ISSN: 1220-3173; E-ISSN: 2457-7618

OZONE THERAPY AS A SUPPORTIVE TREATMENT FOR A MESH SKIN GRAFT IN A PUPPY - A CASE REPORT

OZONOTERAPIA CA TRATAMENT DE SUSȚINERE A UNEI GREFE CUTANATE LA UN CĂȚEL - STUDIU DE CAZ

N.V. OROS[1),*)], Maria Cristina MORARU[1)], Andreea Niculina AȘTILEAN[1)], C.A. OBER[1)], C.C. REPCIUC[1)], L.I. OANA[1)]

ABSTRACT | REZUMAT

Free skin grafts are a viable option for reconstructing limb defects with extensive tissue loss. The success rate of this technique may be improved by using complementary supportive therapies. The aim of this case study was to assess the therapeutic efficiency of topical ozone treatment, applied both preoperatively and postoperatively, in a 4-week-old male puppy with a distal left forelimb degloving injury. Ozone bagging treatment was used to treat the wound for the first five days. Two more sessions were performed three days apart until healthy granulation tissue was observed, and four ozone bagging treatment sessions, were performed every 3 days after grafting surgery was carried out. Twenty days after the surgical procedure, the epithelialization was complete, and the limb no longer required bandaging. According to our knowledge, is the first report of using ozone therapy to accelerate healing in puppy with a distal limb laceration repaired with a free skin graft. Our findings suggest that ozone therapy may be an effective strategy to accelerate the healing process and acceptance rate of free skin without the use of antimicrobial treatments in puppies.

Keywords: mesh skin graft, ozone therapy, puppy, wound

Grefele libere de piele reprezintă o opțiune viabilă pentru reconstrucția defectelor cutanate de la nivelul membrelor. Rata de succes a acestei tehnici poate fi îmbunătățită prin utilizarea terapiilor complementare de susținere. Scopul acestui studiu de caz a fost de a evalua eficiența terapeutică a tratamentului topic cu ozon, aplicat atât pre cat si post grefare la un cățel mascul în vârstă de 4 săptămâni cu o plagă extinsă la nivelul membrului anterior stâng. Metoda "bagging" a fost utilizată pentru a trata plaga în primele 5 zile. Alte două ședințe au fost efectuate la interval de 3 zile până când s-a observat un țesut de granulație sănătos. După operația de grefare, s-au efectuat 4 ședințe de tratament la interval de 3 zile. La douăzeci de zile după procedura chirurgicală, plaga a fost reepitelizată complet fără să mai necesite pansament. Conform cunoștințelor noastre, acesta este primul caz raportat în care terapia cu ozon este utilizată pentru a accelera vindecarea unei plăgi remediate cu o grefă de piele liberă la un cățel. Rezultatele obținute sugerează că terapia cu ozon poate fi o strategie eficientă pentru a accelera procesul de vindecare și a îmbunătății rata de acceptare a grefelor fără utilizarea antibioticelor în cazul cățeilor.

Cuvinte cheie: grefă de piele, ozonoterapie, cățel, plagă

Wound healing is a highly coordinated and complex process involving biological mechanisms to restore the morpho-functional integrity of the skin. The success of wound healing depends on a significant group of biologically active polypeptides that modulate the healing process by transmitting signals to target cells, thereby stimulating their growth, differentiation, and metabolic activation (5). Free skin-grafts are a viable option for reconstructing limb defects with extensive tissue loss. The success rate of this technique may be improved by using complementary supportive therapies. In cases of wounds with a significant loss of substance, the application of a skin graft promotes the patient's recovery by replacing the epithelialization phases and substantially reducing the healing time (3). Ozone therapy is a therapeutic method that has been around for a long time but is still met with some resistance within the medical community. While ozone can have dangerous effects in high concentrations, researchers have observed that when used at appropriate doses, it can have therapeutic effects. As a result, ozone therapy, its various administration methods, and its effects on the human and animal bodies have been extensively studied for over a century worldwide.

Medical ozone (O_3) is a colourless gas with a distinct smell formed by the combination of three oxygen atoms and is produced by special generators. In its gaseous phase, ozone is stable for only 3 seconds, making its storage complicated. For this reason, ozone needs to be generated instantly before direct use (1).

1) University of Agricultural Sciences and Veterinary Medicine, Faculty of Veterinary Medicine, Cluj-Napoca, Romania
*) *Corresponding author: nicusor-valentin.oros@usamvcluj.ro*

Fig. 1. (A) The appearance of the limb upon arrival for consultation; (B) Radiological examination

In the wound healing process, ozone acts by triggering the release of growth factors when the wound is directly exposed to ozone. It also has bactericidal and bacteriostatic effects, helps combat tissue acidosis, and improves the repair process at the dermal level due to a slight increase in oxygenation pressure (7). Ozone stimulates the Krebs cycle by enhancing the oxidative carboxylation of pyruvate and stimulating ATP production (12). This study aimed to evaluate the therapeutic efficiency and feasibility of topical ozone treatment applied both pre- and postoperatively to a puppy with a distal forelimb degloving injury.

