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Restoration of Normoxia by Ozone Therapy May Control Neoplastic Growth: A Review and a Working Hypothesis

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ABSTRACT

In contrast to normal tissues, tumors thrive in hypoxic environments. This appears to be because they can metastasize and secrete angiopoietins for enhancing neoangiogenesis and further tumor spread. Thus, during chronic ischemia, normal tissues tend to die, while neoplasms tend to grow. During the past two decades, it has been shown in arteriopathic patients that ozonated autohemotherapy is therapeutically useful because it increases oxygen delivery in hypoxic tissues, leading to normoxia. Although several oxygenation approaches have been tested, none is able to restore normoxia permanently in patients with cancer. We postulate that a prolonged cycle of ozonated autohemotherapy may correct tumor hypoxia, lead to less aggressive tumor behavior, and represent a valid adjuvant during or after chemo- or radiotherapy. Moreover, it may re-equilibrate the chronic oxidative stress and reduce fatigue.

INTRODUCTION

Tumor hypoxia is a well recognized mechanism of resistance of neoplastic cells to anticancer drugs and radiotherapy and a relevant factor enhancing neoangiogenesis, dedifferentiation, and metastasis (Brahimi-Horn et al., 2001; Bush et al., 1978; Coleman, 1988; Gatenby et al., 1988; Harris, 2002; Hockel and Vaupel, 2001; Fyles et al., 2002; Subarsky and Hill, 2003; Vaupel and Hockel; 2000). Both primary and metastatic tumors thrive in areas where the average pO_2 is lower than in normal tissues and the host appears unable to mount a reaction to reestablish physiologic levels. An anarchic vascularization usually implies anomalous vessels with variable blood flow, edema, hypercoagulability, metastatic progression, and poor prognosis (Brizel et al., 1996; Denko and Giaccia, 2001; Dvorak, 2003; Helczynska et al., 2003; Hockel et al., 1996; Subarsky and Hill, 2003; Young et al., 1988).

In physiologic conditions, at sea level, the pO_2 in the alveolar space (O_2 = 14%) is equivalent to 100 mm Hg (1 atmosphere = 760 mm Hg = 101.3 Pa); the pO_2 of arterial blood is about 98 mm Hg, hemoglobin is fully saturated to oxyhemoglobin (Hb_4O_8), and ~0.3 mL/dL of O_2 is solubilized in the plasma. Depending on their metabolism, tissues (retina, kidney, liver, heart, brain) extract different amounts of O_2 from blood (on average ~25%, or 5 mL O_2/dL blood) so that venous blood has a pO_2 of about 40 mm Hg, with Hb_4O_8 depleted on average of only one molecule of O_2. Thus, the amount of O_2 physically dissolved in the plasma is grossly insufficient for the requirements of the tissues and the normally necessary 5 mL of O_2/dL blood are derived from deoxygenation of Hb_4O_8. The crucial point is that, for reasons mentioned below, erythrocytes of patients with neoplastia are unable to deliver more oxygen to the hypoxic tumor tissue.

Although among different tumors and even within the same tumor there is a marked heterogeneity in terms of O_2 supply (Brizel et al., 1996; Coleman, 1988; Denko and Giaccia, 2001; Dvorak, 2003; Gatenby et al., 1988; Helczynska et al., 2003; Hockel et al., 1996; Vaupel and Hockel, 2000; Young et al., 1988), there is a general consensus that neoplastic tissues prefer a hypoxic and acid microenvironment. The causes seem to be the combination of an aberrant vascular bed, leaky microvessels, elevated interstitial fluid pressure, lack of lymphatics, and reduced blood flow. The average pO_2 in tumors is less than one quarter that of normal cells (2–10 mm Hg versus 40–45 mm Hg). For normal tissues, hypoxemia represents a consistent metabolic disad-

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vantage, whereas experimental observations led to the conclusion that hypoxia is advantageous for growth and expansion of neoplastic cells (Brizel et al., 1996; Gatenby et al., 1988; Harris, 2002; Helczynska et al., 2003; Vaupel and Hockel, 2000; Young et al., 1988). Overexpression of hypoxia-inducing factor (HIF)-1-α was detected in the majority of tumor types compared to the respective normal tissues (Carmeliet et al., 1998; Ryan et al., 1998; Semenza, 2001; Zhong et al., 1999).

HIF-1 is a heterodimer consisting of the hypoxic response factor HIF-a-α and the stably expressed aryl hydrocarbon receptor nuclear translocator (ARNT) or HIF-1-β (Huang and Bunn, 2003; Semenza, 2001; Semenza, 2003). The availability of HIF-1 is determined by HIF-1-α, which is regulated at the protein level in an oxygen-sensitive manner: under hypoxia, HIF-1-α protein is stable, translocates to the nucleus and, after binding to HIF-1-β, activates gene transcription of vascular endothelial growth factor (VEGF), erythropoietin, and glycolytic enzymes that allow neoplastic cells to adapt to hypoxia. In contrast, during normoxia, HIF-1-α binds to the Von Hippel-Lindau tumor suppressor protein, which is one of the components of the multiprotein ubiquitin-E3-ligase complex that targets HIF-1-α for proteosomal proteolysis. Thus, re-establishing normoxia in human tumors inhibits overexpression of HIF-1-α, enhances its degradation, and may limit tumor progression and metastasis.

One of the most studied approaches to blocking the malignant evolution of tumors is to inhibit angiogenesis (Tosetti et al., 2002). This process is clearly stimulated by hypoxia (Brahimi-Horn et al., 2001; Carmeliet et al., 1998; Denko and Giaccia, 2001; Dvorak, 2003; Harris, 2002; Huang and Bunn, 2003; Ryan et al., 1998; Semenza, 2001; Semenza, 2003; Subarsky and Hill, 2003; Zhong et al., 1999), but a direct correction of the hypoxic state seems a more straightforward method to block cancer progression. If this postulation is correct, we now propose a novel approach for constantly restoring normoxia in all tissues.

IS IT FEASIBLE TO CONSTANTLY CORRECT HYPOXIA IN CANCER PATIENTS?

Two questions arise: would it be possible to induce a constant restoration of normoxia, and how will neoplastic cells react to a normal O_2 tension *in vivo*.

