Survival of normothermic microvascular flaps after prolonged secondary ischemia: Effects of hyperbaric oxygen

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Although hyperbaric oxygen has been shown to improve the survival rate of ischemic grafts and flaps of many types, it has not been studied extensively in free tissue transfer. This study was designed to evaluate the effect of hyperbaric oxygen on flap survival when exposed to critical combinations of primary ischemia, reperfusion, and secondary ischemia times. Unilateral abdominal adipocutaneous island flaps based on the superficial inferior epigastric vessels were raised in 133 Sprague-Dawley rats. Primary normothermic ischemia was induced by applying a microvascular clamp to the vascular pedicle for 6 hours. The clamp was removed for 2 hours of reperfusion and then reapplied for a 6-, 10-, or 14-hour period of secondary ischemia. After completion of the secondary ischemia time, the clamp was removed, and the animals were randomly assigned to one of three treatment regimens. The control animals breathed normobaric air, and the others breathed normobaric 100% oxygen or hyperbaric oxygen (100% oxygen at the equivalent of 33 feet of seawater, 2.0 atmospheres absolute), respectively, for two periods of 90 minutes for 7 days. Flap survival was assessed at postoperative day 17 and was found to be an all-or-none phenomenon. Maximum likelihood-derived survival curves were fitted to the data and used to calculate the secondary ischemic time at which 50% of the flaps survived (D50). The D50 for the air and 100% oxygen groups was 6 hours, whereas that for the hyperbaric oxygen group was 10 hours. This difference in D50 was found to be statistically significant (analysis of variance, p < 0.05). Chi-squared statistical evaluation of pooled data reaffirmed a statistically significant increase in flap survival of the animals treated with hyperbaric oxygen vs. those treated with air or 100% oxygen (p < 0.03 and p < 0.01, respectively). Hyperbaric oxygen enhances the tolerance of normothermic, microvascular flaps to prolonged secondary ischemia. A similar effect was not noted in the 100% oxygen group; therefore, the additional expense and technology of a hyperbaric chamber system is necessary to achieve this effect. (Otolaryngol Head Neck Surg 1996;115:360-4.)

**Free tissue transfer techniques have allowed more functional and aesthetic reconstruction of defects in the head and neck region. Despite dramatically improved technique, the failure rate remains between 4% and 20%, resulting in significant rates of morbidity at both donor and recipient sites.** Inherent in the harvesting of a free tissue flap is a primary ischemic time as the tissue is separated from its native blood supply. After transfer, microvascular anastomosis allows reperfusion. Thrombosis, edema, or a kinking of the vessel can interrupt blood flow to the flap. The tissue is, in this case, exposed to a secondary ischemic time and may become compromised. The critical ischemia time (CIT) is the maximum length of time that a tissue can be subjected to ischemia and yet remain viable once the circulation is restored. A previous study has demonstrated the relationship of the CIT to the preceding primary ischemic time and reperfusion time in a rat skin flap model. Tolerance of flaps to secondary ischemic time was
reduced by prolonged primary ischemic times or shortened reperfusion time preceding the onset of secondary ischemia.

Hyperbaric oxygen (HBO) has been shown to effectively improve the survival of ischemic grafts and flaps of many types; however, it has not been studied extensively in application to free tissue transfer. This study was designed to evaluate the effect of HBO on flap survival after exposure to critical combinations of primary ischemia, reperfusion, and secondary ischemia times. It allows for some conclusions concerning the dose response to oxygen and whether the additional technology and expense of a hyperbaric chamber is necessary to achieve an effect.

METHODS AND MATERIAL

One hundred fifty mature female Sprague-Dawley rats weighing between 200 and 250 g were used in this experiment in accordance with the “Guide for the Care and Use of Laboratory Animals” (NIH publication no. 86-23, 1978) and the “Principles of Laboratory Animal Care,” formulated by the National Society for Medical Research, and in conjunction with the veterinarians of the Naval Medical Research Institute. All protocols used in this study were approved by National Naval Medical Center animal care and use committee. The animals were anesthetized with intraperitoneal injections of ketamine hydrochloride (30 mg/kg) and xylazine (12 mg/kg). The skin was shaved and prepared with Betadine, but no depilatory agent was used. A 6 x 3 cm unilateral abdominal adipocutaneous island flap based on the superficial inferior epigastric artery and vein was then raised in the right groin following the surgical method of Strauch and Murray. Six hours of primary ischemia was induced with 2-V microvascular clamps applied to the pedicle midway along its course to occlude the epigastric artery and vein. Visual confirmation of the interruption of flow was obtained under the operating microscope. The flap was then stapled back to its native position. At the end of the 6-hour primary ischemic time, the inferior portion of the flap was raised, and the clamp was removed. The raised portion of the flap was restapled to its bed for the 2-hour period of reperfusion and then lifted to reapply the microvascular clamp to the epigastric pedicle for the duration of the secondary ischemic time. Secondary ischemic times were 6, 10, or 14 hours long. These times were chosen to afford a spectrum of expected flap necrosis based on our experience with this animal model. Because of the high rate of flap necrosis at 14 hours, a second series of 36 animals was performed rather than extending to 18 hours as originally planned. After completion of the secondary ischemia period, the inferior portion of the flap was raised for the final time, the clamp was removed, and the flap was stapled back into position.

