Chapter 5

General Aspects of Ozone Therapy

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Additional information is available at the end of the chapter

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1. Introduction

Ozone is a natural gas consisting of three oxygen atoms that has a distinctive odor. It is intrinsically hazardous over tolerable doses for living organisms. Ozone therapy is a medical therapy that a mixture of oxygen and ozone which is called medical ozone is used as a medical drug, more correctly pro-drug. Medical ozone contains less than 5% of ozone at maximum concentration where rest of it is pure medical oxygen.

The unbelievable versatility of ozone therapy is due to the cascade of ozone-derived compounds able to act on several targets leading to a multifactorial correction of various pathological states. Ozone therapy can improve well-being and delay the negative effects of aging. Aging process basicly related with oxidants and anti-oxidants balance, Advanced Glycoslation End Substances (AGEs), role of genes and immune system, relevance of telomeres and telomerase, hormones, nutrition, environmental factors and some other factors.

2. What is ozone therapy?

Ozone therapy is a general termination of a medical therapy that medical ozone gas is used as drug by several methods. Some of these methods are systemic where many others are local applications. Ozonated autohaemotherapy (O3-AHT) widely known by people firstly described by Wehrli and Steinbart and since 1954 it has been used in millions of patients in different pathologies with apparent clinical benefit. AHT might be applied in two forms, Major AHT simply driving 100-150 ml of venous blood into a sterile bottle made of neutral glass or other ozone resistant material where blood and medical ozone is mixed in therapeutic doses and then reinfused back to the donor without side effects. 3.13 % Natrium Citrate solution is used as an anticoagulant during the procedure with short lasting effect. In some patients Heparin might be used instead of Natrium Citrate depending on the patient's case.

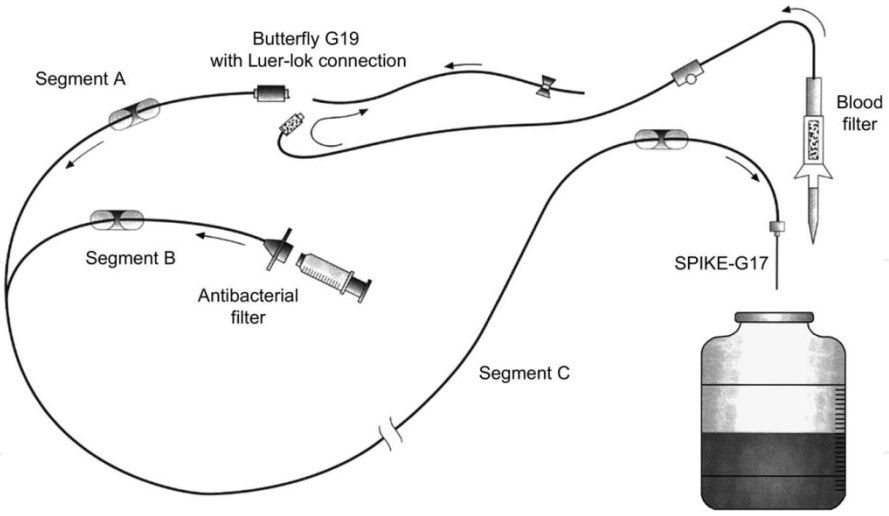

Figure 1. Schematic drawing of the components necessary to perform major autohematherapy

Minor AHT is very similar to major AHT method with a few differences, where 5-10 ml of blood is mixed with precise dose of medical ozone in a syringe and reinjected by intramuscular route to the donor that no anticoagulant is used. Rectal insufflation (RI) of medical ozone gas is another method of systemic ozone therapy that is applied on some cases if others methods cannot be done or this method is preferred over others due to diseases.

Ozone is normally present as 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.

$$3O_2 + 68,400\,\text{cal} \rightarrow 2O_3 \quad (1)$$

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_5 12.076 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

2.1. 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 temperature, pressure and ozone concentration. Only in this situation ozone does not react and in a tightly closed glass bottle, the ozonated water is useful as a disinfectant that 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)

$$O_3 + \text{biomolecules} \rightarrow O_2 + O, \quad (2)$$

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:

$$R-CH=CH-R + O_3 + H_2O \rightarrow R-CH=O + R-CH=O + H_2O_2 \quad (3)$$

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, (hydroxyl radical).

