

# Effect of Hyperbaric Oxygen and Medicinal Leeching on Survival of Axial Skin Flaps Subjected to Total Venous Occlusion

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This study evaluates the effect of hyperbaric oxygen and medicinal leeching on axial skin flaps subjected to total venous occlusion. Axial epigastric skin flaps (3 × 6 cm) were elevated on their vascular pedicles in 40 male Wistar rats. Total venous occlusion was achieved by division of all veins draining the skin flap. Arterial inflow was left intact. Animals were randomly assigned to one of five groups: sham (n = 8); control, total venous occlusion only (n = 8); occlusion with hyperbaric oxygen (n = 8); occlusion with leeching (n = 8); occlusion with leeching and hyperbaric oxygen (n = 8). The hyperbaric oxygen protocol consisted of 90-minute treatments, twice daily, with 100% O<sub>2</sub> at 2.5 atmospheres absolute for 4 days. The leeching protocol consisted of placing medicinal leeches on the congested flaps for 15 minutes, once daily, for 4 days. Laser Doppler measurements of flap perfusion were recorded preoperatively, postoperatively, and on postoperative days 1 and 3. The percentage of flap necrosis was evaluated on postoperative day 3. Mean percentage necrosis and mean laser Doppler readings were compared between both groups. The flaps in the sham group demonstrated 99 percent survival, whereas the flaps in the occlusion-only group demonstrated 100 percent necrosis. The flaps in the occlusion with oxygen, the occlusion with leeching, and the occlusion with oxygen and leeching groups demonstrated 1, 25, and 67 percent survival, respectively. Sham laser Doppler readings remained within normal limits. Laser Doppler readings in the occlusion-only and the occlusion with oxygen groups decreased to negligible levels on postoperative day 1, and on postoperative day 3 no perfusion was demonstrated. In both the occlusion with leeching and the occlusion with leeching and oxygen groups, there was also a significant decrease in laser Doppler measurements after surgery, but perfusion remained stable throughout the remainder of the study. This study demonstrates that hyperbaric oxygen alone is not an effective treatment for skin flaps compromised by total venous occlusion. The combination of leeching and hyperbaric oxygen treatment of total venous occlusion results in a significant increase in flap survival above that found with leeching alone. It appears that hyperbaric oxygen is effective because of the venous out-

flow provided by leeching as demonstrated by laser Doppler flow readings. (*Plast. Reconstr. Surg.* 104: 1029, 1999.)

Total venous occlusion in pedicle flaps, free tissue transfers, and tissue replantation continues to remain a significant and often detrimental clinical problem. Total venous occlusion can result from multiple causes, ranging from hematoma and twisting of the pedicle to anastomotic thrombosis. Regardless of the cause, it is usually the venous anastomosis that clots first, which backs up the microcirculation, resulting in arterial thrombosis and subsequent complete necrosis of the flap.<sup>1</sup> Even after revision surgery for flap salvage, the anastomosis may still not remain patent and the flap becomes subjected to a secondary ischemic event. It has been demonstrated that in both a porcine and rodent model, a secondary ischemic event is less well tolerated than a primary ischemic insult, increasing the likelihood of flap failure.<sup>2,3</sup>

There is some experimental evidence to suggest that hyperbaric oxygen therapy improves survival of random pattern skin flaps,<sup>4,5</sup> as its effects on ischemic axial skin flaps have also been examined. Hyperbaric oxygen therapy was demonstrated to increase flap survival when administered during and after a period of total ischemia.<sup>6</sup> The effect of the therapy in situations of venous congestion has not been scientifically evaluated in controlled animal studies. In a preliminary experiment, we examined the effect of hyperbaric oxygen on perfu-

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sion and survival of skin flaps subjected to total venous occlusion.<sup>7</sup> In this situation, hyperbaric oxygen has no side effect and the flaps went on to develop complete necrosis. Physiologically, hyperbaric oxygen would not be expected to increase oxygenation of tissue that is not perfused.

The medicinal leech *Hirudo medicinalis* has been recognized by surgeons for its ability to reduce venous congestion and to improve blood flow.<sup>8</sup> Even with aggressive leeching, the flap remains at risk for failure and tissue necrosis. If flow can be reestablished with medicinal leeching, then hyperbaric oxygen has the potential to enhance flap survival. The purpose of this study was to evaluate the efficacy of hyperbaric oxygen and medicinal leeching treatments on total venous occlusion in a rodent axial skin flap model.

#### MATERIALS AND METHODS

Forty male Wistar rats, each weighing 300 to 600 g, were used for this experiment. All animals were anesthetized with 50 mg/kg intraperitoneal sodium pentobarbital (Nembutal) during flap elevation and evaluation of flap survival. Supplementation of anesthesia was accomplished with pentobarbital (10 mg/kg) administered intraperitoneally as required.