CASE PRESENTATION

A 4-week-old male puppy was referred for treatment of a large distal left forelimb degloving injury (Fig. 1). Following the evaluation of the lesions, it was decided to debride the necrotic tissue and amputate digits II, III, IV, and V, resulting in a 27.5 cm^2 wound. The bacteriological examination revealed the presence of *Proteus spp.* and *Streptococcus spp.*, both exhibiting susceptibilities solely to marbofloxacin. Considering the patient's age and the potential risks associated with the administration of this antibiotic to younger individuals, the decision was made to abstain from antimicrobial therapy.

OZONE THERAPY AND GRAFT SURGERY

Ozone bagging treatment (Fig. 2A) at a concentration of 60 µg/ml (microgram/millilitre) daily was used to treat the wound for the first 5 days. Two more sessions at 30 µg/ml concentrations were performed 3 days apart until healthy granulation tissue was observed (Fig.2B). A wet to dry simple dressing was used after each ozone therapy session.

On the 11th day, the free skin graft surgery was performed under general inhalation anesthesia. The skin graft was harvested from the left side of the thorax, placed on the granulating tissue, and sutured at the borders (Fig. 2C) of the wound immediately after the ozone bagging session. The grafted wound was covered with a non-adherent neutral paraffin wet dressing, sterile compresses, cotton roll, and elastic vet rap. Four ozone bagging treatment sessions followed the surgical procedure, applied every 3 days at a concentration of 20 µg/ml (Fig. 2D).

RESULTS AND DISCUSSIONS

The first bandage change was performed 3 days after the surgical procedure, with the graft displaying 100% viability, with no signs of bacterial infection (Fig. 3A). Nine days later, the sutures were removed. The

Fig. 2. Ozone therapy (A) First session; (B) Wound on day 11 after 7 sessions of ozone therapy; C) Wound covered with skin graft; (D) Last session of ozone therapy-12 days postoperatively

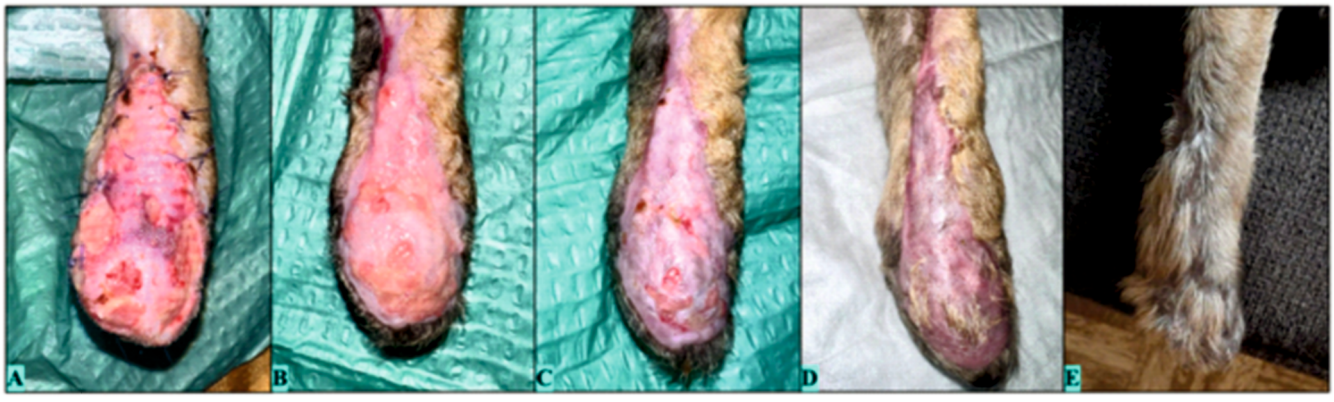

Fig. 3. Mesh skin graft healing evolution. (A) 3 days postoperatively; (B) 9 days postoperatively; (C) 20 days postoperatively; (D) 60 days postoperatively; (E) 120 days postoperatively

graft was adherent to the wound margins, and extensive areas of epithelialization were noted (Fig. 3B).

At 120 days postoperatively, apart from increased hair length in the grafted area, a complete aesthetic recovery was observed (Fig. 3E). The use of ozone as a complementary therapy may be essential in enhancing the acceptance rate of skin grafts. This therapy offers advantages by maintaining the bacterial clearance of both the wound and the skin graft, improving local oxygenation and stimulating fibroblast proliferation and keratinoblasts, - vital processes for proper cutaneous healing (13).

