SUMMARY OF PhD THESIS

Evaluation of ozone therapy in stimulating skin wound healing in dogs and cats

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I. INTRODUCTION

The therapy of skin wounds in dogs and cats is a real challenge in the daily work of the veterinarian. Antibiotic resistance and lack or poor response to widely used conventional therapies make it problematic to successfully treat wounds, ultimately proving time and financial resource-consuming.

For more than a century, ozone (O_3) has been known to be an excellent disinfectant which has to be used with caution due to its oxidizing properties. Only in the last decade, following numerous studies, therapeutic doses have been established. Its therapeutic and regenerative effect results from the chemical reaction of ozone with certain tissue constituents.

There are few clinical studies in the literature that provide information on the type of method and ozone concentration used when adopting this therapy. There is also a lack of clinical comparative studies in evaluating the effectiveness of this method of therapy.

In this paper, well-defined protocols are presented, based on current evidence from the literature and on the clinical experience of the author. Through the results obtained, we encourage veterinarians to adopt this therapy in the management of skin wounds in dogs and cats.

II. PERSONAL CONTRIBUTION

1. Clinical evaluation of ozone therapy in wound healing in dogs and cats

There are few clinical studies in the literature that provide information on the type of method and concentration used when applying this therapy. There is also a lack of comparative clinical studies in evaluating this method of therapy.

Skin wounds in dogs and cats as well as their therapy, are a common problem in veterinary practice. Wound healing is a physiological process mediated by numerous growth factors that are successively released to restore the integrity of the skin. *Secundam intention* management of wound healing involves frequent dressing changes with different properties. Dressings can range from simple ones, such as non-stick dressings, to more complex options, such as foam, hydrocolloid, alginate, or negative pressure dressings. Ozone, used as a complementary therapeutic agent with regenerative properties, has begun to have wider applicability in veterinary medicine.

The objective of this study was to evaluate the clinical efficacy and feasibility of ozone treatment in *per-second healing* protocols of skin wounds in dogs and cats.

To our knowledge, this is the first preliminary controlled study of its kind in dogs and cats. Seven cats with a total of nine wounds and seven dogs with seven wounds of different sizes and locations were included in this study. Three methods of local ozone

administration have been used to cover several properties of ozone in wound treatment: the plastic bagging method, perilesional subcutaneous infiltrations, and lavages with ozonated saline.

Evaluation of the healing process was performed by clinical observation (photography) and planimetry every seven days until complete recovery of patients.

Finally, the use of these three methods of administration could stimulate the achievement of a synergistic effect. The physiological saline solution acts as a lavage element and aims to reduce the superficial bacterial load of the wound, also having immunomodulating, anti-inflammatory and epithelizing properties. Bagging could help deliver oxygen to tissues, controlling infectious processes and stimulating the release of platelet-derived growth factors, which promote healing. Perilesional subcutaneous infiltrates have local anti-inflammatory and analgesic effects since ozone can oxidize pain receptors. Given all these aspects, the presented therapy protocol could cover all the needs of a wound under treatment. Still, this hypothesis requires further research as well as evaluation of their medical benefits. These positive clinical outcomes encourage new prospective controlled trials with more groups and additional assessment methods for even more complex outcomes. Comparing this therapy with other conventional therapies would also be an interesting prospect.

Currently, there are no clinical studies demonstrating that ozone treatment methods are superior to other therapies in wound management in dogs and cats. The results of this study suggest that ozone, used as a complementary therapy in *per-second* wound healing, supports the recovery of patients with extensive acute and chronic wounds, even cats diagnosed with FIV^+. This could be due to ozone's ability to improve local circulation, stimulate the release of growth factors, and decrease wound bacterial load if used according to the protocol described.

In the present study, no statistically assured differences were observed between the two species in wound healing speed.

2. Clinical evaluation of ozone therapy in combination with reconstructive surgery of skin wounds in dogs and cats

Wounds accompanied by considerable loss of substance are common in current practice, and their therapeutic management and choice of closure method represent a challenge for the veterinarian, exerting an important influence on the evolution of healing. This study proposes a new, complementary wound therapy technique that aims to support free skin grafts and local skin flaps using ozone therapy both before and after skin plastic surgery. This approach accelerated the recovery of patients and no complications were observed.

According to literature data consulted, this is the first clinical trial in which ozone therapy is used to support skin grafts and flaps in dogs and cats. This new therapeutic approach could be used pre- and post-operatively to accelerate patients' recovery.

The objective of this study was to clinically evaluate the therapeutic and regenerative effect of applied ozone before and after surgery to support loose skin grafts and local skin flaps in dogs and cats. The hypothesis of this work is based on the antibacterial effect, the oxygenation capacity of tissues and the release of growth factors, following the application of ozone locally. These aspects are crucial for quick and uncomplicated healing.

The results of our study support the hypothesis that ozone therapy accelerates the healing of wounds grafted and remedied by local skin flaps. This is comparable to other conventional treatments used in classical procedures, including negative pressure therapy.

The favorable and accelerated recovery of the presented patients encourages the use of ozone therapy as a protocol to support free skin grafts and local skin flaps in dogs and cats.

