EXPERIMENTAL / LABORATORY STUDIES

The Effect of Hyperbaric Oxygen Therapy (HBO) When Used Postoperatively in Skin Grafting Over Radiated Tissue in the Rat

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Abstract: Under conditions that may risk the success of flap surgery, free skin grafting may be an option for the reconstruction of the irradiated area. Clinical observations reveal that skin grafts applied to irradiated area undergo ulceration, resulting in chronic open wounds. The role of hyperbaric oxygen therapy (HBO) in problem wounds is to increase the tissue oxygen tension to optimize fibroblast proliferation and collagen synthesis, to enhance the oxidative killing capacity of the white blood cells and to stimulate angiogenesis. HBO has also been shown to stimulate angiogenesis in radiation-damaged tissue. In view of this data, an animal model is designed to investigate the effect of HBO therapy on the survival of a skin graft applied on a previously irradiated area. HBO therapy was started on the day of surgery and was continued for 20 days. Graft survival in each rat was calculated as a percentage of the total graft. There was no statistically significant difference between the mean value of the graft survival rate of HBO-treated and HBO-untreated groups.

Key Words: Hyperbaric oxygen therapy, Radiation therapy, Skin graft

Introduction

Reconstruction of an irradiated wound defect is a challenge in reconstructive surgery. Radiation therapy interrupts the normal wound healing mechanism and vascularization of the tissue (1). Local or distant flaps are mostly used as they carry vascularized tissue to the irradiated defect. Random pattern flaps taken from areas adjacent to the defect are least likely to succeed because of the scatter effect of radiation therapy. Axial flaps are preferable but the distal part of the flap is dependent on subdermal plexus. Radiation compromising subdermal plexus may lead to necrosis of the distal part of axial flaps (2). Myocutaneous flaps minimize this risk with the abundant blood supply of the muscle. Among these techniques, a free flap is the main option for reconstruction. It provides an instant augmentation of blood supply, as well as a new population of parenchymal cells critical for healing.

However, under conditions that may risk both the performance and success of the flap surgery, such as poor vascularization of the surrounding tissue, general status of the patient or the surgeon's experience, free skin grafting becomes the optimum treatment for the reconstruction of the irradiated area.

Clinical observations reveal that skin grafts applied to an irradiated area undergo ulceration, resulting in chronic open wounds (3). To overcome this problem, we planned an animal model to investigate the effect of hyperbaric oxygen therapy (HBO) on the skin graft take applied to the irradiated area.

Materials and Methods

Animal model and preoperative irradiation procedure: Thirty randomly selected male Wistar rats, weighing 250 to 350 g, were included in the study. Each

rat was anesthetized with an intramuscular injection of 50 mg/kg ketamine and placed in a prone position. A rectangle of dorsal skin measuring 4 x 5 cm was marked with tattoo dots 1 cm caudal to the line drawn between the ears, on the proximal cervicothoracic spine area.

Each rat was irradiated with an X-ray therapy unit (Stabilipan II, Siemens, Germany). The X-ray unit was operated at 100 kV, 15 mA (filter: 2 mm aluminum, half value layer (HVL) = 2.5 mm aluminum) with a focus skin distance (FSD) of 30 cm. The dose rate was 65.77 cGy/min. A single dose of 10 Gy was given by a 6 x 8 cm tubus through the 4 x 5 cm marked dorsal skin area. A 3 mm lead shield was positioned over the body of the rat during the irradiation to protect the surrounding healthy tissues.

After radiation therapy, the rats were housed in 3 cages, each including 10 rats, for two months. The rats were examined weekly for weight loss and systemic disease. All animals showed loss of hair with thickened skin in the treated area. One rat with a diffuse infection located on the face and 1 rat with systemic infection were excluded from the study during the 2 months postirradiation.

Operative Technique: The surgical procedure was performed under general anesthesia with ketamine. All surgical equipment used was sterile and there was no evidence of postoperative infection. A 4 x 5 cm area of irradiated dorsal skin was removed. A full thickness skin graft with the same diameter was harvested from the abdominal area, which was not irradiated. The donor site was sutured with 4-0 silk primarily. Antibiotic ointment was applied to the incision and it was left open. After defatting and removal of the panniculus carnosus to ensure uniformity, the skin graft was applied to the irradiated defective area on the dorsal side. The graft was sutured with 4-0 chromic cat-gut to the defect and immobilized with a tie-over dressing. After the operation, each rat was housed individually to avoid cannibalism.

