OZONE THERAPY IN VETERINARY MEDICINE

OZONOTERAPIA ÎN MEDICINA VETERINARĂ

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ABSTRACT | REZUMAT

Ozone is a reactive molecule that, in contact with biological substances, has a property to link at the level of double and triple bindings.

Due to its reactivity and instability, it can inactivate microorganisms, stimulate the tissue level oxygen metabolism, it may be efficient in muscular and cutaneous lesions treatment, it can have anti-inflammatory effect by oxidizing some pro-inflammatory mediators, analgesic efficiency by oxidizing some pain mediators and regenerative properties by releasing platelet level specific growth factors.

In veterinary medicine, ozone is used successfully in syndrome of back pain in horse, muscular and cutaneous lesions in all species (antibacterial, anti-inflammatory and stimulant of tissue regeneration effect), lesions of joints, ligaments and tendons (by injection alone or in combination with other substances), in discal hernia in dog, as analgesic (by insufflation or injection in trigger points). In reproduction, it is used by intracavitary insufflations or lavages in mastitis, metritis, endometritis, placentary retentions, vaginitis, uro-vagina in small and big ruminants.

Keywords: Ozone, ozone therapy, regeneration, complementary therapy

Ozonul este o moleculă reactivă, în contact cu substanţele biologice având proprietatea de a se lega la nivelul legăturilor duble şi triple.

Datorită faptului că este o moleculă reactivă, instabilă, inactivează microorganismele, stimulează metabolismul oxigenului la nivel tisular, este eficient în tratarea leziunilor musculo-cutanate, are efect antiinflamator prin oxidarea mediatorilor proinflamatori (acidul arahidonic, prostaglandine derivate), analgezic prin oxidarea mediatorilor durerii şi regenerativ prin eliberarea factorilor specifici de creştere de la nivel trombocitar. Ozonul posedă şi proprietatea de a modula sistemul imunitar prin eliberarea de citochine imunostimulatoare sau imunosupresoare.

În medicina veterinară ozonul se foloseşte cu succes în sindromul durerii spatelui la cal, în plăgi musculo-cutanate la toate speciile (efect antibacterian, antiinflamator şi stimulator al regenerării tisulare), în leziuni articulare, tendinoase şi ligamentare (injectat ca atare sau în combinaţie cu alte substanţe), ca analgezic (prin insuflare sau injectare în puncte trigger) şi în hernii discale la câine. În reproducţie este folosit prin insuflări intracavitare sau lavaje în mastite, metrite, endometrite, retenţii placentare, vaginite şi urovagin la rumegătoare mari şi mici.

Cuvinte cheie: Ozon, ozonoterapie, regenerare, terapie complementară

Ozone is an unstable gas obtained from 95-99% medical oxygen. The gas is generated by a special machine that transforms oxygen (O_2) to ozone (O_3) (Fig 1). Ozone therapy was first mentioned by Tesla and later by Wolff in the 40's during the First World War when he used ozone as ozonized water in treating wounds and gangrenes. Unfortunately, due to the interest of practitioners rather in effects and doses of ozone without research and evaluation of the mechanisms of action, many skeptics succeeded in promoting the idea that ozone is toxic no matter the way or dose it is used in, so it shouldn't be used for medical purposes. Even though the known benefic effects of ozone in medicine are published, the idea of toxicity is still promoted without any solid proof. If in human medicine, the aspects of this therapy are very well known and documented, in veterinary medicine things are not the same. This material has the goal of opening new research objectives in veterinary ozone therapy and of summarizing the present knowledge about already proved good effects of this old yet new treatment.

