The Impact of Ischemic Postconditioning on Ischemic Skin Flap Injuries

Lin Huang, MD

Abstract: Ischemic postconditioning (IPOC) is a useful manipulation to reduce the undesirable effects of ischemia-reperfusion (IR) injury. The effects of IPOC were studied in an axial pattern skin flap model. Methods. The skin flaps of 40 rabbits were randomly divided into four groups. Ischemic postconditioning was performed using six 10-second cycles of repeated ischemia/reperfusion periods. The animals were allocated into four groups: group 1 (control); group 2 (ischemia); group 3 (postconditioning); group 4 (postconditioning 10 minutes later). Flap viability was assessed 1 week after the operation. The surviving flap area was recorded as a percentage of the entire flap area. Fisher’s least significant difference (LSD) test was used for statistical analysis among different groups to evaluate the effects of ischemic preconditioning against ischemia. Results. The mean ± SD of surviving flap areas for groups 1, 2, 3, and 4 were 97.86 ± 0.62, 31.64 ± 1.04, 48.95 ± 0.82, and 30.01 ± 1.12, respectively. Statistical difference did not exist between group 2 and 4, but they were statistically different (P < 0.05) when compared to group 1 or group 3. Conclusion. Ischemic postconditioning has a protective effect on ischemic flaps, but postconditioning should be performed just after the ischemic event.

Ischemia-reperfusion (IR) injury is underlying (partial) flap necrosis. With flap surgery becoming more and more important in reconstructive surgery, new procedures preventing from the flap loss should be provided. Several years ago, the therapeutic strategy of preventing such complications has been focused on improving blood supply and oxygenation in the compromised tissue, whereas, a new concept has emerged over the last few years, which consists of preconditioning or postconditioning the tissue by exposing it to a sublethal degree of environmental stress prior to surgery.

Zhao et al have reported that brief episodes of coronary occlusion and reperfusion at the onset of reperfusion after sustained ischemic insult con-
ferred cardioprotection against IR injury in dogs, known as ischemic postconditioning (IPOC). The author of the present study hypothesized that postconditioning of skin flaps can also minimize flap loss, and designed an experiment to confirm that assumption.

The two goals for the experiment were:
1. To confirm the usefulness of flap postconditioning in minimizing flap loss.
2. To pinpoint one useful postconditioning strategy for better flap survival.

Methods
Forty New Zealand White rabbits weighing 4 kg–5 kg were acclimated to the animal housing facility for at least 48 hours. Rabbits were fed a standard diet of Wayne Rabbit Ration 8600 (Teklad Premier) and were given water ad libitum. Intramuscular injections of LA 200 oxytetracycline (20 mg/kg/day) were given the night before the procedure and once every 7 days thereafter. All animal procedures were approved by the Institutional Animal Care and Use Committee of An Zhen Hospital.

Axial pattern skin flap. The animals were divided into four groups (control group, ischemia group, postconditioning group, and postconditioning 10 minutes later group) with 10 rabbits in each group. Axial pattern island skin flaps were raised on the abdominal wall as Cederna et al. described previously. Abdominal fur was removed with clippers and depilatory cream. A 19 cm² × 15 cm² abdominal cutaneous island flap was elevated in 10 rabbits. The flap consisted of skin, subcutaneous tissue, and panniculus carnosus. All perforators were divided. The only remaining attachment to the flap was the right superficial inferior epigastric pedicle. After flap elevation, a silicon sheet was placed on the muscle bed as a barrier to inhibit vascular invasion and was sutured on the trauma surface with 7-0 polypropylene. Flaps were repositioned and sutured with 6-0 polypropylene suture. Ischemia was induced by clamping the vessel(s) with a microvascular clamp. The same V-type clamp was used both for the artery and the vein.

Experiment protocol.
Group 1: Control group (without ischemia; n = 10). Flaps were prepared as described above and did not undergo induced ischemia.

Group 2: Ischemia group (8-hour ischemia; n = 10). Flaps were prepared and the vascular pedicles were occluded for 8 hours. At the end of the 8-hour ischemia, the animals were anesthetized and the clamps were removed.

Group 3: Postconditioning group (8-hour ischemia + postconditioning; n = 10)

The same procedure in group 2 was performed, but in addition, IPOC was performed just after the induced ischemia. Ischemic postconditioning included 6 cycles of each 10-second ischemia and 10-second reperfusion, which lasted a total of 2 minutes.

Group 4: Postconditioning 10 minutes later group (8-hour ischemia + postconditioning 10 minutes later; n = 10). The entire procedure was similar to group 3, but IPOC was performed 10 minutes after the induced ischemia.

Histologic analysis. Biopsies were collected for histological examination to count cellular infiltration. Tissue samples were taken from the medial border of the flap immediately before dissection. Sections measuring 3.5 mm were cut from the formalin-fixed, paraffin-embedded, full-thickness skin biopsies and dried overnight at 37˚C. All of the sections were evaluated for neutrophil count under x100 microscopy.

Statistical Analysis
Surviving and necrotic parts of the flaps were measured for all groups using a 2-dimensional planimetry in a blinded fashion 1 week after induced ischemia. Surviving portions of the flaps were expressed as a percentage of the entire flap area. One-way analysis of variance was used to compare the significance differences among groups. Significance was set at \( P < 0.05 \).

