Efficacy of hyperbaric oxygen on survival of random pattern skin flap in diabetic rats.

Submitted - 2/6/07; Accepted - 4/3/07

T. ZHANG1,2, W. GONG2, Z. LI1, S. YANG3, K. ZHANG1, D. YIN1, P. XU1, T. JIA2

1Shandong University Medical School, Wenhua West Road 44, Jinan, P.R. China 250012; 2Department of Orthopaedic Surgery, Jinan Central Hospital, Jiefang Road 105, Jinan, P.R. China 250013; 3Department of Orthopaedic Surgery, School of Medicine, Wayne State University, Detroit, MI. U.S.A. 48201

Zhang T, Gong W, Li Z, Yang S, Zhang K, Yin D, Xu P, Jia T. Efficacy of hyperbaric oxygen on survival of random pattern skin flap in diabetic rats. Undersea Hyperb Med 2007; 34(5):335-339. Objectives: This study was designed to determine if hyperbaric oxygen improved random pattern skin flap survival in diabetic rats. Methods: Cranially-based, 4×10cm dorsal skin flaps were raised in 38 diabetic rats induced by streptozocin (STZ). The animals were randomly divided equally into two groups. Group A was a control group observed in the room air and Group B was the experimental group, which received hyperbaric oxygen (HBO2) therapy. The HBO2 regimen consisted of 90 minutes of treatment with 100% O2 at 2.5 ATA (atmosphere absolute ATA) per day for 7 consecutive days. On the 7th postoperative day, we measured the necrotic flap area and the new growth number of capillary vessel and the granulation tissue thickness. Results: The percentage of necrosis flap area for group A was 50.5±10.5%; for group B it was 38.5±9.3%. The reduction in necrosis flap area was highly significant ($p<0.01$) compared with controls. Also, new-growth capillary vessel and granulation tissue thickness were statistically different between the two groups. Conclusions: The findings of this study demonstrated beneficial effects of HBO2 in improving diabetic rat dorsal skin flap survival.

INTRODUCTION

Due to its high prevalence and potentially deleterious effects on a patient’s physical and psychological condition, diabetes mellitus (DM) is a major health care concern (1,2). According to the World Health Organization the number of diabetics has doubled in the past few years and is expected to double once again by the year 2025. In humans, diabetes is one of the most prevalent medical conditions and in China alone there are about 20-40 million diabetics. Poor healing in the diabetic patient is also a challenge for the reconstructive surgeon. Skin flaps are used in all fields of plastic surgery, especially in reconstruction (3,4,5). Because of defective fibroblast/endothelial proliferation, defective epithelization, and reduced collagen deposition wound healing problems are common. In addition, there is a propensity for infection, which can contribute to problem wounds. Finally, microvascular and macrovascular disease that accompanies DM is a major risk factor for flap surgery (6).

Alloxan and STZ (streptozotocin, STZ) are widely used to induce experimental diabetes in animals (7). STZ is an alkylating anti-neoplastic agent used in the treatment of pancreas islet cell and malignant carcinoid tumors. After selective uptake by pancreatic beta cells, it causes alkylation of DNA, which induces activation of poly ADP-ribosylation, a process that leads to depletion of cellular NAD and ATP. Most investigators prefer STZ because of its selectivity for pancreas beta cells and less side effects. We obtained reproducible results with the use of STZ in a diabetic model...
Hyperbaric oxygen (HBO₂) is a method of augmenting oxygen availability to the tissues intermittently in order to promote wound healing (9). The purpose of this experimental study was to objectively evaluate the effects of HBO₂ on the viability of a uniform, random-pattern dorsal skin flap in rats with streptozotocin-induced DM.