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Ozone mechanisms of action

The studies in the last century, according to the properties of ozone, showed its capacity to react to the majority of organic and inorganic substances until they are oxidized completely. In contact with biological substances, it has been shown that it binds at double

and triple binding levels. Some of these substances are proteins, amino acids and fatty acids which are part of the plasmatic lipoprotein complexes of the cell membrane (4). Ozone therapy is based on the reaction of ozone with these elements that have an important role in many disease pathogenesis. Its mechanisms are strongly related to the production of four fundamental species in reaction with membrane phospholipids: ozonide, aldehide, peroxides and hydrogen peroxide (H_2O_2). These will act at cell, biological fluid or tissue level with substances that have double bindings. Ozone also interacts with DNA molecules and cistein residues of proteins.In controlled and adequate quantities, these ozone derivates resulting due to its reaction at double bindings have different biological and therapeutic functions. In their messenger action, they activate enzymes, chemical mediators and immune response mediators (15).

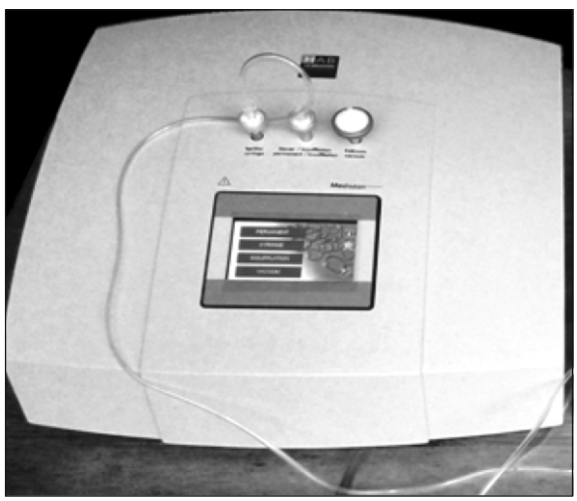

Fig. 1. 21st century touch screen ozone generator

When Ozone interacts with biological fluids (blood, plasma, urine a.s.o.) it dissolves in their water component in seconds. Hydro- and lipophilic antioxidants in these fluids neutralize a significant part of the initial O_3 quantity, but if the concentration and quantity are adequate, a sufficient quantity of reactive oxygen species (ROS) and lipoperoxidation products (LPO) will form. ROS formation in plasma occurs in less then a minute and it generates a transitory decrease of the antioxidant capacity. The normal antioxidant level recovers to normal in about 15-20 minutes.

Hydrogen peroxide and the other mediators diffuse in the cells and activate diverse metabolic mechanisms at erythrocyte, leucocyte and platelet level that will generate many positive biological effects (4). The most important ROS is hydrogen peroxide which is a non-radical oxidant (5), that plays a signaling role in the cells as messenger of the release of the therapeutic ozone dose (14).

Stimulation of the oxygen metabolism

The effects of therapeutic ozone to oxygen metabolism can be explained due to its action to the rheological properties of the blood and has as a result the increase of cellular glycolysis of erythrocytes (15,4). The rheological changes can be explained due to the reversible effects of ozone to erythrocyte aggregation in case of arterial obstructions and an increase of the erythrocyte plasticity (15).

These effects are relevant in explaining the action of ozone at circulatory level. The most probable hypothesis is that this effect occurs after the first treatment. The general effect is similar to post physical training feeling (15).

The increase of glycolysis is evident after every treatment cycle and it can be measured due to an increase of the arterial blood oxygen partial pressure (pO_2) and in the same time a decrease of the venous blood pO_2 (2). All these occur because of a slight decrease of the intracellular pH (Bohr effect) and the stimulation of 2,3-difosfoglicerate and increasing of its concentration, all these determining an increase of oxygen at tissue level (15). Ozone stimulates the reactions of Krebs cycle due to oxidative carboxilation of piruvate release and stimulation of ATP production. Also it significantly reduces NADH through activation of phosphate pentose mechanism. In this reaction, glucose-6-phosphate dehydrogenase (G-6PD) is the main enzyme. Also, C cytochrome is oxidized and the production of glutation peroxidase, catalase and superoxid dismutase is stimulated as they are cell membrane protector (5). Ozone also induces prostacycline production which is a vasodilating element.