The animals received three doses of ceftazidime (50 mg/kg) for prophylaxis against *Pseudomonas aeruginosa*, which causes sepsis and early postoperative death in this animal model. To prevent self-mutilation of the flaps, the animals were sprayed with a nontoxic pepper solution (Chewguard; Summit Hill Laboratories, Navesink, N.J.) twice per day to the surgical site.

After completion of the secondary ischemic period, the animals were randomly assigned to three different groups. Group 1 (control) breathed ambient air throughout the observation period. Group 2 animals were treated with 100% oxygen delivered into an unpressurized hyperbaric chamber (Bethlehem Steel Corp., Bethlehem, Pa.) for 90 minutes twice per day for 7 days, and group 3 animals received HBO treatments (100% oxygen at 2.0 atmospheres absolute [ATA]) at the same time intervals and duration. A calibrated oximeter (model OM-11; Beckman Instruments, Inc., Fullerton, Calif.) ensured 100% oxygen within the chamber, and chamber ventilation at 15-minute intervals prevented carbon dioxide accumulation.

Seven days after surgery the flaps were assessed and found to be either well-healed, pink, and supple or dark, contracted, and indurated. No flaps were equivocal. All of the pedicules were found to be patent when assessed by observation and incision.

Hill equation survival curves (see following equation) were fitted to the data with the maximum likelihood method. Using these curves, for each group we determined secondary ischemia times with 95% confidence limits at which 50% of the flaps failed. These values were compared by use of analysis of variance.

\[ P_{survival} = \frac{\text{Dose}}{\text{Dose} + D_{50}} \]

\[ = \frac{1}{1 + (D_{50}/\text{Dose})^m} \]

where \( P_{survival} \) is the probability of flap survival, \( \text{Dose} \) is the length of secondary ischemic time, \( m \) is the calculated power for the best curve fit, and \( D_{50} \) is the calculated length of secondary ischemic time where flap survival will be 50%.

The data were also analyzed, with intergroup chi-squared statistics first respecting secondary ischemic times and then using pooled data.

RESULTS

Seventeen animals died during the course of the ischemic-reperfusion portion of the experiment and were eliminated from the study. Autopsies of these animals revealed viscous perforation, which likely occurred during intraperitoneal injection of anesthesia, which was the cause of death. After completion of
surgery, all surviving animals were randomly assigned to one of the three experimental groups. The observed necrosis rates and results are listed in Table 1. The maximum likelihood-derived survival curves are demonstrated in Fig. 1. These curves were generated with the data in Table 1. Calculated parameters are listed in Table 2. A 0% flap necrosis rate was predicted for a secondary ischemic time of zero. The secondary ischemic time where 50% of flaps survived (D50) was 6 hours for both groups 1 and 2 (air and 100% O2, respectively) and 10 hours for group 3 (HBO).

This difference was significant when D50 values were compared by use of analysis of variance (p < 0.05). Although the HBO groups had an increased survival trend throughout, intergroup chi-squared analysis performed within each secondary ischemic time yielded significance in only the 14-hour HBO vs. 100% oxygen comparison (p < 0.05). The pooled data chi-squared comparisons demonstrated a significant survival advantage for the HBO-treated flaps vs. air and 100% oxygen at the p < 0.03 and p < 0.01 levels, respectively. There was no significant difference between the air and 100% oxygen groups.

**DISCUSSION**

For the reconstructive surgeon the problem under investigation in this study arises when a free flap demonstrates signs of poor perfusion during the postoperative period. After reestablishment of flow, what can be done to increase the likelihood of flap survival and avoid the “no-reflow” phenomenon? Many pharmacologic agents, hemodilution, and HBO have been examined in the situation of the compromised axial flap, and some have been examined in free tissue transfer. In this study, HBO significantly increased the survival of threatened, simulated free flaps in a previously described rat model. The secondary ischemic time required to induce a 50% rate of necrosis was increased by 170% when HBO was used. By increasing the CIT, flaps that might have otherwise failed can be successfully salvaged. Although Turk et al. demonstrated a prolongation of tolerable primary ischemia time with University of Wisconsin solution, it remains to be seen whether their technique will alter the more clinically relevant critical secondary ischemic time.

