Remote Ischemic Limb Preconditioning After Subarachnoid Hemorrhage
A Phase Ib Study of Safety and Feasibility

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Background and Purpose—Making a limb transiently ischemic has been shown to induce ischemic tolerance in a distant organ. This phenomenon is known as remote ischemic limb preconditioning. We conducted a Phase IB study of remote ischemic limb preconditioning to determine the safety and feasibility of increasing durations of limb ischemia in patients with subarachnoid hemorrhage.

Methods—Patients with aneurysmal subarachnoid hemorrhage underwent limb preconditioning every 24 to 48 hours for 14 days. Limb preconditioning consisted of 3 5-minute inflations of a blood pressure cuff to 200 mm Hg around a limb followed by 5 minutes of reperfusion. In the lead-in phase, we preconditioned the upper extremities, but this proved impractical and we began preconditioning the leg in a similar manner. Ischemia times were then escalated to 7.5 and 10 minutes. After each session, a visual analog scale was obtained and the extremity examined for neurovascular complications.

Results—A total of 33 patients completed the study. Mean age was 53±12 years and mean Hunt Hess score was 2.4±0.9. In the lead-in phase, an average of 7.7±2.4 preconditioning sessions was completed with mean visual analog scale 3.6±3.4. In the dose escalation phase, an average of 8.6±2.1 preconditioning sessions was done with mean visual analog scale 1.8±2.2 and 2.5±2.9 for the 7.5- and 10-minute cohorts, respectively. No session was prematurely terminated due to subject discomfort. No objective signs of neurovascular injury were observed.

Conclusions—We found limb preconditioning to be safe and well tolerated, even at ischemia times of 10 minutes, in critically ill patients with subarachnoid hemorrhage. (Stroke. 2011;42:1387-1391.)

Key Words: delayed cerebral ischemia ■ limb preconditioning ■ remote ischemic preconditioning

Transient exposures to mild ischemia render the brain resistant to subsequently more severe ischemic injury. This is known as ischemic preconditioning and is one of the most powerful anti-ischemic strategies in laboratory models. Similarly, inducing ischemia in one organ also leads to ischemic tolerance in a different, distant organ. A simple example of this phenomenon, referred to as remote ischemic limb preconditioning, is that animals undergoing brief limb ischemia have smaller infarcts than animals not exposed to this intervention before stroke.1-2 Preconditioning induces multiple endogenous anti-ischemia defense mechanisms. These include an upregulation of nitric oxide, improved cerebral blood flow in the ischemic penumbrae, and down-regulation of inflammation and glutaminergic excitotoxicity.3,4 With ischemic preconditioning, cellular energy consumption is optimized and becomes more efficient, rendering the cell more resistant to ischemia.5-8

Only a few ischemic preconditioning trials have been completed. Brief cycles of arm ischemia before cardiovascular surgery or percutaneous cardiac intervention resulted in a reduction of periprocedural myocardial ischemia, fewer myocardial infarctions, and fewer strokes.9-12 However, the therapeutic potential of preconditioning has remained largely unexplored in cerebrovascular disease. Only one randomized trial assessed the effect of two 10-minute cycles of leg ischemia before carotid endarterectomy. The intervention resulted in a statistically nonsignificant reduction of saccadic latency, a surrogate marker for hemispheric ischemia.13

Clinical settings such as carotid revascularization may be very suitable for a study of preconditioning. The risk of cerebral ischemia occurs over a short, well-defined period of time, allowing a preconditioning response to be elicited. Other settings frequently proposed for preconditioning include cardiac bypass surgery or subarachnoid hemorrhage with the ensuing risk of delayed cerebral ischemia (DCI).5,14,15 The high morbidity and mortality associated with DCI has made the investigation of innovative treatment approaches a priority in subarachnoid hemorrhage research.

We report on our preliminary experience with ischemic limb preconditioning after subarachnoid hemorrhage. The scientific rational for such a study is to determine if daily limb preconditioning after subarachnoid hemorrhage induces is-
chemic tolerance and protects from symptomatic vasospasm and DCI. Because the optimal duration of limb ischemia for preconditioning and the safety and tolerability of daily repetitive limb preconditioning in predominantly conscious but critically ill patients is uncertain, we aimed to establish both of these parameters in a preliminary trial of safety and feasibility. The purpose of this particular study was to assess the safety and feasibility of repetitive limb preconditioning and increasing durations of limb ischemia in patients with recent subarachnoid hemorrhage.

