The Effect of an Extended Protocol of Hyperbaric Oxygen Therapy (HBO) on the Rat TRAM Flap Model

IMAN M. AL-LIETHY, M.D.; ABDEL-AZIZ HANAFY, M.D.; MOHAMED ABDEL-AAL, M.D. and BASEM ZAKI, M.D.

The Department of Plastic Surgery, Faculty of Medicine, Ain Shams University.

ABSTRACT

The effect of hyperbaric oxygen is known to increase the survival of skin flaps. Hyperbaric oxygen therapy permits rapid increase of collagen formation which afford more rapid capillary growth and arcading by providing the necessary matrix for capillary support. The purpose of this study is to evaluate the effect of our hyperbaric oxygen protocol on a rat TRAM flap model focusing on the survival of zone 4 which carries a high incidence rate of fat necrosis and partial flap loss specially in high risk group of patients i.e. obese, smokers, and those with abdominal scars. A transverse rectus abdominis myocutaneous flap (TRAM) supplied by the deep inferior epigastric vessel was elevated with a dimension of 6x2.5cm in 20 Sprague Dawley rats. The rats were divided into two groups. Group 1 or the control group (n=10) to show the incidence of necrosis of zone 4 of the TRAM flap and group 2 or the experimental group (n=10) in which the same flap was elevated, the rat was put in a hyperbaric chamber through a protocol of five sessions of hyperbaric oxygen (100% oxygen, 3 atm absolute) on 48 hours. The flap survival was evaluated by gross examination and microangiography of the arborisation of vessels of the flap for both the control group and the rats received the hyperbaric oxygen therapy. Gross examination revealed a necrosis rate of 48.97% of the TRAM of the control group versus 20.89% of the experimental group.

In Conclusion: The results were encouraging showing better outcome of the survival of the TRAM flap of hyperbarically treated animals. More improvement can be achieved through extended protocol of treatment, than we adopted, or combining the oxygen therapy with pharmacological manipulation by different agents as free oxygen radicle scavengers, complamin, basic fibroblast growth factor etc...

INTRODUCTION

The transverse rectus abdominis myocutaneous TRAM flap has evolved as a popular source of tissue in breast reconstruction. Considerable clinical experience with TRAM has demonstrated the reliability of this flap in improving the overall reconstructive and aesthetic outcome of the breast. Although, using the superior-based single pedicle is the preferred method, potential problems can occur with the poorly vascularized contralateral portion of the flap (zone IV) specially in high risk patients [1-5]. It is associated with ischemia-related complications including fat necrosis and partial flap loss in 6-28 percent of reported series [6,7]. Efforts to avoid this relatively high incidence of complications have been directed toward enhancing the nutrient circulation to the flap. Improvement of the viability of the superior-pedicled TRAM flap version has followed an enhanced knowledge of pedicle anatomy, including anomalies and collateral circulations [8,9] relocation of the preferred lower TRAM flap to the midabdomen, where cutaneous perforators predominate [10]. The use of bilateral rectus muscles to serve as a double pedicle is another option which carries a severe donor site morbidity [11]. Ligation of the deep inferior epigastric artery serveral weeks prior to the maim reconstructive procedure, in the form of delay, secondarily provides increased blood flow by means of the superior epigastric arteries when the final procedure is performed [13]. The ‘supercharged’ TRAM flap has been presented as a method where the single superiorly-based pedicle can be augmented by additional flow by means of microvascular anastomosis of the vessels on the opposite random portion of the flap to recipient vessels in the axillae. This procedure represents the ultimate level of complexity [14-16]. Although each of the above methods can reduce the rate of flap necrosis, they all have limitations attributable to the patient and the surgical factors. Therefore, compromised skin flap perfusion in the early postoperative period will continue to be a problem that may lead to flap necrosis and loss. The prevention of tissue ischemia is a challenge in the attempt to improve flap survival. One of the methods advocated for improving the survival of flaps is the use of hyperbaric oxygen therapy. Champion et al., in 1967 achieved 100 percent survival of flaps in rabbits treated with hyperbaric oxygen versus areas of necrosis greater than 40 percent in the control...
group [17]. Other studies demonstrated that the surviving area, of pedicled cutaneous flaps, of hyperbaric oxygen-treated rats was approximately twice that of the control group [18]. Manson and coworkers showed a threefold more distal growth of capillaries, in pedicled flaps of guinea pigs, in hyperbarically treated animals when compared with the controls [19]. The effect of hyperbaric oxygen has hitherto only been studied in skin flaps. Few reports about the effect of HBO on the myocutaneous flaps were found in the literature. The effect of HBO on TRAM flap was studied by Ramon et al., in 1998 [20]. They reported consistent results with the previous investigators [21,22]. They found that the improvement of the survival of the distal area of the flap, using their protocol of HBO, was up to 25 percent. In spite of being statistically significant, the results did not enable them to use the zone 4 of the TRAM clinically. However, they recommended further studies using either an extended protocol with a different pressure, duration, and frequency, or pharmacologic manipulation in combination with HBO hoping for achieving the goal of complete zone IV survival. Searching for better results, we present this study, the purpose of which is to investigate the effect of our HBO therapy protocol on the survival of zone IV of TRAM flap.

