J. Oral Diag. 2017; 02:e20170032.

ORIGINAL ARTICLE

Ozone therapy influence in the tissue repair process: A literature review

Júlia dos Santos Vianna Néri [1]
Erielma Lomba [2]
Alexandre Melo Karam [3]
Silvia Regina de Almeida Reis [4]
Antônio Márcio Teixeira Marchionni [5]
Alena Ribeiro Alves Peixoto Medrado [6*]

Abstract:

Introduction: The ozone therapy is a bio-oxidation therapy, based on a gaseous mixture with oxygen and ozone. It can be considered an alternative therapeutic tool the treatment of many systemic and locals diseases. It induces acute oxygenation stress and is not deleterious, which allows the restoration of oxidation and reduction balance. Within the therapeutic effects, it has been associated to the improvement metabolism and the peripheral tissue oxygenation. In addition, ozone is an important antimicrobial agent against bacteria, fungi, protozoa and viruses. In Dentistry, the ozone therapy has been used in several clinical situations due to its mechanisms of action. **Objectives:** The purpose of this literature review is to discuss the chemical and physical properties of ozone and its mechanisms of action in tissue repair. **Methods:** It is a narrative review based on research articles searched in Pubmed and LILACS database, from December 2015 to May 2016. The following descriptors in English: "ozone", "oxidative stress" and "tissue repair" used. Finally, 36 articles were included in this narrative literature review. **Results and Conclusion:** The use of ozone as treatment seems to be promising in health areas, including dentistry due to its biological and biochemical properties. However, there is a need for more methodologically adequate studies so it can be safely and efficiently applied.

Keywords: Ozone; Soft Tissue Injuries; Wound Healing.

[1] Master in Stomatology from Bahiana School of Medicine and Public Health (Assistent teacher of Bahia Adventist School), Salvador, BA, Brazil
[2] Graduation student of Bahia Adventist College (Graduation student of Bahia Adventist College) , Salvador, BA, Brazil.
[3] Master in Implantodology from University Center of Educacional Fundation of Barretos (Dentist cirurgion), Barretos, SP, Brazil.
[4] PhD in Pathology at Berlin's Free University. Associate Professor of Bahiana School of Medicine and Public Health - Salvador, Bahia, Brazil.
[5] PhD in Laser from Federal University of Bahia (Adjunct teacher of Bahiana School of Medicine and Public Healt), Salvador, BA, Brazil.
[6] PhD in Human Pathology at Federal University of Bahia (Adjunct Professor of Bahiana School of Medicine and Public Health), Salvador, BA, Brazil.

Correspondence to:
Alena Ribeiro Alves Peixoto Medrado.
E-mail: alenamedrado@hotmail.com

Article received on June 7, 2017.
Article accepted on July 14, 2017.

DOI: 10.5935/2525-5711.20170032

INTRODUCTION

Tissue repair involves progressive biochemical and cellular mechanisms in a complex sequence and is initiated at the time of trauma or illness. In the face of any injury, it is through repair that the body recovers and restores its balance[1,2]. Since the last century, the use of ozone therapy has been proposed in the literature because it is able to influence the oxy-reduction equilibrium. It is considered an alternative treatment for many acute and chronic diseases[3-6].

Ozone, which formula is O_3, is a powerful oxidant, and when in contact with organic fluids causes the formation of reactive oxygen molecules (O_2). These molecules influence cellular metabolism, which may be beneficial to tissue repair, in addition to its potent antimicrobial effect[6].

Some other properties of ozone therapy are already described in the literature. It stimulates and regulates the immune system and increases biosynthesis of some organic molecules. Its bio-energetic, antihypoxic, analgesic and hemostatic properties have also been highlighted in literature[3,7]. The aerobic process, increased vasodilators secretion, such as nitric oxide (NO), and acceleration of tissue regeneration have been cited by Seidler et al., in 2008[8]. In addition, ozone therapy is easily systemic or local applied for properly qualified professionals, has low cost, no side effects and has restricted intolerance or contraindication[6].

The only way that ozone cannot be administer by inhalation, as it is a toxic gas to the lungs. There is still much debate and controversy regarding the appropriate therapeutic dose and its effectiveness, because in very low or very high concentrations, it may be useless or bring adverse effects to the individuals[6].