Results
The mean ± SD surviving areas of the flaps for groups 1 , 2 , 3, and 4 were 97.86 ± 0.62, 31.64 ± 1.04, 48.95 ± 0.82, and 30.01 ± 1.12, respectively (Figure 1). Fisher’s least significant difference (LSD) test was used to compare the difference among group 1, 2, 3, and 4. A statistical difference did not exist between group 2 and 4, but they all had a difference \( (P < 0.05) \) when compared to group 1 or group 3. Table 1 summarizes the surviving flap areas in all groups. The results revealed that IPOC has a protective effect on ischemic flaps, but that the postconditioning should be performed just after the ischemic event. Postconditioning, which was performed more than 10 minutes later, did not effect flap survival.

Discussion
From a plastic surgeon’s point of view, IR injury is a problem in microvascular surgery, especially for free tissue transfer and replantation of amputated body parts.

Reperfusion injury is an inflammatory process modulated by complex signaling mechanisms, which ultimately leads to cell death. Restoration of blood flow is essen-
potential for any flap survival; however, such reperfusion can lead to IR injury via numerous inflammatory pathways.\textsuperscript{11}

Zhao et al.\textsuperscript{9} demonstrated that postconditioning was associated with lower plasma levels of malondialdehyde, a product of lipid peroxidation. Furthermore, the Zhao study found that the tissue content of superoxide, as revealed by dihydroethidium staining, was significantly less in the area at risk (myocardium) of postconditioned dogs.

Taken together, these results suggest reduced production of reactive oxygen species and attenuation of lipid peroxidation as possible mechanisms of the postconditioning phenomenon. The author preferred to use a reliable skin flap model in the present study because skin flaps are usually performed in clinical practice, especially after the popularization of perforator flaps. An epigastric skin flap is easy to elevate and handle, and its pedicle has a suitable size for microvascular clamping.\textsuperscript{12} The author could not find a reliable IPOC protocol because a standard protocol for skin flaps in rabbits does not exist. The postconditioning protocol was safe and found to be effective in the author’s preliminary experiments and in other ongoing studies. The present study attempted to mimic the clinical manufacture in this study by doing 8 hours of ischemia (either arterial or venous). In this setting, we explored the effect of IPOC against IR injury.

The experiment suggested IPOC of the skin flap had the advantage of being a way to influence and modify IR injury after it has occurred. It is important to note that ischemia postconditioning should be executed within no more than 10 minutes. Delayed ischemia (more than 10 minutes later) postconditioning will not work against the IR injury. A possible explanation of the phenomenon is that the damage just after ischemia injury is reversible, but it may be irreversible after the reperfusion begins and lasts longer. The exact mechanism of the injury needs more essential exploration.

**Key Points**

- The experiment suggested IPOC of the skin flap had the advantage of being a way to influence and modify ischemia-reperfusion injury after it has occurred. It is important to note that ischemia postconditioning should be executed within no more than 10 minutes.
- Studying the molecular mechanisms of postconditioning may reveal novel pharmacological strategies to protect skin flaps more effectively.
Conclusion

The usefulness of ischemic postconditioning may be a therapeutic alternative in situations of unexpected and uncontrolled ischemic injury, for example, in situations where complications occur during surgery that turn a simple procedure into a complicated one. Furthermore, studying the molecular mechanisms of postconditioning may reveal novel pharmacological strategies to protect skin flaps more effectively.

References

Table 1. Surviving flap portion (%) 1 week after the surgical procedure

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Group 1 (without ischemia)</th>
<th>Group 2 (8-hour ischemia)</th>
<th>Group 3 (8-hour ischemia + IPOC)</th>
<th>Group 4 (8-hour ischemia + IPOC 10 min. later)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>95.20</td>
<td>35.10</td>
<td>45.10</td>
<td>31.80</td>
</tr>
<tr>
<td>2</td>
<td>96.30</td>
<td>25.90</td>
<td>50.50</td>
<td>30.50</td>
</tr>
<tr>
<td>3</td>
<td>100.00</td>
<td>31.20</td>
<td>46.20</td>
<td>31.00</td>
</tr>
<tr>
<td>4</td>
<td>100.00</td>
<td>26.20</td>
<td>48.00</td>
<td>35.50</td>
</tr>
<tr>
<td>5</td>
<td>98.40</td>
<td>32.50</td>
<td>47.30</td>
<td>30.40</td>
</tr>
<tr>
<td>6</td>
<td>99.30</td>
<td>35.10</td>
<td>51.30</td>
<td>25.60</td>
</tr>
<tr>
<td>7</td>
<td>97.20</td>
<td>31.60</td>
<td>52.50</td>
<td>28.50</td>
</tr>
<tr>
<td>8</td>
<td>95.10</td>
<td>33.50</td>
<td>48.40</td>
<td>26.50</td>
</tr>
<tr>
<td>9</td>
<td>97.10</td>
<td>34.00</td>
<td>52.40</td>
<td>34.80</td>
</tr>
<tr>
<td>10</td>
<td>100.00</td>
<td>31.30</td>
<td>47.80</td>
<td>25.50</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>97.86 ± 0.62</td>
<td>31.64 ± 1.04</td>
<td>48.95 ± 0.82</td>
<td>30.01 ± 1.12</td>
</tr>
</tbody>
</table>

LSD test was used to compare the difference among group 1, 2, 3, and 4. Statistical difference did not exist between group 2 and 4, but were statistically different when compared to group 1 or group 3.

IPOC: ischemic postconditioning