Ozone Therapy in Cancer Treatment: State of the Art

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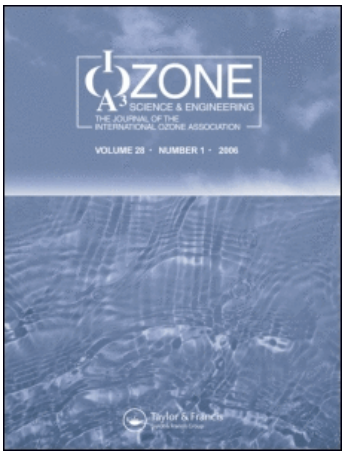

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Ozone Therapy in Cancer Treatment: State of the Art

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Erhlich Ascitic Tumor and Sarcoma 37 were implanted in mice and afterward the animals were treated with ozone (rectally). A significant decrease in the number of metastasis was obtained. In another study, ozone was applied intraperitoneally, before Lewis' lung carcinoma inoculation. A delayed effect in the tumor development kinetics and in the increase rate of tumor volume in the ozone groups was observed. With regard to the clinical trial, patients with prostatic cancer were treated with cobalt-60 therapy and ozone (rectally), decreasing the presence of side effects (due to radiation treatment) and the prostatic specific antigen figures. However, further investigations are necessary to be performed, in order to be considered the ozone therapy as complementary therapy for cancer.

Keywords Ozone, Prostatic Adenocarcinoma, Metastasis, Chemotherapy, Radiotherapy, Lewis' Lung Carcinoma, Sarcoma 37, Erhlich Ascitic Tumor

INTRODUCTION

Cancer is the second leading cause of death behind heart disease. However, deaths from heart disease have declined by 45% in the United States since 1950 and continue to decline, while cancer deaths are increasing. In this century, cancer is projected to be the leading cause of death. A report by the WHO foresees that worldwide cancer rates may double by 2020, unless we take stringent measures for promoting a healthy diet, smoking cessation and improved access to viral immunization (Bailar and Gornih, 1997; Levi et al., 1999; Eaton, 2003).

The development of an effective cancer therapy is a major focus of biomedical research (Giovanni et al., 2000). There is a total consensus that, whenever possible, the primary tumor must be surgically removed (or irradiated) because large tumor load or extensive metastases induce cachexia and an anergic state (Tisdale, 2002; Argiles et al., 2003). However, a complete ablation and cure is rare because haematogenous dissemination of tumor cells in the bone marrow can occur at an early stage of the malignancy (Pantel et al., 1999). Thus, we can presume that, even after a successful operation, the patient, at worse, may have a big dissemination of neoplastic cells that, after overcoming the immunedepression of anesthesia and surgery, may remain dormant or eliminated through the surveillance of the immune system. For that reason, it is not surprising that desperate patients are always looking for other possibilities, particularly in the vast field of complementary medical practices such as diet, nutrition and lifestyle changes, among others (Cassileth and Chapman, 1996; Burstein et al., 1999).

Tumor hypoxia is a well-recognized mechanism for resistance of neoplastic cells to anticancer drugs and radiotherapy. It is also a relevant factor enhancing neoangiogenesis, dedifferentiation and metastasis. Both primary and metastatic tumors thrive in areas where the average pO_2 is lower than normal tissues and the host appears unable to mount a reaction for reestablishing physiological levels (Brahimi-Bruno et al., 2001; Harris, 2002; Subarsky and Hill, 2003).

Neoplasia is a multifactorial process that can be broadly categorized into five etiologies: genetic, viral, chemical, physical and inflammatory. Chemical, physical and inflammatory etiologies are closely linked to reactive oxygen species (ROS), which can readily induce genomic damage (Brauchle et al., 1996; Bauer et al., 1998). Oxygen is required for respiration and the energetic processes that enable aerobic life. Costs associated with oxygen use are ROS formations, which create oxidative stress that has a complex effect on cancer development (Knight, 1995). Under normal physiological conditions, cellular ROS

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generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. The balance between ROS generation and elimination is important for maintaining proper cellular redox states.

Excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein and lipid membrane. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by a variety of anti-oxidant defense mechanisms, including important enzymes, such as superoxide dismutase, catalase and various peroxidases (Winklhofer-Roob, 1994). Although the precise mechanisms responsible for increased ROS stress in cancer cells have not be defined, the increase in ROS generation is attributed to active cellular metabolic activity under the influence of oncogenic signals and/or to mitochondrial malfunction in cancer cells (Szatrowski and Nathan, 1991; Salah-Eldin et al., 2000; Maxwell et al., 2001; Maulik and Das, 2002).

Ozone dissolves in the water of plasma or in water present on the skin surface or in the interstitial fluids and immediately disappears by reacting with organic compounds (hydrosoluble and lipophylic antioxidants, unsaturated fatty acids, etc) generating a number of messengers acting on various blood components and procuring early (by ROS) and late (by LOP) biological effects (Bocci, 2002). It is shown that ozone, via the transitory action of hydrogen peroxide, acts as a mild inducer of cytokines in leukocytes and therefore, primed lymphocytes and monocytes, by releasing cytokines in lymphoid microenvironments, activating the immune system usually suppressed by tumor growth (Larini and Bocci, 2005).

Also, ozone corrects the chronic oxidative stress by upregulating the antioxidant system, achieving an homeostasis redox (Ajamieh et al., 2004, 2005; León et al., 1998), and procures a state of well-being in patients by activating the neuro-endocrine system (Bocci, 2002). Besides these ozone biological effects, experimental findings have indicated that, after ozone therapy, oxygenation increases particularly in the hypoxic tumors (Clavo et al., 2004). Also, on the basis of the clinical improvement in different diseases (Romero et al., 1993; Giunta et al., 2001; Tylicki et al., 2001) after only two months of ozone therapy, it is likely that three–four months therapy may bring about a normal oxygenation of the neoplastic tissues.

In this paper we reviewed the state of the art of the different ozone biological effects that were demonstrated in different animal models, in relation with the possibility to use medical ozone as a therapeutic strategy for cancer treatment. Also, preclinical and clinical studies were performed in order to demonstrate the effects of ozone therapy in the treatment of cancer.

MATERIALS AND METHODS

Preclinical Studies

In both preclinical studies, B6D2F1 and NMRI male mice (18–20 g) were used. All animals were obtained from the National Center for Laboratory Animal production (CENPALAB, Havana Cuba). The animals were housed (10 per cage) under a 12 h light-dark cycle with room temperature maintained at 25°C, humidity at 55–60% and food and water *ad libitum*. Experiments were conducted in accordance with the ethical guidelines established by the Principles of Laboratory Animal care (NIH publication No. 86–23, revised 1985) and were approved by the Ethical Committee for Animal Experimentation of the National Center for Scientific Research, Havana, Cuba. Animals were divided in several groups: positive control (inoculation of the tumor, but without treatment) and experimental (treated with different ozone concentrations) with 10 animals per group.

In the first preclinical study, one million cells of Erhlich Ascitic Tumor and Sarcoma 37 were implanted by the ocular plexus of the mice. After the implantation, animals were treated with 1 mL of ozone, by rectal application, using different ozone concentrations (19, 26 and 42 mg/L), during 12 sessions. The hematogenic dissemination of the neoplastic cells present in the lungs was evaluated.

In the second preclinical study, ozone was applied to mice intraperitoneally at concentrations of 4, 11, 20, 35 mg/L and a volume of 80 ml/kg, daily for 15 days. Twenty four hours after the last ozone treatment, animals were inoculated with 1 million cells of the Lewis' lung carcinoma by subcutaneous way (0.25 mL). The tumor volume increase and the tumor development kinetics of Lewis' Lung carcinoma were evaluated.

Clinical Trial

Seventy patients with prostatic adenocarcinoma, in stage A and B (intracapsular), according to Whitemore Jewet classification (Hernández, 2001), were involved in a phase III controlled, randomized clinical trial. All patients were treated with cobalt-60 therapy, but to 35 patients were added rectal ozone, 6 days per week, at a dose of 8 mg (40 mg/L and 200 ml) during the 6 weeks that lasted the radiotherapy. For the preclinical and clinical trial the ozone was generated by an OZOMED 01 equipment manufactured by the Ozone Research Center obtained from medical-grade oxygen. The ozone concentration was measured by using a UV spectrophotometer at 254 nm.