HBO therapy: Thirteen rats received HBO therapy within 1 h of surgery. Each session of HBO was given in a 0.4 mm³ pressure chamber at 2.5 ATA for 80 min. The rats received 4 sessions of HBO treatment on the operation day and on the 1st postoperative day, 3 sessions on the 2nd and 3rd postoperative days and 2 sessions from the 4th to the 20th postoperative day. The treatment lasted 20 days. The remaining 15 rats were not treated with HBO and were grouped as controls.

Postoperative care: The tie-over dressing was removed on the 5th day following grafting in 28 rats. The grafted area was left open. Antibiotic ointment and 0.9% NaCl were applied every 2 days for 2 weeks. The sutures of the donor site were removed in 10 days without any complication. One rat from the HBO-untreated group was excluded from the study as it was dead on the postoperative 10th day because of a systemic infection.

Evaluation: The grafted area of each rat was photographed with a Sony DSC-F505V digital camera on the postoperative 5th, 11th and 20th days in order to evaluate skin graft take and wound healing. The graft was traced onto a transparent acetate sheet to measure the area of graft take on the 11th postoperative day and a blind evaluation was performed by 2 members of the research team and 1 person who was not involved in the study. The common results were taken into consideration for the evaluation (Figures 1-3). Biopsies were obtained from normal and irradiated skin, and from the graft-normal skin border on the 11th day, after the measurement of the graft survival area and digitally photographs were obtained. Two biopsy specimens of the skin graft taken from each group, and 1 skin sample taken from an unirradiated and untreated rat were processed for electron microscopy.

Results

Graft survival analysis: Graft survival in each rat was calculated as a percentage of the total graft using the following formula: Percent graft survival = area of intact graft x 100 / area of total graft (Tables 1 and 2). The Mann-Whitney U test was used to compare the results. There was no statistically significant difference between the mean values of the percent graft survival for each group (p : 0.607).

Histologic Assessment: Samples of normal rat skin showed keratin in thin orderly lamellae with an epidermis 2 to 4 cells thick and hair follicles under light microscopy.