During the past century several strategies have been proposed for enhancing oxygenation of tumors. The most obvious was breathing pure oxygen, but because of its toxicity, this can only be done for short periods, with only transitory increases of arterial pO_2 (Thomson et al., 2002). Carbogen inhalation on its own or in combination with other therapies is practical and useful at high altitudes (Imray et al., 2003), but has not yet found a definitive role in patients with neoplasias (Bernier et al., 2000; Falk et al., 1992; Griffin et al., 1996; Inch et al., 1970; Rubin et al., 1979; Siemann et al., 1977; Song et al., 1987). Hyperbaric oxygen therapy is a procedure by which almost pure medical oxygen is inhaled in an airtight chamber at about 2.6 atmospheres for 2 hours (Bergo and Tyssebotn, 1999; Dische et al., 1983). During this period the O_2 solubilized in plasma increases up to 5 mL/dL and it becomes sufficient for satisfying tissue requirements so that practically no oxygen molecule is released by Hb_4O_8. In this situation neoplastic tissues may temporarily become normoxic, but only if organ vasoconstriction does not occur (Bergofsky and Bertun, 1966).

Cancer patients are often anemic and recently, in order to improve therapeutic effectiveness as well as to decrease fatigue, recombinant erythropoietin has been widely used (Littlewood et al., 2001; Marrades et al., 1996). Blood transfusion or artificial O_2 carriers can be used (Song et al., 1987; Teicher and Rose, 1984) provided they do not excessively increase blood viscosity; they only correct hypoxic microenvironments temporarily. Vasoactive drugs (Bernier et al., 2000; Honess et al., 1995; Horsman et al., 1989; Siemann et al, 1994; Song et al., 1992) and mild hyperthermia (Dewey et al., 1977; Griffin et al., 1996; Overgaard et al., 1995; Song et al., 1996; Song et al., 1997; Valdagni and Amichetti, 1994) may also be of some help. Although all of these approaches have some merit, they do not solve the problem of constantly correcting tumor hypoxia. Coleman et al. (1988) and later Brown (1999) have proposed that hypoxia could be advantageously used to kill tumor cells by using radiosensitizers, possibly combined with chemotherapeutic drugs, but this approach is beyond the scope of this paper.

IS IT POSSIBLE TO CONSTANTLY IMPROVE OXYGEN DELIVERY TO ISCHEMIC TISSUES?

By serendipity some 14 years ago one of the authors began to examine the biological and clinical effects of a gas mixture composed of 95–98% oxygen and 2–5% ozone on blood (Bocci and Paulesu, 1990). The classical procedure of ozonated autohemotherapy (O_3-AHT) proposed by Wolff (1979) has been optimized (Bocci, 2002) and millions of treatments have been performed with significant clinical efficacy in vasculopathies, without side effects (Bocci, 2002). Our typical autotransfusion, quite simple and safe, consists of 270 mL of blood (anticoagulated with 30 mL 3.8% Na citrate) exposed to a 200 mL gas volume of the O_2–O_3 mixture in a sterile, ozone-resistant, glass bottle with an ozone concentration ranging from 20 to 60 μg/mL per mL of blood (0.42–1.26 mmol/L). Ozone acts as a real chemical drug, ca-

TABLE 1. ENZYME LEVELS (U/G HEMOGLOBIN) IN HUMAN BLOOD AFTER EXPOSING BLOOD SAMPLES ($N = 7$) TO EITHER O_2 OR O_3

Treatment	*SOD*	*GSH-Px*	*GSH-Rd*	*G6PDH*
Control	830.2 ± 249.6	24.4 ± 5.8	2.1 ± 0.5	5.2 ± 2.4
O_2	836.9 ± 293.6	19.1 ± 5.8	2.0 ± 0.5	5.3 ± 2.4
O_3 20 μg/mL	726.8 ± 197.5	25.2 ± 6.4	2.0 ± 0.6	5.7 ± 2.6
O_3 40 μg/mL	694.7 ± 140.9	25.8 ± 6.1	2.1 ± 0.5	5.2 ± 2.7
O_3 80 μg/mL	670.8 ± 185.5	27.2 ± 6.1	2.1 ± 0.4	5.6 ± 3.0

SOD, superoxide dismutase; GSH-Px, reduced glutathione peroxidase; GSH-Rd, reduced glutathione reductase; G6PDH, glucose-6-phosphate dehydrogenase. Values represent mean ± standard deviation. In comparison to oxygen, the ozonated samples did not show significant variations.

pable of activating several biologic mechanisms (Bocci, 1999; Bocci, 2002; Bocci, 2004).

The problem of possible toxicity to blood cell components has been extensively appraised and, depending upon the normal blood antioxidant capacity, the range of the therapeutic window has been determined to lie between ozone concentrations of 20–80 μg/mL per mL of blood (0.42–1.68 mmol/L). Levels of methemoglobin remain normal, the hematocrit value and the osmotic fragility do not change, and the rate of hemolysis is slightly modified (from 0.5% to 1.2%) (Bocci et al., 1998a; Shinriki et al, 1998). Most importantly, no damage to erythrocytic enzymes such as Na/K-ATPase, acetylcholinesterase, superoxide dismutase (SOD), reduced glutathione peroxidase (GSH-Px), reduced glutathione reduxase (GSH-Rd), catalase, and glucose-6-phosphate dehydrogenase (G6PDH) has been noted in blood exposed to concentrations as high as 80 μg/mL both in published data (Cross et al., 1992; Shinriki et al., 1998) and our unpublished data (Table 1), confirming that the potent antioxidant system of blood adequately protects hemoglobin and enzymes.

Patients undergoing ozone therapy do not have adverse effects and most of the patients report a feeling of wellness and euphoria. It is unfortunate that, owing to a number of unfavorable circumstances, including misuse of ozone and a dogmatic assumption that ozone is always toxic, this complementary approach has been either neglected or regarded with skepticism. This problem has been extensively discussed (Bocci, 2002) and it is to be hoped that it will undergo objective scrutiny in the near future. Normally a cycle of 14 to 15 twice weekly treatments significantly improves visual acuity in about 70% of patients with the atrophic form of age-related macular degeneration (ARMD) (Bocci, 2005) and in most of the patients with stage II chronic limb ischemia (Biedunkiewicz et el., 2004; Giunta et al., 2001; Mattassi et al., 1987; Rokitansky et al., 1981; Romero Valdes et al., 1993; Tylicki et al., 2001; Tylicki et al., 2003; Tylicki et al., 2004). These surprising results (Bocci, 2002) are due to constantly improving oxygenation of ischemic tissues and it is worth emphasizing that the responsible agent is ozone and not the transitory oxygenation of a small volume of blood.