MATERIAL AND METHODS

Twenty male Sprague-Dawley rats, each weighing 350-500g were used in the study. We followed the protocol and laboratory standards of Animal Care Comittee of Ain Shams University. The animals were housed in individual cages and fed standard chow and water ad libitum. The room temperature and day-night cycles were kept constant. All rats were anesthetized with intraperitoneal pentobarbital (30mg/kg) and maintained with inhalational flurane.

The hair is depilated from the abdomen then the flap is elevated. For evaluation of the flap survival, reanesthetizing the rats is required with the inhalational method. Animals that died in the first postoperative week are excluded from the study.

TRAM flap model:

It resembles the flap design developed by Ozgentas et al. [23] and Hallock et al. [22]. Under anesthesia and aseptic conditions, the abdominal skin was depilated. We mark the virtual umbilicus (a midpoint between the xiphisternum and the symphysis pubis). A transversely oriented 6x2.5cm rectangular flap was horizontally centered above this point. The tips of the flap reached the midaxillary lines bilaterally. These measurements achieved the same relative width-height ratio as in the clinical TRAM flap (Fig. 1).

After skin preparation, the skin paddle was elevated keeping only the fascial perforators of the right rectus abdominis muscle (Fig. 2). We created an inferiorly-based myocutaneous flap because the superior epigastric is the dominant one in rats in contradictory to the human (Fig. 3). The abdominal wall was closed with 4/0 absorbable sutures. A 6x2.5 of silicone sheet was inserted over the flap donor area. The flap is then sutured to its bed with 5/0 silk or nylon sutures.

After 7 days the rats were anesthetized and the viable skin boundaries of each flap were marked on transparent X-ray plotted film.

Experimental protocol:

Animals were randomly divided into two groups as follows:

Group I (control group, n=10): Flaps were prepared and the animals were returned back to their cages and were observed as controls.

Group II (Hyperbaric oxygen group, n=10): Animals received hyperbaric oxygen therapy five 90-min sessions breathing 100% oxygen at 3 atmosphere absolute in hyperbaric chamber (Figs. 4,5). The first treatment was given within one hour of surgery with a total of three treatments in the first 24 hours and another two in the next 24 hours except the rat number nine which took only two sessions in the first day because he got convulsions.

Evaluation of flap survival:

On the seventh day, the rat is anaesthesised. The evaluation of the flap was determined by the color, refill, Escher and the pin-prick test. The cut line of the viable and non viable part of the flap was traced using a transparent plotted film. The percent of surviving are of each flap was determined by calculating the surface area of the necrosed part by plotting the necrosis on a plotted transparent paper then abstracting it from the total surface area of the flap.

Microangiography:

This procedure was performed on two rats, one in the HBO group and one in the control group. The rat was killed with intracardiac overdose of pentobarbital, the abdominal aorta was cannulated distally, and the proximal segment was ligated (Fig. 6). The dye urographine was injected. Then X-ray film was taken.
RESULTS

Two rats in the control group and one in the experimental group died. They were excluded from the study and replaced by another three rats.

The mean body weight was nearly the same in both groups before surgery, with no significant difference between them (Table 1).