There was no histological difference between the biopsy specimens of skin grafts applied to the irradiated area, and those taken from HBO-treated and HBO-untreated groups. Epidermal hyperplasia, acanthosis, ulceration, decrease in adnexal structures, subepithelial fibrosis and increase in connective tissue, dermoepidermal disjunction and edema were noted in most of the grafts (Figures 4 and 5).
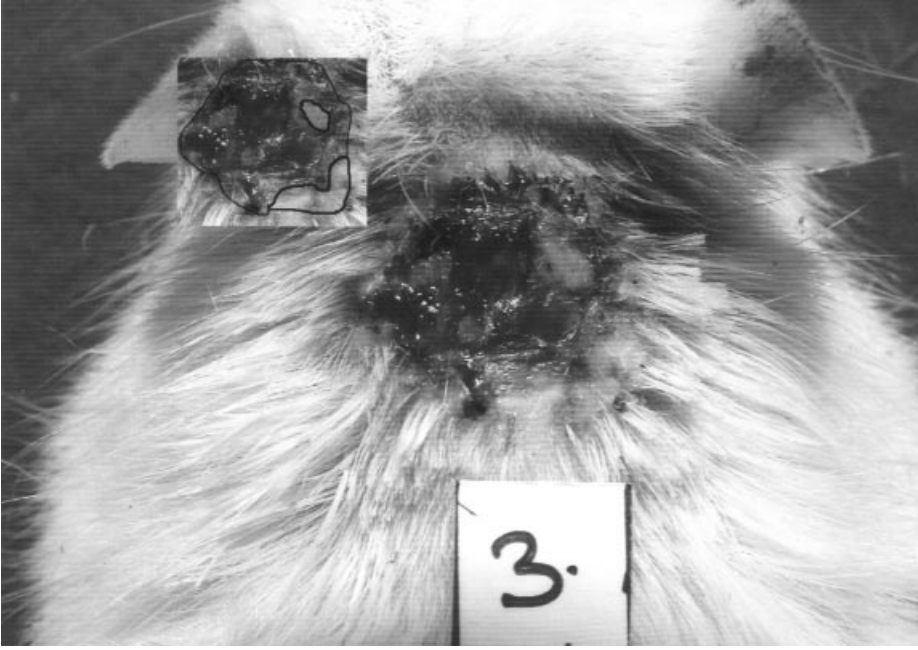
Figure 1. An example of a graft survival area measured on the 11th postoperative day. A 10.87% graft survival rate was observed in this HBO-treated rat. The areas of survival and nonsurvival are delineated in the photograph.
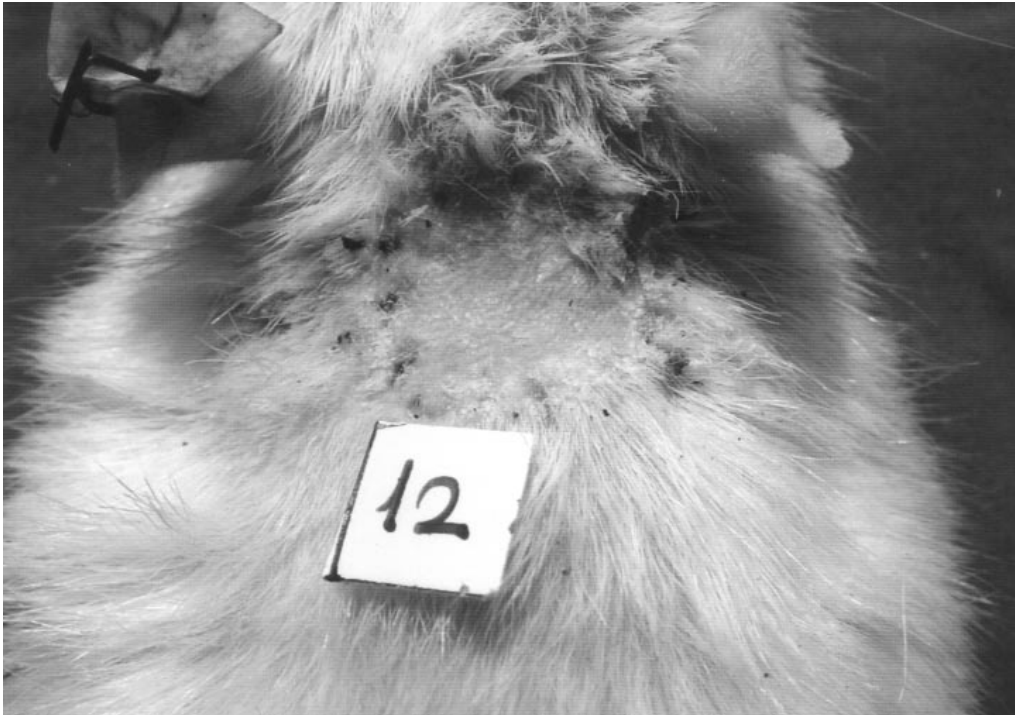

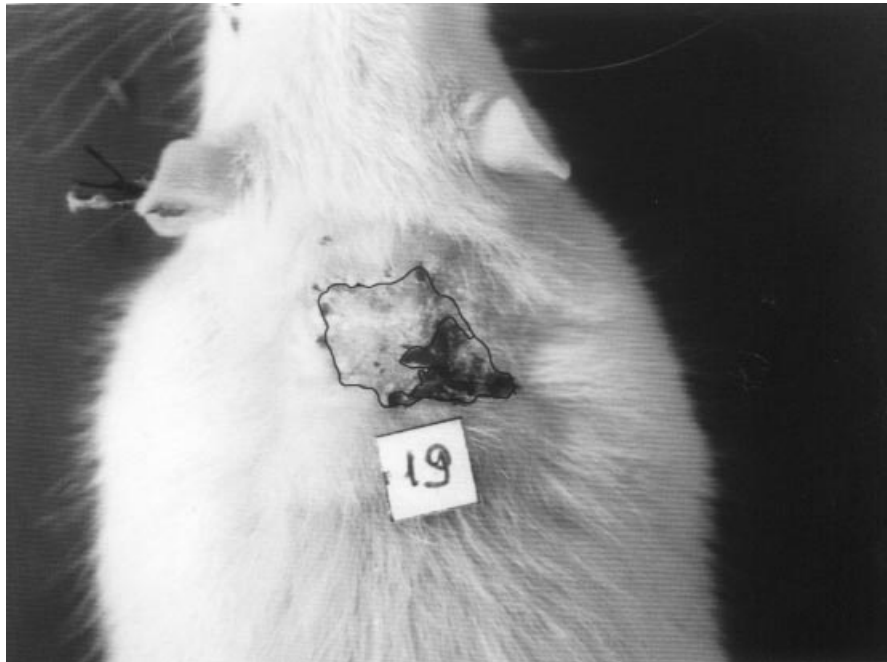Figure 2. An example of a 100% graft take from the HBO-treated group.

