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Scientific and Medical Aspects of Ozone Therapy. State of the Art

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The aim of this review is to dispel misconceptions and skepticism regarding ozone therapy and to clarify the biochemical and pharmacological mechanisms of action of ozone dissolved in biological fluids. The work performed in the last decade in our laboratory allows drawing a comprehensive framework for understanding and recommending ozone therapy in some diseases. It is hoped that this report will open a dialogue among clinical scientists and will inform physicians about the beneficial effects of ozone therapy. © 2006 IMSS. Published by Elsevier Inc.

Key Words: Ozone, Antioxidants, Oxidative stress, Ozone tolerance, Ozone therapy.

Introduction

It is distressing to note that often ozone therapists are more interested in simply knowing the ozone dosage rather than to understand how ozone acts and why we can avoid toxicity. This behavior reveals a lack of knowledge of the fundamental bases regulating a judicious use of ozone and is the result of a superficial preparation acquired during an occasional ozone therapy's course of a few hours. This is not surprising because during the last three decades, on the basis of Wolff's suggestion (1), ozone therapy has been used by practitioners in Europe in an empirical fashion. Unfortunately, even today, most ozone therapists have either a misconception or know only a few technical tips for performing ozone therapy. This problem, associated with the difficulties and cost of performing extensive clinical studies, has hindered real progress, and ozone therapy remains a scarcely known and objected complementary practice. Worst of all, in some countries, often without any medical qualification, quacks continue to inject either ozone intravenously, a procedure prohibited since 1984 in Germany because of the risk of pulmonary embolism and death, or ozonated saline containing a certain toxic amount of hypochloric acid. Moreover, a distinguished American chemist has affirmed the dogma that "ozone is toxic any way you deal with it," reinforcing the concept that ozone should never be used in medicine. This situation has generated a sort of crusade against ozone therapy in spite of the fact that ozone is considered one of the best disinfectants capable of preventing infection outbreaks. This is becoming a crucial advantage because critically ill patients acquire infections while in hospitals and a number of them die every year as a result.

Table 1 summarizes several good reasons for refusing ozone therapy by orthodox medicine. However, problems 1–5 have now been practically overcome, whereas the remaining 6–9 are stumbling blocks hindering progress. During the last 14 years, we have made a great effort to examine ozone therapy in a scientific fashion both at a basic and clinical level, and we now have some ideas how ozone acts, how and why its toxicity can be controlled and how therapeutic effects can be exerted (2–11). There is no need to invoke philosophical speculations because the mechanisms of action are in the realm of classical biochemistry, physiology and pharmacology.

This review aims to give the reader the essential information and the frame of mind to operate as a real physician. An extensive description is available in three recent books (9–11).

What Is Ozone and How Can We Use It?

Ozone is normally present as a gas made of three atoms of oxygen with a cyclic structure. The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (5–13 mV) according to the reaction:

$$3O_2 + 68,400\,\text{cal} \rightarrow 2O_3$$

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Table 1. Why oxygen ozone therapy has not yet been accepted by orthodox medicine

1. Excessive empiricism
2. Lack of standardization
3. No precise ozone generator
4. Lack of solid scientific biological and clinical data
5. Ozone toxicity
6. The problem of charlatans
7. Lack of regulation and disinterest of health authorities
8. Lack of financial support
9. Skeptical and uninformed scientists

Consequently, we always collect a gas mixture comprising no less than 95% oxygen and no more than 5% ozone. Air must be excluded because toxic nitrogen dioxide (N_2O_2) will be formed as well as ozone and it is imperative that generators are made of high quality, ozone-resistant materials such as stainless steel, neutral glass and Teflon.

Ozone is 1.6-fold denser and 10-fold more soluble in water (49.0 mL in 100 mL water at 0°C) than oxygen. Although ozone is not a radical molecule, it is the third most potent oxidant ($E^\circ = +2.076$ V) after fluorine and persulfate. Ozone is an unstable gas that cannot be stored and should be used at once because it has a half-life of 40 min at 20°C.

Ozone is a controversial gas because, although it is very useful in the stratosphere by absorbing dangerous B and C ultraviolet radiations, it is toxic for the pulmonary tract in the troposphere, particularly mixed with carbon monoxide (CO), N_2O_2 and traces of acids as occurs in photochemical smog.

It must be clear that if we want to use ozone in medicine, we must avoid its toxicity that can be controlled only if we operate cautiously by 1) using a precise ozone generator equipped with a well-standardized photometer, which allows us to determine the ozone concentration in real time, 2) by collecting a precise gas volume with a defined ozone concentration. The total dose is simply calculated by multiplying the ozone concentration with the gas volume. As an example, if we ozonate a blood volume of 225 mL with 225 mL of gas with an ozone concentration of 30 μg/mL, the total dose is equivalent to 6.75 mg of ozone. 3) We must know the optimal dose for achieving a therapeutic effect without any toxicity.

At variance with blood, the eyes and the lungs are very sensitive to ozone because they have minimal antioxidant and neutralizing capabilities and therefore ozone should never contact these organs.

What Is the Behavior and Fate of Ozone after Coming in Contact with Body Fluids?

The essential concepts to bear in mind are the following: a) as any other gas, ozone dissolves physically in pure water according to Henry's law in relation to the temperature, pressure and ozone concentration. Only in this situation does ozone not react and, in a tightly closed glass bottle, the ozonated water (useful as a disinfectant) remains active for a couple of days; b) on the other hand, at variance with oxygen, ozone reacts immediately as soon as it is dissolved in biological water (physiological saline, plasma, lymph, urine):

$$O_3 + \text{biomolecules} \rightarrow O_2 + O^{\bullet}$$

where atomic oxygen behaves as a very reactive atom. Contrary to the incorrect belief that ozone penetrates through the skin and mucosae or enters into the cells, it is emphasized that, after the mentioned reaction, ozone does not exist any longer.

In order of preference, ozone reacts with polyunsaturated fatty acids (PUFA), antioxidants such as ascorbic and uric acids, thiol compounds with -SH groups such as cysteine, reduced glutathione (GSH) and albumin. Depending upon the ozone dose, carbohydrates, enzymes, DNA and RNA can also be affected.

All of these compounds act as electron donor and undergo oxidation. c) The main reaction:

$$\text{R-CH=CH-R}' + O_3 + H_2O \rightarrow \text{R-CH=O} + \text{R}'\text{-CH=O} + H_2O_2$$

shows the simultaneous formation of one mole of hydrogen peroxide (included among reactive oxygen species, ROS) and of two moles of lipid oxidation products (LOPs) (12).

The fundamental ROS molecule is hydrogen peroxide, which is a non-radical oxidant able to act as an ozone messenger responsible for eliciting several biological and therapeutic effects (13,14). The concept that ROS are always harmful has been widely revised because, in physiological amounts, they act as regulators of signal transduction and represent important mediators of host defense and immune responses.

Presence of traces of Fe^{++} should be avoided because, in the presence of hydrogen peroxide, via the Fenton's reaction, they will catalyze the formation of the most reactive $OH^{\bullet}$ (hydroxyl radical).

