

OZONE THERAPY IN TWO GERIATRIC MALE DOGS WITH POOR VASCULARISED CHRONIC WOUNDS OZONOTERAPIA ÎN TRATAMENTUL PLĂGILOR CRONICE SLAB VASCULARIZATE LA DOI CÂINI GERIATRICI

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ABSTRACT | REZUMAT

Chronic wound management in geriatric patients is still a challenge for veterinary practitioners. Because of less or no physical activity, prolonged decubital positions, lack of peripheral blood flow and efficient immune system these patients are predisposed to a slower healing time of cutaneous lesions and perhaps to other complications like secondary infections, phlegmonas and finally chronic ulcerations with antibiotic-resistant germ development. Even if not studied in veterinary medicine, direct topical and injectable ozone therapy is a frequently used treatment for human patients with type 2 diabetic ulcerations, which motivated us to perform this study. A 10 years old Great Dane with an ulcerated atonic 8/5 cm wound on the lateral side of the left distal tibia respectively a 7 years old German Shepard with an atonic 20/25 cm wound on the dorsal side of the neck were taken under study. After an unsatisfactory subantibiotic treatment, the patients were infiltrated subcutaneously with ozone in a 15µg/ml dose, 0,5-1 ml/ point of inoculation at a distance of about 3 cm between points, followed by a topical treatment with 50 µg/ml of ozone with the plastic bag method in one of the cases. The healing was evaluated by photographic means. Decreases of the wound dimensions were very evident after the very first treatment and complete healing was registered after 16 days for the Great Dane and 46 days for the German Shepherd.

Keywords: ozone infiltrations, atonic wound, epithelisation, phlegmona

Managementul plăgilor cronice la pacienții geriatrici este o provocare pentru orice practician în medicina veterinară. Datorită diminuării sau lipsei activității fizice, a decubitului prelungit, a aportului deficitar de sânge în zonele periferice și a sistemului imun slăbit acești pacienți sunt predispuși la o prelungire a timpului de vindecare a leziunilor cutanate ba chiar la complicații cu infecții suprapuse, flegmoane și în final ulceratii cronice rezistente la orice formă topică de tratament. Ozonoterapia topică directă, puțin studiată în medicina veterinară, face minuni în tratamentul ulcerelor întâlnite la pacienții cu diabet de tip 2 în medicina umană, aspect care ne-a sugerat utilizarea acestei metode în cazul de față. În materialul de față am luat în studiu un câine din rasa Great Dane, în vârstă de 10 ani, sex M care prezenta o plagă ulcerativă, atonă la nivelul feței laterale a jaretului stâng cu dimensiunea de 8/5 cm și adâncimea de 1,5 cm. După o serie de tratamente clasice fără un răspuns favorabil, pacientul a fost supus din două în două zile tratamentului prin infiltrare subcutanată cu ozon în doză de 15 mcg/ml 0.5 ml/punct de infiltrație, iar punctele de infiltrație la o distanță de 3 cm între ele, urmat de tratamentul topic cu ozon prin metoda pungii de plastic în doză de 50 mcg/ml la presiune atmosferică și un pansament steril de protecție. Vindecarea a fost evaluată prin monitorizarea fotografică a dimensiunilor plăgii la fiecare sedință de tratament și prin aprecierea gradului de șchiopătură înainte de fiecare tratament. Reducerea dimensiunilor plăgii a fost evidentă încă după prima sedință de ozonoterapie, țesutul de granulație și linia de epitelizeare au apărut de asemenea după prima sedință. Vindecarea completă a plăgii s-a produs după 6 ședințe de ozonoterapie.

Cuvinte cheie: ozonoterapie, plagă atonă, epitelizeare, flegmon, geriatrie

Considering the high costs associated with treating of chronic infected wounds in dogs, the development of better treatment strategies is needed.

The medical use of ozone (also known as triatomic oxygen or trioxxygen) was initiated in the 19th century. Ozone has multiple therapeutic effects in wound healing due to the property of releasing nascent oxygen, which has been shown to have bactericidal/bacteriostatic properties and to stimulate some antioxidant enzymes (1, 6, 13, 14).

