Effects of hyperbaric oxygen on full-thickness meshed sheet skin grafts applied to fresh and granulating wounds in horses

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Objective—To determine the effects of hyperbaric oxygen therapy (HBOT) on full-thickness skin grafts applied to fresh and granulating wounds of horses.

Animals—6 horses.

Procedures—On day 0, two 4-cm-diameter circular sections of full-thickness skin were removed from each of 2 randomly selected limbs of each horse, and two 4-cm-diameter skin grafts were harvested from the pectoral region. A skin graft was applied to 1 randomly selected wound on each limb, leaving the 2 nongrafted wounds to heal by second intention. On day 7, 2 grafts were harvested from the pectoral region and applied to the granulating wounds, and wounds grafted on day 0 were biopsied. On day 14, 1 wound was created on each of the 2 unwounded limbs, and the wounds that were grafted on day 7 were biopsied. All 4 ungrafted wounds (ie, 2 fresh wounds and 2 wounds with 1-week-old granulation beds) were grafted. The horses then received HBOT for 1 hour daily at 23 PSI for 7 days. On day 21, the grafts applied on day 14 were biopsied.

Results—Histologic examination of biopsy specimens revealed that grafts treated with HBOT developed less granulation tissue, edema, and neovascularization, but more inflammation. The superficial portion of the graft was also less viable than the superficial portion of those not treated with HBOT.


Free skin graft, the type of graft most commonly applied to wounds of horses, can be either full-thickness or partial thickness. A full-thickness graft is not as readily accepted as a partial-thickness graft, and if accepted, its upper layers are more likely to slough because a full-thickness graft requires more nourishment and has fewer exposed blood vessels available for imbibition of plasma and for inosculation. For these reasons, full-thickness skin grafting in humans is usually reserved for fresh, uncontaminated wounds.

Treating patients with cutaneous wounds by use of HBOT increases the partial pressure of oxygen at the wound, which in turn enhances microbial killing by leukocytes, replication of fibroblasts, formation of collagen, and neovascularization of ischemic tissue. Treatment of human wounds with hyperbaric oxygen improves acceptance of partial-thickness and full-thickness skin grafts when the grafted wound is compromised by infection or its vascularity is inadequate to support the graft. A full-thickness graft applied to a granulating wound on a horse could be considered compromised because full-thickness grafts often require more nourishment than can be supplied by a granulating recipient wound.

Infection is reported to be the most common cause of graft failure in horses, but chronic inflammation, inherently present during second intention healing of wounds on the distal portion of limbs of horses, may be at least as important because it reduces the quality of the granulation bed and results in the production of moderate amounts of purulent exudate, both of which negatively influence acceptance of grafts. Therefore, grafts applied to wounds of horses may be at greater risk of failure than are grafts applied to wounds of other species.

The purpose of the study reported here was to determine the effects of HBOT on full-thickness skin grafts applied to fresh and granulating wounds of horses. We wished to test the hypothesis that oxygenation of a full-thickness graft could be enhanced after graft-
ing by use of HBOT, survival of the graft, including its superficial layers, would be improved.

**Materials and Methods**

Six healthy mixed-breed 4- to 15-year-old mares were used in this study. All procedures were approved by the University of Tennessee’s Institutional Animal Care and Use Committee.

On day 0, a 5 × 10-cm, elliptical piece of full-thickness skin was excised from the pectoral area of each horse, with the horse standing and sedated with detomidine HCl (0.02 mg/kg, IV), after desensitizing the surgical site with local anesthetic solution. After excising subcutaneous fascia from the piece, two 4-cm-diameter circular skin grafts were excised. Each graft received eight 3- to 4-mm-long fenestrations with a No. 15 scalpel blade. Subcutaneous tissue of the pectoral wound was apposed with size 0 polydioxanone in a simple continuous pattern, and skin was apposed with skin staples. A Stent bandage, comprised of gauze swabs, was sutured to the wound with size 2 polypropylene sutures.

After the pectoral wound was sutured, the horses were sedated with xylazine HCl (1.1 mg/kg, IV) and anesthetized with ketamine HCl (2.2 mg/kg, IV). Anesthesia was maintained with a combination of xylazine, guaifenesin, and ketamine (700 mg of xylazine and 1.5 g of ketamine in 1 L of guaifenesin) infused at a rate of 2 to 3 mL/kg/h. Two circular full-thickness sections of skin, each 4 cm in diameter, were excised from the randomly selected metatarsus and from 1 randomly selected metacarpus; these wounds were on the lateral aspect of the limb and approximately 5 cm apart. A full-thickness skin graft was placed on 1 randomly selected wound on each limb, leaving the other 2 wounds open to form granulation tissue. The grafts were attached to the wounds with skin staples and 8 simple interrupted size 0 polypropylene sutures(196,625),(806,649), placed at equidistant points around the margin of the wound. The wounds were covered with a sterile nonadherent synthetic pad, which was secured with sterile elastic gauze. This pad was impregnated with 500 mg of cefotaxime sodium (500 mg/pad). An absorbent cotton pad was applied to each wounded limb and secured with an elastic adhesive bandage.

