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

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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. (Stroke. 2015;46:2691-2694. DOI: 10.1161/STROKEAHA.115.009070.)

Key Words: brain ischemia ▪ infarction ▪ ischemic postconditioning ▪ neurogenesis ▪ stroke
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 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).

**Figure 1.** Ischemic volume and behavioral outcomes. A. Ischemic volume was significantly smaller in postconditioning (Postc) group. B. Neuroscores were decreased after postconditioning treatment compared to controls (CTL; 100 min ischemia). *P<0.05. Mean±SD.

**Figure 2.** Ischemic postconditioning (Postc) increased neurogenesis. DCX/BrdU cell positive number increased in the peri-infarct area after postconditioning treatment indicating that postconditioning activates neurogenesis. *P<0.05. Mean±SD. CTL indicates controls, 100 min ischemia.
Discussion
After brain injury, tolerance mechanisms are activated as part of the endogenous neuroprotective program. It is increasingly recognized that finding ways to boost these endogenous mechanisms may provide novel avenues for stroke therapy. Accumulating studies in various experimental models now suggests that ischemic postconditioning may provide acute protection. 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. 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, 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. 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.

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Disclosures
None.

References


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