Effects of Postconditioning on Neurogenesis and Angiogenesis During the Recovery Phase After Focal Cerebral Ischemia

Elga Esposito, PhD; Kazuhide Hayakawa, PhD; Takakuni Maki, MD, PhD; Ken Arai, PhD; Eng H. Lo, PhD

Background and Purpose—Postconditioning may be a clinically feasible way to protect the brain after a stroke. However, its effects during the recovery phase post stroke remain to be fully elucidated. Here, we examine the hypothesis that ischemic postconditioning amplifies neurogenesis and angiogenesis during stroke recovery.

Methods—Male Sprague–Dawley rats were subjected to 100-minute transient middle cerebral artery occlusion (MCAO) or postconditioning (100-minute middle cerebral artery occlusion plus 10-minute reperfusion plus 10-minute reocclusion). After 2 weeks, infarct volumes, behavioral outcomes, and immunohistochemical markers of neurogenesis and angiogenesis were quantified.

Results—Postconditioning significantly reduced infarction and improved neurological outcomes. Concomitantly, brains subjected to postconditioning showed an increase in doublecortin/BrdU and collagen-IV/Ki67-positive cells.

Conclusions—These results suggest that therapeutic effects of postconditioning may involve the promotion of neurogenesis and angiogenic remodeling during the recovery phase after focal cerebral ischemia.

Key Words: brain ischemia • infarction • ischemic postconditioning • neurogenesis • stroke

In a recent consensus workshop, leaders in the innate tolerance field identified postconditioning as a potentially powerful and clinically relevant approach for stroke. Experimental studies suggest that ischemic postconditioning interferes with cell death mechanisms and reduces infarction during the acute phase after focal cerebral ischemia.

In previous studies, 2 key animal models of ischemic postconditioning were mainly used. The permanent distal occlusion of the middle cerebral artery (MCA) followed by a series of occlusion of both common carotid arteries and a 100-minute MCA occlusion followed by 10 minutes of reperfusion and 10 minutes of reocclusion. Ischemic postconditioning was also investigated in animals subjected to global ischemia induced by occlusion of the common carotid arteries and of the 2 vertebral arteries, 4-vessel occlusion, followed by different cycles of noninjurious common carotid artery occlusion.Taken together, these studies suggest that postconditioning may offer positive protective effects during the acute phase of stroke. However, how postconditioning may provide longer lasting benefits during stroke recovery remains unclear.

During the recovery phase after stroke, an increase in angiogenesis and neurogenesis occurs. It has been proposed that these neurovascular responses provide the required signals and substrates for plasticity and remodeling as damaged brain tissue attempts to reorganize and recover. In this proof-of-concept study, we used a rat model of focal cerebral ischemia to assess the hypothesis that the beneficial effects of postconditioning may involve the amplification of neurogenesis and angiogenesis during the delayed periods after initial injury.

Methods

Middle Cerebral Artery Occlusion and Ischemic Postconditioning

All experiments were performed following protocols approved by Massachusetts General Hospital Institutional Animal Care and Use Committee in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. We used 2 groups of stroke animals: one group exposed to postconditioning and the other group exposed to standard poststroke care. Transient focal ischemia was induced by introducing a 5.0 surgical monofilament nylon suture (Doccol) into the MCA for 100 minutes in male Sprague–Dawley rats. Ischemic postconditioning was induced as previously described. Briefly, the postconditioning technique involves 100 minutes of occlusion, and then reperfusion was established for 10 minutes after which the MCA was reoccluded for another 10 minutes.

To analyze cell renewal processes, BrdU was dissolved in PBS at 10 mg/mL and injected intraperitoneally to a dose of 50 mg/kg every second day for 2 weeks. Animals were recovered for 14 days. Neurological scores were graded on a scale of 0 to 4 with a higher score indicating more severe sensory-motor deficits. All procedures and measurements were performed in a blinded and randomized manner.

Received February 9, 2015; final revision received June 10, 2015; accepted June 17, 2015.

From the Departments of Neurology and Radiology, Massachusetts General Hospital, Harvard Medical School, Boston.

Guest Editor for this article was Miguel A. Perez-Pinzon, PhD.

Correspondence to Eng H. Lo, PhD, or Elga Esposito, PhD, Massachusetts General Hospital, E Bldg 149, 13th St, Charlestown, MA 02129. E-mail lo@helix.mgh.harvard.edu or esposito@partners.org

© 2015 by American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.115.009070
Physiological parameters and regional cerebral blood flow did not change between the 2 groups (data not shown).

Histology and Immunohistochemistry

Infarction volumes were quantified on Nissl-stained sections using the indirect morphometric method. Immunohistochemistry was performed as described before. To assess microvessel remodeling, double labeling of anti-type IV Collagen (1:10; SouthernBiotech) with anti-Ki67 (1:500; Abcam; a general cell proliferation marker) was pursued as a surrogate marker of angiogenic-related events. To study neurogenic-related events, we double stained anti-DCX (1:100; Abcam) with anti-BrdU (1:50; Invitrogen) as a surrogate marker of neurogenesis. To clarify that Ki67-positive cells are not proliferating microglia/macrophages, we double stained Ki67 and Iba1.