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Ozone stimulates and regulates the immune system activity augmenting the synthesis of certain organic molecules. Its bio-energetic, anti-hypoxic, analgesic and homeostatic properties are also mentioned in the literature (8). Ozone-therapy is a successful method used in the treatment of wounds that tend to heal abnormally like diabetic ulcers, distal limb wounds in horses, high surface extended lesions which are difficult to be protected by dressings, which tend to be exposed to infections and wounds that do not heal by conventional therapies because maybe of antibioresistance. Ozone in precise doses acts like a local antiseptic, tissue oxygenating agent and epithelisation promoter by accelerating the release of many growth factors (4, 5, 7). At the early stage of treatments (including debridement), wound healing is managed by platelet-derived growth factors (PDGFs) and transforming growth factors- β (TGF- β). Then tissue macrophages supply the key multiple growth factors for healing (4, 5). However, limited research has been conducted because it is difficult to obtain the tissue specimens in clinical trials to verify the correlation of the treatments and the expressions of growth factors in the wounds.

MATERIALS AND METHODS

The biological material consisted in two male dogs, a 10-year-old Great Dane with an ulcerated atonic 8/5 cm wound on the lateral side of the left distal tibia and a 7-year-old German Shepard with an atonic 20/25 cm wound on the dorsal side of the neck. The non-biological materials consisted in conventional dressing materials, 5-10 ml syringes and an ozone generator (Medozon Compact, Hermann Aparateau GmbH).

Case 1

The 10 years old Great Dane came to our clinic with a small abscess on the lateral side of the distal left tibia and no deep pain in the area distal to the abscess. The abscess was drained by standardised means and a bacteriological exam and antibiogram were performed from the secretion obtained. The bacteria population obtained after the exam was *Protheus* spp. and *E coli*.

The drainage incision turned into an ulceration of about 5/4 cm (20 cm²) and necrotic processes kept advancing even after the classical treatment by Metronidazol 15 mg/kg (Metronidazol, Infomed Fluids), enrofloxacin 5mg/kg (Enroxil®, KRKA) and marbofloxacin 80 mg (Marbofloxin®, Krka) for 5 days. We carried out another bacteriological exam with an antibiogram and the resulting germs (*protheus* spp.) showed sensitivity to marbofloxacin which was administered for another 10 days.

Even under antimicrobial treatment the wound had

an unsatisfactory evolution, the contraction phase was delayed and the ischemic and necrotic processes advanced. In this situation we decided to introduce ozone-therapy in the fourth day after the marbofloxacin treatment began and to stop antibiotic medication.

Ozone was used topically and by perilesional and intralesional infiltrations. The topic therapy was made with the plastic bag technique (Fig. 1) in which 50 µg/ml of ozone at atmospheric pressure were introduced and maintained for 30 minutes every treatment session. The infiltrations were realised on the entire circumference of the wound at a dose of 12 µg/ml in 1 ml volumes in each infiltration point. The infiltration points had a 3 cm distance between them.



Fig. 1. Topical treatment, plastic bag technique

Case 2

The 7 years old German Shepherd came with a posttraumatic wet gangrene on the dorsal cervical region acquired while he disappeared for 5 days (Fig. 2).

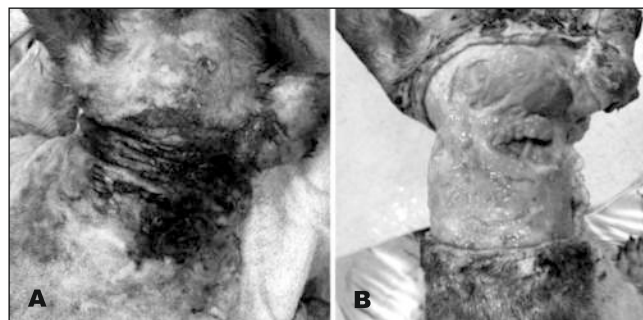


Fig. 2. A. Appearance of the gangrene when the dog was first time presented to the hospital, **B.** Appearance of the wound after debridement and 10 days of antimicrobial treatment