It is determined [15] that the formation of nitrogen monoxide (NO,) 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 mg/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,), alkoxyl radicals (LO,), 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++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 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.

2.2. 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 dosedependent 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]. H2O2 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 H2O2 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, H2O2 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. It is determined that 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 it was 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 kB [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, it was 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], it is postulated that LOPs, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stres [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 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 celldegeneration.

3. A concise summary of biological effects observed after ozone therapy

3.1. Erythrocytes

These cells respond with an activation of glycolysis due to activation of the pentose hosphate pathway. It is found that increased adenosine triphosphate levels (from 13899/260 to 19689/232 mM) in patients with age-related macular degeneration (ARMD) (atrophic form) after a therapeutic cycle (14 sessions) of O3-AHT.26 Moreover, Viebahn [27] reported the same effect in athletes and elderly patients after rectal insufflation of O2_/O3. Ozonation implies a small but consistent oxidation of GSH to glutathione disulfide, and GSH reductase utilizes the reduced form of the coenzyme nicotinamide adenine dinucleotide phosphate supplied by G6PDH to reduce glutathione disulfide to GSH, which indeed returns rapidly to the original level. [28] The increase of 2,3-diphosphoglycerate varies depending on the basic level in ARMD patients and only those who had a low level showed a marked increase with therapy. Viebahn, [27] after a longer cycle of therapy in elderly people, observed a significant increase. An increase of 2,3-diphosphoglycerate level in oxyhemoglobin shifts to the right (p50 value increases); its dissociation curve implies an increased delivery of O2 into the hypoxic tissues. The life-span of ozonated 99Tc-labeled erythrocytes and their uptake by liver and spleen are comparable with oxygenated erythrocytes.(3)

A problem still under study regards the generation of biochemically improved erythrocytes during prolonged ozone therapy. While ROS have an extremely short life, LOPs, during the reinfusion of ozonated blood, return into the donor's circulation. While they are fairly stable in vitro, they rapidly disappear from blood in vivo owing to considerable dilution into body fluids, degradation by aldehyde dehydrogenases, excretion into bile and urine, and uptake in various organs including bone marrow cells. This process is crucial for explaining the mechanism of ozone tolerance: during erythrogenesis, submicromolar LOP concentrations can upregulate the synthesis of antioxidant enzymes and indeed, after appropriate density gradient separation, it is found that young (lighter) erythrocytes contain more G6PDH than older (heavier) cells generated before the therapy. [25] This result suggests that ozone therapy enhances the generation of erythrocytes with improved metabolic characteristics, a sort of 'supergifted erythrocytes' able to correct hypoxia in vascular diseases.

3.2. Leukocytes

These were the cells that were examined first as it is hypothesized that ozone could act as an IFN-g inducer.[29] Since then it is shown [30,31] that ozone behaves as a weak (compared with mitogens) cytokine (such as tumor necrosis factor-a, interleukin-2, interleukin-6, interleukin-8, transforming growth factor-b [TGF-b]) inducer. Several studies [32 - 35] have confirmed that ozone can stimulate bronchoalveolar cells to release proinflammatory cytokines and eicosanoids. Thanks to parallel progress in understanding the role of antioxidants and redox