There are several chronic or acute lesions that affect the oral cavity, both linked to systemic and local changes. Thus, the study and use of a therapeutic agent such ozone, with several beneficial properties for humans, is an important tool in the treatment of these oral diseases.

The aim of this narrative review is to discuss the chemical and physical characteristics of ozone as well as its mechanisms of action in tissue repair.

MATERIALS AND METHODS

The present study is a narrative literature review and it was based on research articles searched in Pubmed and LILACS database, from December 2015 to May 2016. It was used intersection of the following descriptors in English: "ozone", "oxidative stress" and "tissue repair".

The selection of the articles was based on the titles that addressed how the ozone therapy can influence wound healing, and its biological effects, including the role of reactive oxygen species and the general aspects of the ozone. Its clinical applicability and outcomes, as well as cellular changes induced by its administration considered.

First, it was collected the summaries that were available in database. 76 articles were found in Pubmed and 34 in LILACS. Only articles that provided evidence about the use of ozone in Dentistry and its therapeutic potential on tissue repair were selected. The following inclusion criteria were stablished: articles relevant to the theme, written in English and that had been published between 1987 to 2015. Articles that did not have fully abstracts were excluded from the study.

After prior examination, only 36 articles were included in this narrative literature review.

LITERATURE REVIEW

Physical and chemical properties of ozone

Ozone has a molecular weight of 47.98 g/mol, and is a gas composed of three oxygen atoms, present in abundance in the upper atmosphere. At room temperature, it has a strong odor, a blue color and has the ability to filter harmful ultraviolet rays, which plays a fundamental role in maintaining the biological balance of the biosphere[4,9]. Normally, the concentration of ozone in atmospheric air rotates around 0.1 ppm[10]. O_3 is one of the most important oxidants present in nature and is considered the third most powerful oxidant after fluoride and persulfate. It can act as a precursor of many radicals. It is highly reactive with organic compounds and highly thermodynamically unstable because, depending on the conditions of temperature and pressure, it easily decomposes by exothermic reaction in pure oxygen, with short survival (40 minutes at 20 °C). Therefore, it cannot be stored and should be used quickly[8,11,12].

In nature, its formation occurs through the combination of oxygen in the air, along with influencing factors such as ultraviolet (UV) radiation, electric shocks and intense physical stress in water[9]. Artificially, it is necessary a generator capable of promoting electric discharges for ozone formation. Through a high voltage gradient, these specific generators produce O_3 from the passage and photo dissociation of dioxygen in oxygen atoms ($O_2 \rightarrow 2O$), which react with other dioxygen molecules, forming the ozone ($O_2 + O \rightarrow O_3$)[3].

Depending on its concentration, pressure and temperature, O_3 dissolves physically in pure water.

Some of its molecules decompose rapidly, in a matter of seconds, and the other part remains stable for hours, with a half-life about 10 hours (at pH = 7 and 20°C). So, it can remain in water for a few days, if stored in a tightly closed glass vessel[13,14]. In addition to ozonized water, ozone can also be mixed with oils by means of electric discharges. When ozonized, the unsaturated acids present in the oils form ozonizes, and hydrolysis of these can generate aldehydes, ketones and hydrogen peroxides (H_2O_2), which are responsible for triggering biochemical reactions in living tissues[13,14].

Tissue repair

Skin integrity and mucous membranes function as a flexible mechanical barrier, which is intended to protect humans from infections. When this barrier is broken, either by surgery or trauma, there is a need for repair of connective tissue and regeneration of epithelium, through an intriguing healing process that begins immediately after injury[15]. The repair can occur through two mechanisms: By first intention, when the tissues are approximated, or by second intention, characterized when the lesion is extensive and infected, with great tissue destruction and uncounted edges[16]. Immediately after injury, there is extravasation of substances from the blood, derived from injured vessels, which have the purpose of filling the lesion. This extravasation is mainly composed of platelets, fibrin and fibronectin, which act in the release of inflammatory signaling substances, such as cytokines and coagulation system proteins, which eventually trigger the inflammatory response cascade in association with prostaglandins, interleukins (IL) and NO[17]. All stages of repair are coordinated by growth factors and specific cytokines[18]. Initially, there is vasodilation of adjacent vessels, with exudative phenomena and defense cells, mainly polymorph nuclear leukocytes, which arrive at the site in an attempt to destroy the external agent or remove cellular debris. Neutrophils then secrete cytokines, with the goal of attracting other cells, such as leukocytes and fibroblasts.