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^{\bullet} + OH^{-}.$$

Interestingly, we (15) have also determined the formation of nitrogen monoxide ($NO^{\bullet}$) in human endothelial cells exposed to ozonated serum. Attention should be paid to the fact that an excess of ROS can lead to the formation of other toxic compounds such as peroxynitrite ($O=NOO^{-}$) and hypochlorite anion (ClO^{-}).

Although ROS have a lifetime of less than a second, they can damage crucial cell components and, therefore, their generation must be precisely calibrated to achieve a biological effect without any damage. This can be achieved by regulating the ozone dose (ozone concentration as μg/mL of gas per mL of blood in 1:1 ratio) against the antioxidant capacity of blood that can be measured and, if necessary,

strengthened by oral administration of antioxidants before and throughout ozone therapy. d) LOPs production follows peroxidation of PUFA present in the plasma: they are heterogeneous and can be classified as lipoperoxides ($LOO^{\bullet}$), alkoxyl radicals ($LO^{\bullet}$), lipohydroperoxides (LOOH), isoprostanes and alkenals, among which are 4-hydroxy-2,3 transnonenal (HNE) and malonyldialdehyde (MDA). Radicals and aldehydes are intrinsically toxic and must be generated in very low concentrations. They are *in vitro* far more stable (6) than ROS but fortunately, upon blood reinfusion, they undergo a marked dilution in body fluids, excretion (via urine and bile), and metabolism by GSH-transferase (GSH-Tr) and aldehyde dehydrogenases. Thus, only submicromolar concentrations can reach all organs, particularly bone marrow, liver, central nervous system (CNS), endocrine glands, etc., where they act as signaling molecules of an ongoing acute oxidative stress (16).

If the stage of the disease is not too far advanced, these molecules can elicit the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), GSH-peroxidases (GSH-Px), GSH-reductase (GSH-Rd) and catalase (CAT). Interestingly, Iles and Liu (17) have just demonstrated that HNE, by inducing the expression of glutamate cysteine ligase, causes an intracellular increase of GSH, which plays a key role in antioxidant defense. Furthermore, LOPs induce oxidative stress proteins, one of which is heme-oxygenase I (HO-1 or HSP-32) which, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin (18). Bilirubin is a significant lipophilic antioxidant and a trace of CO cooperates with NO in regulating vasodilation by activating cyclic GMP. Fe^{2+} is promptly chelated by upregulated ferritin. The induction of HO-1 after an oxidative stress has been described in hundreds of papers as one of the most important antioxidant defense and protective enzyme. Moreover, LOPs exert a neuroimmunomodulatory effect highlighted by a feeling of well being reported by patients during ozone therapy.

Although it remains hypothetical, it is possible that LOP, throughout the treatments, acting as acute oxidative stressors in the bone marrow microenvironments activate the release of metalloproteinases, of which MP-9 particularly may favor the detachment of staminal cells (11). These cells, once in the blood circulation, may be attracted and home at sites where a previous injury (a trauma or an ischemic-degenerative event) has taken place. The potential relevance of such an event would have a huge practical importance and will avoid the unnatural, costly and scarcely effective practice of the bone marrow collection with the need of the successive and uncertain reinfusion (19).

It is emphasized that submicromolar LOPs levels can be stimulatory and beneficial, whereas high levels can be toxic. This conclusion, based on many experimental data (16), reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result: too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue) so that they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity.

In conclusion, it must be clear to the reader that the ozonation process either happening in blood, or intradiscal or in an intramuscular site represents an acute oxidative stress. However, provided that it is precisely calculated according to a judicious ozone dosage, it is not deleterious but is actually capable of eliciting a multitude of useful biological responses and, possibly, can reverse a chronic oxidative stress due to aging, chronic infections, diabetes, atherosclerosis, degenerative processes and cancer. Indeed, the ozonotherapeutic act is interpreted as an atoxic but real ''therapeutic shock'' able to restore homeostasis.

Which Are the Biological Effects Elicited by ROS and LOPs?

The ozonation process is therefore characterized by the formation of ROS and LOPs acting in two phases. This process happens either *ex vivo* (as a typical example in the blood collected in a glass bottle) or *in vivo* (after an intramuscular injection of ozone) but, while ROS are acting immediately and disappear (early and short-acting messengers), LOPs, via the circulation, distribute throughout the tissues and eventually only a few molecules bind to cell receptors. Their pharmacodynamics allow minimizing their potential toxicity and allows them to become late and long-lasting messengers.

Formation of ROS in the plasma is extremely rapid and is accompanied by a transitory and small ozone dose-dependent decrease (ranging from 5 to 25%) of the antioxidant capacity. Importantly, this return to normal within 15–20 min owes to the efficient recycling of oxidized compounds such as dehydroascorbate to ascorbic acid (20). H_2O_2 diffuses easily from the plasma into the cells and its sudden appearance in the cytoplasm represents the triggering stimulus: depending upon the cell type, different biochemical pathways can be concurrently activated in erythrocytes, leukocytes and platelets resulting in numerous biological effects. It must be noted that between the plasma and the cytoplasm compartments there is a gradient and the intracellular H_2O_2 concentration is only about 1/10 of the plasmatic one (21). The rapid reduction to water is operated by the high concentration of GSH, CAT and GSH-Px; nonetheless, H_2O_2 must be above the threshold concentration for activating several biochemical pathways.

Let us now examine how hydrogen peroxide, now universally recognized as one of the main intracellular signalling molecules (13), acts on the different blood cells. The mass of erythrocytes mops up the bulk of hydrogen peroxide: GSH is promptly oxidized to GSSG and the cell, extremely sensitive to the reduction of the GSH/GSSG

ratio, immediately corrects the unbalance by either extruding GSSG or reducing it with GSH-Rd at the expense of ascorbate or of the reduced nicotinamide adenine dinucleotide phosphate (NADPH), which serves as a crucial electron donor. Next, the oxidized NADP is reduced after the activation of the pentose phosphate pathway, of which glucose-6-phosphate dehydrogenase (G-6PD) is the key enzyme. We have determined a small but significant increase of ATP formation (10,11), but whether this is due to the activation of the pentose cycle or to phosphofructokinase or to both remains to be clarified. 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, due either to a slight decrease of intracellular pH (Bohr effect) or/and an increase of 2,3-diphosphoglycerate (2,3-DPG) levels. Obviously, one AHT treatment has a minimal effect and we need to ozonate at least 2.5–4 L of blood within a period of 30–60 days. During this period, LOPs act as repeated stressors on the bone marrow and these frequent stimuli cause the adaptation to the ozone stress during erythrogenesis with upregulation of antioxidant enzymes. As a consequence, a patient with chronic limb ischemia undergoing ozone therapy can have a clinical improvement due to the formation of successive cohorts of erythrocytes progressively more capable of delivering oxygen to his/her ischemic tissues. However, the final improvement is also due to the localized release of NO, CO and growth factors released from platelets and endothelial cells.