regulation of gene transcription, it has been clarified that, among several signals, H2O2 is one of the most significant cytokine inducers.[36] As already mentioned, after ozonation H2O2 freely diffuses into the leukocyte cytoplasm and activates specific protein kinases that, by phosphorylating IkB bound to the nuclear factor-kB allows the migration of the transcription heterodimer p50_/p65 into the nucleus where it activates gene expression.[37] Obviously H2O2 must reach a concentration able to activate the kinase without being instantaneously reduced by intracellular antioxidants. [38] Therefore the relevance of the response depends on the levels of H2O2, which can act as either 'life or death' signals. The fact that ozone can either be a toxic or a useful signal depends on the minimal antioxidant capacity of the respiratory tract lining fluid, whereas blood has a very potent capacity. The data in fact indicate that too little ozone (hence H2O2) is ineffective and too much (or too little antioxidants) can be toxic. During recent years it is addressed the following questions: first, as ozone acts as a mild cytokine inducer, does reinfusion of ozonated blood modify the plasma cytokine level in vivo? Second, does the induction of oxidative stress proteins, particularly of heme-oxygenase I (HO-I), and of adaptation to the therapeutic oxidative stress have an immunomodulatory effect? And third, can we devise an optimal schedule for improving the immune reactivity in immunodepressed patients?

The classical O3-AHT, usually consisting of 225 ml of blood (plus 25 ml of 3.8% sodium citrate solution) treated with 225 ml of gas (O2_/O3) with ozone concentrations ranging from an initial 20 mg/ml slowly scaled up to 40_/50 mg/ml per ml of blood, continued for several months, twice weekly, is ideal for this purpose. A probable explanation is that, after each blood reinfusion, a small percentage of immune cells are activated and home in several organs: these cells release into the microenvironment cytokines that, in turn, prime or activate neighboring cells thus slowly reinforcing immune responses. Modifications of cytokine plasma levels are hardly detectable so that side effects like the flu-like syndrome, typically observed after administration of immunoadjuvants, are absent [39] and actually most of patients report a sense of well-being during the therapy. There is a wealth of experimental data [40 - 44] showing that both animals and plants can develop ozone tolerance by upregulating the expression of antioxidants, which can correct a chronic imbalance between excessive endogenous oxidation due to viral infections, cancer, chronic inflammations and depressed antioxidants. Both chronic hepatitis C virus and cancer patients have shown a marked improvement of their clinical conditions after several months of O3-AHT treatments, suggesting that this 'calculated and brief oxidative stress' truly merits the term 'therapeutic shock'.

3.3. Platelets

It is known that ROS can induce platelet activation and it was obvious to assume that blood ozonation, by generating H2O2, could cause it. [45 – 46] Moreover H2O2 or other ROS can activate phospholipase C, phospholipase A2, cyclo-oxygenases and lipo-oxygenases and thromboxane synthetase, allowing a step increase of intracellular Ca2_, release of prostaglandin E2, prostaglandin F2a and thromboxane A2 with irreversible platelet aggregation. For these reasons it is studied the behavior of either human platelet rich-plasma anticoagulated with heparin or citrate, either untreated or simply oxygenated, or ozonated at three concen-

trations (20, 40 and 80 mg/ml). Because the plasmatic Ca2_ level potentiates the ozone effect, we were not surprised to observe a rapid platelet aggregation in heparinized plasma particularly at the highest concentration. [47] Consequently the release of several growth factors like platelet-derived growth factor AB, TGF-b1, interleukin-8 and thromboxane_/2 were significantly higher from heparinized platelets than Ca2_-free platelets. [48] These results taught us that it is better to chelate Ca2_ for performing a safe autohemotherapy. Nonetheless the release of growth factors from Ca2_-free platelets is still important because the reinfusion of ozonated blood implies an elevation of plasma levels of TGF-b that may explain why the healing of necrotic ulcers in hind limb ischemia due to atherosclerosis and diabetes markedly quickens during treatment with both parenteral (O3-AHT) and topical treatments with ozonated water and oil. [49 – 50]

3.4. Endothelial cells and the vascular system

During the reinfusion of ozonated blood, the endothelium comes in contact with traces of LOPs that soon disappear in vivo. It is [51] investigated the effect of addition of ozonated (40 and 80 mg/ml) human plasma to human endothelial cells in culture and it is measured a significant increase of the critical relaxing factor NO+that was ozone dose dependent.