HOW DOES OZONE ACT?

Ozone dissolves in the water of plasma and immediately disappears by reacting with organic compounds (including hydrosoluble and lipophylic antioxidants, and unsaturated fatty acids), generating a number of messengers acting on various blood components and producing biologic effects early, by reactive oxygen species (ROS), and late, by lipid oxygenation products (LOP). While we were assessing the range of the therapeutic window, we found that the ozone concentration must reach a critical threshold to be effective as otherwise it results only in a placebo effect characterized by the lack of ROS and LOP. An early effect is due to a sudden increase of hydrogen peroxide that switches on a number of biochemical pathways in erythrocytes, leukocytes, platelets, and endothelial cells (Bocci, 2002; Stone and Collins, 2002). The late effects are due to a number of LOP with a half-life far longer than ROS. Upon blood reinfusion into the donor, which begins 5 to 10 minutes after blood ozonation, LOP undergoes extensive dilution, catabolism, and excretion. Nonetheless the residual LOP will activate endothelial cells and parenchymal cells of several organs, among which bone marrow is particularly relevant (Fig. 1).

Each day ~0.8% of the erythrocyte pool (a fraction corresponding to about 40 mL of blood including 2×10^{11}, 4-month old erythrocytes) (Young et al., 1988) is taken up by erythrocatheretic organs. A rational schedule of ozone therapy envisaged for cancer patients includes 3 sessions weekly for 6 months, allowing the ozonation of ~20 L of blood. Bearing in mind the axiom that more ozone is not necessarily better (Bocci, 2002), this volume is most likely sufficient for correcting the hypoxic state. Ozone causes two important modifications, of which the first happens *ex vivo* and the second *in vivo*.

The first occurs in the glass bottle while ozone dissolves in the water of plasma and generates hydrogen peroxide and lipoperoxides which behave as secondary messengers: almost instantaneously, they enter into the erythrocytes and activate a number of biochemical pathways. These ROS are almost immediately reduced (H_2O_2 to H_2O and peroxides [ROO] to hydroperoxides [ROH]) at the expense of reduced glutathione (GSH).

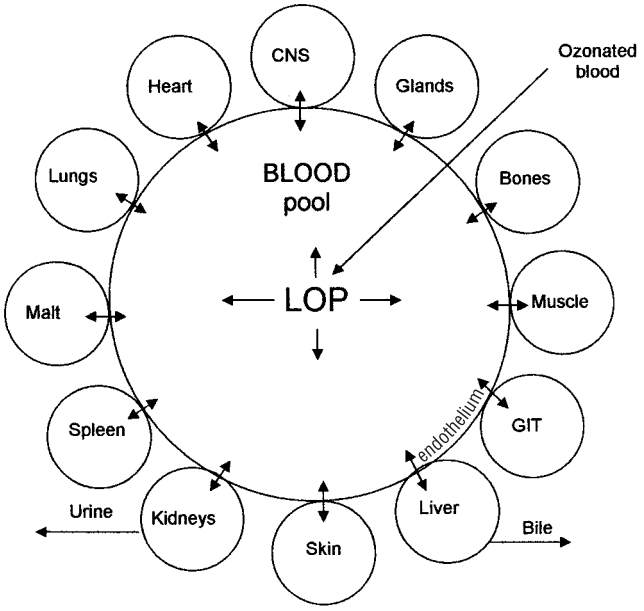

FIG. 1. After reinfusion of ozonated blood into the donor and dilution in the blood pool, lipid oxygenation products (LOP) will be distributed in all organs. Besides catabolism and excretion via bile and urine, traces of LOP will act as bioregulators eliciting a number of biological effects. CNS, central nervous system; GIT, gastrointestinal tract; Malt, mucosa-associated lymphoid tissue.

While GSH-Rd utilizes the coenzyme nicotinamide adenenine dinucleotide phosphate in reduced form (NADPH) to recycle GSSG to the original level of GSH, the oxidized NADP is reduced after the activation of the pentose phosphate pathway, of which G6PDH is the key enzyme. Glycolysis is accelerated, with a consequent increase of ATP levels. Moreover, for a brief period, the reinfused erythrocytes enhance the delivery of oxygen into ischemic tissues because of a shift to the right of the oxygen-hemoglobin dissociation curve, as a result either of a slight decrease of intracellular pH (Bohr effect) or/and an increase of 2,3-diphosphoglycerate (2,3-DPG) levels.

The second and more important modification occurs in the bone marrow, when submicromolar amounts of LOP present in the reinfused blood, acting as a weak stress factor, are able to influence the differentiation of the erythroblastic lineage.

It is emphasized that each O_3-AHT represents a calculated, very transitory oxidative stress that, by activating the adaptive mechanism, results in the generation of erythrocytes with improved biochemical characteristics. These supergifted erythrocytes, as we called them, due to a higher content of 2,3-DPG and antioxidant enzymes, become able to deliver more oxygen into ischemic tissues (Bocci, 2002; Clavo et al., 2003; Giunta et al., 2001; Mattassi et al., 1987; Rokitansky et al., 1981; Romero Valdes et al., 1993; Tylicki et al., 2001; Tylicki et al., 2003; Tylicki et al., 2004). The consequence of repeated treatments, depending upon the volume of ozonated blood, the ozone concentration, and the schedule, is that after a few initial treatments, a cohort of supergifted erythrocytes will enter the circulation daily and will relentlessly substitute for old erythrocytes generated before the therapy. This means that, during prolonged ozone therapy, the erythrocyte population will include not only cells of different ages but, most importantly, erythrocytes with different biochemical and functional capabilities.

In 4 patients with ARMD, after a short cycle of 14 O_3-AHT treatments (in which ~3.8 L of blood was ozonated over 7 weeks), density-gradient separation of old and young erythrocytes (Micheli et al., 1985) showed a marked increase of G6PDH in the young erythrocytic fraction generated during the course of ozone therapy (Micheli et al., in preparation) (Table 2). Other relevant biochemical changes such as glycolysis activation with increased ATP and 2,3-DPG levels, particularly in patients with low basal levels, have been measured in erythrocytes at the end of the cycle (Bocci, 2002; Mattassi et al., 1987; Rokitansky et al., 1981). While the enzymatic activity does not change during the ozonation procedure, it does significantly increase *in vivo* after a therapeutic cycle: we have found that GSH-Px, GSH-Rd, GSH-Tr, and SOD increase by 210%, 147%, 164%, and 141%, respectively, amply confirming previous data reported by Hernandez et al. (1995).