Statistical Analysis

The OUTLIERS preliminary test for detection of error values was initially applied. Afterward, data were analyzed by one-way analysis of variance (ANOVA) followed by homogeneity variance test (Bartlett-Box). In addition, a multiple comparison test was used (Duncan test). Results are presented as mean ± standard error of the mean. The level of statistical significance employed was $p < 0.05$.

RESULTS

Preclinical Studies

In the first preclinical trial, the hematogenic dissemination of the neoplastic cells present in the lungs was evaluated for Erhlich Ascitic Tumor (Figure 1) and Sarcoma 37 tumor (Figure 2). A decrease of the number of cells per mice in relation with the increase of ozone concentration was observed. These results demonstrated that in both tumors a significant decrease in the number of metastasis was obtained.

In the second preclinical study, the results of the tumor volume increase and the tumor development kinetics of Lewis' lung carcinoma were evaluated (Figures 3 and 4). The main results of the antitumor indirect answer evaluation showed a delayed effect in the tumor development kinetics, as well as in the increase rate of tumor volume in the ozone pre-treatment groups in comparison with the control group, with a trend to obtain better results using the lower ozone concentration. Also, after 30 days, an animal without signs of tumor development in each one of the different ozone groups was observed, while in the positive control group all animals developed the tumor.

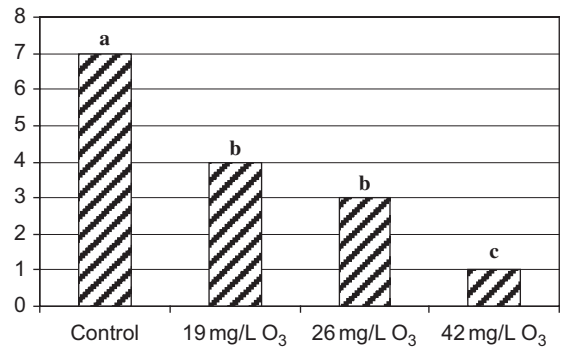

FIGURE 1. Tumor multiplicity in mice inoculated by the ocular plexus with 1 million cells of Ehrlich Ascitic Tumor, using 1 mL of ozone at different concentrations (19, 26 and 42 mg/L) by rectal application, during 12 sessions after the inoculation. Data are mean ± SEM. Means having different letters indicate significant difference ($p < 0.05$) between groups.

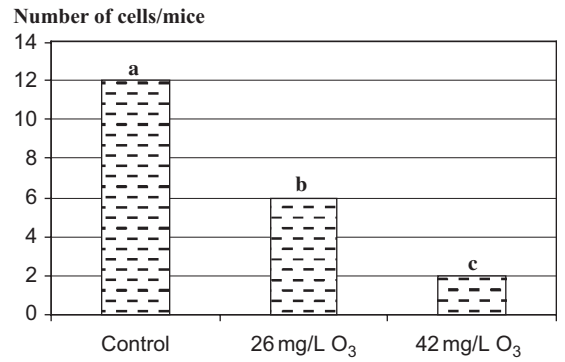

FIGURE 2. Tumor multiplicity in mice inoculated by the ocular plexus with 1 million cells of Sarcoma-37, using different ozone concentrations (26 and 42 mg/L) by rectal application, during 12 sessions after the inoculation. Data are mean ± SEM. Means having different letters indicate significant difference ($p < 0.05$) between groups.

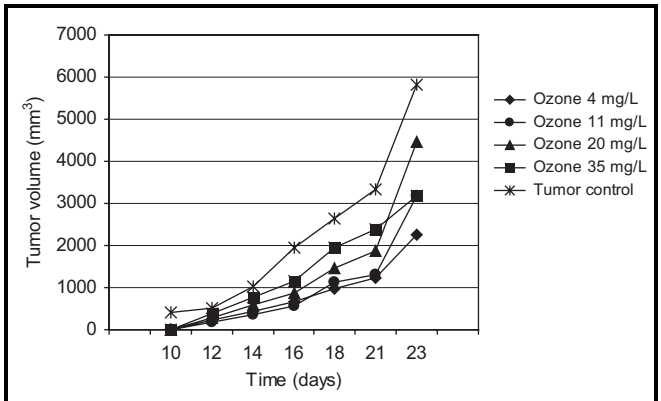

FIGURE 3. Tumor volume increase of Lewis' lung carcinoma. Ozone was applied to mice intraperitoneally at concentrations of 4, 11, 20, 35 mg/L and a volume of 80 ml/kg, daily for 15 days. Twenty-four hours after the last ozone treatment, animals were inoculated with 1 million cells of the Lewis' lung carcinoma by subcutaneous way (0.25 mL).