Although ozone is one of the most potent disinfectants, it cannot inactivate bacteria, viruses and fungi *in vivo* because, paradoxically, the pathogens are well protected, particularly inside the cells, by the powerful antioxidant system. Thus, as I proposed a long time ago (22,23), ozone acts as a mild enhancer of the immune system by activating neutrophils and stimulating the synthesis of some cytokines (2,5–7). Once again, the crucial messenger is hydrogen peroxide, which after entering into the cytoplasm of blood mononuclear cells (BMC) by oxidizing selected cysteines, activates a tyrosine kinase, which then phosphorylates the transcription factor nuclear factor κB (24), allowing the release of a heterodimer (p50+p65). This complex moves on to the nucleus and switches on some hundred genes eventually responsible for causing the synthesis of several proteins, among which are the acute-phase reactants and numerous interleukins. In the past, we have measured the release of several cytokines from ozonated blood upon *in vitro* incubation (2–7). Once the ozonated leukocytes return to the circulation, they home in lymphoid microenvironments and successively release cytokines acting in a paracrine fashion on neighboring cells with a possible reactivation of a depressed immune system (25). This process, described as the physiological cytokine response, is part of the innate immune system and helps us to survive in a hostile environment.

During ozonation of blood, particularly if it is anticoagulated with heparin, we have noted an ozone dose-dependent increase of activation of platelets (8,26) with a consequent release of typical growth factors, which will enhance the healing of chronic ulcers in ischemic patients. Whenever possible, the use of heparin as an anticoagulant is preferable to sodium citrate because, by not chelating plasmatic Ca^{++}, it reinforces biochemical and electric events.

During reinfusion of the ozonated blood into the donor, the vast expanse of the endothelial cells will be activated by LOPs, resulting in an increased production of NO, plasma S-nitrosothiols and S-nitrosohemoglobin (15,27). Whereas NO has a half-life of less than 1 sec, protein-bound-NO can exert vasodilation also at distant ischemic vascular sites with relevant therapeutic effect.

Moreover, on the basis of the phenomenon of ozone tolerance that says the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response (28,29), we have postulated that LOPs, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress (10,11). The bone marrow is particularly relevant because it can upregulate antioxidant enzymes during erythrogenesis and allows the release of staminal cells for possibly regenerating infarcted organs. Moreover, the stimulation of the endocrine and central nervous systems may help to understand why most patients during prolonged ozone therapy report a feeling of euphoria and wellness, probably due to an improved metabolism as well as to an enhanced hormonal or neurotransmitter release.

The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom and there is good experimental evidence (30–34) that this phenomenon is present in the animal and vegetal kingdom. Moreover, it is already supported by our findings of an increased level of antioxidant enzymes and HO-1 during ozone therapy (10,11). It also suggests that a judicious use of ozone, in spite of acting as an oxidant, enhances the antioxidant capacity, which represents the critical factor for overcoming chronic viral infections, ischemia and cell degeneration.

Which Are the Routes of Ozone Administration?

Table 2 shows that ozone can be administered with great flexibility but it should not be injected intravenously as a gas because of the risk of provoking oxygen embolism, given the fact that the gas mixture contains always no less than 95% oxygen.

So far the most advanced and reliable approach has been the major ozonated AHT because, on the basis of the patient's body weight, a predetermined volume of blood (200–270 mL) can be exposed to an equal volume of gas

Table 2. Routes of ozone administration

Parenteral
Intravenous, intra-arterial,[a] intramuscular, subcutaneous, intraperitoneal, intrapleural, intra-articular, periarticular, myofascial, intradiscal, intraforaminal, intralesional[b]
Topical or locoregional
Nasal,[c] tubal,[c] auricular, oral,[c] vaginal, urethral and intrabladder, rectal, cutaneous, dental

[a]No longer used for limb ischemia. Hepatic metastasis could be embolized via the hepatic artery.
[b]Intratumoral or via a fistula.
[c]To be performed during 30–40 sec apnea.

(O_2–O_3) in a stoichiometric fashion, with the ozone concentration precisely determined. Figure 1 shows a schematic drawing of the components necessary to perform AHT with an ozone-resistant glass bottle (plastic bags must be avoided because they are not ozone resistant and contaminate blood with pthalates and plastic microparticles). Blood, drawn from a cubital vein via a G19 Butterfly needle, is rapidly sucked inside the bottle under vacuum via Segment A. Then a precise volume of gas is delivered via segment B. With gentle mixing to avoid foaming, ozonation of blood is completed in 5–10 min and the ozonated blood is reinfused, via suitable tubing with blood filter, into the donor in about 15 min. This simple, inexpensive (all the necessary disposable material costs about 12 US$) procedure has already yielded therapeutic results in vascular diseases superior to those achieved by conventional medicine. Moreover, the therapeutic modalities, until now restricted to major AHT and to the empirical and imprecise rectal insufflation of gas (11), have been extended: they include the quasi-total body exposure to O_2–O_3 (35) and the extracorporeal blood circulation against O_2–O_3 (36). The latter procedure is rather invasive because blood collected from a vein circulates through an ozone-resistant gas exchanger and, with the help of a peristaltic pump, returns to the circulation via a contralateral vein. On the other hand, the partial cutaneous exposure to oxygen-ozone does not need any venous puncture and, owing to the vast expanse of the skin, allows a generalized and beneficial effect. Clearly, today we can select the most suitable method for different pathologies, their stage and the patient's condition.

A discussion on its own is needed for the minor AHT, which basically consists of withdrawing 5 mL of blood to be immediately and vigorously mixed for 1 min with an equal volume of O_2–O_3 at an ozone concentration ranging between 80 and 100 µg/mL of gas per mL of blood. It has been extensively described in Bocci (11). The strongly oxidized blood, including the foam, is promptly injected into the gluteus muscle without the need of any anesthetic. As an unspecific immunomodulatory approach, I have used this treatment since 1953 and, during the last two decades, several ozone therapists have successfully treated herpetic infections (for review, see Reference 11). I have speculated that blood infiltrated into the muscular tissue will undergo coagulation due to platelet and prothrombin activation.