Fibroblasts differentiate into myofibroblasts and migrate to the lesion after two or three days, and through their contractile capacity, promote a retraction of the wound, which can reach 50 to 70% of the initial size. Over time, they secrete substances that fill the injured area, corresponding to extracellular matrix substances, such as hyaluronic acid and type III collagen.

Within 3 to 5 days, takes place granulation tissue deposition. It is a rich tissue fulfilled by vascular endothelial growth factors (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), epithelial growth factor (EGF), and transform growth-beta factor (TGF-β). The granulation tissue is composed of neoformed vessels, leukocytes, macrophages and fibroblasts[18-20].

The remodeling corresponds to the final phase of the repair, in association with biosynthesis and degradation of damaged substances, through metalloproteinase, whose secretion is influenced by several stimuli, such as PDGF and FGF, cytokines, IL-1 and Tumor necrosis factor alpha (TNF-α). Type III collagen is replaced by much more resistant collagen type I, which confers tension to the newly formed tissue[21]. At the end of the repair process, myofibroblasts and same fibroblasts disappear by apoptosis, mainly induced by NO[19,20]. In 2005, Kandler et al.[21] reported the need for a balance between the production of reactive oxygen species (ROS) and their neutralization in the healing process. A study by Sen et al.[1], in 2002, demonstrated that H_2O_2 induce the expression of VEGF in macrophages and keratinocytes, acting on angiogenesis, and stimulating collagen production, which corroborates the idea that tissue repair is subject to oxidation and reduction, known as redox control.

According to Sem and Roy[2], in 2007, oxygen is considered as a fundamental agent in tissue repair. Hemostasis, inflammation, re-epithelialization, vascularization and nitric oxide activity are sensitive to redox balance. Therefore, the study of ROS in repair is fundamental for the investigation of new therapies. Thus, ozone therapy, acting in the redox balance and in the correct concentration, can be effective in the treatment of wounds[6].

Ozone therapy and tissue repair

When in contact with organic fluids such as saliva, plasma, urine and lymph, ozone interacts intensely with all tissue components. This interaction depends on its concentration, and can react with polyunsaturated fatty acids, antioxidants, cysteine, glutathione, and albumin, even with carbohydrates, enzymes, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).

All these reagents act as donor electrons and are oxidisable, and participate in the ozonation process and the consequent formation of ROSs and lipid oxidation products (LOPs). These molecules are

responsible for the biochemical actions of ozone, and function as biochemical regulators of inflammation at distinct times and physiological concentrations[13,14]. Of these molecules, ROS are characterized by being highly unstable, rapidly forming, immediate action and half-life less than one second. They are derived from the oxygen molecule, generated by endogenous and exogenous sources and function as signaling molecules of innumerable cellular processes. Through interactions with cellular components and depending on the concentration of ozone in the tissue, these molecules can trigger biological effects that are either therapeutically or detrimental to health[13,14].

During normal metabolism, osteoclasts, platelets, lymphocytes, neutrophils, monocytes and fibroblasts can induce the formation of ROS. When in excess, these reactive oxygen species can trigger damage to cellular constituents, extracellular components and affect the metabolism of the cells responsible for extracellular matrix synthesis, fundamental in tissue repair, which leads to apoptosis and cellular necrosis[22].

Hydrogen peroxide is the main ROS. It is characterized as a potent oxidizer, and has the ability to activate different biochemical pathways. It functions as an ozone messenger with distinct cellular effects[14]. In cellular components, as in erythrocytes, H_2O_2 generated by ozone can alter glycolysis, with increased energy formation and consequent improvement of O_2 transport to tissues. In neutrophils and leukocytes, it stimulates the synthesis of cytokines and interleukins, which are beneficial to immunosuppressed patients. In platelets, there is an increase in platelet activity, which leads to an improvement in autacoids and growth factors release[13,14]. Bocci[23], since 1996, has already reported that ozone therapy can accelerate tissue repair.