Future studies should focus on establishing therapeutic dosages and timing of application during the healing process. Furthermore, the biological effects of ozone therapy need to be evaluated *in vitro*. We consider prospective studies with a larger number of patients including a control group necessary to validate our results.

3. Evaluation of the antimicrobial effect of ozonized olive oil

Bacterial superinfection is the most common complication in patients who have skin lesions with impaired healing. As antibiotic resistance has become a major concern in recent years, research has been done for alternative treatment options. Research has focused on finding a molecule with antiseptic and antimicrobial potential, structurally different from classical antibiotics, effective on microorganisms, with the ability not to generate resistance after multiple applications and producing minimal collateral effects.

The objective of this study was to develop a local product based on ozonized olive oil obtained with the help of a generator for medical use. The applicability of this product can be found in the therapy of skin wounds in animals. The in *vitro* evaluation of the antibacterial effect of ozonized olive oil on different species of bacterial cultures and fungi was performed, as well as its physicochemical analysis. 4 samples of ozonised extra virgin olive oil were tested for different periods of time (1, 3, 6 and 12 hours respectively at an ozone concentration of 80 µg/ml with a mixture flow rate of 4 l/min) alongside a blank sample of non-ozonised oil. For evaluation, reference strains of fungi, *Candida albicans* DMSZ 1386, Gram positive bacteria: *Staphylococcus aureus* ATCC 6538P and *Enterococcus faecalis* ATCC 29212 and Gram negative: *Escherichia coli* ATCC 13076, *Pseudomonas aeruginosa* ATCC 27853 and *Klebsiella pneumoniae* NCTC 13438 were used. The bacteria were grown on Nutrient Agar (NA) medium, and for *Candida albicans* a Sabouraud Dextrose medium (SAB) was used with the addition of chloramphenicol. Also, the oil samples obtained were physico-chemically characterized to establish viscosity, iodine index, peroxide index and acidity index.

The obtained results validate the use of ozone generators, for medical use, in the production of ozonized olive oil, the antibacterial effect being directly proportional to the peroxide value in the oil samples, which confirms that the peroxide contained in ozonized oil is responsible for antibacterial activity.

A good antibacterial effect was also observed at a low peroxide index value (224 mEq/1000g), which corresponds to ozonization of oil for 12 hours using the described parameters. Susceptibility was more pronounced to Gram positive bacterial strains, and the antimycotic effect (*Candida albicans*) was high.

Integrating ozonized olive oil into therapeutic wound management in animals could be an effective and affordable option.

III. GENERAL CONCLUSIONS AND RECOMMENDATIONS

The general conclusions deriving from the studies carried out can be summarized as follows:

1. Ozone therapy used complementarily, in *the per-second* healing of skin wounds in dogs and cats, accelerates the recovery of patients;
2. Complementary ozone therapy has also been shown to be effective in cats with wounds, diagnosed with FIV^+;
3. Ozone therapy has proven its effectiveness and clinical feasibility in *per-second healing* protocols of skin wounds in dogs and cats;
4. Ozone therapy decreases the time required to obtain granulation tissue in order to apply a skin graft or make a local skin flap;
5. Ozone therapy applied in the postoperative management of free skin grafts and local skin flaps, improves their acceptance rate;
6. Favorable and accelerated recovery of patients encourages the use of ozone therapy as a protocol to support free skin grafts and local skin flaps in dogs and cats.
7. The antimicrobial effect was directly proportional to the peroxide value in the oil samples, which confirms that the peroxide contained in ozonized oil is responsible for antimicrobial activity;
8. Ozone generators for medical use having a flow rate of at least 4 l/min can be used to produce ozonized extra virgin olive oil.

Taking into account the results of the studies carried out within this research project, we recommend:

1. Complementary ozone therapy by its local application in *secondary intention* management of skin wounds in dogs and cats;
2. Application of local ozone therapy both before and after remedying skin defects by reconstructive surgery in dogs and cats;
3. Favorable and accelerated recovery of patients encourages the use of ozone therapy as a protocol to support loose skin grafts and local skin flaps in dogs and cats.
4. Integration of ozonized olive oil into the therapeutic management of wounds in animals, as an an effective option.

IV. ORIGINALITY AND INNOVATIVE CONTRIBUTIONS OF THE THESIS

The studies conducted and presented within this doctoral thesis reveal their element of originality in the evaluation of the efficiency and clinical feasibility of ozone therapy in the healing process of skin wounds in dogs and cats, as well as the development and improvement of a method for the ozonization of olive oil with antibacterial potential by using a generator for medical use.

The innovative elements of the doctoral thesis are represented by:

1. Contouring and implementing an ozone administration technique in *secondary intention* healing of skin wounds in dogs and cats, combining the synergistic effect of the three methods of topical application;
2. Evaluation of clinical efficacy of ozone therapy, by planimetry, namely quantifying the speed of epithelization, contraction and total healing, in the *secondary intention* management of skin wounds in dogs and cats;
3. Application of ozone therapy, as a complementary method of supporting reconstructive surgery performed by free skin grafts and local skin flaps, used before and after these surgical procedures.
4. Development of an efficient ozonization technique of extra virgin olive oil, using a generator for medical use;
5. Obtaining an antibacterial and antimycotic effect of ozonized olive oil at a low peroxide index value (224 mEq/1000g).