This controlled increase of oxidative stress finally has, as result, an increase of the antioxidant system (4). According to the ozone tolerance theory, we can say that the exposure of organism to small dose of substance that may be harmful in large doses induces a benefic adaptative response. It's been proved that LPO, due their long time messenger action, can send an acute controlled oxidative stress to all organs (5).

LPO acts also as stress factor for bone marrow adapting the erythrogenesis and increasing the level of antioxidant enzymes. The new formed erythrocytes posses a much bigger G-6PD activity comparing to the elder ones, fact that determined their "super-active erythrocytes" name (5).

Immune system activation

Many studies have demonstrated that ozone has a modulator action to the immune system due to the synthesis or release of immune stimulating or suppressive cytokines at a controlled level without exciding the normal necessary values. Good results were observed in patients with immunological dysfunctions treated with ozone (15).

"Immunological" action of ozone in the blood is focused on monocytes and lymphocytes which release small quantities of cytokines. Ozone is a modulator of the immune system by neutrophils activation and stimulation of synthesis of some cytokines (6). Ozone, in a concentration of 30-55 mcg/cc, stimulates the release of interferon in high quantities, the release of TNF and IL-2. IL-2 release also starts a whole immune response chain (6).

Ozone mechanisms of action in pain management

Some studies admit that ozone has an analgesic and anti-inflammatory double mechanism of action.

Ozone decreases the level of some pro inflammatory mediators (IL 1, 8, 12, 15), oxidizes the pain mediators and improves local perfusion with a better tissue oxygenation (15). Ozone "relieves" the pain.

As an anti inflammatory drug, ozone oxidizes arachidonic acid, prostaglandins (E2) and derivate prostaglandins which, in high concentrations, are involved in maintaining inflammatory processes (13).

Another mechanism that might explain the analgesic effect of ozone is the reflex mechanism described in acupuncture for example that activates other endogenous mechanisms and the result might be a high endorphin secretion level (15).

In lumbar pain treatment generated by diverse factors (in human, the most studied is discal hernia), paravertebral or intralesional, ozone treatment has very good results (15).

Ways of using ozone in animals

According to the Madrid Declaration regarding ozone therapy (2014), the medical ways of using ozone accepted in humans and animals are the following:

- Major Autohaemotherapy (MAHT);
- Minor Autohaemotherapy (MiHT);
- Subcutaneus, intramuscular, paravertebral, peridural, intraarticular injections (Fig. 2);
- The ozone bag technique;
- Fistula insufflation;
- Vaginal insufflation;
- Urethral and bladder insufflation;
- Rectal insufflation;
- Trigger points micro-dose injections;
- Topic treatment with ozonized water, oil, gel and ointments.

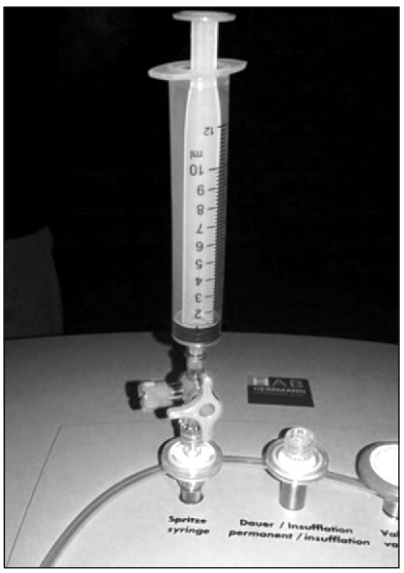

Fig. 2. Automatic syringe charging

Ozone using in veterinary medicine

Ozone is used in veterinary medicine for around 30 years. Although its mechanisms of action hasn't been studied in animals.