Graft revascularization is a critical phase in the graft's acceptance process. In support of this process, ozone appears to act by releasing essential growth factors such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and vascular endothelial growth factor (VEGF), as demonstrated in an experimental study on guinea pigs carried out by Kim in 2009 (6). Additionally, ozone's ability to release nitric oxide and induce prostacyclin production, leading to vasodilatation, may play a crucial role in the accelerated recovery of the patient. The hydrogen peroxide resulting from the reaction between ozone and polyunsaturated fatty acids increases the expression of 2,3- diphosphoglycerate in erythrocytes, thereby improving oxygen transport and release at the skin graft site (2). The acceptance rate of a skin graft is significantly influenced by the recipient's graft site health status. Graft site infection is a major factor that often leads to graft rejection. In our case, no clinical signs of bacterial infection were observed; this may be attributed to the bactericidal and bacteriostatic effects of ozone (4).

In a retrospective study evaluating skin grafts as a technique for closing cutaneous defects in dogs and cats, the average time between the appearance of the skin defect and the graft placement in dogs was 31.1 days (11). Another case report involving the use of a skin graft to close two skin defects on the hind limbs of a 2-month-old puppy reported a graft acceptance rate of 75% and complete epithelialization after 30 days, with the patient benefiting from only non-adherent absorbent dressing with neomycin and bacitracin (10). In our case, wound management, from patient presentation to skin graft placement took 11 days and 20 days after graft application, epithelialization exceeded 90% (Fig. 3C). In two earlier studies, we reported the effectiveness of this therapy in the ongoing management of wounds in cats, including a case involving skin grafting in a cat (8, 9). Based on clinical evidence, these considerations lead us to posit that the complementary use of ozone therapy in wound management accelerates the restoration of tissue integrity.

CONCLUSIONS

To the best of our knowledge, this is the first report of using ozone treatment to accelerate healing in a puppy with a distal limb laceration repaired with a free skin graft. Our findings suggest that ozone therapy may be an effective strategy to accelerate the healing process and acceptance rate of free skin grafts without the use of antimicrobial therapy in puppies. However, further controlled prospective studies are required to validate these results.

ACKNOWLEDGMENTS

We would like to thank Dr. Pauline Durieux for taking care of this patient during therapy.

REFERENCES

1. *Bocci V.*, (2010), OZONE, Springer, Dordrecht, Netherlands, 17-24
2. *Bocci V.A.,* (2006), Scientific and medical aspects of ozone therapy. State of the art. Archives of Medical Research, 37(4):425-435
3. *Corr S.,* (2009), Intensive, Extensive, Expensive: Management of Distal Limb Shearing Injuries in

Cats. Journal of Feline Medicine and Surgery, 11 (9):747-757
4. *Doroszkiewiaz W., Sikorsica J., Jankowskis P.,* (1994), Studies on the influence of Ozone on complement mediated killing of bacteria. Clin Immunol Med Microbiol, 9(4):281-228
5. *Johnston S.A., Tobias K.M.,* (2018), Veterinary surgery:small animal, (Ed.)Elsevier, St. Louis, USA
6. *Kim H.S., Noh S.U., Han Y.W., Kim K.M., Kang H., Kim H.O., Park Y.M.,* (2009), Therapeutic effects of topical application of ozone on acute cutaneous wound healing. Journal of Korean Medical Science, 24(3):368
7. *Lim Y., Phung A.D., Corbacho A.M., Aung H.H., Maioli E., Reznick A.Z., Cross C.E., Davis P.A., Valacchi G.,* (2006), Modulation of cutaneous wound healing by ozone: Differences between young and aged mice. Toxicology Letters, 160(2): 127-134
8. *Oros N.A., Repciuc C. Ober C., Mihai M., Oana L.I.,* (2023), Combined oxygen-ozone therapy for mesh skin graft in a cat with a hindlimb extensive wound. Animals, 13(3):513-513
9. *Oros N.A., Repciuc C., Ober C., Peștean C., Mircean M., Oana L.I.,* (2023), Clinical evaluation of medical ozone use in domestic feline cutaneous wounds—a short case series. Animals, 13(17):2796-2796
10. *Rahal S.C., Mortari A.C., Morishin Filho M.M.,* (2007), Mesh skin graft and digital pad transfer to reconstruct the weight-bearing surface in a dog. The Canadian Veterinary Journal, 48(12):1258-1260
11. *Riggs J., Jennings J.L.F., Friend E.J., Halfacree Z., Nelissen P., Holmes M.A., Demetriou J.L.,* (2015), Outcome of full-thickness skin grafts used to close skin defects involving the distal aspects of the limbs in cats and dogs: 52 cases (2005-2012). Journal of the American Veterinary Medical Association, 247(9):1042-1047
12. *Rilling S., Viebahn R.,* (1987), The Use of Ozone in Medicine; (Ed.) Haug Publishers, Heidelberg, Germany, 17
13. *Travagli V., Zanardi I., Valacchi G., Bocci V.,* (2010), Ozone and Ozonated Oils in Skin Diseases: A Review. Mediators of Inflammation, 2010:610 418.