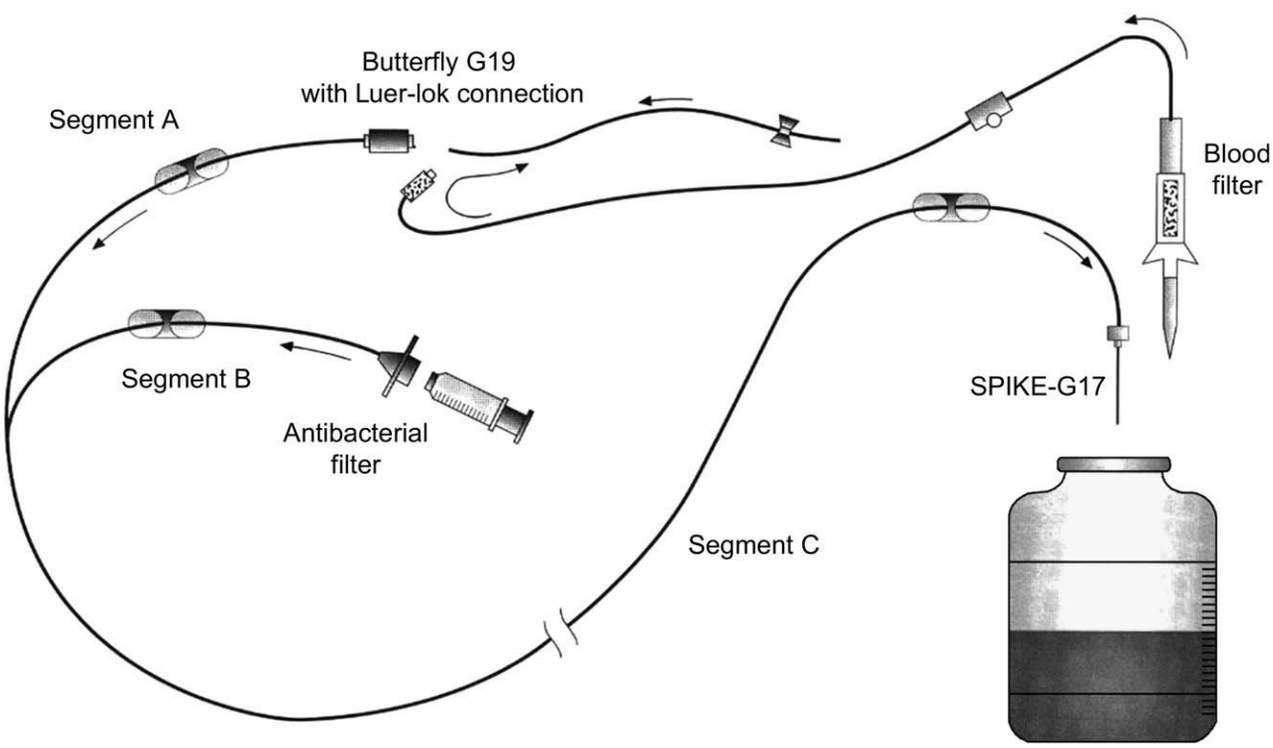

Figure 1. Schematic drawing of the components necessary to perform the ozonated autohemotherapy with an ozone-resistant glass bottle under vacuum.

Although patients rarely report a slight swelling and pain at the injection site, a mild sterile inflammatory reaction may take place with infiltration of monocytes and neutrophils scavenging denatured proteins, lysed erythrocytes and apoptotic cells. If plama contains some free virions (HCV, HBV, HHV, HIV and so on), these will be inactivated by the high ozone concentration and may act as an autovaccine. At the same time a moderate release of cytokines will modulate the physiological response (25), and the abundance of heme will upregulate the synthesis of both antioxidant enzymes and oxidative stress proteins, particularly of heme oxygenase I. It is wonderful that such a simple and autologous treatment can act as a powerful enhancer of several biological responses.

A variant and unnecessarily complicated procedure proposed in the 1990s consists of treating a similarly small volume of citrated blood with ozone, ultraviolet light (obviously generating more ozone and ROS) and heat (42.5°C) for 3 min. To my knowledge, without clarifying the rationale of using three physicochemical stresses, this method appears superfluous because ozone, as an oxidizer, is more than enough and the addition of other stresses makes the interpretation of the response very difficult. A first pilot study by Garber et al. (37) testing this technique in HIV patients was badly conceived and showed neither toxicity nor efficacy, but it has amply discredited the use of ozone. This approach has been subsequently used in patients with either vasculitis (38) or advanced chronic heart failure (39). As might be expected, two biological studies (40,41) have shown the possibility of controlling a chronic oxidative stress (33) and of activating regulatory T cells for downregulating a chronic inflammation. In conclusion, while I am not using this variant, I systematically couple the major and minor AHT as above described in all patients because I have noticed a potentiation of the biological and therapeutic effects. My opinion is that only by using a double-focused approach (it is less expensive than the variant minor AHT), able to simultaneously expand the interaction of ozonated messengers with both blood and muscular tissue, one can achieve a more rapid and intense therapeutic efficacy.

On the basis of experimental data obtained during the last decade (3–11) and on the average antioxidant capacity of human blood, we have determined the so-called 'therapeutic window,' that is the range of ozone concentrations (expressed as μg/mL of gas per mL of blood) within which ozone can exert therapeutic effects without toxicity with regard to major AHT. The range is surprisingly wide: 10–15 μg/mL as a minimum and 80 μg/mL as a maximum. Above 90 μg/mL, an incipient hemolysis (4–5%) warns about toxicity. The threshold level varies between 15 and 20 μg/mL, depending upon the individual antioxidant capacity. The scheme presented in Figure 2 is meant to illustrate the breadth of action expressed by the ozonated blood throughout the whole organism.

It is clear that the ozone oxidative activity is efficiently counteracted by the wealth of plasmatic and intracellular antioxidants so that an ozone concentration of 5–10 μg/mL per mL of blood is practically neutralized: only a trace of ROS and LOPs become detectable and therefore, at this very low level of ozonation, AHT may only have a placebo

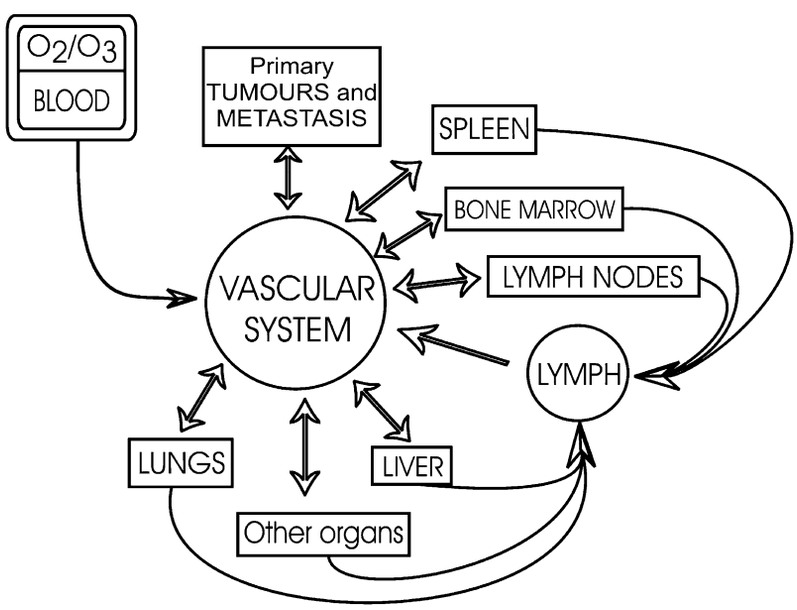

Figure 2. Ozonated blood, after reinfusion into the donor patient, is distributed throughout the whole organism. Erythrocytes continue to circulate in the vascular system delivering more oxygen into ischemic areas while leukocytes, migrated through post-capillary venules into various organs, slowly induce an immune response. Platelets will release their hormonal contents into the blood and will disappear. The reinfused LOPs undergo dilution into about 3 L plasma and 9–11 L interstitial fluid but will deliver the message of an acute oxidative stress to the whole body.

effect. As we are particularly conscious of ozone toxicity, we always apply the strategy "start low go slow" and, depending on the stage of the disease and the patient's condition, we usually scale up the concentrations from 15, then 20, 30 and 40 μg/mL, and more when necessary, during the 1st, 2nd, 3rd and 4th weeks, respectively. By using this strategy, after many thousands of autotransfusions, we have never recorded any acute or chronic toxicity. The venous puncture is usually well tolerated because it is performed with a G19 Butterfly needle (quite suitable for withdrawing blood into the glass bottle under vacuum) that remains inserted throughout the 35–40 min treatment. However, a small percentage of women have a very poor venous access: in this case we can select one of the following three options: rectal insufflation of gas, body exposure to gas, or the slow infusion into a visible vein on the hand dorsum, via a G25–27 needle, of an isotonic glucose solution containing a final concentration of 0.03–0.06% (8.8–17.6 mM) hydrogen peroxide (11,14). This last approach cannot be as effective as the classical ozonated AHT, but it is useful. We absolutely discourage the use of ozonated saline because it contains sodium hypochlorite and can cause phlebitis (14).