Statistical Analysis

Values are expressed as mean±SD. Infarct volumes and cell counts of immunopositive cells were assessed with Student’s t test. Neurological outcomes were analyzed using Mann–Whitney test. P<0.05 were considered statistically significant.

Results

At 2 weeks, Nissl staining revealed well-defined infarcts in all control animals subjected to 100 minutes of MCA occlusion (181.9±17.68 mm³, n=8). In rats that were subjected to ischemic postconditioning, infarct volumes were markedly reduced (132.8±10.74 mm³, n=9, P<0.05; Figure 1A). Postconditioning had also a positive effect on neurological outcomes. Rats treated with postconditioning had significantly better scores (0.4±0.4, n=9) compared with controls (1.2±0.7, n=8; Figure 1B).

Markers of neurogenesis were analyzed in the peri-infarct regions at 2 weeks. Immunohistochemistry demonstrated that enhanced signals for DCX/BrdU were detected in the ipsilateral hemisphere. Compared with untreated controls (19.67±5.5 positive cells/mm²), DCX/BrdU-positive cells appeared to be increased in animals subjected to ischemic postconditioning (53±10.5 positive cells/mm²; Figure 2).

As a marker for angiogenesis, immunostaining was performed to quantify microvessels that were double positive for collagen-IV and Ki67. As expected, peri-infarct regions appeared to show an increase in microvessels. The density of collagen-IV-Ki67 microvessels was significantly higher in the postconditioning group (80.74±22.49 positive cells/mm²) compared with controls (30.09±14.07 positive cells/mm²). We did not detect any increase in Ki67/Iba1 double staining in postconditioning group compared with controls, suggesting that microglia may not provide a large contribution to our signals (Figure 3).
Discussion

After brain injury, tolerance mechanisms are activated as part of the endogenous neuroprotective program.\(^7,8\) It is increasingly recognized that finding ways to boost these endogenous mechanisms may provide novel avenues for stroke therapy.\(^1,9\) Accumulating studies in various experimental models now suggests that ischemic postconditioning may provide acute protection.\(^10\) But what may be missing is a full understanding of the mechanisms responsible for postconditioning and long-term neuroprotection. In this proof-of-concept study, we used a rat model of transient focal ischemia to confirm that beneficial effects of postconditioning may last for up to 2 weeks. Our main goal in this study was to show that potential benefits of postconditioning were accompanied by augmentation of neurogenic- and angiogenic-related markers in recovering brain tissue.

Ischemic brain insults potently stimulate progenitor proliferation in both the subgranular zone and subventricular zone of adult rodents.\(^11\) Neuron progenitors are then able to migrate to injury sites, perhaps as part an endogenous repair response after stroke and brain injury. Similarly, angiogenesis, that is, the growth of blood vessels from the existing vasculature, may also contribute to the recovery phase after stroke. Proangiogenic genes are upregulated within minutes of the onset of cerebral ischemia in rodents,\(^12\) and within the peri-infarct zone, angiogenesis may significantly participate in neurovascular remodeling and recovery. Increasingly, it has been suggested that delayed effects of many stroke therapies may involve the augmentation of neurogenesis and angiogenesis.\(^13\) Our findings here raise the possibility that postconditioning may also recruit these endogenous protective mechanisms.

Taken together, the present study suggests that beneficial effects of ischemic postconditioning can be maintained up to 2 weeks post ischemia, and the underlying mechanisms may be consistent with improvements in poststroke neurogenesis and angiogenesis. However, there are several caveats to keep in mind. First, we only examined a single protocol for postconditioning. Whether outcomes can be further improved with different doses and timing remains to be tested. Extending the present single and 100-minute poststroke postconditioning regimen closer to or even beyond the 4.5 hours of tissue-type plasminogen activator window may be an important next step. Second, besides neuronal and vascular cells, other cell types may also be involved. For example, immune cells, such as macrophages and microglia, are known to contribute to neurogenic and angiogenic phenomenon. In our study, Ki67-positive cells did not double stain for Iba1, suggesting that postconditioning may not affect this response in our model. However, further studies to assess effects of postconditioning on immune profiles are warranted. Third, sustained clinical benefit must be more rigorously explored. Two weeks end points may be a reasonable timeframe for exploring delayed effects in experimental models. But for translational assurance, longer-term studies are needed and more behavioral studies specific for long-term outcomes should be considered. Finally, this remains a proof-of-concept study and our results cannot prove causality. Whether the markers of neurogenesis and angiogenesis are causative or correlational must be carefully assessed in future gain and loss-of-function experiments. Postconditioning may provide a promising therapeutic approach for stroke. Continued investigation into its potential mechanisms is warranted.

Sources of Funding

This work was supported in part by grants from the National Institutes of Health and the Rappaport Foundation.

Disclosures

None.

References


Effects of Postconditioning on Neurogenesis and Angiogenesis During the Recovery Phase After Focal Cerebral Ischemia
Elga Esposito, Kazuhide Hayakawa, Takakuni Maki, Ken Arai and Eng H. Lo

Stroke. published online August 4, 2015;
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/early/2015/08/04/STROKEAHA.115.009070

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/