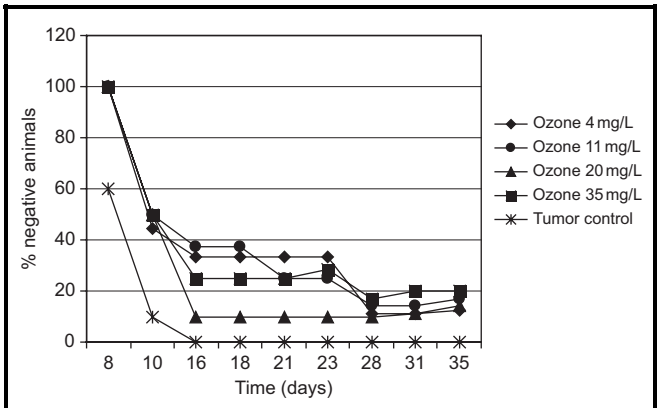

FIGURE 4. Tumor development kinetics of Lewis' Lung carcinoma. Ozone was applied to mice intraperitoneally at concentrations of 4, 11, 20, 35 mg/L and a volume of 80 ml/kg, daily for 15 days. Twenty-four hours after the last ozone treatment, animals were inoculated with 1 million cells of the Lewis' lung carcinoma by subcutaneous way (0.25 mL).

Clinical Trial

In the clinical, the appearance of side effects (radiodermatitis, cystitis, proctitis) occurred, since the first 2 weeks of treatment, in patients treated only with cobalt therapy. However, ozone application decreased these side effects, even they do not appear during this time of irradiation. At the end of the treatment, in 84% of the

patients treated only with cobalt therapy and in 52% of the ozone group, referred the presence of side effects, with significant differences between both groups. Only 5 patients (14%) finished the cobalt-60-therapy, while 17 patients (49%) of the ozone group, could finished the irradiation treatment, with significant differences between both groups (Figure 5).

To all patients prostatic specific antigen (PSA) was measured. At the beginning of the treatment no significant differences between both groups were observed (Figure 6A). One month after having finished the treatment, the figures of PSA decreased, with less than 10 ng/mL, in 92% of patients treated with ozone and in 52% of the control group, with significant differences between both groups (Figure 6B).

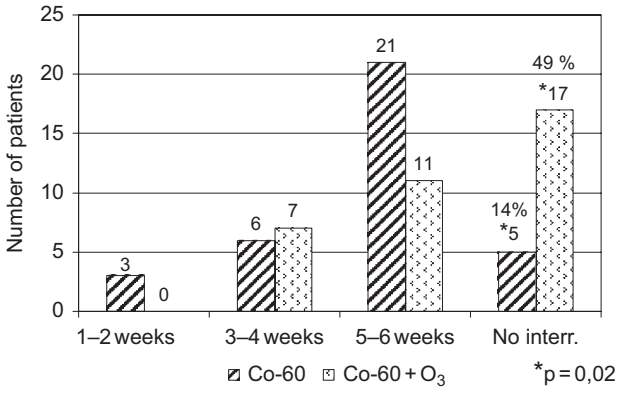

FIGURE 5. Patient distribution according to the appearance of side effects, as well as the total number of patients that do not interrupt the irradiation treatment in both groups. All patients were treated with cobalt-60 therapy, but to 35 patients were added rectal ozone, 6 days per week, at a dose of 8 mg (40 mg/L and 200 mL) during the 6 weeks that lasted the radiotherapy.

DISCUSSION

The results obtained in this study have demonstrated the potentialities of ozone as an antimetastatic agent, as well as an adjuvant for the treatment of cancer patients. Ozone therapy can exert influence in certain points of the complex tumor process. First, it has been demonstrated its effect in the regulation of the oxygen metabolism and oxygenation, for example, in the use of the aerobic pathway for the production of energy and re-establishing the normal metabolic functions, controlling the lactic acidosis (Hernández et al., 1995; Bocci, 2002; Larini and Bocci, 2005).