Normally we perform the treatment bi-weekly but, if necessary, we can do it every day or even three times daily.

When Ozone Therapy Should Be Used?

Whenever orthodox medicine fails to solve the medical problem, the physician has the duty to fully inform the patient of all valid options available before beginning ozone therapy. I dislike antagonizing ozone therapy to orthodox medicine because I believe that there is only good medicine, which is the one that is able to cure the patient.

So far our experience is ample only for chronic limb ischemia (11,42–45), cutaneous chronic ulcers due to ischemia and diabetes (10,11), and in the atrophic form of age-related macular degeneration (ARMD) (11). In chronic limb ischemia, the orthodox treatment is performed by prostanoid infusions, but the benefit is inferior and far more expensive than ozone therapy. Ozone therapy really helps about 70% of the ARMD (dry form) patients (11) because there is no other conventional option. The neovascular, exudative (or wet form) must be first treated with photodynamic therapy (46) or radiation (47) or with other experimental approaches based on blocking the activity of extracellular vascular endothelial growth factor (48).

I will then enumerate other pathologies where ozone therapy can be proficiently combined with orthodox therapies: 1) Acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi (11). Even parasitic infections such as giardiasis and cryptosporidiosis have been treated in children by Cuban physicians after administration of ozonated oil (11). 2) Osteomyelitis, pleural empyema, peritonitis, abscesses with fistulae, bed sores, chronic ulcers, diabetic foot, burns, insect and jellyfish stings, infected wounds, onychomycosis and candidiasis. These infections, often supported by antibiotic-resistant bacteria, like methicillin-resistant *Staphyloccocus aureus* and poor penetration of antibiotics into infected areas, are responsible for too many cases of death occurring in hospitals of even the most advanced countries. In such cases, ozonated AHT associated with the topical application of ozonated olive or sunflower oils allows a rapid disinfection and enhances healing tremendously. Unfortunately, the use of ozonated oils is hardly known and a detailed description of their preparation, application and results is reported in my most recent book (11). It is most interesting that ozone, an unstable gas, can be stably trapped as an ozonide between a double bond of a PUFA: $-(CH_2)_7-O_3-(CH_2)_7CH_3$. When the ozonated oil is layered over the ulcer's exudate at the oil−water interface, the ozone moves slowly into the water and, by reacting with biomolecules, generates a steady flow of H_2O_2. The effects of sterilization and improved oxygenation are responsible for the accelerated cicatrization. In comparison to pharmaceutical creams often containing useless antibiotics and growth factors, once ozonated oil is known and used, it will be extremely beneficial to millions of patients. 3) Herpetic infections (HHVI and II), herpes zoster and papillomavirus infection. The modality of the intramuscular injection of minor ozonated AHT, used as an autovaccine and associated with the topical therapy with ozonated oil, is very effective in preventing relapse of herpetic infections. This approach, particularly when used in combination with the acyclovirs, can cure herpetic infections in the majority of patients (11). It must be mentioned that a new vaccine can significantly reduce the incidence of herpes zoster infection and post-herpetic neuralgia (49). Chronic hepatitis-C and HIV infections, whenever possible, must be basically treated with either PEG-interferon alpha + ribavirin or highly active anti-retroviral therapy, respectively, because these drug combinations usually lower the viral load rapidly. However, ozone therapy could be simultaneously performed as a useful adjuvant treatment (11). 4) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Crohn's disease): results with AHT seem encouraging but are anecdotal. 5) Other chronic ischemic diseases (cerebral and heart ischemia). Ozone therapy exerts beneficial effects because it can a) increase oxygen, glucose and ATP delivery within ischemic tissues, b) enhance neoangiogenesis and possibly facilitate the implantation of bone marrow stem cells, which can provide neovascularization and tissue regeneration, c) induce the preconditioning phenomena by upregulating the expression of antioxidant enzymes and heme oxygenase I and d) trigger a neurohumoral response for improving quality of life. Our preliminary study (11) in end-stage cardiopathic patients, when either transplantation or

