography. The intervention was safe and well tolerated.

Remote Limb Conditioning in Focal Ischemia Models

There is an extensive preclinical literature on the effectiveness of ischemic preconditioning and postconditioning in both global hypoxia-ischemia and focal ischemia using direct (cerebral artery) occlusion.\textsuperscript{11–13} However, preclinical reports of remote limb conditioning in focal cerebral ischemia are recent and more limited (Table 1).

Remote hindlimb preconditioning performed immediately before the onset of focal cerebral ischemia reduced infarct size measured at 48 hours by ≈80%; infarct size was also reduced when the conditioning was performed at 12 or 48 hours before ischemia, although the infarct reduction was less.\textsuperscript{14} Hindlimb preconditioning delivered just before ischemia had a long-term benefit because there was a reduction in infarct size at 2 months and improved long-term functional outcomes.\textsuperscript{15} Both hexamethonium and pretreatment with capsaicin blocked the protective effective of preconditioning, indicating an involvement of local nerve afferents and the autonomic nervous system.\textsuperscript{15} In a middle cerebral artery (MCA) suture occlusion model, preconditioning 24 hours before MCA occlusion with bilateral femoral ischemia reduced infarct size and improved short-term outcomes; the effect was blocked by hexamethonium.\textsuperscript{16} However, no effect was observed when the MCA occlusion was delayed to 48 or 72 hours after the preconditioning.

In a rat temporary MCA suture occlusion model, both remote limb preconditioning and postconditioning with 4 cycles of 5 minutes or occlusion followed by 5 minutes of reperfusion applied with a tourniquet on the hindlimb reduced infarct size.\textsuperscript{17} Remarkably, the postconditioning applied 40 minutes before reperfusion was significantly more effective at reducing infarct size than preconditioning (before onset of MCA occlusion). The authors suggested that these findings might reflect the kinetics of a humoral factor that decays with time, indicating that the timing of pairing the hindlimb ischemia stimulus to the cerebral ischemia may be critical. In a rat temporary MCA suture occlusion model, bilateral femoral occlusion with 3 cycles of 10-minute occlusions at the time of MCA occlusion reduced infarct size at 2 days in a transient MCA occlusion model. However, there was no difference in infarct size at 2 months.\textsuperscript{19} Bilateral femoral occlusion for 3 cycles of both 5 and 8 minutes was effective at reducing infarct size when delayed 3 hours and as late as 6 hours after reperfusion, in a rat temporary MCA suture occlusion model.\textsuperscript{20} Mechanical stroke occlusion models may have limited application to the human clinical situation, in which
spontaneous recanalization and tissue plasminogen activator (tPA)–induced recanalization lead to a more gradual return of cerebral blood flow (CBF), different than that which occurs with an abrupt withdrawal of a suture. In a mouse thromboembolic model, remote preconditioning with 5 cycles of 5-minute occlusion applied to the femoral artery at 2 hours after ischemia improved relative CBF, reduced infarct size, and improved functional outcome at 48 hours after stroke.

There was an additive effect of this regimen to late IV tPA administered at 4 hours, suggesting that remote preconditioning may be a combination therapy to use with IV tPA.

**Mechanism of Remote Ischemic Conditioning**

How is the protective effect transmitted and communicated from the distant ischemic-reperfused muscle to the target organ: the brain and the brain vasculature? Three theories