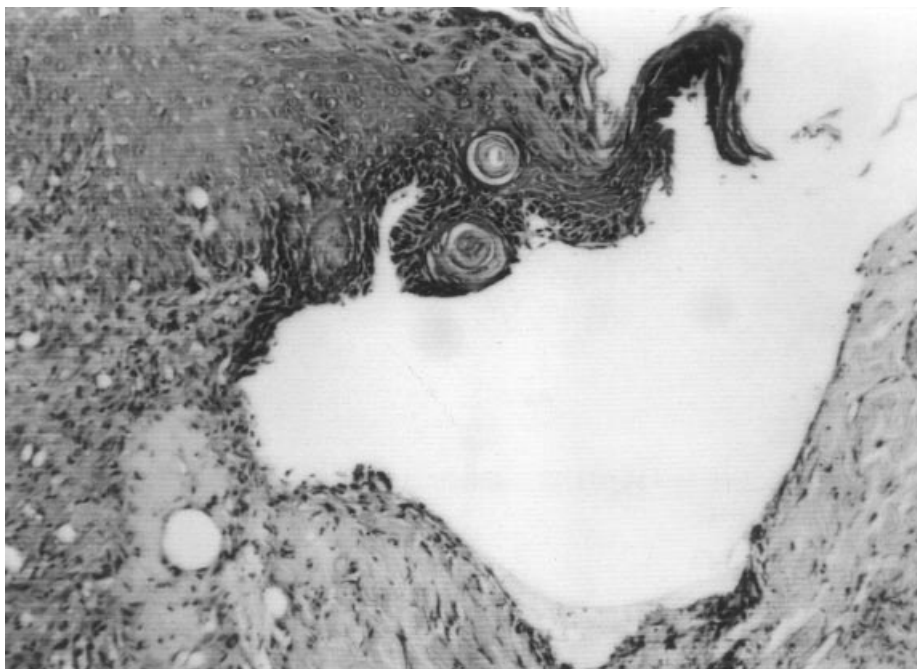Figure 3. A 70% graft survival rate is measured in this HBO-untreated rat.

Figure 4. Histopathologic view of the sample obtained from the ulcerated area of the graft in the HBO-treated group (HE x125). Epithelial hyperplasia and ulceration are seen.

Electron microscopic assessment of the 2 skin grafts obtained from each group revealed irregular and scattered collagen fibrils and fibroblasts having an irregular cell membrane and nucleus with peripherally distributed rough chromatin. These were regarded as nonspecific findings for irradiated tissue (Figure 6).

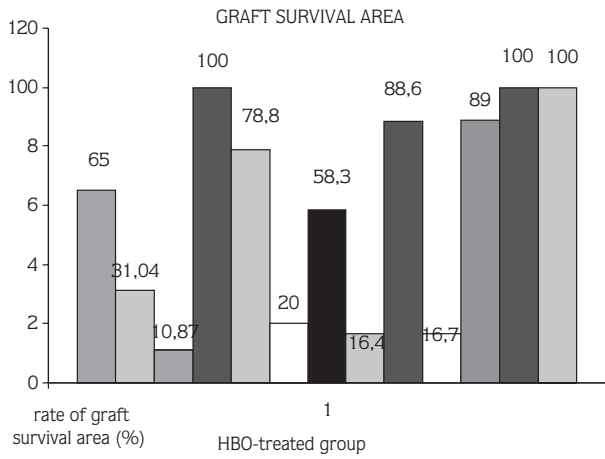Table 1. The rate of graft survival area in each HBO-treated rat.