surgical revascularization was no longer feasible, has already shown that ozone therapy combined with the conventional best medical therapy can improve a gloomy prognosis. 6) Degenerative disorders: AHT helps patients in the early phase of senile dementia. On the other hand, it is rarely and minimally useful in diabetic retinopathy, retinitis pigmentosa, sudden hearing loss and chronic tinnitus. 7) Pulmonary diseases: emphysema, asthma, chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome. COPD is becoming the fourth cause of death in spite of orthodox therapy based on the inhaled combination of corticosteroids plus long-acting β2-agonists and antibiotics, when necessary (50). Unfortunately these drugs, if prolonging the patient's life, do not arrest the progression of the disease. The rationale for using ozone therapy is briefly based upon a) blood reinfusion, LOPs, present in low concentrations act on the vast endothelial surface and enhance the release of prostacyclin and NO while release of endothelin-1 is depressed (8,15). It is known that the release of NO and S-nitrosothiols represents the physiological mechanism for vasodilation (51,52) and contrasts the release of the anion superoxide, which causes vasoconstriction and deploys negative influences on platelets and endothelial cells. Secondly, the delivery of oxygen in ischemic tissues is enhanced and the progressive increase of antioxidant enzymes and heme oxygenase-1 counteracts the chronic oxidative stress, typical of pulmonary diseases. Moreover, the mild stimulation of the immune system helps to contain recurrent and chronic pulmonary infections. Recently, I have been able to treat advanced COPD patients with very encouraging results demonstrated by a marked improvement of the respiratory parameters and the walking test (11). 8) Terminal nephropathies are progressively worsened by a chronic oxidative stress not yet controllable by orthodox medicine and therefore ozone therapy could stabilize this serious dysfunction and improve the quality of life of these patients (11). 9) In a similar manner, ozonated AHT combined with topical application of ozonated oil is proving to be very useful in the metabolic syndrome well exemplified in patients with type 2 diabetes suffering from chronic ulcers with no tendency to heal (11). There is no doubt that patients prefer ozone therapy to hyperbaric oxygen and local larval (maggot) therapy (53). Needless to say, we must continue to strictly control the glycemic level. 9) Skin diseases (psoriasis, atopic dermatitis): available data seem positive but there are no randomized studies. 10) Chemoresistant metastatic cancer; therapy of cancer-related fatigue: we have reported (11,54) that a 6-month, biweekly, ozone therapy session in preterminal patients previously heavily treated with chemo- or/and radiotherapy does improve their quality of life but is unable to block cancer progression. On the other hand, ozone therapy may be far more useful immediately after surgery, possibly combined with chemo- or/and radiotherapy. Not only could it potentiate the effect of the cytotoxic drugs but by inducing the antioxidant response, it could reduce chemotoxicity (55). It is deplorable that oncologists do not want to cooperate and want to apply only their protocols. Meantime, even if survival is moderately prolonged at the cost of a poor quality of life, the mortality remains very high. Peter Boyle, Director of the International Agency for Cancer Research in Lyon, France has communicated that in Europe, in 2004, new cancer cases amounted to 2.9 million with over 1.7 million deaths. These impressive numbers indicate that the war on cancer remains wide open and that a skeptical attitude against the use of ozone therapy is unjustified. 11) Orthopedic diseases (the problem of backache): the direct intradiscal injection of oxygen-ozone is a great success in about 75% of patients (11,56) and is one of the few modern techniques able to solve the problem of a hernial disc with a mini-invasive approach. The indirect procedure that I defined as a ''chemical acupuncture'' consists of injecting 10-20 mL of gas into the paravertebral muscle corresponding to the metameres of the disc; it is also effective in about two thirds of patients but, in this case, the mechanism of action is linked to the activation of the antinociceptor system. The gas injection appears also effective in alleviating osteoarthrosis and several other joint–tendinitis affections. 12) Chronic fatigue syndrome and fibromyalgia: AHT has been found beneficial in the majority of patients (11). 13) Dentistry and stomatology: ozone has been found very useful for treating primary root carious lesions (57). Moreover, local application of ozonated oil in aphthous ulcers (cold sores) occurring on the tongue, lips and cheeks of many people allows an extremely rapid healing and disappearance of pain (11). 14) Emergency situations such as those occurring after extensive trauma, burns, acute peritonitis and toxic sepsis often lead to multiple organ failure and death. The combination of the best orthodox therapy with three to four daily mild ozonated major AHT can prevent or reduce the worsening of the metabolic impairments and reduce mortality. Moreover, patients waiting for organ (particularly heart) transplantation may improve resistance to infections and immunesuppression (due to anesthesia and surgery) if they could undergo six to eight major and minor AHTs presumably during 6–15 days before surgery. During heart transplantation, organs such as the brain and kidneys may be damaged by the ischemia reperfusion syndrome that can be attenuated by previous adaptation to oxidative stress. A similar concept could be adopted for scheduled complex operation or application of joint implants. This sort of prophylactic ozone therapy, with little effort and expense, may reduce the risk of infections, shorten the hospitalization and save money. However, the implementation of the prophylactic ozone therapy remains a dream in so far as World Health Authorities remain aloof and entangled in economic and political problems.

Hyperbaric Oxygen Therapy (HOT) and Ozone Therapy

It appears relevant to briefly clarify the validity and scopes of these two different approaches. In the hyperbaric chamber, the breathing of pure oxygen at 2.6 atmospheres greatly increases the solubilization of oxygen in the plasma (about 5 mL/dL) so that the dissolved oxygen is sufficient to satisfy the cellular requirements even in ischemic tissues. That is the reason why patients with chronic limb ischemia, or with diabetic foot, or ARMD often undergo HOT. Unfortunately, this is only a palliative treatment because, after 2 h, as soon as the patient comes out of the chamber, hypoxia resumes in the ischemic areas and the therapeutic effect is minimal and temporary. On the other hand, during ozone therapy, while the hyperoxygenation of the reinfused blood has a negligible relevance, the ozone triggers a series of biological mechanisms that lead to normalizing the delivery of oxygen for several days with consequent therapeutic effects. Two excellent reviews (58–59) clarify the exclusive role of HOT in air embolism, decompression sickness, CO-poisoning and clostridial myonecrosis but, regrettably, do not examine the relevance of ozone therapy. Indeed, they objectively report that HOT may be useful in chronic limb, heart and cerebral ischemia, autoimmune colitis, sickle cell anemia, chronic osteomyelitis, ARMD, diabetic foot, thermal burns, extensive chronic ulcers and bed sores, but the actual evidence is flawed and anecdotal. All of these latter conditions can instead greatly benefit by the use of parenteral (and when necessary topical) ozone therapy because the multiple mechanisms of action of ozone can correct pathologies linked to ischemia, infections, delayed healing and chronic oxidative stress (reviewed in Reference 11). In conclusion, both HOT and ozone therapy are important, but it is necessary to understand that their respective field of application is different and each approach must be used profitably only in selected pathologies.

Conclusions and Perspectives

I often ask myself if ozone therapy is obsolete or worthwhile being pursued. Our many treated patients answer for me and they loudly say that it is very beneficial. The compliance is excellent and the patients, as soon as the therapeutic effect declines, ask for a new cycle. This is an excellent proof that provided we are using judicious ozone concentrations, there is neither acute nor chronic toxicity. It has been unfortunate that, in the past, the direct intravenous injection of the gas, now prohibited, and misuse of ozone by incompetent quacks has generated the dogma that ozone is toxic and should not be used in medicine. This concept is wrong and has also been based first on non-physiological studies (60) performed in washed erythrocytes, hence unprotected by the plasma antioxidants and second, in not recognizing the profound difference between the endogenous chronic oxidative stress, occurring every day during a lifetime or during a chronic disease, and the calculated, extremely brief and exogenous oxidative stress that we induce on blood by using a precise and small ozone dose. We know that any drug, depending upon its dosage, can be either therapeutic or toxic. The following elementary observation is even more compelling: the normal glucose concentration in the plasma ranges between 0.7 and 1 mg/mL and is essential for survival. However, when this concentration falls below 0.4 mg/mL, the consequent hypoglycemic coma can be deadly. On the other hand, if the glucose concentration remains constantly above 1.3 mg/mL, it induces the metabolic syndrome, as is well exemplified by the current diabetic epidemic. Thus, the dogma about ozone toxicity is futile because, after millions of treatments, we have never observed any acute or chronic toxicity. Moreover, most of the patients report a feeling of wellness.

Needless to say, ozone therapy does not ‘‘cure’’ ARMD or other chronic pathologies but, by performing the maintenance therapy, it does improve the condition and maintain a good quality of life. On the other hand, even orthodox medicine, with the exception of several infectious diseases thanks to antibiotics, antivirals, antibodies and vaccines and far less frequently of cancer thanks to surgery/chemotherapy, is unable to "cure" most human diseases such as atherosclerosis, advanced cancer, diabetes, degenerative, metabolic and autoimmune diseases.