<table>
<thead>
<tr>
<th>Reference</th>
<th>Stroke Model</th>
<th>Time of Conditioning</th>
<th>Limb Ischemia Protocol</th>
<th>Infarct Size Reduction</th>
<th>Behavioral Testing</th>
<th>Quality*</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ren et al, Neuroscience 2008</td>
<td>Bilateral CCA occlusion (30 min) with distal MCA occlusion, male SD rat</td>
<td>PreC 48 h, 12 h, or immediately before ischemia</td>
<td>Femoral occlusion: 2–3 cycles of 5 or 15 min</td>
<td>Immediate preC: infarct size reduced from 48% of cortex to 9% with 3 cycles/15 min; to 25% with 2 cycles of 15 min; and no reduction with 2 cycles of 5 min</td>
<td>Not done</td>
<td>Randomized</td>
<td>PreC at 48 h: infarct reduced to 23% with 3 cycle/15 min; none with 2 cycles of 15 min; PreC at 12 reduced to 25% and none with 2 cycles/15 min with 3 cycles of 15 min</td>
</tr>
<tr>
<td>Ren et al, Brain Res 2009</td>
<td>Bilateral CCA occlusion with distal MCA occlusion, male SD rat</td>
<td>PostC at reperfusion, 3, 6 h postreperfusion</td>
<td>Femoral occlusion: 3 cycles of 15 min</td>
<td>Infarct size at 2 days reduced by 67% at reperfusion, 43% at 3 h, not at 6 h; no reduction in infarct size at 2 months</td>
<td>VISIBLE-forearm placement improved out to 60 days</td>
<td>Randomized, blinded</td>
<td>Blocked by capsaicin and cycloheximide</td>
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<tr>
<td>Wei et al, PLOS 2012</td>
<td>Bilateral CCA occlusion with distal MCA occlusion, male SD rat</td>
<td>PreC before onset</td>
<td>Femoral occlusion: 3 cycles of 15 min</td>
<td>Infarct size reduced at 2 days and 2 months (40%)</td>
<td>Improvement in 3 behavioral tests</td>
<td>Randomized, blinded</td>
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<td>Malhotra et al, Brain Res 2011</td>
<td>MCA suture occlusion (120 min), male Wistar rat</td>
<td>PreC 24, 48, or 72 h before ischemia</td>
<td>Infrarenal aortic occlusion (bilateral leg ischemia) 3 cycles/10 min</td>
<td>Infarct size (24 h) reduced with preC 24 h prior; no effect with preC 48 or 72 h prior</td>
<td>Improvement in graded neurological examination scores with preC 24 h prior</td>
<td>Randomized, blinded</td>
<td>Blocked by hexamethonium</td>
</tr>
<tr>
<td>Ren et al, Neuror Res 2011</td>
<td>MCA suture occlusion (90 min), male SD rat</td>
<td>PerC at time of MCA occlusion</td>
<td>Bilateral femoral occlusion: 3 cycles of 10 min</td>
<td>Infarct size at 24 h reduced by 66%</td>
<td>Not done</td>
<td>Randomized, blinded</td>
<td>PerC reduced cerebral edema and blood–brain barrier permeability</td>
</tr>
<tr>
<td>Hahn et al, Stroke 2011</td>
<td>MCA suture occlusion (120 min), male SD rat</td>
<td>PreC (before occlusion) and PerC (before reperfusion)</td>
<td>Femoral occlusion with tourniquet: 4 cycles of 5 min</td>
<td>Infarct size reduced; perC superior to preC in infarct size reduction</td>
<td>Not done</td>
<td>Randomized</td>
<td></td>
</tr>
<tr>
<td>Sun et al, JCBFM 2012</td>
<td>MCA suture occlusion (90 min), male SD rats</td>
<td>PostC at reperfusion, 3 or 6 h</td>
<td>Femoral occlusion for 15 s, 2/3 cycles of 15 min; 3 cycles of 5 min</td>
<td>Infarct size at 72 h reduced most with postC at 6 h, 3 cycles of 5 min</td>
<td>Garcia scores improved at 24, 48, and 72 h</td>
<td>Randomized, blinded</td>
<td>Effect abolished by mitochondrial KATP channel blocker</td>
</tr>
<tr>
<td>Hu et al, Brain Research</td>
<td>MCA suture occlusion (120 min), male SD rats</td>
<td>PreC 1 h before</td>
<td>Femoral tourniquet: 3 cycles of 5 min</td>
<td>Infarct size by TTC and MRI-DWI at 24 h reduced with preC</td>
<td>Neurological deficit scores improved at 24 h with preC</td>
<td>Randomized, blinded</td>
<td>PreC effect abolished with selective adenosine A1 receptor antagonist</td>
</tr>
<tr>
<td>Hoda et al, Stroke 2012</td>
<td>Thromboembolic clot with/without tPA at 4 h, male C57Bl mouse</td>
<td>PerC at 2 h post occlusion</td>
<td>Femoral occlusion: 5 cycles of 5 min</td>
<td>Infarct size reduced by 25% with perC at 2 h; additive effect with tPA at 4 h (50%)</td>
<td>Neuro scores improved with perC and with tPA perC</td>
<td>Randomized, blinded, sample size estimation</td>
<td>Increase in relative CBF with perC</td>
</tr>
</tbody>
</table>

CBF indicates cerebral blood flow; CCA, common carotid artery; MCA, middle cerebral artery; MRI-DWI, Magnetic Resonance Imaging-Diffusion; perC, preconditioning; postC, postconditioning; preC, preconditioning; SD, Sprague Dawley; tPA, tissue plasminogen activator; and TTC, triphenyltetrazolium chloride.

