Local Cooling Provides Muscle Flaps Protection from Ischemia-Reperfusion Injury in the Event of Venous Occlusion During the Early Reperfusion Period

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Abstract Clinicians often place patients in heated rooms following muscle flap transfers. We hypothesize that exposure of flaps to heated room temperatures could result in an unnecessary hyperthermic ischemic insult if the flaps were to be compromised by venous outflow obstruction, while exposure of elective flaps to local cooling during early perfusion may provide protection in the event of venous occlusion. The rat rectus femoris muscle flap was elevated and clamped for 1 h. The muscle was then exposed to various temperatures for 1 h of perfusion followed by complete venous occlusion for 3 h. Occlusion clamps were removed and flaps were allowed to reperfuse for 24 h. Flaps were assessed for muscle necrosis and edema. Venous occluded muscles demonstrated decreased muscle necrosis and edema in the locally cooled group ($8.5 \pm 6.7\%$, 3.06 ± 0.14 ; $P < 0.001$) compared to the room temperature group ($76.2 \pm 23.0\%$, 3.73 ± 0.13), and the local warming group ($97.3 \pm 1.4\%$, 3.84 ± 0.29) respectively. No difference was noted in muscle necrosis nor edema amongst non-ischemic muscles irrespective of temperature exposure. These results suggest a beneficial role for exposure of elective flaps to local cooling during the early perfusion period in order to provide protection from ischemia reperfusion injury in the event of a venous occlusion insult. The prophylactic exposure of flaps to local cooling is further supported by

the lack of a harmful effect when flaps were not compromised by venous occlusion.

Keywords Muscle flaps · Ischemia-reperfusion injury · Rectus femoris · Free flaps · Surgical flaps · Temperature · Hypothermia · Reperfusion injury · Microsurgery

Introduction

Clinicians often place patients in heated rooms following elective muscle flap transfers in order to improve flap perfusion by encouraging vasodilation of the feeding recipient vessels, yet there is no proof in literature to support this practice. There is however a substantial body of evidence demonstrating the benefit of hypothermia during free flap ischemia [3, 4, 6, 9, 24, 27, 29–31]. Vascular compromise occurs in 3–6% of free flaps. Unfortunately, subsequent salvage surgery with revision of the anastomosis has a success rate of only 36–70% [32]. Seventy-eight percent of these cases result from venous occlusion (thrombosis) however no literature was identified to evaluate the effects of hypothermia on flaps with venous occlusion [16, 32]. We hypothesized that exposure of elective flaps to heated room temperatures (30°C) could result in an unnecessary hyperthermic ischemic insult if the flaps were to be compromised by venous outflow obstruction, while exposure of elective flaps to local cooling (10°C) during the early perfusion period could provide protection during a venous occlusion insult. In addition, we hypothesized that local cooling would not affect the outcome of muscle flaps if not compromised by venous occlusion with respect to room temperature and local warming.

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Methods

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Rectus Femoris Muscle Flap Model

Male Wistar rats (300–350 g) were anesthetized with pentobarbital sodium (42 mg/kg intraperitoneally) and with periodic supplementation (10 mg/kg intraperitoneally) as needed. A right thigh incision was made to expose the upper leg musculature and femoral vessels. The rectus femoris muscle was dissected free on its dominant vascular pedicle using standard microvascular technique. Ischemia was then induced by a microvascular clamp (O'Brien, Link America, Denville, NJ, USA) carefully placed across the dominant vascular pedicles (artery and vein) to the rectus femoris for 1 h to mimic intraoperative ischemia during free flap transfer.

The rectus femoris dissection was performed on a sterile sheet laid over the microscope table. Asepsis during the initial ischemic period was maintained by providing a local sterile environment. Following 1 h of ischemia, the microvascular clamp was removed and good vascular inflow and outflow through the pedicle was verified under direct magnified vision. The muscle flap was allowed to perfuse for 1 h and venous occlusion was then induced by a microvascular clamp (O'Brien, Link America, Denville, NJ, USA) carefully placed across the dominant venous pedicle for 3 h. During the early reperfusion period muscle groups were exposed to various temperatures (local cooling, room temperature, and/or local warming). Local cooling (10°C) was introduced by wrapping the rectus femoris muscle around a glass tubing as described by Mowlavi et al. [22]. Contact cooling was provided by continuous irrigation of this glass tubing with water cooled to 10°C using a thermostatic pump. Room temperature (23°C) was maintained by exposing the raised muscle flap to the external environment while being covered by a moist gauze to prevent desiccation. Local warming was maintained by contact heating provided by continuous irrigation of this glass tubing with water warmed to 30°C using a thermostatic pump [22]. Each of these temperatures were verified by use of a needle probe thermometer.

Following the 3 h of early venous occlusion, venous clamps were released thus mimicking salvage surgery. Muscle flaps were sutured down using 6–0 Vicryl to avoid muscle twisting and pedicle kinking. Wound incisions were re-approximated using a running skin stitch with 4–0 nylon. Body temperature was attained by re-approximating the skin incision over the dissected muscle using interrupted

skin stitches. Intramuscular temperatures were verified in all groups using a needle probe thermometer. Animals were allowed to awaken for the remaining period of perfusion. These muscle flaps were assayed for muscle necrosis and edema formation following a total of 24 h of perfusion. Each trial consisted of six rats which were stained for muscle necrosis and muscle edema.