##### *Skin Flap*

A 3 × 6 cm rectangular skin flap in the left abdominal groin area was raised in each animal by using the midline as the medial border. This flap has a blood supply in the axial distribution of the inferior epigastric artery and vein. The blood supply to each flap was isolated in the groin and the inferior epigastric vein was ligated with 5-0 silk then divided. All other veins draining the flap were also ligated or cauterized to provide total venous occlusion. The inferior epigastric artery was left intact. The edges of the flap were sutured back into place with 6-0 nylon.

##### *Experimental Protocol*

The animals were randomly assigned to one of five experimental groups: sham ( $n = 8$ ), which consisted of the elevation of the epigastric flap only; control, total venous occlusion only ( $n = 8$ ); occlusion with hyperbaric oxygen ( $n = 8$ ); occlusion with leeching ( $n = 8$ ); and occlusion with leeching and hyperbaric oxygen ( $n = 8$ ). The hyperbaric oxygen protocol consisted of twice-daily treatments 8 hours apart

for 90 minutes with 100% oxygen at 2.5 atmospheres absolute on the day of surgery and for 3 postoperative days. The first oxygen treatment each was started within 10 minutes after leeching. The hyperbaric chamber used was a monoplace chamber manufactured by Sechrist Industries, Inc., Anaheim, Calif. (model 2500 B). Calcium carbonate crystals were used during treatments to prevent carbon dioxide accumulation. Oxygen concentrations were checked with a calibrated oximeter at half-hour intervals to ensure 100% oxygen concentrations within the chamber. No seizure activity or deaths occurred from hyperbaric oxygen treatments at the above exposures. The leeching protocol consisted of placing medicinal leeches (*Hirudo medicinalis*) on the congested flap for 15 minutes once a day immediately after surgery and for 3 postoperative days.

##### *Laser Doppler Flowmetry*

The instrument used was the Laserflo BPM<sup>2</sup> (Vasamedics, Inc., St. Paul, Minn.). Laser Doppler flow readings were recorded in two standardized locations (central, midline, 3 cm from proximal flap edge and distal, midline, 5 cm from proximal edge) on the flap to eliminate regional variation previously reported in this model.<sup>9</sup> Laser Doppler recordings were taken preoperatively, immediately postoperatively, and on postoperative days 1 and 3 before placement of the leech. The laser Doppler was zeroed on a neutral colored surface. All measurements were performed after the probe had been placed on shaved skin for at least 60 seconds. One leech per flap per day of treatment was used. The leeches were not weighed but were noted to be visibly engorged after 15 minutes of attachment. Observation of the flaps revealed a decrease in venous congestion during leech attachment and for a variable time afterward (range, 1 to 8 hours).

##### *Flap Necrosis Analysis*

Flap necrosis was determined on postoperative day 3. The percentage of flap necrosis was determined by clinical examination and measured by digital photography and computerized planimetry.

##### *Statistics*

All values are shown as the mean ± SEM. Statistical analysis comparing the percentage of flap necrosis was performed by using two-way factorial analysis of variance. Statistical com-

parison of the difference in flap perfusion was performed by using analysis of variance for repeated measures. Duncan's post hoc comparison was used to evaluate specific mean comparisons. A *p* value of  $\leq 0.05$  was accepted as significant.

RESULTS

The flaps in the sham group demonstrated  $1 \pm 1$  percent necrosis. This result demonstrate the reliability of this rodent axial skin flap model. All flaps in the total venous occlusion only group exhibited 100 percent necrosis. When hyperbaric oxygen was administered to the occlusion-only group, the flaps demonstrated  $99 \pm 0.6$  percent necrosis. The flaps in the occlusion with leeching group exhibited  $75.2 \pm 5.5$  percent necrosis, and the occlusion with leeching and oxygen group demonstrated  $33.4 \pm 4.2$  percent necrosis, *p* < 0.05 (Fig. 1). Laser Doppler readings (Fig. 2) of the sham group remained at baseline levels. For the control (occlusion-only) and the occlusion with oxygen groups, laser Doppler readings decreased to negligible levels on postoperative day 1, and by postoperative day 3, no perfusion was demonstrated. For both the occlusion with leeching and the occlusion with leeching and oxygen groups, laser Doppler readings decreased significantly after surgery but stabilized throughout the remainder of the study.