Then, improving tumor oxygenation by significantly and constantly increasing oxygen availability and microcirculation may slow down tumor growth and inhibit metastatization. Second, the slight and transient oxidative stress produced by this therapy stimulates the increase of cellular antioxidant enzymes capable of inhibiting chronic oxidative stress (Hernández et al., 1995; Bocci, 2002; Ajamieh et al., 2004, 2005; Larini and Bocci, 2005). In cancer, a persistent oxidative stress has been noted as a factor favoring the progression of invasion and metastasis (Toyokuni et al., 1995).

The fact that cancer cells live better in a hypoxic environment may imply that they have a rudimentary antioxidant system to get rid of ROS. Then, ozone could exert important cytotoxic effects on neoplastic cells if they have a poor defensive system. Third, ozone modulates the immune system making possible the recovery of the immunological response against the tumor cells (Hernández et al., 1995; Bocci, 2002; Larini and Bocci, 2005; Ajamieh et al., 2004; Ajamieh et al., 2005).

It has been demonstrated that the growth of human cancer is inhibited by ozone in culture, suggesting that cancer cells have an impaired defense system against ozone damage (Sweet et al., 1980; Washüttl et al., 1990).

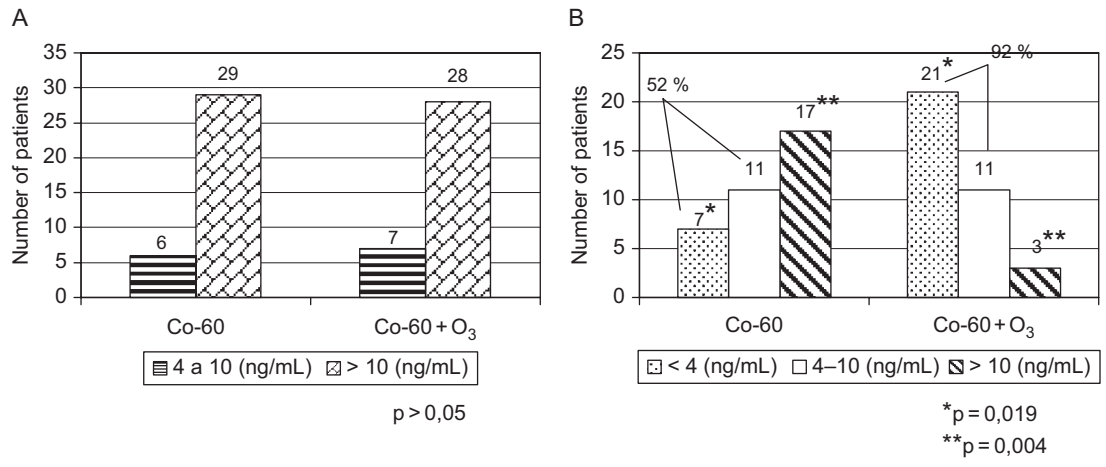

FIGURE 6. Patient distribution according to the prostatic specific antigen (PSA) figures, at the beginning (A) and 30 days after having finished the treatment (B) of cobalt-60-therapy or cobalt-60-therapy + ozone.

Also, it was found that incubating neoplastic cells in the continuous presence of a low dose of ozone (< 0.5 ppm) for 24 h was cytotoxic. Moreover, ozone was able to potentiate the cytotoxicity of 5-fluorouracil (5-FU) and to increase the sensitivity in a 5-FU-resistant colon carcinoma variant *in vitro* (Zänker and Kroczek, 1990). In conjunction with these results, combining ozone therapy with radiotherapy, an increase of the cytotoxic activity produced by the irradiation of the neoplastic cells was observed (Zänker and Kroczek, 1989).

Cisplatin is an effective chemotherapeutic agent commonly used in the treatment of a variety of solid organs cancer, including those of the head, neck, testis, ovary and breast, however, nephrotoxicity is an important side effect of this drug (Lebwohl and Canetta, 1998). In preclinical studies, it has been clearly demonstrated that oxidative preconditioning with ozone exerts protective effect in cisplatin induced acute nephrotoxicity in rats (Borrego et al., 2004). Furthermore, the data provide strong evidence that rectal ozone therapy effectively prevented a decrease in the renal antioxidant defense system and certainly avoided the deleterious effect of cisplatin on it. Also, it has been proved that ozone treatment reversed the damage generated by the chemotherapy (González et al., 2004; Calunga et al., 2004).