**Methods**

The study was conducted at a large community-based teaching hospital, which serves as a referral center for patients with subarachnoid hemorrhage. We enrolled subjects with acute subarachnoid hemorrhage due to a ruptured cerebral aneurysm, secured by either coiling or surgical clipping, within 96 hours from symptom onset. Subjects with the following were excluded: (1) impaired prehospitalization functional status defined as modified Rankin Scale score >2; (2) subarachnoid hemorrhage from a lesion other than cerebral aneurysm; (3) Hunt Hess scale >4 at presentation; (4) history of peripheral vascular disease and ankle brachial index <0.7; (5) any extremity soft tissue, orthopedic, or vascular injury which in the clinical judgment of the investigator may pose a contraindication to preconditioning, for example, superficial wounds, cellulitis, fracture, deep vein thrombosis; (6) patients with symptomatic vasospasm at the time of enrollment; (7) neurogenic pulmonary edema or cardiac failure requiring inotropic support; or (8) severe or unstable concomitant chronic condition or disease.

All patients received standard treatment for subarachnoid hemorrhage, cerebral vasospasm, and DCI. In addition, all subjects underwent limb preconditioning throughout the study period for cerebral vasospasm.

**Limb Preconditioning Protocol**

Subjects underwent a limb preconditioning session every 24 to 48 hours from the time of enrollment to Day 14 (or discharge if before Day 14). We initially induced ischemia in the upper extremity according to a method shown to protect the myocardium during cardiac surgery. A blood pressure cuff was placed over the arm and inflated to 200 mm Hg (or 20 mm Hg above systolic pressure if pressure >200 mm Hg). The cuff remained inflated for 5 minutes followed by 5 minutes of reperfusion. Each preconditioning session consisted of 3 5-minute cycles of ischemia/reperfusion. We also included a control group who underwent a sham preconditioning procedure. Sham preconditioning consisted of minimal inflation to systolic pressure 30 mm Hg, just enough to cause some pressure on the extremity. Subjects were initially randomly assigned to true or sham preconditioning. After completion of each preconditioning session, subjects were asked (if possible) to rate pain on a visual analog pain scale (VAS), which ranges from 0 (no pain) to 10 (worst imaginable pain). After each session, the extremity was examined for tissue breakdown or signs of neurovascular injury.

The lead-in phase of the study describes our initial experience with the first 21 subjects. After enrolling the first 6 subjects, we had to modify our protocol because upper extremity preconditioning proved impractical due to the number of intravenous lines and continuous drips required by critically ill patients. We subsequently preconditioned the lower extremity in an identical manner; a large cuff (45.0 to 56.5 cm) was placed over the upper thigh and inflated to 200 mm Hg (or 20 mm Hg above systolic pressure if systolic pressure was >200 mm Hg) with confirmation of loss of pedal pulsations by palpation. An ankle brachial index was performed to screen for peripheral vascular disease and patients with an index <0.7 were excluded. In subjects with leg paresis, the nonparetic leg was preconditioned.

After completing the lead-in phase, we began the dose escalation phase of the study. The objective was to determine if limb ischemia times of 10 minutes were tolerated and safe. The rational for this was that almost all clinical studies to date have used limb ischemia times of 5 minutes, whereas preclinical data suggest that longer ischemia times of 10 to 15 minutes induce cerebral protection. Escalation proceeded in 2 cohorts of 6 patients. The first cohort assessed ischemia times of 7.5 minutes and the second tier ischemia times of 10 minutes.

**Safety Monitoring**

We defined the following as primary safety end points: (1) VAS >8 and patient unwillingness to proceed with the preconditioning intervention; (2) objective signs of tissue or neurovascular injury felt to be due to cuff inflation, which in the judgment of the investigators’ precluded continuation. This included palpation of distal pedal pulses, visual inspection (for edema, redness, skin breakdown), and palpation for tenderness; and (3) development of any symptomatic and asymptomatic deep vein thrombosis in the preconditioned extremity. Lower extremity ultrasound was obtained only if clinically indicated by the attending neurocritical care team.

Subjects were monitored for cerebral vasospasm and delayed cerebral ischemia. We defined vasospasm by transcranial Doppler as mild (mean flow velocities >120 cm/s) and severe (mean flow velocity >200 cm/s) with Lindegaard ratio >3. Symptomatic vasospasm was defined as the development of a new neurological deficit or deterioration in level of alertness (Glasgow Coma Scale reduction ≥2 points) attributable to vasospasm with exclusion of other potential complications (hydrocephalus, seizures, fever, metabolic derangement).DCI was defined as symptomatic vasospasm or in patients whose neurological examination is unreliable (lethargy, stupor) as new areas of infarction on CT or MRI attributable to vasospasm. A modified Rankin Scale score was obtained at 3 months by telephone interview.