The mean surviving area of the flap on the last day of the experiment is shown in Table (2) and illustrated in Figs. (7-10). It was calculated according to the rat’s weight. Survival of the TRAM flap in our study is better than reported in previous studies [20]. The flap was examined clinically to determine the viable from non viable parts. The absence of refill and bleeding on pin-prick are evident signs of ischemia, while the presence of Escher is a sure late sign. The mean surviving area of the control group was (51.03%) while in the HBO group reached (79.11%) which had a significant difference.

Microangiography:

X-ray from one animal in each group was taken (Fig. 11). The animal treated with HBO has an extensive arborisation of capillaries.

Table (1): Average body weight of the rats in each group.

<table>
<thead>
<tr>
<th>Weight (gm)</th>
<th>Control</th>
<th>HBO</th>
</tr>
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<tbody>
<tr>
<td>400</td>
<td></td>
<td>410</td>
</tr>
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Table (2): Surviving skin areas (percentage).

<table>
<thead>
<tr>
<th>Rat number</th>
<th>Control group</th>
<th>HBO group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surface area of surviving portion (cm²)</td>
<td>Percent of surviving portion</td>
</tr>
<tr>
<td>1</td>
<td>7.5</td>
<td>50.0</td>
</tr>
<tr>
<td>2</td>
<td>9.825</td>
<td>65.5</td>
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<td>3</td>
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</tr>
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<td>6</td>
<td>7.575</td>
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<td>7</td>
<td>5.31</td>
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</tr>
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<td>8</td>
<td>4.5</td>
<td>30.0</td>
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<td>80.0</td>
</tr>
<tr>
<td>10</td>
<td>10.35</td>
<td>69.5</td>
</tr>
</tbody>
</table>

Mean 76.545 51.03% 118.665 79.11%

Fig. (1): Rat TRAM flap design.

Fig. (2): TRAM flap elevation from left to right. The arrows indicate the musculocutaneous perforators of the left rectus abdominis muscle.

Fig. (3): The inferiorly-based rat TRAM has been elevated and reflected.

Fig. (4): Experimental HBO chamber.
Fig. (5): The rat is in the HBO chamber.

Fig. (6): Cannulation of the rat aorta and injection of the dye during microangiography.

Fig. (7): Typical pattern of flap necrosis in the control group.

Fig. (8): Typical pattern of flap necrosis in the HBO group.

Fig. (9): Typical pattern of flap necrosis in the control group (another case).

Fig. (10): Improvement of zone IV of TRAM flap under the effect of HBO.

Fig. (11): Microangiography of the TRAM flap of the control.

Fig. (12): Microangiography of the HBO group (Note better arborisation of the vessels of the flap).
DISCUSSION