TABLE 2. EVALUATION OF G6PDH ACTIVITY IN TOTAL, YOUNG, AND OLD RED BLOOD CELLS (RBC) IN BLOOD SAMPLES FROM 4 PATIENTS WITH AGE-RELATED MACULAR DEGENERATION, BEFORE AND AFTER AN OZONE THERAPY CYCLE OF 13 TREATMENTS

	G6PDH activity[a]			*Young RBC*[b] *(%)*	*Old RBC*[b] *(%)*
	Total RBC	*Young RBC*	*Old RBC*		
Before treatment ($n = 4$)	356.8 ± 90.7	550.3 ± 157.5	310.7 ± 127.3	3.1 ± 2.8	96.9 ± 2.8
After treatment ($n = 4$)	406.2 ± 40.4	748.2 ± 181.9	434.8 ± 86.7	3.4 ± 3.0	96.6 ± 3.8

[a]G6PDH activity expressed as nmoles/h/mg hemoglobin in whole erythrocyte population and in young and old fractions before and after 13 O_2/O_3 treatments.

[b]Percentage of young ("light") and old ("heavy") erythrocytes obtained from whole blood by isopycnic centrifugation.

G6PDH, glucose-6-phosphate dehydrogenase.

Values represent mean ± standard deviation.

That ozone can induce the release of erythrocytes with improved functional activity is not surprising, as the phenomenon of adaptation to chronic oxidative stress (De Maio, 1999; Jolly and Marimoto, 2000), also defined as oxidative preconditioning (Barber et al., 1999; Bocci, 1996a; Bocci, 1996b; Kume et al., 1996; León et al., 1998) or hormesis (Calabrese, 2002; Goldman, 1996), implies that the repeated treatments induce the synthesis of several oxidative stress proteins among which heat shock protein 32, identical to heme-oxygenase-1 (HO-1), is a prototypical example. This happens in all organisms from plants to humans, and has also been termed ozone tolerance (Bocci et al., 1999; Burkey and Eason, 2002; Sharma et al., 1996). Our calculated therapeutic stress on blood *ex vivo* must be clearly distinguished from the lifelong, endogenous, oxidative stress due to oxygen, because, although it seems a paradox, ozone therapy can upregulate the antioxidant defenses.

On the basis of the clinical improvement in patients with ARMD (Bocci, 2005) and chronic limb ischemia (Clavo et al., 2003; Giunta et al., 2001; Mattassi et al., 1987; Romero Valdes et al., 1993; Tylicki et al., 2001) after only 2 months of therapy, it is likely that 3 or 4 months of therapy may bring about normal oxygenation of the neoplastic tissues. This possibility is supported by recent experimental findings that indicated that, after ozone therapy, oxygenation increases, particularly in the most hypoxic tumors (Clavo et al., 2004a; 2004b).

The treatments need to be continuously maintained but this is not a problem given the excellent patient compliance shown in other diseases (Bocci, 2002). ROS and LOP not only increase erythrocytic function (Bocci et al., 1998a) but activate leukocytes (Bocci et al., 1993a; 1993b; Bocci et al., 1994; Bocci et al 1998b; Paulesu et al., 1991), platelets (Bocci et al., 1999; Valacchi and Bocci, 1999), and endothelial cells (Valacchi and Bocci, 2000). This multidirectional and simultaneous activation leads to an increased release of NO, adenosine, and autacoids, and contributes to improved tissue vascularization (Jia et al., 1996). HO-1 will enhance heme breakdown yielding a higher level of bilirubin (a potent lipophylic antioxidant like a-tocopherol) and CO (Bak et al., 2002; Dore, 2002; Lee and Chau, 2002; Snyder and Baranano, 2001; Zuckerbraun and Billiar, 2003). HO-1 indirectly reduces vascular constriction because it suppresses the gene expression of endothelin-1 and inhibits the proliferation of smooth muscle cells (Duckers et al, 2001; Morita and Kourembanas, 1995). It has been shown that traces of CO cooperate with NO in favoring vascular relaxation (Bak et al., 2002).

Reinfusion of ozonated blood does not involve intravenous infusion of gas, which has been prohibited since 1984, because oxygen can cause a deadly embolism (Jacobs, 1982). On the other hand, ozone reacts instantaneously and disappears: the reaction cascade generates the compounds responsible for eliciting a variety of biologic effects that allow considering ozone as a multipotent bioregulator. Briefly expanding this concept, the result that ozone could directly and selectively inhibit neoplastic cells growth (Sweet et al., 1980) is irrelevant *in vivo* unless ozone is directly injected into a neoplastic nodule, which is a rare event. In addition to the normalization of hypoxia, ozone therapy can display other interesting biologic effects that may enhance the therapeutic result. First, reinfused LOP are heterogeneous but include cytotoxic aldehydes such as malonyldialdehyde and 4-hydroxy-2,3-alkenals (Esterbauer et al., 1991). These compounds undergo extensive dilution and are partly excreted and partly catabolized by enzymes such as GSH-Tr and ALDH. However, they also bind to cells and it is possible that neoplastic cells are sensitive to LOP that nonetheless must only reach submicromolar levels to avoid toxic effects in normal cells. Second, in a series of papers (Bocci et al., 1993a; Bocci et al., 1993b; Bocci et al., 1994; Bocci et al., 1998b; Paulesu et al., 1991), we showed that ozone, via the transitory action of hydrogen peroxide, acts as a mild inducer of cytokines in leukocytes and therefore primes lymphocytes and monocytes by releasing cytokines in lymphoid microenvironments, and may slowly bring about a concerted activation of the immune system usually suppressed by tumor growth. This is an interesting possibility because an endogenous and balanced cytokine production is conceptually more effective and free of toxicity than the exogenous administration of a single cytokine (Bocci, 1988; Bocci, 1998).

In October 2003 we initiated an open study applying ozone therapy to chemotherapy-resistant cancer patients. The initial observation was that patients with a Karnofsky performance status $<40\%$ who underwent excessive treatments, in spite of excellent compliance, continue to show disease progression and die in a few weeks. On the other hand, after 30 to 45 treatments, patients with a Karnofsky status $\geq 70\%$, even with diffused metastasis (usually liver or lungs), report a net improvement of their quality of life. Although a definitive conclusion, based upon evaluations of objective parameters, cannot be achieved before an extensive study on the latter category of patients, a preliminary study on advanced head and neck tumors supports our contention (Clavo et al., 2004b).