The general status of the animal was very depressed with permanent lateral recumbency, severe anaemia (HCT 15%) and advanced sepsis (WBC – 23.45*10³/mm³, LYM 28.3%, NEU 21.08*10³/mm³, Pro-T 3,4, ALT- 635). A slight sedation was performed with medetomidin 10 µg/kg (Domitor®, Orionpharma) and ketamine 2 mg/kg (Ketaminol®, Intervet)

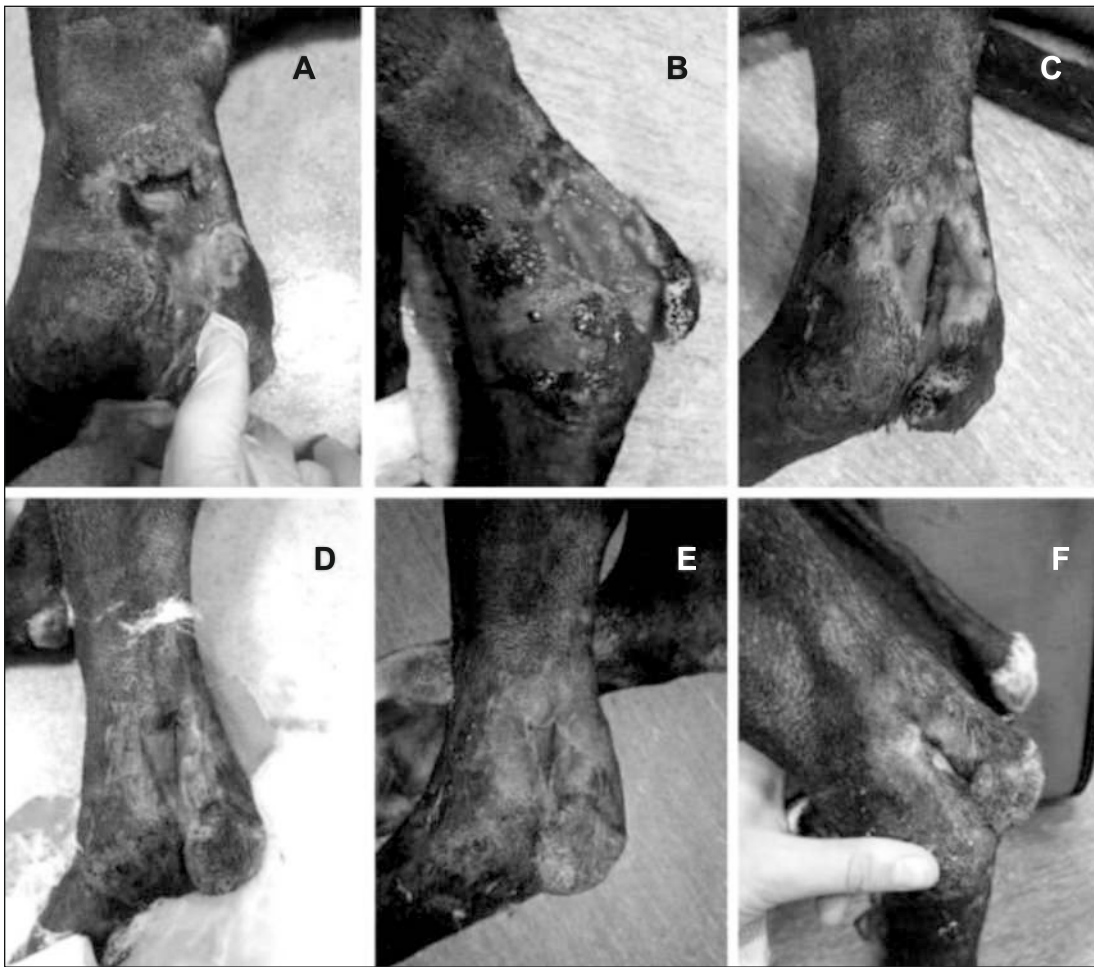


Fig. 3. Case 1 wound evolution under ozone therapy. **A** - Wound before the first treatment session, **B** - Granulating tissue and good vascularisation after the second treatment session, **C** - Wound epithelizing after 3rd session, **D** - 4th session of treatment, **E** - 6th session of treatment, **F** - Closed wound after 16 days and the last treatment session