Written informed consent was obtained from all subjects (or proxies) and the study was monitored by the local Institutional Review Board and a safety monitoring committee.

**Statistical Analysis**

Statistical analyses include summary statistics presented as mean and SD for continuous variables and frequency and percentages for categorical variables. Between-group comparisons were made with analysis of repeated measures with significance set as P<0.05. Postanalysis of variance was done with Bonferroni multiple comparisons tests. Categorical variables were compared by means of a χ² test or Fisher exact test as appropriate. Analysis was performed with SPSS Version 17 (Chicago, IL).

**Results**

A total 34 patients were enrolled. One subject refused continued study participation due to headaches. The baseline characteristics of the 34 subjects are outlined in Table 1. The most frequent site of aneurysm was the anterior communicating artery (46%) followed by the posterior communicating artery (21%) and middle cerebral artery (12%). During the course of the study, mild transcranial Doppler vasospasm was
found in 22 of 33 (67%). Severe transcranial Doppler vasospasm was diagnosed in 8 of 33 (24%) and DCI in 6 of 33 (18%). Two subjects (6%) died during the course of the study. The mean National Institutes of Health Stroke Scale score at 14 days was 1.7±2.6. The mean 3-month modified Rankin Scale score was 1.3±1.4.

**Lead-In Phase**
In our initial experience, we enrolled 21 subjects. The first 6 subjects (including 3 with sham preconditioning) underwent arm preconditioning. Arm preconditioning proved impractical because monitoring devices and intravenous lines interfered with our ability to induce limb ischemia. The next 15 subjects (including 4 with sham procedure) underwent leg preconditioning, which proceeded without difficulties. Those subjects had on average 7.7±2.4 preconditioning sessions. In full treatment patients who could respond, the mean VAS per patient session was 3.6±3.4. For sham-conditioned patients, the VAS was 0. No session was prematurely terminated due to subject discomfort. No objective signs of neurovascular injury were observed with no subjects experiencing skin breakdown, prolonged discoloration, or temperature or pulse disparities in the treated limb. Among the 21 patients, 9 lower extremity Duplex studies were obtained. Deep vein thrombosis was found in 3; 2 were asymptomatic, 1 in a patient undergoing arm preconditioning and 1 in the leg of a patient who had the contralateral leg preconditioned. These were felt to be unrelated to the intervention. An asymptomatic partial occlusion occurred in 1 patient in the preconditioned leg.

**Dose Escalation Phase**
We then escalated limb ischemia times to 7.5 and 10 minutes, respectively, in cohorts of 6 subjects in each tier. Those subjects underwent an average of 8.6±2.1 preconditioning sessions. In those able to respond, the VAS was 1.8±2.2 and 2.5±2.9 for the 7.5- and 10-minute cohorts, respectively. The VAS was higher in the 5-minute ischemia group, but this did not differ significantly from the other 2 groups. No session was prematurely terminated due to subject discomfort. No objective signs of neurovascular injury were observed. There were no cases of deep vein thrombosis.

**Outcome Data**
The main focus of our study was safety and subject tolerability of the procedure, but we explored a potential efficacy signal. Table 2 shows stroke severity and transcranial Doppler data among the 4 groups. Given the small sample size, imbalances between the groups due to chance are expected and were found. Although none of the group differences were statistically significant, the 10-minute limb ischemia group was older, more likely to have hypertension, and had a higher modified Rankin Scale score at 3 months. In that group, the 3-month outcome data were influenced by a smaller sample size because 1 patient could not be contacted (the only loss of follow-up in the study) and by the death of 1 subject (81 years old). Excluding that outlier, the 3-month modified Rankin Scale score (n=4) was 1.5±1.

**Discussion**
Ischemic preconditioning was first demonstrated in the myocardium where transient occlusions of a canine coronary artery resulted in a decrease in eventual myocardial infarct size after permanent coronary occlusion. It is now well recognized to confer ischemic protection across many different organs and species. In the myocardium, ischemic preconditioning remains one of the most powerful protective strategies with reductions in infarct size between 50% and 70%.

Similar tolerance to ischemia is also achieved in the brain. Animals pretreated with 2 minutes of global ischemia showed marked protection against CA1 neuronal loss after 5 minutes of global ischemia. Simulating human stroke more closely are mouse models that show brief occlusions of the middle cerebral artery decrease final infarct size after temporary or permanent middle cerebral artery occlusion. Cross-tolerance is also observed between different organs. Pretreatment with 15-minute bilateral forelimb ischemia resulted in a 54% increase in normal-appearing CA1 neurons in comparison to an untreated control group in an asphyxial cardiac arrest model.