Table 2. The rate of graft survival area in each HBO-untreated rat.
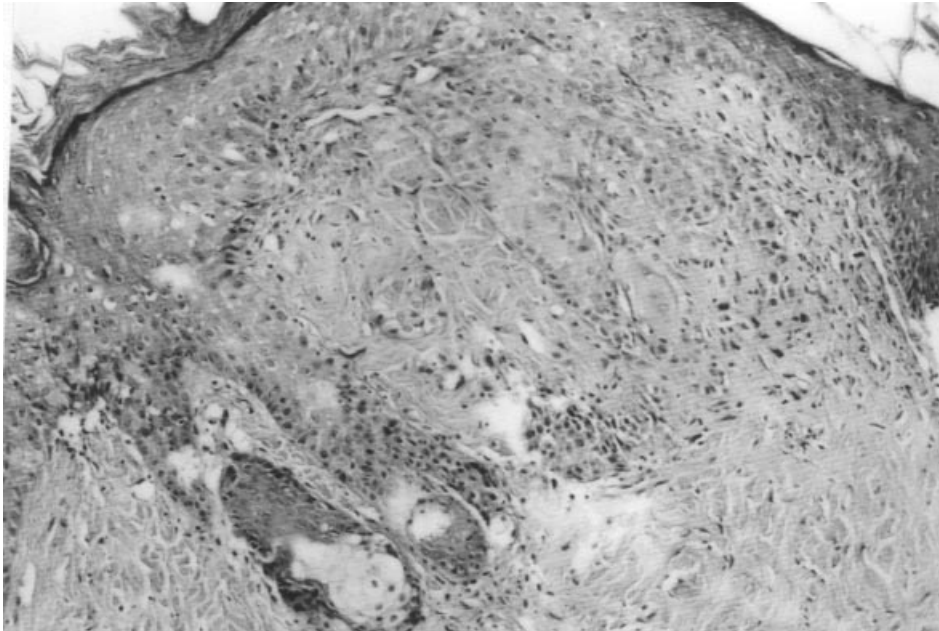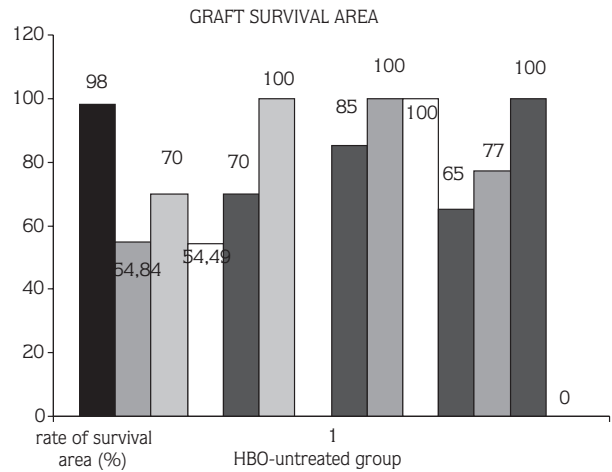

Figure 5. Histopathologic view of the graft sample from the HBO-untreated group (HE x125). Epithelial hyperplasia leads to papillary proliferation through the subepithelial area. Ulceration, dermoepidermal dysjunction, increase in fibrosis and a decrease in adnexal structures are observed.

Discussion

The goal in radiation therapy is to irradiate tumors while causing minimal adverse effects on the surrounding normal tissue. However, in practice there is usually some degree of residual damage to tissue after radiotherapy. Radiation has been shown to produce degeneration and necrosis of the vascular endothelium causing progressive obliterative endarteritis. The destruction of the microvasculature results in 3 H's, that is hypoxia, hypocellularity and hypovascularity (4). Moreover, radiation predominantly affects fibroblast function. These negative effects on vascularity and collagen synthesis cause wound healing problems (1,5). The rationale for HBO therapy in problem wounds is to increase tissue
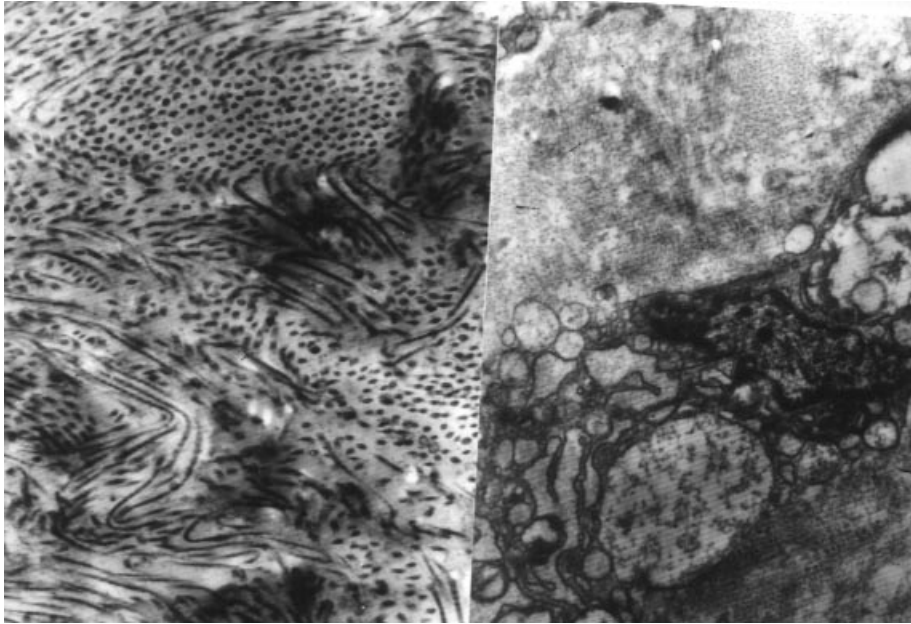
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Figure 6. Electron microscopic assessment of the 2 skin grafts obtained from each group revealed irregular and scattered collagen fibrils and fibroblasts having an irregular cell membrane and a nucleus with peripherally distributed rough chromatin.