and rigorous debridement of the wound was made. After debridement a 20/25 cm wound resulted (500 cm²). Postop treatment was metronidazole 15 mg/kg (Metronidazol, Infomed Fluids), amoxicillin cla-vulanic acid 12.5 mg/kg (Synulox®, Pfizer) and fluids Ringer constant rate infusion (CRI) 10 ml/kg/h. The debridement of the wound and dressing change were performed daily for about 10 days. For the next 8 days granulation was stimulated with colloidal dressings (Hydrotac®, Hartmann) but still the granulation phase was slow. To accelerate the entire process, we decided to introduce ozone therapy by local perilesional infiltrations at a 12 µg/ml dose in 1 ml volumes in each infiltration point at 3 cm distance between the points.

RESULTS AND DISCUSSIONS

Case 1 had a good evolution, the cutaneous tissue in the affected area developed a better peripheral cir-

culation and an acceleration in granulation tissue expression which could be inferred from the blood drops, resulted after the retraction of the treatment needles and the red colour of the granulation tissue. Also, the contraction of the wound was visible between treatments (Fig 3). Complete epithelisation was achieved after 16 days of treatment, 6 sessions of topic treatment and infiltrations. We also noticed that after the treatment the patient recovered proprioception of the affected limb and began walking and putting weight on it, but for the assessment of any influences of ozone infiltrations over neurologic disorders more exact research is needed. The wound epithelisation speed was of approximately 1.25 cm² daily (Fig. 3).

Case 2 had a good evolution as well. Granulation tissue developed quickly and the contraction phase was visible between treatments. The borders of the wound got adherent to the underlying muscle tissue after the first 2 sessions and the epithelisation line was