In this clinical trial, we have found a protection conferred by ozone treatment in patients with prostate cancer submitted to radiotherapy, decreasing the side effects and making possible that more patients can end the cobalt therapy treatment. Also, a synergism between radiotherapy and ozone was achieved because the figure of PSA significantly decreases when ozone is added to the radiation treatment. We have not measured any biochemical parameter in this study, but we think that the capacity of ozone to maintain the homeostasis redox (Hernández et al., 1995; Bocci, 2002; Larini and Bocci, 2005; Ajamieh et al., 2004, 2005) is one of its main effects to take into account in the benefits achieved in the treatment of cancer. Also, it has been demonstrated in the preclinical study using the Lewis' lung carcinoma a certain antitumor indirect answer showing a delayed effect in the tumor development kinetics, as well as in the increase rate of tumor volume in the ozone pre-treatment groups in comparison with the control group.

Two important processes, that are favored in the tumor development, are the induction of angiogenesis and the inhibition of apoptosis and both present the common characteristic of being dependent of the generation of ROS (Bauer et al., 1998; Brauchle et al., 1996). Tumor cells produce ROS, including hydrogen peroxide, which can be one of the elements that triggers the angiogenic process in the microenvironment of the tumor (Bauer et al., 1998; Brauchle et al., 1996; Knight, 1995; Maulik et al., 2001; Szatrowski and Nathan, 1991; Toyokuni et al., 1995). For that reason, it is evident that an agent able to regulate the antioxidant-prooxidant balance and the generation of ROS will present potentialities to lead the destiny of the malignant cell to apoptosis and to inhibit the angiogenesis.

We have demonstrated in the preclinical trial using the Erhlich Ascitic Tumor and the Sarcoma 37 an ozone antimetastatic effect. Angiogenesis and apoptosis are dependent of the generation of ROS, among other factors, and we know the capacity of ozone to maintain the homeostasis redox (Hernández et al., 1995; Bocci, 2002; Larini and Bocci, 2005; Ajamieh et al., 2004, 2005), then, it can influence in the fate of the cell. Of course, there are others factors involved in this regulation.

For example, some apoptosis regulatory proteins relevant to renal pathology have been characterized, which seems to be strongly related in the balance between factors that contribute to survival growth or lethality in renal cells. Bax is a Bcl-2 like protein that binds and antagonizes the protective effect of Bcl-2 and Bcl-X_L, rendering cells more sensitive to death. In these sense, the ratio of expression of Bcl-2 or Bcl-X_L to Bax appears to determine cell fate in an adverse environment (Ortiz et al., 2000). In cisplatin model, ozone conferred a renal cell protection (Borrego et al., 2004; González et al., 2004). Nephroprotection and beneficial effects conferred by ozone in cisplatin induce acute renal damage are associated not only to the possibility to increase the antioxidant defense system, achieving an homeostasis redox, but also it has to be considered changes in the renal expression of Bax.

In fact, it was demonstrated (Borrego et al., 2006) in this cisplatin model that ozone modulates the expression of Bax protein. Recent studies revealed that large amounts of ROS suppressed the expression of Bcl-2 increasing the expression of Bax and the heterodimerization between pro- and antiapoptotic proteins, decreasing the ubiquitination and degradation of proapoptotic proteins (Dechao et al., 2004). Ozone application under controlled conditions may generate an appropriate amount of ROS capable of generating a cytoprotective response which could include an increase in the ubiquitination and degradation of Bax, in a similar way to what was reported for N-acetylcysteine and pyrrolidine dithiocarbamate (Wu et al., 2005). Also, it has been observed an increase in Bcl-X_L expression in ozone treated renal tissue, which correlates with a decrease in Bax expression in the same tissue (unpublished data), favoring a cellular ratio Bax/Bcl-X_L promoting a cytoprotective pathway in renal cells.

CONCLUSIONS

In spite of the positive ozone biological effects, its potential usefulness as an adjuvant in chemo-radiotherapy and its antimetastatic effect, further investigations are necessary to be performed, in order to be considered the ozone therapy as complementary therapy for cancer.

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