Breasts reconstructed with a natural living tissue have a realistic form, remain soft, and are generally trouble free over a lifetime. The most widely used method of breast reconstruction with autogenous tissue is the TRAM island flap. However, ischemia-related complications including fat necrosis and partial flap loss continue in 5 to 28 percent of reported series [6]. This complication rate is higher in patients with risk factors which include obesity, cigarette smoking, radiation and abdominal scars [24]. So, many efforts were focused on methods that could potentially increase flap safety and reliability by improving blood supply. Several variables have been identified that may affect flap circulations, including patient risk factors, tissue volume requirements and the vascular anatomy of the rectus abdominis musculocutaneous territory. Consideration of these variables has resulted in surgical modifications of the unipedicled flap specially the superiorly-pedicled version. The double pedicle TRAM was one of these modifications which based on a study of Hartrampf et al., [25] who believed that a single pedicle would reliably nourish only 60% to 70% of the total lower abdominal flap in a healthy patient and overdependence on a single pedicle to nourish the abdominal tissue beyond the midline would lead to high incidence of fat necrosis and partial flap loss. They recommended the use of double-pedicled TRAM flap in patients who need additional tissue volume. However, it resulted in high incidence of hernia and abdominal wall weakness [26]. Ligation of the deep inferior epigastric artery several weeks prior to the main reconstructive procedure in the form of delay provides increased blood flow. However, the indications for flap delay include patients with multiple risk factors who are prohibitively high risk for conventional or microsurgical reconstruction. In addition, the delay procedure is used when the flap volume requirements are large and associated with radiation or significant scars. In a series of Godner et al., reasonable results of delay of TRAM flap were found [27]. However, the delay procedure is a perplexing option because of related questions which wait answers about the efficacy of delay, what vessels should be divided and the optimal interval prior to elevation of the flap [21]. Adding to this the inherent risk of two closely spaced surgical procedure. The supercharged TRAM flap has been presented as a method where the single superiorly-based pedicle can be augmented by additional flow by means of microvascular anastomosis of vessels on the opposite random portion of the flap to the recipient vessels in the axillae [28]. Although the supercharged and the free TRAM have evolved as reliable choices for breast reconstruction, the patients requiring a large volume of tissue or have damaged or absent recipient vessels in the axillae or chest area may present as a dilemma. Even the modification done by Semple [29], in which he augmented the random portion of the flap with a bilateral deep inferior epigastric artery and vein loop (turbocharging). The sufficient retrograde flow may be present only in selected group of patients and violation of the opposite rectus muscle pedicle may occur. Each of the above methods can reduce the incidence of the flap necrosis but they have limitations attributable to patient and surgical factors. Therefore, compromised skin flap perfusion in the early postoperative period will continue to be a problem that may lead to flap necrosis. Numerous animal studies have suggested the value of HBO in enhancing flap and graft survival [30,31]. Champion et al. [17] using a rabbit model, achieved 100 percent survival in pedicle flaps treated with hyperbaric oxygen versus areas of necrosis greater than 40 percent in all the control. Manson and coworkers used histochemical staining with ATPase to show the smaller blood vessels in pedicled flaps of guinea pigs [19]. They demonstrated a threefold more distal growth of capillaries in hyperbarically treated animals when compared with the controls. The mechanisms of action of HBO are either mechanical or due to elevated partial pressure of oxygen in the tissues. Mechanical therapeutic effects of HBO are limited to the compression reduction in size of gas-containing spaces within the body, primarily in the vascular tree. When oxygen is breathed at 2 atm of pressure it causes vasoconstriction with about 20% decrease in the blood flow to muscles [32]. However, this is generally more than compensated for by the increased oxygen tension in that tissue, which may reach 250-300mmHg when HBO is applied. Normal tissue PO2 is 30 to 40mmHg, while in ischemia, oxygen levels fall much lower below 30mmHg, which leads to severe compromise of fibroblast and leukocytic functions [33]. Although hypoxia (15mmHg) is optimal for stimulation of capillary budding, the increase in collagen formation afforded by hyperbaric oxygen permits more rapid capillary growth and areading by providing the necessary matrix for capillary support. The hypoxia necessary for capillary budding is present between HBO treatments. HBO therapy enables fibroblast replication, collagen formation and increased bactericidal function of leukocytes. There is only one previous study which evaluated the effects of HBO on the viability of TRAM flap by Ramon et al. [20]. They selected a protocol of five 90-minute sessions at 2.5 atmospheres absolute.
Three treatments were given within 24 hours of flap elevations and two treatments during the next 24 hours. The first treatment was given within an hour of flap elevation. They selected this protocol on the bases of previous studies on island skin flaps [34] and axial pattern skin flaps survival under the effect of the HBO [38]. They demonstrated marginal improvement in skin survival area in a rat model of the TRAM flap using their hyperbaric protocol. The difference was about 25% in the area of surviving skin, although statistically significant, would not enable them to use zone 4 of the TRAM flap clinically. So, they had got encouraging results but they recommended further studies to improve the outcome which can achieve the goal of complete zone 4 survival. We conducted this study on 20 rats. Ten of them served as a control group to show the incidence of necrosis in zone 4 of inferiorly-based TRAM flap. It was 62.7% while in the experimental group it reached 21.8. The effect of HBO depends on certain variables such as the delay from the surgery to the beginning of treatments, the length of each exposure, the frequency of treatments, the pressure, and the overall duration of each treatment. The type of the animal and the kind of the flap also interfere with the results. The type, pressure, and the overall duration of each exposure, frequency of treatments, the length of each exposure, the frequency of treatments, the overall duration of each treatment. The type of the animal and the kind of the flap also interfere with the results. The type of the animal and the kind of the flap also interfere with the results.

REFERENCES