Surgical use of ozone

Vigliani A. et al (2005) publishes therapeutic success in the treatment of back pain syndrome in horses by paravertebral ozone administration (19). Ballardini (2005) makes a study about subcutaneous administration of ozone in painful dorsal muscles problems of sport horses obtaining good results (3). Ozone can also be used in facet joint diseases of horses by eco-guided intraarticular injections (8). Ozone has a great effect on wounds due to its antibacterial, anti-inflammatory, analgesic and regenerative properties (9). In skin wounds, ozonized water, oils or gels can be used.

Another topic regarding ozone therapy is the ozone bag method which seems to be a good choice in limb wounds. In 2010, Garcia C. proves good effects of this method applied to horses with limb wounds due to habronemosis (9). In articular, tendinous or ligamentar problems, ozone can be applied in place as pure gas or as an enhancer of PRP action in horses (1), dogs (12). Analgesic effects of ozone rectally insufflated or injected into trigger points in dogs has been proved by Teixeira L.R. (2013), by comparison with effects of

meloxicam (17). Another study in dogs with discal hernias proved that intradiscal ozone administration under fluoroscopic guidance is a minimum invasive way to reduce medullar compression (11).

Reproductive use of ozone

Intracavity gaseous insufflation ozone therapy or cavitary lavages with ozonised saline solution has been used successfully in mastitis (16), metritis, endometritis (16), fetal membranes retention (7), vaginitis and uro-vagina (20) in cattle and goats. As good effects of these therapies have been shown in ruminants, it is justified to consider a great opportunity to research the activity of ozone in reproductive diseases of mares and bitches.

Internal medicine use of ozone

In animals, systemic infections, just like in human systemic infections, major autohaemotherapy and rectal insufflations are the most used and recommended techniques. Alves G.E.S. et al. (2004) proves the benefic effects of major ozonized autohaemotherapy in intestinal reperfusion lesions of horses (2).

Perspectives and limitations of ozone in veterinary medicine

In human medicine, ozone therapy is used successfully in infectious diseases, immunosupressive diseases, ischemic conditions, degenerative diseases related to ageing (hormetic effect of O_3), acute or chronic joint pathology including discal herniation, diabetic ulcers, with regenerative purposes in muscular-tendinous lesions of athletes and in wounds of many categories. There are many products on the market based on ozonized vegetal oil in various presentations. These products have emollient, scaring, antibacterial and local disinfection properties (18). This products are sold in conformity with the CEMD (European Conformity Medical Device) requirements and registered under European Directive 93/42/CEE.

Studies published about ozone therapy in small animals are very few or inexistent. In horses and dogs, ozone therapy has been researched in empirical ways without relevant and detailed proof that could sustain the obtained clinical effects. A relevant and well documented study has been made by Haddad M.A. (2009) about the biochemical status of horse blood following ozonized autohaemotherapy.