We are certainly not blinded by ozone therapy but the great strides of molecular biology and gene therapy during the last decade have not yet been paralleled by comparable advances in therapeutic innovations and many unforeseen difficulties still have to be overcome (61). I do not want to diminish scientific achievements but simply to point out that we are often unable to predict the pitfalls when new treatments are applied from mice to patients. This is probably one reason for the worldwide boom of complementary medicine, not only in underdeveloped countries but also in the U.S. Patients, as human beings, are often disappointed by the high-tech therapist. Moreover, conventional therapy often has side effects, and about 55,000 Americans may have died as a result of taking the now infamous Vioxx (62,63).

Ozone therapy is capturing increasing attention all over the world, since our studies reported in two books (10,11) have clarified the main biochemical mechanisms of action and the real possibility of taming ozone toxicity. We now have the first comprehensive framework for understanding and recommending ozone therapy in a few diseases as a first choice and in combination with orthodox therapy in many others. Indeed, one important characteristic of ozone therapy is that it can be experimentally verified both at the biochemical and clinical levels.

So far, the most advanced and reliable approach has been the major ozonated AHT but today we also have other technical possibilities and we can select the optimal method for different pathologies. As far as chronic diseases are

concerned, the problem is that official medicine tends to treat symptoms rather than the cause(s) of the disease. Besides the fact that the etiology is too complex or remains obscure, the treatment is often limited and remains unsatisfactory. On the other hand, a simple gaseous molecule like ozone, that probably is even produced *in vivo* (64), by acting on many targets, at least in part can recover functional activities that have gone astray. We have good reasons to believe that the therapeutic power of ozone therapy consists of simultaneously improving circulation and oxygen delivery, in enhancing the release of autacoids, growth factors and cytokines and in reducing the endogenous, chronic oxidative stress. In other words, ozone therapy seems to act as a biological response modifier.

Finally, I cannot omit mentioning some drawbacks. Although the cost of ozone is very low, it represents an impractical drug because it is unstable and cannot be stored in any form. However, by using a portable ozone generator we can perform domiciliary AHT treatments, useful for the elderly and for those patients with chronic diseases. Moreover, rectal insufflation of gas can be easily done by the patient at home, under the ozone therapist's supervision. Topical therapy of chronic ulcers and infectious wounds with ozonated oil is very practical and easy because we have standard and stable preparations. The last, but certainly not the least, problem is the lack of financial support for performing controlled and randomized clinical trials, whose results are critical and urgently needed to prove the validity and atoxicity of ozone therapy in various diseases. Objective results from clinical studies represent the unique possibility of convincing the biased opponents of this approach. The private ozone therapist, or even the small existing national associations, in comparison to the pharmaceutical industries that can register an annual profit of 340 billion dollars, have no financial power and how can an ant compete with an elephant? Really, we do not want to compete with official medicine, but only help patients to regain health.

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References

1. Wolff HH. Die behandlung peripherer durchblutungsstorungen mit ozon. Erfahr Hk 1974;23:181–184.
2. Bocci V, Paulesu L. Studies on the biological effects of ozone: 1. Induction of interferon gamma on human leucocytes. Haematologica 1990;75:510–515.
3. Bocci V, Luzzi E, Corradeschi F, Paulesu L, Di Stefano A. Studies on the biological effects of ozone: 3. An attempt to define conditions for optimal induction of cytokines. Lymphokine Cytokine Res 1993;12: 121–126.
4. Bocci V, Luzzi E, Corradeschi F, Paulesu L, Rossi R, Cardaioli E, et al. Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. J Biol Regulat Homeost Agent 1993;7:133–138.
5. Bocci V, Luzzi E, Corradeschi F. 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.
6. Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, et al. 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 1998;12:67–75.
7. 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. Mediat Inflamm 1998;7:313–317.
8. Bocci V, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci AM, et al. Studies on the biological effects of ozone: 9. Effects of ozone on human platelets. Platelets 1999;10:110–116.
9. Bocci V. Ossigeno-ozono terapia, Comprensione dei meccanismi di azione. Milano: Casa Editrice Ambrosiana;2000.
10. Bocci V. Oxygen-Ozone Therapy. A critical evaluation. Dordrecht, The Netherlands: Kluwer Academic Publishers;2002.
11. Bocci V. OZONE. A New Medical Drug. Dordrecht, The Netherlands: Springer;2005.
12. Pryor WA, Squadrito GL, Friedman M. The cascade mechanism to explain ozone toxicity: the role of lipid ozonation products. Free Radic Biol Med 1995;19:935–941.
13. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS Lett 2000;486:10–13.
14. Bocci V, Aldinucci C, Bianchi L. The use of hydrogen peroxide as a medical drug. Riv Ital Ossigeno Ozonoterapia 2005;4:30–39.
15. Valacchi G, Bocci V. Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. Mediat Inflamm 2000;9:271–276.
16. Dianzani MU. 4-Hydroxynonenal and cell signalling. Free Radic Res 1998;28:553–560.
17. Iles KE, Liu R-M. Mechanisms of glutamate cysteine ligase (GCL) induction by 4-hydroxynonenal. Free Radic Biol Med 2005;38: 547–556.
18. Snyder SH, Baranano DE. Heme oxygenase: a font of multiple messengers. Neuropsychopharmacology 2001;5:294–298.
19. Wollert KC, Drexler H. Clinical application of stem cells for the heart. Circ Res 2005;96:151–163.
20. Mendiratta S, Qu Z-C, May JM. Erythrocyte ascorbate recycling: antioxidant effects in blood. Free Radic Biol Med 1998;24:789–797.
21. Stone JR, Collins T. The role of hydrogen peroxide in endothelial proliferative responses. Endothelium 2002;9:231–238.
22. Bocci V. A reasonable approach for the treatment of HIV infection in the early phase with ozone therapy (autohemotherapy). How inflammatory cytokines may have a therapeutic role. Mediat Inflamm 1994; 3:315–321.
23. Bocci V. Autohaemotherapy after treatment of blood with ozone. A reappraisal. J Intern Med Res 1994;22:131–144.
24. Baeuerle PA, Henkel T. Function and activation of NF-kB in the immune system. Annu Rev Immunol 1994;12:141–179.
25. Bocci V. Roles of interferon produced in physiological conditions. A speculative review. Immunology 1988;64:1–9.
26. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10: Release of factors from ozonated human platelets. Mediat Inflamm 1999;8:205–209.
27. Frehm EJ, Bonaventura J, Gow AJ. S-nitrosohemoglobin: an allosteric mediator of NO group function in mammalian vasculature. Free Radic Biol Med 2004;37:442–453.
28. Goldman M. Cancer risk of low-level exposure. Science 1996;271: 1821–1822.