The no-reflow state leads to an irreversible loss of the flap even if perfusion is reestablished. Ischemia-induced hypoxia leads to a reduction in cellular energy stores and disruption of the Na+/K+ pump, whereas reperfusion is associated with the generation of oxygen free radicals. On a microscopic level the no-reflow phenomenon is associated with endothelial swelling, increased capillary permeability, vasoconstriction, and focal thrombosis in the microvasculature.
Table 1. Flap necrosis rates

<table>
<thead>
<tr>
<th>Secondary ischemic time</th>
<th>Group*</th>
<th>n</th>
<th>Survival</th>
<th>Necrosis</th>
<th>% Necrosis</th>
</tr>
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<tbody>
<tr>
<td>6 hours</td>
<td>1</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>50</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>3</td>
<td>12</td>
<td>9</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>10 hours</td>
<td>1</td>
<td>13</td>
<td>4</td>
<td>9</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>3</td>
<td>7</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>46</td>
</tr>
<tr>
<td>14 hours</td>
<td>1</td>
<td>22</td>
<td>1</td>
<td>21</td>
<td>95</td>
</tr>
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<td></td>
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<td>21</td>
<td>0</td>
<td>21</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>18</td>
<td>4</td>
<td>14</td>
<td>78</td>
</tr>
</tbody>
</table>

Based on 6 hours of primary ischemia, 2 hours reperfusion and x hours of secondary ischemia.

*1: Air; 2: O₂; 3: HBO.

Although this study did not specifically address the mechanism by which HBO improves free flap survival, previous articles suggest that HBO may intervene at several points along the pathway to a no-reflow state. Zamboni et al. demonstrated a significant decrease in vasoconstriction and neutrophil adherence to endothelium in an in vivo skeletal muscle preparation using intravital microscopy. This effect, combined with other findings by the same group, demonstrates significantly improved distal microvascular perfusion with laser Doppler measurements in HBO-treated rat skin flaps. Zamboni et al. also noted increased survival of axial pattern skin flaps when HBO treatments were performed during reperfusion after 8 hours of primary ischemia.

Oxygen-derived free radicals have been implicated as important mediators of reperfusion injury after ischemia of free flaps, leading potentially to the irreversible no-reflow phenomenon. The neutrophil is a known source of oxygen free radicals and has been demonstrated to play a central role in events associated with reperfusion injury. In spite of this, HBO has been beneficial in improving the survival rate of ischemic flaps and grafts, as previously discussed. Several studies have shown that free radical scavengers administered to ischemic skin and muscle provide a protective effect by decreasing reperfusion oxygen radical-mediated tissue injury. Preliminary results from Kaelin et al. suggest that superoxide dismutase levels are significantly increased in rat skin flaps after HBO therapy compared with skin flaps not exposed to HBO. In addition, they demonstrated improved survival of adipocutaneous free tissue flaps after HBO. Thom and Elbuen studied lipid peroxidation and demonstrated that hyperoxgenation alters these biochemical pathways in ways that antagonize and significantly decrease lipid peroxidation and free radical formation. Zamboni et al. hypothesized that reduction of free radical formation by any or all of the above mechanisms might reduce endothelial acti-

Table 2. Maximum likelihood model parameters

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose50</th>
<th>SE</th>
<th>Power (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (Air)</td>
<td>6.37</td>
<td>1.06</td>
<td>3.04</td>
</tr>
<tr>
<td>2 (O₂)</td>
<td>8.34</td>
<td>0.85</td>
<td>3.87</td>
</tr>
<tr>
<td>3 (HBO)</td>
<td>9.54</td>
<td>1.18</td>
<td>2.76</td>
</tr>
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</table>

SE: Standard error.

HBO refers to the breathing of 100% oxygen while at a pressure greater than 1 ATA. The delivery of HBO requires a recompression chamber as well as an oxygen delivery system. Justification of the additional expense of a recompression facility can be made only when comparisons are made between HBO and 100% oxygen at 1 ATA (sea level) in addition to air. In this study, flap survival in the 100% oxygen–treated group (group 2) was no different from that in the air group (group 1), whereas HBO at 2.0 ATA significantly improved the survival rate. Further study to develop a true oxygen dose-response curve might suggest that higher pressures of oxygen may yield even greater tolerances of free flaps to ischemia. We chose 2 ATA of pressure for our HBO treatments because of the low likelihood of oxygen toxicity at this depth in human beings and the design limitations of clinical monoplace chambers.

This study and those referenced herein would suggest that HBO treatments significantly increase the tolerance of free flaps to prolonged secondary ischemic times. Anecdotal clinical reports of HBO’s effects on threatened free flaps and the tolerance of this treatment by the general population are encouraging; however, only a prospective, randomized, and well-controlled clinical trial can provide definitive proof of its efficacy.

In summary, we have noted a statistically significant increase in the tolerable secondary ischemic time when
normothermic, microvascular flaps were treated with HBO in this rat model. The increase in tolerance of ischemia, on the order of 170%, was not noted in the 100% normobaric oxygen-breathing group, thus justifying the need for a recompression facility. The interaction of HBO with the systems responsible for the creation and removal of oxygen free radicals and the no-reflow phenomenon merits further study.

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REFERENCES