In contrast to the dogmatic assertion that ozone is always toxic, after three decades of correct ozone therapeutic practice in Europe, it can be affirmed that properly used ozone does not produce any adverse effects but actually improves the quality of life of the patient with cancer. The mechanisms producing the state of wellness and euphoria are not yet experimentally clear but a complex hormonal and neurotransmitter modification is likely to occur during the "therapeutic shock" due to ozone action (Bocci, 2002). Conventional cancer treatments often cause fatigue (Gutstein, 2001; Servaes et al., 2002) and attenuating this serious symptom may help to improve the psychologic, behavioral, and metabolic state of the patient.

CONCLUSIONS

In order to render the tumor microenvironment normoxic, several approaches have been pursued; although they are correct in theory, they are not practical and above all transitory. Although the use of oxygen-ozone therapy in metastatic cancer was postulated five years ago (Bocci, 1998), it has been neglected by oncologists and we could not perform a study. However, the clinical application of ozone therapy in ischemic vasculopathies (Giunta et al., 2001; Mattassi et al., 1987; Rokitansky et al., 1981; Romero Valdes et al., 1993; Tylicki et al., 2001) has shown that it can permanently correct the hypoxia in ischemic tissues. Anecdotal reports have claimed excellent results in cancer but unpublished results cannot be assessed and remain valueless. We have garnered considerable experience with the effect of short cycles of O_3-AHT in ARMD (Bocci, 2005) and vasculopathic patients. Laboratory evaluation has shown that ozone therapy with time can modify the biochemistry of mature circulating erythrocytes. This is not due to the very high but absolutely transitory oxygenation of blood *ex vivo*, but exclusively to the reaction of ozone with blood components. The novel point to bear in mind is that ROS and then LOP messengers, generated during blood ozonation, reach all microenvironments after blood reinfusion into the donor: In the bone marrow, they influence the differentiation of the erythroblastic lineage, so that cells mature with an improved biochemical machinery that will improve their function in the circulation and will result in increased oxygen delivery into ischemic tissues. If we want to change the erythrocytic population permanently, we have to program an intensive cycle of at least 6 months, followed by maintenance therapy, to preserve the benefit, as we have observed in ARMD patients (Bocci, 2005). After each O_3-AHT a new cohort of young erythrocytes will replace old and inefficient cells. The progressive substitution of a poorly functioning cell population with an increasing majority of supergifted erythrocytes may be capable of normalizing oxygen levels in neoplastic areas.

Normoxia, by inhibiting HIF-1 activity, may reduce tumor growth and metastasis (Semenza, 2003). LOP, by interacting with the endothelium, enhances NO and NO-thiol formation, which will further increase the oxygen supply by improving the tumor microcirculation. Upregulation of HO-1, which is the norm during ozone therapy, will also increase the release of traces of CO that act in concert with NO. The generalized improvement of metabolism, the mild stimulating effect on the immune system, and a positive effect on neurotonic neurotransmitters and hormone secretion may constitute a great help for patients with cancer who are often plagued by fatigue. In contrast to the opinion of those scientists who, without any direct experience, claim that ozone is toxic, it can be stated that properly performed ozone therapy carried out for years in thousands of patients has not yielded any acute or chronic side effects. Ironically for the detractors, most patients report a feeling of wellness and are well compliant.

In conclusion, it has been proved that ozone therapy can:

- Improve blood circulation and oxygen delivery to ischemic tissues
- Correct the chronic oxidative stress by upregulating the antioxidant system
- Induce a mild activation of the immune system
- Procure a state of wellbeing in patients.

Can ozone therapy be useful in metastatic cancer either as the last resort or as a complementary therapy during chemo- or radiotherapy? Only unbiased, randomized (in comparison to the best orthodox therapy) clinical studies for several solid tumor types carried out in several oncologic treatment institutions can answer this question. Despite our efforts, so far conventional oncologists have disregarded this approach. This seems unfair for the patient because chemotherapy is not always curative and several other approaches, although promising and fashionable, such as gene therapy, cancer vaccines, angiogenesis-inhibitors, telomerase-inhibitors, and matrix metalloproteinase-inhibitors have not yet yielded a real advantage.

An exciting aspect of ozone therapy is the range of biologic effects induced by the interaction of ozone messengers with many targets, first in blood *ex vivo* and then in many organs after reinfusion. This novel therapeutic approach may profoundly modify the biochemistry and behavior of neoplastic cells and a good test will be the evaluation of HIF-1-α that, if down-regulated, may inhibit neoangiogenesis and further metastasization. In spite of the skepticism of others, the time has come to scientifically evaluate the ability of ozone therapy to stabilize tumor progression and improve the quality of life of patients with neoplastic diseases.

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REFERENCES

Bak I, Papp G, Turoczi T, Varga E, Szendrei L, Vecsernyes M, Joo F, Tosaki A. The role of heme oxygenase-related carbon monoxide and ventricular fibrillation in ischemic/reperfused hearts. Free Radic Biol Med 2002;33:639–648.

Barber E, Menéndez S, León OS, Barber MO, Merino N, Calunga JL, Cruz E, Bocci V. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediat Inflamm 1999;8:37–41.

Bergo GW, Tyssebotn I. Cardiovascular effects of hyperbaric oxygen with and without addition of carbon dioxide. Eur J Appl Physiol Occup Physiol 1999;80:264–275.

Bergofsky EH, Bertun P. Response of regional circulations to hyperoxia. J Appl Physiol 1966;21:567–572.

Bernier J, Denekamp J, Rojas A, Minatel E, Horiot J, Hamers H, Antognoni P, Dahl O, Richaud P, van Glabbeke M. ARCON: accelerated radiotherapy with carbogen and nicotinamide in head and neck squamous cell carcinomas. The experience of the cooperative group of radiotherapy of the European organization for research and treatment of cancer (EORTC). Radiother Oncol 2000;55:111–119.

Biedunkiewicz B, Tylicki L, Nieweglowski T, Burakowski S, Rutkowski B. Clinical efficacy of ozonated autohemotherapy in hemodialyzed patients with intermittent claudication: An oxygen-controlled study. Int J Artif Organs 2004;27:29–34.