29. Calabrese EJ, Baldwin LA. Hormesis: U-shaped dose responses and their centrality in toxicology. Trends Pharmacol Sci 2001;22:285–291.
30. Bocci V. Does ozone therapy normalize the cellular redox balance? Med Hypotheses 1996;46:150–154.
31. Sharma YK, Davis KR. The effects of ozone on antioxidant responses in plants. Free Radic Biol Med 1997;23:480–488.
32. Leon OS, Menendez S, Merino N, Castillo R, Sam S, Perez, et al. Ozone oxidative preconditioning: a protection against cellular damage by free radicals. Med Inflamm 1998;7:289–294.
33. Barber E, Menendez S, Leon OS, Barber MO, Merino N, Calunga JL, et al. Prevention of renal injury after induction of ozone tolerance in rats submitted to warm ischaemia. Mediat Inflamm 1999;8:37–41.
34. Larini A, Bianchi L, Bocci V. The ozone tolerance: I. Enhancement of antioxidant enzymes is ozone dose-dependent in Jurkat cells. Free Radic Res 2003;37:1163–1168.
35. Bocci V, Borrelli E, Valacchi G, Luzzi E. Quasi-total body exposure to an oxygen-ozone mixture in a sauna cabin. Eur J Appl Physiol 1999; 80:549–554.
36. Bocci V, Di Paolo N. Oxygenation-ozonation of blood during extracorporeal circulation (EBOO). III. A new medical approach, ozone. Science 2004;26:195–205.
37. Garber GE, Cameron DW, Hawley-Foss N, Greenway D, Shannon ME. The use of ozone-treated blood in the therapy of HIV infection and immune disease: a pilot study of safety and efficacy. AIDS 1991;5:981–984.
38. Cooke ED, Pockley AG, Tucker AT, Kirby JDT, Bolton AE. Treatment of severe Raynaud's syndrome by injection of autologous blood pretreated by heating, ozonation and exposure to ultraviolet light (H-O-U) therapy. Int Angiol 1997;16:250–254.
39. Torre-Amione G, Sestier F, Radovancevic B, Young J. Effects of a novel immune modulation therapy in patients with advanced chronic heart failure. J Am Coll Cardiol 2004;44:1181–1186.
40. Fadok VA, Bratton DL, Konowal A, Freed PW, Westcott JY, Henson PM. Macrophages that have ingested apoptic cells in vitro inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF-β, PGE 2 and PAF. J Clin Invest 1998;101:890–898.
41. Tremblay J, Chen H, Peng J, Kunes J, Vu MD, Sarkissian SD, et al. Renal ischemia-reperfusion injury in the rat is prevented by a novel immune modulation therapy. Transplantation 2002;74:1425–1433.
42. Tylicki L, Niew GT, Biedunkiewicz B, Burakowski S, Rutkowski B. Beneficial clinical effects of ozonated autohemotherapy in chronically dialysed patients with atherosclerotic ischemia of the lower limbs. Int J Artif Organs 2001;24:79–82.
43. Clavo B, Perez JL, Lopez L, Suarez G, Lloret M, Rodriguez V, et al. Effect of ozone therapy on muscle oxygenation. J Altern Compl Med 2003;9:251–256.
44. Biedunkiewicz B, Tylicki L, Niewegloski T, Burakoski 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.
45. De Monte A, van der Zee H, Bocci V. Major ozonated autohemotherapy in chronic limb ischemia with ulcerations. J Alt Compl Med 2005; 11:363–367.
46. Chan WM, Lam DS, Wong TH, Lai TY, Kwok AK, Tam BS, et al. Photodynamic therapy with verteporfin for subfoveal idiopathic choroidal neovascularization: one-year results from a prospective case series. Ophthalmology 2003;110:2395–2402.
47. The AMDRT Research Group. The age-related macular degeneration radiotherapy trial (AMDRT): one year results from a pilot study. Am J Ophthalmol 2004;138:818–828.
48. Gragoudas ES, Adamis AP, Cunningham ET, Feinsod M, Guyer DR. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 2004;351:2805–2816.
49. Oxman MN, Levin MJ, Johnson GR, Schmader KE, Straus SE, Gelb LD, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. N Engl J Med 2005;352:2271–2284.
50. Barnes PJ. Scientific rationale for inhaled combination therapy with long-acting β2-agonists and corticosteroids. Eur Respir J 2002;19:182–191.
51. Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. Immunol Today 1995;16:128–130.
52. Stamler JS. S-nitrosothiols in the blood: roles, amounts and method of analysis. Circ Res 2004;94:414–417.
53. Lipsky BA. Medical treatment of diabetic foot infections. Clin Infect Dis 2004;39:S104–S114.
54. Bocci V, Larini A, Micheli V. Restoration of normoxia by ozone therapy control neoplastic growth: a review and a working hypothesis. J Alt Compl Med 2005;11:257–265.
55. Gonzalez R, Borrego A, Zamora Z, Romay C, Hernandez F, Menendez S, et al. Reversion by ozone treatment of acute nephrotoxicity induced by cisplatin in rats. Med Inflamm 2004;13:307–312.
56. Andreula CF, Simonetti L, De Santis F, Agati R, Ricci R, Leonardi M. Minimally invasive oxygen-ozone therapy for lumbar disk herniation. Am J Neuroradiol 2003;24:996–1000.
57. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone-generating device on micro-organisms associated with primary root carious lesions in vitro. Caries Res 2000;34:498–501.
58. Cianci P. Advances in the treatment of the diabetic foot: is there a role for adjunctive hyperbaric oxygen therapy? Wound Repair Regen 2004; 12:2–10.
59. Gill AL, Bell CN. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. Q J Med 2004;97:385–395.
60. Goldstein BD, Balchum OJ. Effect of ozone on lipid peroxidation in the red blood cell. Proc Soc Exp Biol Med 1967;126:356–359.
61. Couzin J, Kaiser J. As Gelsinger case ends, gene therapy suffers another blow. Science 2005;307:1028.
62. Maxwell SRJ, Webb DJ. COX-2 selective inhibitors-important lessons learned. Lancet 2005;365:449–451.
63. Beardsley S. Avoiding another VIOXX. Sci Am 2005;292:9–10.
64. Babior BM, Takeuchi C, Ruedi J, Gutierrez A, Wentworth P Jr. Investigating antibody-catalyzed ozone generation by human neutrophils. Proc Natl Acad Sci USA 2003;100:3031–3034.

Short Communication

Is it true that ozone is always toxic? The end of a dogma

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Keywords: ozone, lungs, antioxidants, acute oxidative stress; 4-hydroxynonenal,ozonetherapy

Abstract

There are a number of good experimental studies showing that exposure by inhalation to prolonged tropospheric ozone damages the respiratory system and extrapulmonary organs. The skin, if extensively exposed, may also contribute to the damage. The undoubtful strong reactivity of ozone has contributed to establish the dogma that ozone is always toxic and its medical application must be proscribed. Although it is less known, judiciously practiced ozonetherapy is becoming very useful either on its own or applied in combination with orthodox medicine in a broad range of pathologies. The opponents of ozonetherapy base their judgment on the ozone chemistry, and physicians, without any knowledge of the problem, are often skeptical. During the last 15 years, a clear understanding of the action of ozone in biology and medicine has been gained, allowing today to argue if it is true that ozone is always toxic. The fundamental points that are discussed in this paper are: the topography, anatomical and biochemical characteristics of the organs daily exposed to ozone versus the potent antioxidant capacity of blood exposed to a small and precisely calculated dose of ozone only for a few minutes. It is becoming clear how the respiratory system undergoing a chronic oxidative stress can release slowly, but steadily, a huge amount of toxic compounds able to enter the circulation and cause serious damage. The aim of this paper is to objectively evaluate this controversial issue.

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