oxygen tension intermittently to optimize fibroblast proliferation and collagen synthesis to enhance the oxidative killing capacity of the white blood cells during periods of hypoxia and to stimulate angiogenesis during periods of relative hypoxia (6,7)

HBO has been shown to stimulate angiogenesis in radiation-damaged tissue as a result of the establishment of a steep oxygen gradient across the affected area (4,8). These steep oxygen gradients trigger the recognition of radiated tissue as a wound and initiate angiogenesis (4). Tissue oxygen studies have shown that angiogenesis becomes measurable after 8 treatments with HBO, reaches a plateau at 80-85% of nonradiated tissue vascularization after 20 treatments and remains at this level whether or not HBO is continued (5). HBO therapy has known value in the treatment of radiation related injuries in head and neck surgery and radiation cystitis refractory to conventional therapy (9).

In view of the beneficial effects of HBO on irradiated tissue, we planned an animal model to investigate the effect of HBO on the take of a free skin graft applied to the irradiated area. Similar protocols for HBO therapy after skin grafting have been used in previous experimental studies (10,11). In accordance with those

protocols we utilized 2.5 ATA for 80 min for a period of 20 days.

The effect of graft bed irradiation on the healing of rat skin grafts was investigated by Wang et al. Their study confirmed significant decreases in granulation tissue formation and a delay in the healing process. Graft bed irradiation reduced fibrinogen and fibronectin deposition in the wound and the diameter of collagen fibrils in the granulation tissue. This might be caused by either a direct effect of radiation on epidermal cell proliferation or by an indirect cellular response to radiation-induced environmental changes (12).

One experimental study in rats and one clinical study about skin grafting suggested the beneficial effect of HBO treatment (13,14). However, Hosgood et al. showed the negative effects of HBO on the viability of free skin grafts in dogs (10). They explained their results by the detrimental environment created by HBO administration conducive to production of oxygen-derived free radicals and by the inability of the free skin graft to adequately counter these oxidative stresses. Oxygen-derived free radicals are generated during reperfusion when oxygen is reintroduced to ischemic tissue. A free skin graft is completely avascular and dependent on nutrients imbibed

from the graft bed for survival and may lack the cellular protective mechanism to tolerate hyperoxia and oxidative stress (10). McCarthy et al. studied the pattern of accumulation of products of lipid peroxidation in free full-thickness skin graft in rats over 10 days after grafting (15). They proposed that the accumulation of lipid products in skin grafts can be explained by oxygen-derived free radical-induced injury attritutable to postischemic reperfusion during the revascularization of the skin graft.

Lemarie et al. documented that HBO treatment may increase the accumulation of lipid peroxidation products in the free skin graft (11).

In our study, we did not observe a statistically or clinically significant effect of HBO therapy on the take of free skin grafts applied to the irradiated area in rats. Although it is logical to expect that the administration of pure oxygen under high pressure, especially to an area with low vascularity due to radiation, enhances free radical formation, there are studies that seem to show that hyperoxia can increase the biochemical defense mechanisms against free radicals (12).

We did not evaluate lipid peroxidation products. Therefore it is not appropriate to explain our results with oxygen-derived free radicals that impair the wound healing process. As the beneficial effects of HBO on radiation injuries are well established, we think that further experimental studies to investigate the timing of HBO administration after both irradiation and skin grafting as well as the use of antioxidant therapy should be considered before a definite conclusion is drawn about the effect of HBO on skin graft take on irradiated areas.

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