Bocci V. Roles of interferon produced in physiological conditions. A speculative review. Immunology 1988;64:1–9.

Bocci V. Does ozone therapy normalize the cellular redox balance? Med Hypotheses 1996a;46:150–154.

Bocci V. Ozone as a bioregulator: Pharmacology and toxicology of ozonetherapy today. J Biol Regulat Homeost Agent 1996b;10:31–53.

Bocci V. Ozonetherapy as a possible biological response modifier in cancer. Forsch Komplementärmed 1998;5:54–60.

Bocci V. Ozone as Janus: This controversial gas can be either toxic or medically useful. Mediators Inflamm 2004;13:3–11.

Bocci V. Biological and clinical effects of ozone. Has ozonetherapy a future in medicine? Brit J Biomed Sci 1999;56:270–279.

Bocci V. Oxygen-ozone therapy: A critical evaluation. Dordrecht, Netherlands: Kluwer Academic Publishers, 2002.

Bocci V. Ozone: A New Medical Drug. Dordrecht, The Netherlands: Springer, 2005:132–144.

Bocci V, Luzzi E, Corradeschi F, Paulesu L, Rossi R, Cardaioli E, Di Simplicio P. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res 1993a;12:121–126.

Bocci V, Luzzi E, Corradeschi F, Paulesu L, Di Stefano A. Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. J Biol Regulat Homeost Agent 1993b;7:133–138.

Bocci V, Luzzi E, Corradeschi F, Paulesu L. Studies on the biological effects of ozone: 5. Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy. Biotherapy 1994;7:83–90.

Bocci V, Paulesu L. Studies on the biological effects of ozone: 1. Induction of interferon gamma on human leucocytes. Haematologica 1990;75:510–515.

Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, Gerli R. Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone. J Biol Regulat Homeost Agent 1998a;12:67–75.

Bocci V, Valacchi G, Corradeschi F, Fanetti G. Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. Mediators Inflamm 1998b;7:313–317.

Bocci V, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci AM, Di Simplicio P. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. Platelets 1999;10:110–116.

Brahimi-Horn C, Berra E, Pouyssegur J. Hypoxia: The tumor's gateway to progression along the angiogenic pathway. Trends Cell Biol 2001;11:S32–S36.

Brizel DM, Scully SP, Harrelson JM, Layfield LJ, Bean JM, Prosnitz LR, Dewhirst MW. Tumor oxygenation predicts for the likelihood of distant metastases in human soft tissue sarcoma. Cancer Res 1996;56:941–943.

Brown JM. The hypoxic cell: A target for selective cancer therapy (18th Bruce F. Cain Memorial Award lecture). Cancer Res 1999;59:5863–5870.

Burkey KO, Eason G. Ozone tolerance in snap bean is associated with elevated ascorbic acid in the leaf apoplast. Physiol Plant 2002;114:387–394.

Bush RS, Jenkin RD, Allt WE Beale FA, Bean H, Dembo AJ, Pringle JF. Definitive evidence for hypoxic cells influencing cure in cancer therapy. Br J Cancer Suppl 1978;37:302–306.

Calabrese EJ. Hormesis: Changing view of the dose-response, a personal account of the history and current status. Mutat Res 2002;511:181–189.

Carmeliet P, Dor Y, Herbert JM, Fukumura D, Brusselmans K, Dewerchin M, Neeman M, Bono F, Abramovitch R, Maxwell P, Koch CJ, Ratcliffe P, Moons L, Jain RK, Collen D, Keshert E, Keshet E. Role of HIF-1-alpha in hypoxia-mediated apoptosis, cell proliferation and tumour angiogenesis. Nature 1998;394:485–490.

Clavo B, Perez JL, Lopez L, Suarez G, Lloret M, Rodriguez V, Macias D, Santana M, Morera J, Fiuza D, Robaina F, Gunderoth M. Effect of ozone therapy on muscle oxygenation. J Altern Complement Med 2003;9:251–256.

Clavo B, Perez JL, Lopez L, Suarez G, Lloret M, Rodriguez V, Macias D, Santana M, Hernandez MA, Martin-Oliva R, Robaina F. Ozone therapy for tumor oxygenation: A pilot study. Evid Based Complement Altern Med 2004a;1:93–98.

Clavo B, Ruiz A, Lloret M, Lopez L, Suarez G, Macias D, Rodriguez V, Hernandez MA, Martin-Oliva R, Quintero S, Cuyas JM, Robaina F. Adiuvant ozonetherapy in advanced head and neck tumors: A comparative study. Evid Based Complement Alternat Med 2004b;1:321–325.

Coleman CN. Hypoxia in tumors: A paradigm for the approach to biochemical and physiologic heterogeneity. J Natl Cancer Inst 1988;80:310–317.

Cross CE, Motchnik PA, Bruener BA, Jones DA, Kaur H, Ames BN, Halliwell B. Oxidative damage to plasma constituents by ozone. FEBS Lett 1992;298:269–272.

De Maio A. Heat shock proteins: Facts, thoughts, and dreams. Shock 1999;11:1–12.

Denko NC, Giaccia AJ. Tumor hypoxia, the physiological link between Trousseau's syndrome (carcinoma-induced coagulopathy) and metastasis. Cancer Res 2001;61:795–798.

Dewey WC, Hopwood LE, Sapareto SA, Gerweck LE. Cellular responses to combinations of hyperthermia and radiation. Radiology 1977;123:463–474.

Dische S, Anderson PJ, Sealy R, Watson ER. Carcinoma of the cervix: Anaemia, radiotherapy and hyperbaric oxygen. Br J Radiol 1983;56:251–255.

Dore S. Decreased activity of the antioxidant heme oxygenase enzyme: implications in ischemia and in Alzheimer's disease. Free Radic Biol Med 2002;32:1276–1282.