*Studies reviewed for reporting of 3 measures of study quality: randomization, blinding of end points, and whether sample size analysis was performed for a hypothesized effect size.
have been advanced, mostly from work in the heart: (1) humoral factors acting via the systemic circulation; (2) neurogenic transmission with involvement of muscle afferents and the autonomic nervous system; and (3) effects on leukocytes or circulating immune cells. In elegant studies, Shimizu et al found that dialysate of plasma from rabbits and humans with remote ischemic limb preconditioning protected an isolated perfused heart (Langendorff heart) and cardiomyocytes in vitro. The dialysate of plasma was obtained using a 15-kDa cutoff dialysis membrane, indicating the protective factors to be of low molecular mass (<15 kDa). The protection in an isolated perfused heart system showed that there was no requirement for neural innervation in the heart. However, further work using this isolated heart model to separate out the effects of the remote stimulus in the target organ, the heart, indicated that peripheral innervation of the ischemic limb was required; femoral nerve transection abolished release of the protective humoral factors and cardioprotection. Moreover, both topical capsaicin on the abdomen (via activation of C fiber afferents) and femoral nerve stimulation elicited release of the protective humoral factors in the circulation and reduced infarct size. Denervation of afferents with pretreatment with capsaicin abolished the protective effect of remote limb postconditioning in a focal cerebral ischemia model. In an in vivo study in mice, both femoral vein occlusion and sciatic and femoral transection abolished the cardioprotective effect of remote limb preconditioning, implying the requirement for both humoral and neural pathways.

Neural Mechanism

The protective effects of remote conditioning on the heart and brain are blocked by hexamethonium, a ganglion blocker, suggesting a requirement for activation of the autonomic nervous system. Moreover, bilateral vagotomy blocked the protective effect of remote conditioning in the heart. In a rat myocardial ischemia model, cell-specific silencing of dorsal motor nucleus of the vagus nerve by genetic targeting abrogated the protective effect of remote conditioning, demonstrating a requirement for the efferent limb of the vagus nerve; conversely, optogenetic activation of the dorsal motor nucleus of the vagus nerve mimicked the effect of remote limb conditioning and reduced infarct size. The vagus nerve is part of the cholinergic anti-inflammatory pathway, and activation of the vagus is associated with reduced inflammation after sepsis and ischemia-reperfusion via effects on the liver and spleen. Activation of parasympathetic efferents is known to have neuroprotective effects; both vagal and sphenopalatine ganglion stimulation reduce cerebral infarct size.

Although the precise identification of the humoral mediators with remote conditioning is not known, autacoids, such as adenosine, opioids, and bradykinin, have all been implicated. These autacoids may be released by the muscle and, via a paracrine effect, stimulate local nerve afferents or elicit the release of other humoral factors (Figure 2). They may also enter the systemic circulation and bind to receptors on the cerebral endothelium, neurons, or other cells.

Effects on Immune Cells

Ischemic limb conditioning reduces neutrophil activation and adherence to endothelium and inflammatory gene expression. In humans, remote limb conditioning reduced CD11b expression, as measured by flow cytometry and modified leukocyte inflammatory gene expression. Immune cell activation and regulation may also be affected by release and binding of endogenous opioids.

Cerebral Blood Flow

Remote ischemic conditioning may also be vasculoprotective and improve CBF. Remote limb perconditioning increased relative CBF after thromboembolic stroke. In the liver, remote limb preconditioning protected against ischemia-reperfusion injury and increased hepatic microvascular blood flow. This effect was mediated by NO and dependent on endothelial NO synthase. CBF increased in the late phase of ischemic preconditioning of the brain after brief MCA occlusion 72 hours after MCA occlusion. Ischemic preconditioning also increased CBF and perfusion when delivered 24 hours before permanent MCA occlusion. Moreover, NO seems to play a role in rapid ischemic preconditioning because the neuroprotective effect of preconditioning is lost in endothelial NO synthase knockout mice. Preconditioning with lipopolysaccharide before MCA occlusion is neuroprotective through an effect on improved CBF and preservation of microvascular perfusion. Therefore, part of the protective effect of remote limb conditioning may be related to improved CBF and microvascular perfusion and mediated by NO.

Final Common Pathways

From work in the heart, a large body of evidence suggests that the final common pathway of protection in the target organ involves activation of the reperfusion injury salvage kinases or survival activating factor enhancement pathways that ultimately converge on the mitochondria to open mito-KATP channels and thereby prevent opening of the mitochondrial permeability transition pore. Remote limb conditioning activates and upregulates the Akt pathway in cerebral ischemia models. Moreover, blocking the mito-KATP channel abolished the protective effect of remote limb conditioning in focal cerebral ischemia. These pathways may be operative not only in brain but also in the brain endothelium.