DISCUSSION

Venous congestion is one of the most frequent complications leading to necrosis in

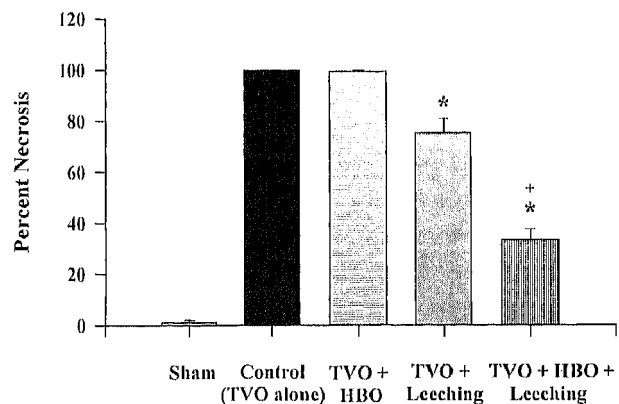


FIG. 1. Muscle necrosis (mean percent  $\pm$  SEM) in sham, control (total venous occlusion alone, TVO-alone), total venous occlusion plus hyperbaric oxygen (TVO + HBO), total venous occlusion plus leeching (TVO + Leeching), and total venous occlusion plus leeching plus hyperbaric oxygen (TVO + Leeching + HBO) groups. *n* = 8 in each group. Asterisks, *p* < 0.05 versus control (TVO alone); plus sign, *p* < 0.05 versus TVO + Leeching.

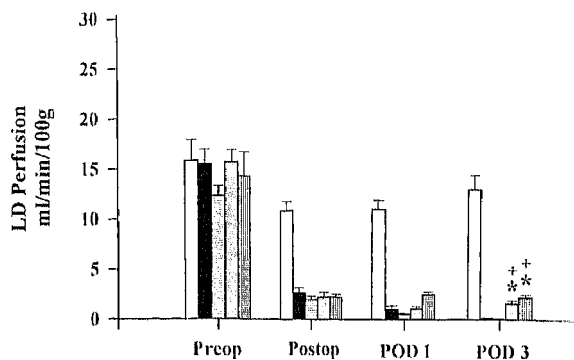


FIG. 2. Laser Doppler skin blood perfusion (mean ml/min per 100 g  $\pm$  SEM) at preoperative (Preop), immediately postoperative (Postop), and postoperative days 1 (POD 1) and 3 (POD 3) in sham (open bars), control (total venous occlusion alone, TVO-alone) (filled bars), total venous occlusion plus hyperbaric oxygen (TVO + HBO) (horizontal shaded bars), total venous occlusion plus leeching (TVO + Leeching) (diagonal hatched bars), and total venous occlusion plus leeching plus hyperbaric oxygen (TVO + Leeching + HBO) (checked bars) groups. *n* = 8 in each group. Asterisks, *p* < 0.05 versus control (TVO alone); plus sign, *p* < 0.05 versus TVO + HBO.

pedicle flaps, free tissue transfers, and tissue replantation. Thrombosis usually occurs at the venous anastomosis, which damages the microcirculation and results in arterial thrombosis and eventual tissue loss.<sup>1</sup> Previous studies have demonstrated that acute venous occlusion is more detrimental than global arteriovenous occlusion.<sup>2,10</sup> Congestion can be divided into total or partial lack of venous outflow. The primary treatment for partial venous occlusion is medicinal or chemical leeching. There are multiple reports of the benefits of medicinal leeching in the treatment of surgical uncorrectable venous insufficiency that may be encountered after microsurgical free flap and replantation procedures.<sup>11-13</sup> The anticoagulant in the saliva of the leech (*Hirudin*) is a highly potent anticoagulant that may allow the wound to bleed for up to 48 hours. The laser Doppler flow measurements in this study demonstrated an increase in flap perfusion during leeching, which tapered off after the cessation of leeching but remained at low but stable levels as the wound continued to bleed.

At the present time, the primary treatment for total venous occlusion is surgical reexploration to provide venous outflow. If the anatomic problem is not correctable, then leeching is indicated. This study demonstrates that hyperbaric oxygen treatment alone is ineffective. However, if perfusion can be established by leeching, then hyperbaric oxygen becomes a valuable adjunct.

These results are logical in light of the fact that the effects of hyperbaric oxygen are known to be systemic and require blood flow to the injured tissues to be effective.<sup>6</sup> Postoperative day 3 was the time period selected to terminate therapy because further treatment did not alter flap survival. In human subjects, the duration of therapy to improve flap survival is not known but would probably require anywhere from 3 to 10 days, whereas neovascularization of the flap results in sufficient venous drainage. Although from this experimental study, it seems that the combination of medicinal leeching and hyperbaric oxygen is a viable option in the treatment of tissues subjected to venous occlusion, human trials will be necessary to determine the efficacy of this combination therapy in clinical practice.

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