Duckers HJ, Boehm M, True AL, Yet SF, San H, Park JL, Clinton Webb R, Lee ME, Nabel GJ, Nabel EG. Heme oxygenase-1

protects against vascular constriction and proliferation. Nat Med 2001;7:693–698.
Dvorak HF. Rous-Whipple Award Lecture. How tumors make bad blood vessels and stroma. Am J Pathol 2003;162:1747–1757.
Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med 1991;11:81–128.
Falk SJ, Ward R, Bleehen NM. The influence of carbogen breathing on tumour tissue oxygenation in man evaluated by computerised pO2 histography. Br J Cancer 1992;66:919–924.
Fyles A, Milosevic M, Hedley D, Pintilie M, Levin W, Manchul L, Hill RP. Tumor hypoxia has independent predictor impact only in patients with node-negative cervix cancer. J Clin Oncol 2002;20:680–687.
Gatenby RA, Kessler HB, Rosenblum JS, Coia LR, Moldofsky PJ, Hartz WH, Broder GJ. Oxygen distribution in squamous cell carcinoma metastases and its relationship to outcome of radiation therapy. Int J Radiat Oncol Biol Phys 1988;14:831–838.
Giunta R, Coppola A, Luongo C, Sammartino A, Guastafierro S, Grassia A, Giunta L, Mascolo L, Tirelli A, Coppola L. Ozonized autohemotransfusion improves hemorheological parameters and oxygen delivery to tissues in patients with peripheral occlusive arterial disease. Ann Hematol 2001;80:745–748.
Goldman M. Cancer risk of low-level exposure. Science 1996;271:1821–1822.
Griffin RJ, Okajima K, Barrios B, Song CW. Mild temperature hyperthermia combined with carbogen breathing increases tumor partial pressure of oxygen (pO2) and radiosensitivity. Cancer Res 1996;56:5590–5593.
Gutstein HB. The biologic basis of fatigue. Cancer 2001;92: 1678–1683.
Harris AL. Hypoxia: A key regulatory factor in tumour growth. Nat Rev Cancer 2002;2:38–47.
Helczynska K, Kronblad A, Jogi A, Nilsson E, Beckman S, Landberg G, Pahlman S. Hypoxia promotes a dedifferentiated phenotype in ductal breast carcinoma in situ. Cancer Res 2003;63:1441–1444.
Hernandez F, Menendez S, Wong R. Decrease of blood cholesterol and stimulation of antioxidative response in cardiopathy patients treated with endovenous ozone therapy. Free Radic Biol Med 1995;19:115–119.
Hockel M, Schlenger K, Aral B, Mitze M, Schaffer U, Vaupel P. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. Cancer Res 1996;56:4509–4515.
Hockel M, Vaupel P. Tumor hypoxia: Definitions and current clinical, biologic, and molecular aspects. J Natl Cancer Inst 2001;93:266–276.
Honess DJ, Andrews MS, Ward R, Bleehen NM. Pentoxifylline increases RIF-1 tumour pO2 in a manner compatible with its ability to increase relative tumour perfusion. Acta Oncol 1995;34:385–389.
Horsman MR, Chaplin DJ, Brown JM. Tumor radiosensitization by nicotinamide: A result of improved perfusion and oxygenation. Radiat Res 1989;118:139–150.
Huang LE, Bunn HF. Hypoxia-inducible factor and its biomedical relevance. J Biol Chem 2003;278:19575–19578.
Imray CH, Walsh S, Clarke T, Tiivas C, Hoar H, Harvey TC, Chan CW, Forster PJ, Bradwell AR, Wright AD, Birmingham Medical Research Expeditionary Society. Effects of breathing air containing 3% carbon dioxide, 35% oxygen or a mixture of 3% carbon dioxide/35% oxygen on cerebral and peripheral oxygenation at 150 m and 3459 m. Clin Sci 2003;104:203–210.
Inch WR, McCredie JA, Sutherland RM. Effect of duration of breathing 95 percent oxygen plus 5 percent carbon dioxide before x-irradiation on cure of C3H mammary tumor. Cancer 1970;25:926–931.
Jacobs M-T. Side-effects and typical complication due to ozonetherapy [in German]. OzoNachrichten 1982;1:5.
Jia L, Bonaventura C, Bonaventura J, Stamler JS. S-nitrosohaemoglobin: A dynamic activity of blood involved in vascular control. Nature 1996;380:221–226.
Jolly C, Morimoto RI. Role of the heat shock response and molecular chaperones in oncogenesis and cell death. J Natl Cancer Inst 2000;92:1564–1572.
Kume M, Yamamoto Y, Saad S, Gomi T, Kimoto S, Shimabukuro T, Yagi T, Nakagami M, Takada Y, Morimoto T, Yamaoka Y. Ischemic preconditioning of the liver in rats: Implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. J Lab Clin Med 1996:128:251–258.
Lee TS, Chau LY. Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. Nat Med 2002;8: 240–246.
León OS, Menéndez S, Merino N, Castillo R, Sam S, Perez L, Cruz E, Bocci V. Ozone oxidative preconditioning: A protection against cellular damage by free radicals. Mediators Inflamm 1998;7:289–294.
Littlewood TJ, Bajetta E, Nortier JW, Vercammen E, Rapoport B, Epoetin Alpha Study Group. Effects of epoetin alpha on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: Results of a randomized, double-blind, placebo-controlled trial. J Clin Oncol 2001;19:2865–2874.
Marrades RM, Roca J, Campistol JM, Diaz O, Barbera JA, Torregrosa JV, Masclans JR, Cobos A, Rodriguez-Roisin R, Wagner PD. Effects of erythropoietin on muscle O2 transport during exercise in patients with chronic renal failure. J Clin Invest 1996;97:2092–2100.
Mattassi R, D'Angelo F, Bisetti P, Colombo R, Vaghi M. Parenteral ozonetherapy in peripheral obstructive arteriopathies: Biochemical mechanism and clinical results [in Italian]. Il Giornale Di Chirurgia 1987;8:109–111.
Micheli V, Ricci C, Taddeo A, Gili R. Centrifugal fractionation of human erythrocytes according to age: Comparison between Ficoll and Percoll density gradients. Quad Sclavo Diagn 1985;21: 236–248.
Morita T, Kourembanas S. Endothelial cell expression of vasoconstrictors and growth factors is regulated by smooth muscle cell-derived carbon monoxide. J Clin Invest 1995;96:2676–2682.
Overgaard J, Gonzalez GD, Hulshof MC, Arcangeli G, Dahl O, Mella O, Bentzen SM. Randomized trial of hyperthermia as adjuvant to radiotherapy for recurrent or metastatic malignant melanoma. European Society for Hyperthermic Oncology. Lancet 1995;345:540–543.
Paulesu L, Luzzi E, Bocci V. Studies on the biological effects of ozone: 2. Induction of tumor necrosis factor (TNF-α) on human leucocytes. Lymphokine Cytokine Res 1991;10:409–412.
Rokitansky O, Rokitansky A, Steiner J, Trubel W, Viebahn R, Washüttl J. Ozonetherapy in peripheral arteriopathies: Clinic, biochemist and gas-analysis determinations [in German]. In: Water. Berlin: Ozon-Weltkongress, 1981:53–75.