**MATERIALS AND METHODS**

**Animal Model**

Approval for animal work was obtained from the ethical committee of Shandong University Medical School. Forty male Wistar rats weighing 220 to 330 g, aged 10 weeks, were used in the study. Standard rat chow and water were given ad libitum, and animals were housed in groups of 4 to 5 rats at standard conditions (24 ± 2°C and 50 ± 5% relative humidity) with 12 h light–dark cycles. All animals were received a single intraperitoneal (IP) injection (60 mg/kg) of STZ (Sigma Chemical Co., St. Louis, Mo.) dissolved in 0.1 mol/L citrate buffer (pH 4.5). To prevent hypoglycemia, 2 ml of 5% glucose was administered orally and drinking water was supplemented with 10% sucrose for the first 24 h after STZ injection. Blood glucose levels were determined with a blood glucose meter (Hypoguard Supreme, Minneapolis, Minn.) 3 days after IP injection of STZ and rats were considered diabetic if blood glucose levels were greater than 250 mg/dl (10). We collected 38 rats that met the criterion and randomly divided them into two groups of nineteen animals four weeks before the operation (8). Group A served as controls. Group B, receiving the HBO₂, served as the experimental group.

After determination of the glycemic state, the rats were weighted again and after 24 hours, anesthetized with Tiletamin Chloridrate (25 mg/kg) and Zolazepam Chloridrate (25 mg/kg), intraperitoneally. Then, the animals were positioned over a flat surface (figure 1a), with extended limbs, their backs were shaved and a random-pattern, cranially-based dorsal skin flap was elevated. Delineation of the flap was done in the dorsum of the rats by means of a transparent plastic pattern, cut in the standard dimensions (4×10 cm). The flap was then incised with scalpel, being elevated in a plane superficial to the deep muscular fascia, including the superficial fascia, panniculus carnosus, subcutaneous tissue and skin (figure 1b) (11). After flap elevation, an impermeable plastic barrier, cut in the same dimensions, was placed between the flap and its donor bed. After that, the rats were placed in individual cages, receiving food and water ad libitum. The percentage of skin flap necrosis area was calculated on the seventh postoperative day via the paper-template method.

Fig. 1A. The diabetic rat shaved for operation.

Fig. 1B. A random skin flap is raised.
For HBO$_2$ treatment, the animals were placed in a ball pressure chamber (Jinan Iron Works, China.) with a volume of 20 l and a continuous oxygen flow of 2 l/min. Four or five animals at a time were put into the chamber and exposed to 100% oxygen daily at a temperature of 25-26°C. Animals receiving HBO$_2$ (group B) were subjected to 90-minutes of 100% O$_2$ treatment at 2.5 ATA per day for 7 consecutive days. The first treatment began within 4.5 hours after flap elevation. After HBO$_2$, the animals were returned to their cages.

**Histology**

To assess the pathologic changes of the surviving flaps, we performed hematoxylin-eosin staining in a set of sections that were examined by light microscopy. The number of the new capillary vessel and the height of granulation tissue were counted in 5 sections per animals. An observer, unaware of animal group and pathologic outcome, examined each slide (data not shown). By hematoxylin-eosin staining, the large vessels were excluded as not new-growth capillary vessel.

**Necrosis area of the flap**

At postoperative day 7, the regions of survival and necrosis were clearly defined in all of the flaps: the surviving skin appeared pink and tender, whereas the distal necrotic portion was black and rigid (figures 2a and 2b).

After 7 days, paper templates of the flaps were made, and the flap and skin necrosis areas were calculated using computer-assisted imaging equipment (KS 300 Image Analysis System).

**Statistical analysis**

Parametric group data are presented as means ± SD. Comparisons of the percentage necrosis of the flap, new capillary vessel and granulation tissue thickness between the two groups was performed with the unpaired $t$ test. Differences were considered statistically significant at a $p$-value of <0.05.

**RESULTS**

All the characteristic alterations of diabetic rats were observed in both groups, including polydipsia, polyuria, asthenia, dehydration and weight loss. Figure 1 and 2 show details of representative flap preparation and evaluation.

The main results are summarized in Table 1. The HBO$_2$ group measurements compared with the control group include the granulation tissue thickness, capillary vessel and percentages of necrosis on the seventh postoperative day. The percentages of necrosis in the HBO$_2$ animals varied from 26.2 to 47.8% (mean 38.5%) and in the control animals ranged...
between 34.5 and 58.9% (mean 50.5%; Table 1). The granulation tissue thickness, capillary vessel number are also highly statistical significant when compared with controls.