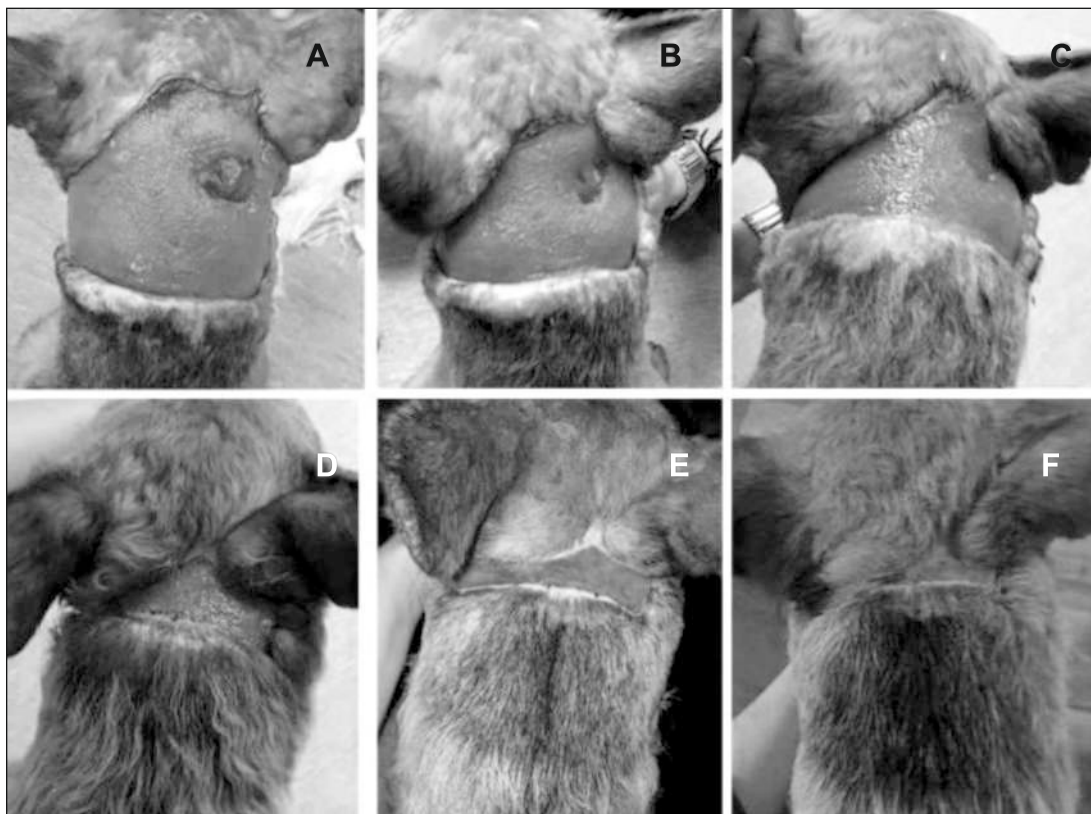


Fig. 4. Case 2 wound evolution under ozone therapy. **A** - Before the first treatment session, **B** - before the 4th treatment session, **C** - Before the 8th treatment session, **D** - Before the 12th treatment session, **E** - Before the 13th treatment session, **F** - Before the 15th treatment session

visible after the first ozone treatment. Granulation tissue filled up the deeper crevasses very well and the wound healed completely after 46 days of treatment, 15 infiltration sessions respectively. The wound epithelisation speed was of approximately 10 cm² daily (Fig. 4). Ozone therapy seems to accelerate the granulation process of muscular-cutaneous defects, a fact that can be macroscopically observed by a quick levelling of the deeper wounds. The contraction phase is also accelerated due to the significant reduction of the wound surface, even after a few treatment sessions (from 1.25 to 10 cm² daily). Our results support the amelioration of the peripheral blood flow theory due to a brighter red colour of the granulation tissue after infiltrations and due to a higher quantity of blood expressed after the retraction of the infiltrating needles. The balance between the surface of the wound and the healing time in our study was satisfactory and better than in the previous treatments given to these patients which consisted in topic and systemic antimicrobial treatments. Case 1 was treated 15 days without satisfying results. The ozone treatment led to complete healing in 16 days. Case 2 was also treated for 18 days before the introduction of ozone therapy and the results didn't satisfy our expectancies.

Ozone infiltrations healed the wound in 46 days.

We consider that amongst the regenerative process and stimulative effect, antibacterial effect had a great influence in our patients' wounds, especially in case 1 where we used topical treatment. The bactericidal effect of ozone was demonstrated by Rilling (11) and Doroszkiewicz (9). This bactericidal mechanism consists in the reaction of ozone with the unsaturated fatty acids present in the phospholipid layer of the cellular membrane, hydrofoil peroxides being obtained. Usually infected cells have their own production of hydrogen peroxide as a defence mechanism. The concentration of hydrogen peroxide is so high that cells are not capable of accepting other peroxides, so the cell membrane undergoes a lysis process or a synergic process which destroys microorganism by ozonolysis. Also, ozone therapy, is a well-known solution for a faster cicatrization of wounds with a prolonged evolution (2, 3). Intra- and perilesional infiltration treatment and topic gaseous treatment has shown its benefits in atonic, over infected and poor vascularized wounds in the case of our canine subjects. Soares et al (2019) demonstrated that injectable ozone therapy promotes a quicker epithelisation, better cell proliferation and increased matrix deposition in wounds obtained by in-

ducing cutaneous defects in rats (12).

Kim et al (2009) observed by immunohistochemical examination an increased expression of PDGF and TGF- β in keratinocytes after the ozone treatment, facts that might be of high influence in wound healing. (10, 15). Unfortunately, samples from the two cases in the study weren't collected for histological and immunohistochemical evaluation due to the fact that the owners of the dogs didn't agree.

Transient treatment and small O₃ doses can reactivate useful body functions and might display therapeutic activity (5). These results suggest the positive effect on wound healing of ozone, which might be associated with its anti-inflammatory and anti-apoptotic properties (5).

The evolution of the two cases after ozone treatment was satisfactory; the wounds had a very good epithelisation rhythm. We consider that the perilesional ozone infiltration is a great method of stimulating the granulation and contraction phases of chronic wounds and the topic plastic bag technique is a good technique for infected atonic wounds like case 1. To our knowledge, there are no studies in the literature about the effect of ozone infiltrations in dogs with chronic wounds. In order to consolidate our results, we recommend further studies on clinical trials with wounds of same localization, similar dimensions and developing stages in order to standardize and stabilize the stimulative effects of ozone in the healing processes of canine chronic wounds.

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