Clinical Trials in Stroke

A phase Ib trial of remote ischemic preconditioning in subarachnoid hemorrhage patients to target and reduce delayed ischemia from vasospasm demonstrated safety, tolerability, and feasibility, using a blood pressure cuff on the leg with inflations as long as 10 minutes. In another small clinical trial in subarachnoid hemorrhage, cuff inflations for 5 minutes were associated with transient elevation of increased CBF, and brain microdialysis indicated persistent (up 54 hours) improved lactate/pyruvate ratios and reduced glucose levels, suggesting increased membrane preservation. A striking reduction in new strokes in a population of patients with intracranial arterial stenosis with repetitive bilateral arm
preconditioning suggests that chronic use of remote limb conditioning may have a role in high-risk stroke-prone patients.49

A randomized clinical trial of remote limb conditioning in acute stroke patients in the prehospital setting has been conducted in Denmark.50 Subjects were randomized in a single-blind fashion to 4 cycles of 5 minutes occlusion with a blood pressure cuff on the arm or no intervention. The target population was those patients with a positive magnetic resonance imaging-diffusion weighted image lesion who received IV tPA within 4.5 hours at the hospital. The primary outcome was penumbral salvage; the mismatch between perfusion imaging and diffusion imaging that does not progress to infarction at 30 days on T2 fluid attenuated inversion recovery. Although the results of this trial have not yet been reported, these clinical trials to date show that limb conditioning in humans is safe, well tolerated, and feasible, with durations of occlusion as long as 10 minutes in patients with subarachnoid hemorrhage and twice daily for 300 days in both upper extremities in patients with intracranial arterial stenosis.47,49

Next Steps
The Stroke Treatment Academic Industry Roundtable (STAIR) recommends that promising stroke therapies be tested in animals of both sexes, in aged mice, in animals with comorbidities, and in multiple species.51 The preclinical studies of remote limb conditioning in acute ischemic stroke are limited to young male rodents. There are no data on whether remote limb conditioning is effective in females, in aged animals, or in animals with comorbidities, such as diabetes mellitus and hypertension. Remote limb preconditioning is effective in uremic animals at reducing myocardial infarct size.52 However, diabetes mellitus and aging may attenuate the cardioprotective effect of ischemic preconditioning and remote conditioning in the heart.53,54 In diabetics, the stimulus for ischemic preconditioning of the heart may need to be increased because diabetics have defective phosphatidylinositol 3-kinase (PI3K)-Akt signaling.55,56 The remote limb conditioning protocols require optimization before clinical trial (Table 2). Although most clinical trials of ischemic preconditioning to date have used tourniquets or blood pressure cuffs on the arm, the preclinical studies used hindlimb ischemia as the stimulus. The greater mass of ischemic muscle in the leg compared with the arm may be important to generate a sufficient conditioning effect, especially in aged humans with comorbidities. Most preclinical studies and clinical trials have used the 4 cycles of 5 minutes of occlusion first described by Murry et al.5 However, subsequent
preclinical limb conditioning studies have performed unilateral or bilateral femoral ischemia of varying time durations and cycle number (Table 1). Factors such as the total duration of occlusion (5–15 minutes) and the number of cycles need further optimization. Moreover, combining preconditioning with additional postconditioning may enhance the neuroprotective effects. Adding daily limb postconditioning to postconditioning in the heart led to added benefit with improved left ventricular remodeling and reduced mortality.97

There is also a need for biomarkers of the conditioning response in humans. Putative biomarkers include CBF, autocoids, such as adenosine, bradykinin, and endogenous opioids, anti-inflammatory and proinflammatory cytokines, NO and nitrite, and other yet to be defined humoral factors. These would help ensure that the threshold for a conditioning response has been reached with the limb conditioning regimens. Finally, because remote limb conditioning targets reperfusion injury, the target population in acute stroke clinical trials should only include patients with a high likelihood of reperfusion, either with IV tPA or with mechanical intraarterial interventions.

Conclusions

Remote limb conditioning represents a promising, safe, well-tolerated, feasible, and low-cost therapy to target reperfusion injury. In clinical trials to date in patients undergoing cardiac and vascular procedures, there are hints of activity and protection. To increase the chance for success of future acute stroke clinical trials, further preclinical testing is needed according to STAIR criteria. Because of its feasibility, low cost, and safety, remote limb conditioning could become a routine procedure in the prehospital setting, in emergency departments, in rural and community hospitals, and in neurological intensive care units. However, it is important that clinical trials be based on solid and rigorous preclinical testing, and that the trial design uses optimized conditioning protocols.

Table 2. Optimization of Protocols of Remote Limb Conditioning

<table>
<thead>
<tr>
<th>Site(s) of limb conditioning</th>
<th>Timing and frequency</th>
<th>Perconditioning</th>
<th>Postconditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arm</td>
<td>Prehospital</td>
<td>In-hospital, before or concurrent with IV tPA or IA intervention</td>
<td></td>
</tr>
<tr>
<td>Leg</td>
<td></td>
<td>Immediately on reperfusion, 3 h, 6 h, 24 h, Repeated dosing daily</td>
<td></td>
</tr>
<tr>
<td>Bilateral leg</td>
<td></td>
<td>Combination of per- and postconditioning</td>
<td></td>
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</tbody>
</table>

IPA indicates tissue plasminogen activator.

Acknowledgments

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Disclosures

None.

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