On the other hand, LOPs are molecules that are distributed easily through the tissues, which are more diffused, more stable, and have the function of minimizing potential toxicities. They characterized as long-distance late messengers, and their metabolism mainly regulated through glutathione transferase and aldehydes dehydrogenases. Its production occurs after the oxidation of the polyunsaturated fatty acids of the cell membrane[14]. These are important molecules, capable of generating acute oxidative processes and function as signals of other existing oxidative stresses when in a low concentration[13,14]. When the living tissue is attacked by oxidative stress, activation of release systems of antioxidant agents occurs, which corresponds to the first line of defense of living tissue. Among the compounds of this release system of antioxidants, are the enzymes superoxide dismutase, glutathione peroxidase, glutadionereductase, catalase and hemeoxygenase-I.

Through the action of these enzymes, there is an increase in the release of stem cells by bone marrow cells, beneficial to tissue reconstitution, as well as increased NO production, fundamental in angiogenesis, through endothelial cells[13,14]. Valacchi et al.[10,24] in 2002 and 2003, observed increased expression of hemeoxygenase-I, involved in cell proliferation and apoptosis, and metalloproteinase that participate in the balance between synthesis and reabsorption of the extracellular matrix when the skin was exposed to ozone.

Patients often report the sensation of well-being and euphoria during treatment with ozone therapy, and this can be explained by the account of the LOPs, because they stimulate the endocrine and central nervous system, with consequent improvement of metabolism, hormonal production and neurotransmitters release[13,14].

When there is overproduction of ROS, oxidative stress occurs, which is the cause of many diseases, such as cancer (CA), and consequently there is worsening of these diseases evolution. It is fundamental that the therapeutic dose of ozone in the tissues is respected, so as not to make it a toxic agent. However, the therapeutic use tends to be beneficial to the organism, since it represents an acute, non-toxic oxidative stress, which consequently induces modifications at the cellular level capable of reversing another preexisting oxidative stress[14,25].

Ozone therapy can, when in therapeutic doses, act as a non-toxic "therapeutic shock" to the body because it tends to restore the balance of the redox state through changes in the physiological response, which may be useful in the treatment of metabolic, inflammatory, infectious and neoplastic diseases[14,23]. Schulz et al.[26], in 2008, used ozone therapy from peritoneal insufflation of rabbits with advanced stage of squamous cell carcinoma. The authors observed that six of the seven surviving rabbits had total remission of the tumor with absence of local or distant metastasis. These data corroborate the idea that ozone therapy may be effective in the treatment of neoplasms. The authors suggested that, although the exact mechanism of action of ozone for tumor regression is still uncertain, peritoneal O_3/O_2 insufflation is considered a promising strategy in cancer therapy.

In a study carried out by Re et al.[27] in 2008, it was proven that ozone is capable of promoting the increase

and preservation of endogenous antioxidant systems, thus promoting an oxidative precondition. Similar results were found in the study by Gracer et al.[28] in 2005, which described that the application of ozone in inflamed tissue may favor the synthesis of extracellular matrix, as well as cell proliferation and metabolic normalization. Traina[6], in 2008, evaluated tissue repair in rat wounds, and ozone provided a greater reduction in wound area and tissue formation. In 2000, Cardoso et al.[29] observed that ozonized water can attenuate the appearance and severity of gastric ulcers, as well as the edema of induced dermal lesions, as it acts as a modulator of the inflammatory process by the induction of oxidative stress. Martinez-Sanchez et al.[30] in 2005, evaluated adult diabetic patients who had foot ulcers located at the extremities of the body and observed that in relation to the group treated with antibiotics, the group that received ozone therapy had a decrease of lesion area and perimeter, as well as a statistically significant difference in relation to the healing time.

Patel et al.[31], in 2011, evaluated the effects of ozonized oil on the healing of wounds on the palate. 18 patients were divided between the test group (n=8) and the control group (n=10) and were treated with 2mL ozonized oil and a placebo one, respectively, in the same concentration daily for 1 week. The authors observed that, in the test group, there was an improvement in wound size when compared to control.

The authors also evaluated epithelial keratinization, regeneration and degeneration through the cytological technique, where the surface of the sterilized interproximal brush was firmly supported on the wound and rotated for collection of cellular material. There was a significant improvement of the ozone group in relation to epithelial regeneration.

In 2009, Kim et al.[32] carried out a study with the objective of evaluating the therapeutic effect of ozonized oil on wound healing in pigs. The ozone group obtained a significant reduction of wounds in relation to the control group. The tissues were stained, showing the increase in the intensity of collagen fibers and fibroblasts in the ozone group. Immunohistochemically, increased expression of PDGF, TGF-β and VEGF was observed.