REFERENCES

1. *Alonso Roberto M.V.,* (2013), Plasma rico en plaquetas ozonizado en lesiones de los ligamentos colaterales de la articulacion interfalangiana distal, Trabajo de graduacion, Universidad de Buenos Aires, Facultad de Sciencias Veterinarias.
2. *Alves G.E.S., Abreu J.M.G., Ribeiro Filho J.D., Muzzi L.A.L., Oliveira H.P.,Tannus R.J., Buchanan T.,* (2004), Efeitos do ozônio nas lesões dereperfusao do jejuno em eqüinos. Arq.Brasil.Med.Vet. Zootec. 56:433–437.
3. *Ballardini E.,* (2005), Oxygen-Ozone Therapy for Spinal Muscle Disorders in the Horse. Riv. It. Ossigeno Ozonoterapia ,4: 70-73.
4. *Bocci V.,* (2005), General mechanism of action of ozone therapy and mechanism in pain treatment. Rev. Soc. Esp. Dolor, 12: 24-36.
5. *Bocci V.,* (2005), Ozone A New Medical Drug, Dordrecht, The Nederlands. Springer: 295.
6. *Bocci V., Luzzi E., Corradeschi F., Paulesu L., Di Stefano A.* (1993), Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymph. and Cyt. Res., 12:121-126.
7. *Djuricic D., Vince S., Ablondi M., Dobranic T., Samardzija M.,* (2012), Intrauterine ozone treatment of retained fetal membrane inSimmental cows. Anim. Reprod. Sci. 134:119–124.
8. *Garcia Liñeiro J.A. , Argibay T., Scipioni H., Duarte E.,* (2009), Eco guide oxygen peroxidum injection (ozone therapy) as treatment of intervertebral lesions in horses, description of the evolution of 15 cases. World Equine Veterinary Association WEVA Congress 2009 Guaruya – Brasil.
9. *Garcia C.A, Eurides D, Prado B.R, Neves Rodrigues, M M; Nara Neves, S M; Queiroz Rinaldi, F C,* (2010) Skin healing in one equine by therapy with ozone Revista CENIC. Cien. Biol., 41: 1-6.
10. *Haddad MA, Souza M.V, Hincapie J.J, Ribeiro Jr JI, Ribeiro Filho JD, Benjamin LA,* (2009), Comportamento de componentes bioquímicos do sangue em equinos submetidos à ozonioterapia. Arq. Brasil. Med. Vet. Zootec. 61: 539-546.
11. *Han H. J., Kim J. Y., Jang H. Y., Lee B., Yoon J. H., Jang S. K., Jeong S. W.*, (2007), Fluoroscopic-guided intradiscal oxygen-ozone injection therapy for thoracolumbar intervertebral disc herniations in dogs. In vivo, 21: 609-613.

12. *Hernandez Aviles Mercedes,* (2013), Utilización del ozono y factores de crecimiento ozonizados en patologías musculo-esqueléticas en la especie canina. Rev. Esp. Ozonoterapia, 3: 1: 91-94.
13. *Lamberto R., Gregorio M., Nabil M.,* (2011), Clinical evidence of ozone interaction with pain mediators. Saudi. Med. J., 32: 1363-1367.
14. *Martinez-Sanchez G., Al-Dalain S.M, Menendez S., Re L., Giuliani A., Candelario-Jalil E., Alvarez H., Fernandez-Montequin J.I, Leon O.S,* (2005), Therapeutic efficacy of ozone in patients with diabetic foot. Eur. J. Pharmacol., 523: 151-61.
15. *Schwartz A., Kontorchnikova K., Malesnikov O*., (2011), Guía para el uso médico del ozono. Fundamentos terapéuticos e indicaciones. Espana, Madrid, AEPROMO.
16. *Scrollavezza P., Abbondi M., Pogliacomi B., Guareschi D., Dall'Aglio R., Poldi R., Pezzoli G.,* (1997), Ozone treatment in mastitis, metritis and retention of fetal membranes in the dairy cow. In Abstr. in Proc. 2nd Int. Symp. Ozone Applications, Havana, Cube, 35.
17. *Teixeira LR, Luna SPL, Taffarel MO, Lima AFM, Sousa NR, Joaquim JGF, Freitas PMC,* (2013), Comparison of intrarectal ozone, ozone administrated in acupoints and meloxicam for postoperative analgesia in bitches undergoing ovariohysterectomy. Vet. J, 197:794–799.
18. *Travagli V., Zanardi I., Valacchi G., Bocci V.,* (2010), Ozone and Ozonated Oils in Skin Diseases: A Review. Med. of Infl., 9:2-7.
19. *Vigliani A., Boniperti E., Scudo E., (*2005), Paravertebral O2-O3 Treatment in Mechanical Lumbar Pain in Riding Horses. Riv. It. Ossigeno Ozonoterapia 4: 64-69.
20. *Zobel R., Tkalcic S., Stokovic I., Pipal I., Buic V.,* (2012), Efficacy of ozone as a novel treatment option for urovagina in dairy cows. Reprod. Dom. An. 47:293–298.