<table>
<thead>
<tr>
<th>Group</th>
<th>Granulation tissue thickness (mm)</th>
<th>Capillary vessel number (10 MF)</th>
<th>Necrosis area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>73 ±12.63</td>
<td>125 ±16.3</td>
<td>50.5 ±10.5</td>
</tr>
<tr>
<td>HBO2</td>
<td>141 ±14.56</td>
<td>417 ±20</td>
<td>38.5 ±9.3</td>
</tr>
<tr>
<td>p value</td>
<td>0.005</td>
<td>0.003</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 1. Granulation tissue thickness, capillary vessel and percentages of necrosis on the seventh postoperative day.

DISCUSSION

STZ, once administered intravenously, leads to DM by means of destruction of the pancreatic insular tissue, including the beta cells that produce insulin, which suffer degenerative alterations and necrosis. The resulting diabetic animal is an excellent experimental model to study organ alterations arising from insulin deficiency (8, 12).

Among the complications of diabetes is wound healing impairment. In diabetic patients, protein synthesis is reduced while catabolism is increased, with changes in the tissue growth process, regeneration and repair. Healing is altered due to problems of protein metabolism and fibroblasts depression. Skin flaps often represent the only adequate option for surgical repair after removal of skin lesions and tumors, especially when larger reconstructions are necessary, demanding adequate functional and aesthetic results. The most common complication after reconstructions with flaps is partial necrosis. This undesirable event leads to additional operative procedures, increasing hospitalization time and delaying the patient’s return to normal activities. Failure of the operation may make reparation very difficult and undermine the surgeon-patient relationship. The most important aspect that governs the viability of a skin flap is adequate nutritive blood flow in the microcirculation.

HBO2 delivers oxygen dissolved in plasma in proportion to its partial pressure according to Henry’s Law. The dissolved gas enhances oxygen transport to hypoxic tissue, promotes oxygen diffusion through tissue interstitial fluids, and reduces edema. Clinical applications of HBO2 include the treatment of chronic refractory osteomyelitis, anaerobic infections, compartment syndromes, and as an adjunct for healing of problem wounds after radiation and in diabetic patients. Wang et al (13) have also recommended the use of HBO2 following musculoskeletal injuries in conjunction with physiotherapy and exercise until healing is completed.

This study clearly demonstrates efficacy of HBO2 in improving surviving flap area in diabetic rats. HBO2 given within 5 hours after the operation significant improved surviving skin flap area, which agrees with the data of Erdmann et al (14). The additional benefit of providing HBO2 for 7 days postoperative may be due to sustaining the tissue oxygen tension in poorly-perfused, ischemic, but viable cells until blood flow to the flap increases. This has been shown to occur within 36 to 48 hours in delayed flaps in pigs (15). Alternatively, the improved flap survival may be attributed to enhanced capillary in-growth occurring within four days, as described by Clark and Moon (16). Some authors also report that HBO2 could significantly increase VEGF (vascular endothelial growth factor) expression (17). VEGF is an endothelial cell-specific mitogen in vitro and an angiogenic inducer in a variety of in vivo models. We also found the number of capillary vessel in the HBO2 treatment group was improved compared with the control group. The HBO2 group’s granulation tissue thickness,
which is integral to healing, was also found to be thicker than the control group.

In summary, we employed a diabetic animal model that closely approximates a DM human skin flap reconstruction, and used it to examine the effects of HBO₂ therapy on the healing of ischemic skin flaps. The findings demonstrate beneficial effects of the therapy in this diabetic wound model. The intermittent using of HBO₂, at 2.5 ATA per day for 90 minutes for 7 consecutive days, is effective in allaying the necrosis of the skin flap and in wound healing. Further studies of mRNA expression for VEGF and other angiogenic factors after HBO₂ will assess their roles on flap neovascularization associated with DM.

REFERENCES