Muscle necrosis was assessed by measuring non-nitro blue tetrazolium (NBT) staining areas. NBT reacts with an electron acceptor in the citric acid cycle and is a good indicator of cellular oxidative capacity and has been shown to be a reliable test in the identification of non-viable muscle [12]. The NBT specimen was dried for 24 h at 30°C and placed in NBT for 5–7 min followed by formaldehyde. Evaluation was completed microscopically by Sigmascan planimetry software to determine the percent NBT stain. NBT reacts with an electron acceptor in the citric acid cycle and is a good indicator of cellular oxidative capacity. Edema was defined as a wet-to-dry weight ratio by measuring the tissue weight immediately following harvest and then measuring the dry weight 24 h after convection oven drying at 38°C temperature for 24 h.

Statistical Analysis

All values were expressed as the mean±standard deviation for percent non-NBT staining and wet-to-dry muscle weight ratio. Outcome measures between each of the experimental groups were compared using one-way analysis of variance and post Tukey test (Sigma Stat, Version 2.0, Jandel Scientific, San Rafael, CA, USA). Significant values were considered for *p* values <0.05.

Results

The regional local cooling [10°C] group showed significant decreased muscle necrosis ($8.5\pm 6.7\%$, $p<0.001$) as compared to venous occluded groups at room temperature [23°C] ($76.2\pm 22.9\%$) and local warming [30°C] ($97.3\pm 1.4\%$) (Fig. 1). Furthermore, the local cooling venous occluded group was found to have less muscle edema (3.06 ± 0.14 , $p<0.001$) as measured by a wet-to-dry weight ratio as compared to the room temperature (3.73 ± 0.12) and local warming (3.84 ± 0.29) groups (Fig. 2).

In the non-occluded muscle groups, no significant difference was noted between groups. Muscle necrosis was $5.8\pm 2.0\%$ (in local cooling groups), $3.1\pm 2.4\%$ (in room temperature groups), and $4.6\pm 3.4\%$ (in local warming groups) each of which is not significant ($p>0.05$). Furthermore, muscle edema was 2.35 ± 0.12 (in local cooling), 2.29 ± 0.18 (at room temperature), and 2.36 ± 0.25 (in local warming) ($p>0.05$).

Figure 1 This figure illustrates the degree of muscle necrosis in the local cooling, room temperature, and local warming groups.

Conclusions

Free flap transfer patients are frequently placed in a heated room to improve flap perfusion. In this study we observed that local warming exposure of elective flaps during reperfusion did not provide protection to muscle flaps in the event of venous compromise. Instead, these results suggested a benefit for exposure of elective flaps to local cooling during the early perfusion period. The prophylactic exposure of flaps to local cooling was further supported by the lack of a harmful effect in flaps that were not compromised by venous occlusion.

Ischemia reperfusion injury is implicated as the cause of tissue failure in the 10% of microvascular repairs that fail despite patent vessel anastomosis [10]. There are many pathways that result in response to ischemia reperfusion injury which ultimately lead to necrotic and apoptotic cell death [2, 7]. Initially cytokines, coagulopathy, and reactive oxygen species have a substantial contribution to ischemia/reperfusion injury [21, 26]. Further up-regulation of apoptotic ligands such as Fas and tumor necrosis factor- α as well as a long list of Bcl-2-related proteins, caspase family cell death proteases, and signaling kinases have been implicated as well [23, 25].

Free radical production has been shown to lead to endothelial cell damage which in turn creates a thrombogenic state and thrombus formation [13, 17, 18]. This results in subsequent venous and microcirculatory occlusion [14]. In skeletal muscle, 3 h of ischemia results in nearly complete venous occlusion with substantial microcirculation thrombosis [10]. During early venous occlusion, arterial flow has been shown to continue, which leads to microvascular hemorrhage and edema in the extravascular space [11, 15, 19, 28]. This increased pressure further collapses the capillaries. Pressure continues to increase until arteriolar collapse occurs leading to further tissue ischemia

[8]. Furthermore, edema also limits oxygen diffusion into the extravascular space [1, 20].

Venous occlusion has been observed to be more injurious to tissue than arterial occlusion. Studies have shown that as little as 10 min of venous occlusion can be harmful to tissues and 60 min can cause up to 75% necrosis of capillary beds that may never regain function [5, 8]. In a previous replant model, the benefits of local cooling were observed when muscle flaps were exposed to local hypothermia complete pedicle occlusion (artery and vein) [22]. It has been found that cells with limited metabolic activity are more resilient during both arterial and venous occlusive periods [8]. By limiting the damage produced during this ischemic insult we may be able to lengthen this reversible period and limit tissue damage. The findings of this study further support the use of local cooling to slow metabolic activity and provide improved outcomes in flap salvage procedures in a free flap model. Local cooling during this period may extend the critical ischemia time and thus improve salvage procedure success.

A rectus femoris muscle flap model served to simulate the venous occlusive state of reperfusion injuries that occur in free flap procedures. By simulating venous occlusion we were able to evaluate the effects of local cooling, room temperature, and local warming during this critical period. The results of this study established that local cooling in the rectus femoris muscle flap model improves viability and decreases muscle edema in the event of venous occlusion. We recognized that it would be clinically difficult to predict which muscle flaps were at risk for venous occlusion; this acknowledgement would thus support the exposure of all muscle flaps to local cooling prophylactically in

Figure 2 This figure illustrates degree of muscle edema (wet to dry muscle weight ratios) in the local cooling, room temperature, and local warming groups.

the early reperfusion period. Thus, we confirmed that local cooling did not have a deleterious effect on non-venous occluded flaps. In fact, no difference in muscle necrosis or edema was observed amongst venous competent flaps exposed to local cooling, room temperature, and local warming.

Patients may benefit from local cooling following muscle free flap transfers during the early reperfusion period. Further clinical studies are warranted to establish the benefit of regional hypothermic exposure of muscle flaps in the early reperfusion period as observed in this animal study. It would also be of interest to evaluate these findings in the event of arterial occlusion as the primary initiating event in flap failure.

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