Bandages were changed daily for 21 days. Wounds received cefotaxime at each bandage change. Phenylbutazone (2.2 mg/kg) was administered PO, once daily, for 3 days. On day 7, 2 additional 4-cm-diameter full-thickness grafts were collected as described. The horses were anesthetized for a third time, and 2 circular 4-cm-diameter full-thickness cutaneous wounds were created, 1 on each ungrafted metacarpus and metatarsus, and the wounds grafted on day 7 were biopsied. All 4 wounds, 2 fresh wounds and two 1-week-old granulating wounds, received a graft, and the limbs were bandaged. Horses received phenylbutazone (2.2 mg/kg) PO, once daily, for 3 days.

Horses received HBOT in a sealed, monoplace hyperbaric oxygen chamber after recovering from anesthesia and daily thereafter for 6 more days. All wounds were covered with a dry bandage during HBOT. The chamber was gradually brought to a pressure of 23 psi (2.6 ATA) during a period of 20 minutes and maintained at this pressure for 60 minutes. At the end of this 60-minute period, the chamber was returned to ambient pressure during a 20-minute period. The concentration of oxygen within the chamber remained between 77% and 80% (highest possible concentration achievable by available chamber).

The grafted limbs were rebandaged daily, and percentage acceptance of the grafts was assessed subjectively for 7 days. On day 21, wounds being assessed for the effects of HBOT (ie, wounds grafted on day 14) were biopsied. Biopsy specimens were harvested with a 5-mm-diameter skin biopsy punch at a randomly selected site on the grafted wound. Biopsy specimens, which were all harvested from wounds 7 days after grafting, were examined histologically by 1 pathologist (RLD) who was unaware of treatment. Specimens were scored subjectively for graft edema, superficial viability, inflammation, neovascularization, and formation of granulation tissue at the graft-wound interface. Histologic changes were characterized by use of multiple variables (eg, inflammation, hemorrhage, and edema) on the basis of intensity of change as none (0), mild (1), moderate (2), marked (3), or severe (4) or the distribution of the change as none (0), local (1), multifocal (2), coalescing (3), or diffuse (4).

**Statistical analysis**—A mixed-model ANOVA was used to test the effects of treatment, graft thickness, preparation (fresh or granulating wound), and interaction between treatment and preparation (fresh or granulating bed) on percentage of granulation tissue, edema, superficial viability, inflammation, and neovascularization of the grafts. Limb (right or left, metacarpal or metatarsal) and horse were included as random factors in the model. Adjustment for multiple levels of the interaction term was made by use of the Tukey method. The distribution of residuals from the model was evaluated to determine the fit of the model to the data. All statistical tests were 2 tailed, and values of P < 0.03 were considered significant. A software program was used to analyze all the data.

**Results**

Dehiscence occurred at all 4 donor sites on the pectoral region of each horse, but all healed with little or no scarring by about 6 weeks. At the time of biopsy (day 7 after grafting), all the grafts appeared to be free of infection and were firmly adhered. No grafted wound had
gross evidence of infection. All grafts appeared to have been accepted at 7 days. Granulation tissue protruded slightly through fenestrations of the grafts placed on granulating wounds, regardless of whether the grafted wound received HBOT.

At the time of biopsy, which was 7 days after graft application for all wounds, grafted skin applied to fresh wounds and that received HBOT had significantly (P = 0.007) reduced capillary density, compared with grafted skin applied to fresh wounds that did not receive HBOT. Superficial portions of the grafts were less viable when the horse was treated with HBOT after grafting, compared with no HBOT, regardless of whether the wound was fresh or granulating when grafted, but the difference was not significant (P = 0.24).

Histologic examination revealed that the graft-wound interface of the grafted fresh and granulating wounds developed less granulation tissue when the horse received HBOT than when the horse did not receive HBOT, but the difference was significant (P = 0.02) only for the grafted fresh wounds. Grafts had less edema (P = 0.08) but more inflammation (P = 0.08) when the horse received HBOT, regardless of whether the wound was fresh or granulating, but these differences were also not significant.

Discussion

In this study, HBOT appeared to decrease the viability of full-thickness skin grafts applied to either fresh or granulating wounds of horses. Grafts that received HBOT subjectively appeared, at 7 days, to be less edematous than those that did not receive HBOT, but even though HBOT reduced graft edema, it negatively affected neovascularization and superficial viability. A decrease in neovascularization was likely responsible for the decrease in superficial viability. These results differed from what others have observed in dermal wound models, but were similar to the findings observed by Kalns et al, who determined that partial-thickness skin grafts of pigs that received HBOT had reduced neovascularization and hence reduced viability.