In another study, where ozonation of platelet-rich plasma samples was performed, the results demonstrated the increase of IL-8, which allows the leukocytes to exit the circulation into the tissues in order to facilitate the phagocytosis of bacteria and necrotic tissue of ulcers and wounds. It may be a plausible justification for the acceleration of tissue repair in patients with chronic ulcers and treated with systemic ozone therapy. In addition to IL-8, the results also demonstrated an increase in growth factors, such as PDGF, transforming growth factor beta1 (TGF-β1) and IL[33]. In 2006, Lim et al.[34] found that the exposure of dermal wounds to ozone increased the activity of factor nuclear kappa B (NF-κB), an important immunomodulatory of inflammation, as well as the expression of TGF-β, which is fundamental for remodeling tissue. Similar results described by Huth et al.[35] These authors observed that aqueous ozone influenced the NF-κB system, which corroborates with the idea that ozone does indeed play an anti-inflammatory role and immunomodulatory.

Ozone therapy has been strongly related to increased expression of nitric oxide. If there is NO deficiency, this may negatively influence tissue repair and its role related to collagen synthesis, through regulation of fibroblasts[36].

CONCLUSION

Although ozone therapy shows to be a promising and effective treatment in different areas of dentistry because of its peculiar properties, such as its oxidative and antimicrobial potential, more researches, with adequate methodological designs, randomized samples and standardized methods that support their use in a safe and permanent manner, are need.

REFERENCES

1. Sen CK, Khanna S, Babiot BM, Hunt TK, Ellison EC, Roy S. Oxidant-induced vascular endothelial growth factor expression in human keratinocytes and cutaneous wound healing. J Biol Chem. 2002;277:33284-90.
2. Sen CK, Roy S. Redox signals in wound healing. Biochim Biophys Acta. 2007;1780:1348-61.
3. Gopalakrishnan S, Parthiban S. Ozone- a new revolution in dentistry. J Bio Innov. 2012;1:58-69.
4. Kumar A, Bhagawati S, Tyagi P, Kumar P. Current interpretations and scientific rationale of ozone usage in dentistry: A systematic review of literature. Eur J Gen Dent. 2014;3:175-80.
5. Maiya A. Applications of ozone in dentistry. Int J Clin Dent Sci. 2011;2:23-7.
6. Traina AA. Biological effects of ozone in water on dermal wounds healing in rats [Thesis]. São Paulo: Faculty of Dentistry, University of São Paulo; 2008.
7. Das S. Application of ozone therapy in dentistry. Indian J Dent Adv. 2011;3:538-42.
8. Seidler V, Linetskiy I, Hubálková H, Staňková H, Šmucler R, Mazánek J. Ozone and its usage in general medicine and dentistry. A review article. Prague Med Rep. 2008;109:5-13.