Rendering a limb transiently ischemic to protect a distant organ such as the brain is of great clinical importance because this can be readily and noninvasively achieved in humans. A study of 82 patients undergoing abdominal aneurysm repair showed that 2 10-minute cycles of intraoperative crossclamping of the iliac artery led to a 27% reduction in troponin, 22% a decrease in myocardial infarction, and 23% reduction in renal impairment. In 2 studies of patients undergoing coronary artery bypass surgery, myocardial injury was reduced in patients who underwent 3 cycles of arm ischemia, induced with a blood pressure cuff, just before surgery. Perioperative troponin levels were decreased by 43% in patients preconditioned in this manner. In 242 subjects, 3 5-minute cycles of arm ischemia before percutaneous cardiac intervention has been associated with reduced troponin re-

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**Table 2. Clinical Findings by Group**

<table>
<thead>
<tr>
<th>Age, Years</th>
<th>Hypertension, No. (%)</th>
<th>Hunt Hess</th>
<th>TCD Spasm Mild, No. (%)</th>
<th>TCD Spasm Severe, No. (%)</th>
<th>DCI, No. (%)</th>
<th>3-Month mRS Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 minute, n=7</td>
<td>49±29</td>
<td>1 (14)</td>
<td>2.7±0.5</td>
<td>4 (57)</td>
<td>0 (0%)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>5 minutes, n=14</td>
<td>53±14</td>
<td>6 (43)</td>
<td>2.4±1.0</td>
<td>9 (64)</td>
<td>4 (29)</td>
<td>1.6±1.8</td>
</tr>
<tr>
<td>7.5 minutes, n=6</td>
<td>47±12</td>
<td>2 (33)</td>
<td>2.5±1.0</td>
<td>5 (83)</td>
<td>2 (33)</td>
<td>1 (17)</td>
</tr>
<tr>
<td>10 minutes, n=6</td>
<td>62±10</td>
<td>5 (83)</td>
<td>2.2±1.2</td>
<td>4 (67)</td>
<td>0 (0%)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

TCD indicates transcranial Doppler; DCI, delayed cerebral ischemia; mRS, modified Rankin Scale.
lease, less electrocardiographic ST elevation, and fewer major adverse cardiovascular events.11 However, in a recent study with a larger population, a protective effect of remote ischemic preconditioning before coronary surgery could not be confirmed.27

Only a few clinical reports have assessed the preconditioning potential of the brain. In a small study of 12 patients undergoing cerebral aneurysm clipping for subarachnoid hemorrhage, direct preconditioning by temporary occlusion of a cerebral artery attenuated tissue hypoxia during subsequent prolonged arterial occlusion.28 A small case series reported that during carotid stenting, patients who became symptomatic at the time of carotid occlusion were able to tolerate subsequent occlusions during the procedure without any recurrent symptoms.29 In 70 patients randomized to 2–10minute cycles of leg ischemia or sham preconditioning before endarterectomy, there was a nonsignificant reduction of saccadic latency (a surrogate for hemispheric injury) in patients who were preconditioned (32% versus 53%).13

Several questions remain regarding the best method to induce a preconditioning response. The optimal duration of limb ischemia remains uncertain. Is preconditioning a leg better than preconditioning an arm? The potentially larger volume of tissue affected by preconditioning a leg may translate into a greater biological effect. Clinical studies in cardiac medicine have examined 5-minute cycles of arm ischemia,19,11,126 However, it is not clear if this is the optimal method to induce a preconditioning response. In laboratory studies, limb ischemia times have typically exceeded 5 minutes (in the range of 10 to 30 minutes) and the hind leg of the animal was preconditioned.1,2,25,30−32

These questions led us to report our preliminary experience with limb preconditioning for neuroprotection. We were particularly concerned if repetitive and longer limb ischemia times, approaching durations achieved in laboratory animals, would be tolerated safely by patients with subarachnoid hemorrhage. Previous studies of limb preconditioning induced limb ischemia immediately before surgery with subjects already under general anesthesia. Our patient population was predominantly conscious, which raised additional concerns about potential tolerability. Our experience strongly suggests that this is the case. We did not find a single case of objective neurovascular injury and at no time did a subject refuse continued participation due to pain or discomfort.

In conclusion, we report the first clinical experience with remote ischemic preconditioning after subarachnoid hemorrhage. We found that a trial of repetitive ischemic limb preconditioning is feasible, safe, and well tolerated in awake patients with subarachnoid hemorrhage. The safety of limb preconditioning at ischemia times of at least 10 minutes needs further assessment in larger clinical trials.

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Disclosures
None.

References


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