Intermittent HBOT of patients that have received a skin graft has been advocated if the graft is considered to be compromised because HBOT, by providing high oxygen tension in the graft, reduces edema, ischemia, and infection, all of which contribute to graft rejection. Hyperbaric oxygen does not speed healing of normal wounds but may induce problematic wounds to heal more like normal ones.3,5 Because HBOT is of great value in controlling surface infection, this treatment may substantially benefit a grafted wound when the recipient wound is at risk of becoming infected.4,13 Even though wounds seen in clinical practice are usually contaminated or infected, we avoided infection of the wounds in the present study by topically applying an antimicrobial because infection is almost impossible to standardize.

McFarlane and Wermuth found that intermittent treatment of wounded rats with 100% oxygen at 2 to 3 ATA for 2 hours once daily for 5 days significantly increased survival of cutaneous flaps and composite, free skin grafts, compared with wounded rats that did not receive HBOT. Perrins reported improved viability of partial-thickness skin grafts in a small number of human patients treated with HBOT twice daily for 3 days after grafting, and Bowersox et al found, in a clinical study, that HBOT was a useful adjunct in the management of ischemic skin flaps and partial-thickness skin grafts in human patients. Champion et al observed that the entire cutaneous pedicle flap of rabbits that received HBOT survived, whereas the pedicle flap of rabbits that did not receive HBOT had areas of necrosis > 40%. Jurell and Kajser found that cutaneous pedicle flaps of rats that received HBOT survived significantly longer than did pedicle flaps of rats that did not receive HBOT and that the surviving area of the flaps of the group that received HBOT was approximately twice that of the control group. By use of histochemical staining to detect small blood vessels in cutaneous pedicle flaps of guinea pigs, Mason et al found 3-times the ingrowth of capillaries in the pedicle flap of guinea pigs treated with HBOT, compared with that of control flaps.

The effects of HBOT are based on the gas laws and the physiologic and biochemical effects of hyperoxia. Most oxygen carried in the blood is bound to hemoglobin, which is 97% saturated at atmospheric pressure. Some oxygen is carried in solution, and this portion, according to Henry’s law, increases as atmospheric pressure increases. Breathing normobaric air results in PaO₂ of approximately 100 mm Hg. Breathing 100% oxygen at 3 ATA causes PaO₂ to increase to 2,000 mm Hg, and oxygen tension in tissue to rise to about 500 mm Hg, which provides a blood concentration of 60 mL of oxygen/L (compared with 3 mL/L at atmospheric pressure), which is sufficient oxygen tension to adequately oxygenate tissue without the contribution of oxygen from hemoglobin.  Normal oxygen tension of tissue is 30 to 40 mm Hg, but ischemia caused by infection, trauma, or edema causes the oxygen tension to decrease much lower, and at < 30 mm Hg, functions of fibroblasts and leukocytes are severely compromised. Although the greatest increase in PaO₂ occurs during administration of HBOT, increased oxygen tension in skin persists for at least 4 hours after the termination of each treatment, and this hyperoxygenated tissue maintains a metabolic environment conducive to synthesis of collagen, proliferation of fibroblasts, and destruction of microbes by leukocytes.3

The ideal exposure to HBOT for healing of wounds of humans has yet to be determined, but to salvage compromised skin grafts or skin flaps of human patients, HBOT is usually applied at 2 to 2.4 ATA for 90 to 120 minutes twice daily for 3 to 10 days at 100% oxygen. Different treatment protocols may result in different results, which may explain, at least in part, differences obtained between studies using different wound models. The authors’ regimen of HBOT for 1 hour daily for 5 days was chosen because it is a regimen commonly used to treat humans with wounds.

No detrimental adverse effects of this regimen of HBOT were observed in the horses. Barotrauma to the middle ear is the most common adverse effect of HBOT of human patients. Oxygen breathed under pressure is toxic, primarily to the brain and lungs, but intermittent exposure to oxygen at 2 to 2.4 ATA for 90 to 120 minutes twice daily presents little, if any, pulmonary or neurologic hazard to human patients.5
Tissue hypoxia is an important stimulus for neovascularization in wounded tissue, and so by creating hyperoxia in wounded tissue, HBOT removes the stimulus for neovascularization. Although HBOT supports wounded tissue by increasing oxygen tension within it, concomitant stimulation of neovascularization is important in maintaining high tissue oxygen tension. Neovascularization is essential for graft acceptance, and a high oxygen gradient between the wound and the graft is essential for neovascularization of the graft. When this gradient is reduced, as occurs with HBOT, neovascularization is retarded.

Results of the present study suggested that HBOT is not indicated for full-thickness skin grafts on fresh or granulating wounds in horses when the treatment is administered immediately after grafting. Studies to determine the effectiveness of HBOT after neovascularization is established may result in different findings.

References