Romero Valdes A, Menendez Cepero S, Gomez Moraleda M, Gomez Moraleda M, Ley Pozo J. Ozone therapy in the advanced stages of arteriosclerosis obliterans. Angiologia 1993;45:146–148.
Rubin P, Hanley J, Keys HM, Marcial V, Brady L. Carbogen breathing during radiation therapy: The Radiation Therapy Oncology Group Study. Int J Radiat Oncol Biol Phys 1979;5: 1963–1970.
Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J 1998; 17:3005–3015.
Semenza GL. Angiogenesis in ischemic and neoplastic disorders. Annu Rev Med 2003;54:17–28.
Semenza GL. Hypoxia-inducible factor 1: Oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001;7:345–350.
Semenza GL. Targeting HIF-1 for cancer therapy. Nat Rev Cancer 2003;3:721–732.
Servaes P, Verhagen C, Bleijenberg G. Fatigue in cancer patients during and after treatment: Prevalence, correlates and interventions. Eur J Cancer 2002;38:27–43.
Sharma YK, León J, Raskin I, Davis KR. Ozone-induced responses in Arabidopsis thaliana: The role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. Proc Natl Acad Sci U S A 1996;93:5099–5104.
Shinriki N, Suzuki T, Takama K, Fukunaga K, Ohgiya S, Kubota K, Miura T. Susceptibilities of plasma antioxidants and erythrocyte constituents to low levels of ozone. Haematologia 1998;29:229–239.
Siemann DW, Hill RP, Bush RS. The importance of the pre-irradiation breathing times of oxygen and carbogen (5% CO2: 95% O2) on the in vivo radiation response of a murine sarcoma. Int J Radiat Oncol Biol Phys 1977;2:903–911.
Siemann DW, Horsman MR, Chaplin DJ. The radiation response of KHT sarcomas following nicotinamide treatment and carbogen breathing. Radiother Oncol 1994;31:117–122.
Snyder SH, Baranano DE. Heme oxygenase: A font of multiple messengers. Neuropsychopharmacology 2001;25:294–298.
Song CW, Hasegawa T, Kwon HC, Yang DH, Cho BH, Lee ST. Increase in tumor oxygenation and radiosensitivity caused by pentoxifylline. Radiat Res 1992;130:205–210.
Song CW, Lee I, Hasegawa T, Rhee JG, Levitt SH. Increase in pO2 and radiosensitivity of tumors by Fluosol-DA (20%) and carbogen. Cancer Res 1987;47:442–446.
Song CW, Shakil A, Griffin RJ, Okajima K. Improvement of tumor oxygenation status by mild temperature hyperthermia alone or in combination with carbogen. Semin Oncol 1997;24:626–632.
Song CW, Shakil A, Osborn JL, Iwata K. Tumor oxygenation is increased by hyperthermia at mild temperatures. Int J Hyperthermia, 1996;12:367–373.
Stone JR, Collins T. The role of hydrogen peroxide in endothelial proliferative responses. Endothelium 2002;9:231–238.
Subarsky P, Hill RP. The hypoxic tumor microenvironment and metastatic progression. Clin Exp Metastasis 2003;20:237–250.
Sweet F, Kao M-S, Lee S-CD, Hagar WL, Sweet WE. Ozone selectively inhibits growth of human cancer cells. Science 1980;209:931–933.
Teicher BA, Rose CM. Perfluorochemical emulsions can increase tumor radiosensitivity. Science 1984;223:934–936.
Thomson AJ, Webb DJ, Maxwell SR, Grant IS. Oxygen therapy in acute medical care. BMJ 2002;324:1406–1407.
Tosetti F, Ferrari N, De Flora S, Albini A. Angioprevention: Angiogenesis is a common and key target for cancer chemopreventive agents. FASEB J 2002;16:2–14.
Tylicki L, Niewglowski T, Biedunkiewicz B, Burakowski S, Rutkowski B. Beneficial clinical effects of ozonated autohemotherapy in chronically dialysed patients with atherosclerotic ischemia of the lower limbs: Pilot study. Int J Artif Organs 2001;24:79–82.
Tylicki L, Nieweglowski T, Biedunkiewicz B, Chamienia A, Debska-Slizien A, Aleksandrowicz E, Lysiak-Szydlowska W, Rutkowski B. The influence of ozonated autohemotherapy on oxidative stress in hemodialyzed patients with atherosclerotic ischemia of lower limbs. Int J Artif Organs 2003;26:297–303.
Tylicki L, Biedunkiewicz B, Nieweglowski T, Chamienia A, Slizien AD, Luty J, Lysiak-Szydlowska W, Rutkowski B. Ozonated autohemotherapy in patients on maintenance hemodialysis: Influence on lipid profile and endothelium. Artif Organs 2004;28:234–237.
Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. Mediators Inflamm 1999;8:205–209.
Valacchi G, Bocci V. Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. Mediators Inflamm 2000;9:271–276.
Valdagni R, Amichetti M. Report of long-term follow-up in a randomized trial comparing radiation therapy and radiation therapy plus hyperthermia to metastatic lymph nodes in stage IV head and neck patients. Int J Radiat Oncol Biol Phys 1994;28: 163–169.
Vaupel P, Hockel M. Blood supply, oxygenation status and metabolic micromilieu of breast cancers: Characterization and therapeutic relevance. Int J Oncol 2000;17:869–879.
Wolff HH. Ozone in Medicine: Theoretical Basis and Therapeutic Advantages [in German]. Heidelberg, Verlag für Medizin, 1979.
Young SD, Marshall RS, Hill RP. Hypoxia induces DNA overreplication and enhances metastatic potential of murine tumor cells. Proc Natl Acad Sci U S A 1988;85:9533–9537.
Zhong H, De Marzo AM, Laughner E, Lim M, Hilton DA, Zagzag D, Buechler P, Isaacs WB, Semenza GL, Simons JW. Overexpression of hypoxia-inducible factor 1alpha in common human cancers and their metastases. Cancer Res 1999;59:5830–5835.
Zuckerbraun BS, Billiar TR. Heme oxygenase-1: A cellular Hercules. Hepatology 2003;37:742–744.

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