9. Sujatha B, Kumar MG, Pratap MJS, Raja V. Ozone therapy - a paradigm shift in dentistry. Health Sci. 2013;2:1-10.
10. Valacchi G, van der Vliet A, Schock BC, Okamoto T, Obermuller-Jevic U, Cross CE, et al. Ozone exposure activates oxidative stress responses in murine skin. Toxicology. 2002;179:163-70.
11. Yilmaz S, Algan S, Gursoy H, Noyan U, Kuru BE, Kadir T. Evaluation of the clinical and antimicrobial effects of the Er:YAG laser or topical gaseous ozone as adjuncts to initial periodontal therapy. Photomed Laser Surg. 2013;31:293-8.
12. Eregowda NI, Poornima P. Ozone in dentistry. Indian J Dent Adv. 2015;7:36-40.
13. Bocci V. Ozone as Janus: this controversial gas can be either toxic or medically useful. Mediators Inflamm. 2004;13:3-11.
14. Bocci VA. Scientific and medical aspects of ozone therapy. State of the Art. Arch Med Res. 2006;37:425-35.
15. Gál P, Vasilenko T, Kostelníková M, Jakubco J, Kovác I, Sabol F, et al. Open Wound Healing In Vivo: Monitoring Binding and Presence of Adhesion/Growth-Regulatory Galectins in Rat Skin during the Course of Complete Epithelialization. Acta Histochem Cytochem. 2011;44:191-9.
16. Kumar V, Abbas AK, Fausto N, Robbins SL, Cotran RS. Robbins and Cotran pathologic basis of disease. 7th ed. Philadelphia: Elsevier Saunders; 2005.
17. Mendonça RJ, Coutinho-Netto J. Cellular aspects of healing. An Bras Dermatol. 2009;84:257-62.
18. Kwon YB, Kim HW, Roh DH, Yoon SY, Baek RM, Kim JY, et al. Topical application of epidermal growth factor accelerates wound healing by myofibroblast proliferation and collagen synthesis in rat. J Vet Sci. 2006;7:105-9.
19. Grotendorst GR, Rahmanie H, Duncan MR. Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation. FASEB J. 2004;18:469-79.
20. Hinz B, Pahn SH, Thannickal VJ, Galli A, Bochaton-Piallat M, Gabbiani G. The myofibroblast: one function, multiple origins. Am J Pathol. 2007;170:1807-16.
21. Kandler B, Maitz P, Fischer MB, Watzek G, Gruber R. Platelets can neutralize hydrogen peroxide in an acute toxicity model with cells involved in granulation tissue formation. Bone. 2005;36:671-7.
22. Bhusari BM, Mahajan R, Rajbhoj S, Shah P. Reactive Oxygen Species & Its Role in Periodontal Disease. J Dental Med Sci. 2014;13:52-9.
23. Bocci V. Does ozone therapy normalize the cellular redox balance? Implications for the therapy of human immunodeficiency virus infection and several other diseases. Med Hypotheses. 1996;46:150-4.
24. Valacchi G, Pagnin E, Okamoto T, Corbacho AM, Olano E, Davis PA, et al. Induction of stress proteins and MMP-9 by 0.8 ppm of ozone in murine skin. Biochem Biophys Res Commun. 2003;305:741-6.
25. Hernández FA. To what extent does ozone therapy need a real biochemical control system? Assessment and importance of oxidative stress. Arch Med Res. 2007;38:571-8.
26. Schulz SS, Häussler U, Mandic R, Heverhagen JT, Neubauer A, Dünne AA, et al. Treatment with ozone/oxygen-pneumoperitoneum results in complete remission of rabbit squamous cell carcinomas. Int J Cancer. 2008;122:2360-7.
27. Re L, Maysouf MN, Menéndez S, León OS, Sánchez GM, Hernández F. Ozone therapy: clinical and basic evidence of its therapeutic potential. Arch Med Res. 2008;39:17-26.
28. Gracer RI, Bocci V. Can the combination of localized "proliferative therapy" with "minor ozonated autohemotherapy" restore the natural healing process? Med Hypotheses. 2005;65:752-9.
29. Cardoso CC, Carvalho JC, Ovando EC, Macedo SB, Dall'Aglio R, Ferreira LR. Action of ozonized water in preclinical inflammatory models. Pharmacol Res. 2000;42:51-4.
30. Martínez-Sánchez G, Al-Dalain SM, Menéndez S, Re L, Giuliani A, Candelario-Jalil E, et al. Therapeutic efficacy of ozone in patients with diabetic foot. Eur J Pharmacol. 2005;523:151-61.
31. Patel PV, Kumar V, Kumar S, Gd V, Patel A. Therapeutic effect of topical ozonated oil on the epithelial healing of palatal wound sites: a planimetrical and cytological study. J Investig Clin Dent. 2011;2:248-58.
32. Kim HS, Noh SU, Han YW, Kim KM, Kang H, Kim HO, et al. Therapeutic effects of topical application of ozone on acute cutaneous wound healing. J Korean Med Sci. 2009;24:368-74.
33. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human plates. Mediators Inflamm. 1999;8:205-9.
34. Lim Y, Phung AD, Corbacho AM, Aung HH, Maioli E, Reznick AZ, et al. Modulation of cutaneous wound healing by ozone: differences between young and aged mice. Toxicol Lett. 2006;160:127-34.
35. Huth KC, Saugel B, Jacok FM, Cappello C, Quirling M, Paschos E, et al. Effect of aqueous ozone on the NF-kappaB system. J Dent Res. 2007;86:451-6.
36. Luo J, Chen AF. Nitric oxide: a newly discovered function on wound healing. Acta Pharmacol Sinica. 2005;26:259-64.