The induction of nitric oxide synthase and the release of NO+was reinforced in the presence of 20 mm of arginine and was abolished by the addition of 20 mM of L-N-omega-nitro-L-arginine methyl ester. In physiological conditions the endothelium regulates the vascular tone [52] by producing some 1_/10 mM of NO+and 1 nM of anion superoxide (one of the contracting factors). The intravascular half-life of NO+is about 2 msec with a strictly localized consumption so that the likelihood of improving vasodilation in remote ischemic areas (the macula or the limbs) seems negligible. However, NO+readily reacts with GSH, cysteine, albumin and hemoglobin (cysteine residue b 93) and the formed S-nitrosothiols and S-nitrosohemoglobin have half-lives of 5_/50 min, allowing a pharmacological effect at distant sites. [53 – 54] It remains to be ascertained whether ozonated blood enhances the release of prostacyclin (PGI2) and angiopoietins, both important factors for improving ischemic vasculopathies.

3.5. Parenchymal cells in other organs

Upon reinfusion of ozonated blood, LOPs can reach other organs such as the hypothalamus, endocrine glands, liver, kidneys and bone marrow. The phenomenon of adaptation to the repeated and acute oxidative stress imposed by O3-AHT is most interesting and able to elicit crucial therapeutic responses. During prolonged treatment, cells throughout the body receive small and gradual pulses of LOPs that are responsible for:

1. Neuro-endocrine responses explaining the reported feeling of wellness, [55]
2. The upregulation of antioxidant enzymes in several cell types that is an excellent way to re-equilibrate the oxidant_/antioxidant unbalance
3. Inducing a number of stress or heat shock proteins (HSPs) such as HSP27, HO-1 (HSP 32), HSP72 and HSP [90 – 56 – 59]

It is observed that HO-1 is a protective enzyme allowing the formation of Fe2_, bilirubin (an antioxidant) and carbon monoxide (CO), a vasodilator that, like NO+, increases the level of cyclic guanosine monophosphate, the reaction catalyzed by guanylate cyclase. Besides gases produced by the gut flora, it is truly remarkable that cells can release other gaseous molecules (NO+, CO and CO2), and it is even more surprising that even ozone can be produced by activated antibody-coated neutrophils. [60] These gases can now be considered as molecules able to deliver crucial physiological and pharmacological effects.

Excessive amounts of these molecules are toxic, causing serious pathological events and possibly death. Nature teaches us that these gases, depending on their concentrations, can be either friends or foes and similarly ozone therapy can be either useful or toxic. If this reasoning is correct, ozone therapy, when judiciously performed, is a simple, inexpensive and atoxic approach with the advantage of activating several biomechanisms in different cells unusually leading to an integrated and often incredible response.

4. The extreme versatility of ozone therapy

The sarcastic comment of the opponents is that ozone therapy looks like a panacea for all diseases. Indeed it seems so, but in reality this is due to the multitude of compounds originated at first from the reaction of ozone with body fluids, and eventually able to display pleiotropic effects delivered by different organs. For the sake of brevity we can only summarize the therapeutic effects so far reported

Specialization	Pathology
Dermatology	Herpes Zoster and simplex, acne, eczema, lipodystrophy (cellulite), mycosis, psoriasis, atopic dermatitis
Internal Medicine	Hepatitis, diabetes, atherosclerosis, arterial hypertension, osteoarthritis, asthma, chronic bronchitis, gastritis, gastric ulcer, Crohn's disease, chronic constipation, hypothyroidism.
Nephrology / Dialysis	Adjuvant in the treatment of ischemic-metabolic pathologies.
Neurology	Migraines, depression, vasomotor cephalea, neuro-vascular disorders.
Dentistry	Treatment of cavities, disinfection of cavities during surgery and post-operatory period. Periodontitis, aphthas.
Orthopedic Rheumatology	Disc-radicular conflicts, disc herniation, articular rheumatism, lumbago, osteoarthritis, arthropathy, periarthritis, rheumatoid arthritis.
Angiology	Venous insufficiency, diabetic ulcer, arthropathy, coronaropathy, gangrene, postphlebitic ulcer, peripheral vasculopathy.
Gynecology	Bacterial infections by protozoa or mycosis, Bartholin's cyst, vaginitis, menopause, chronic pelvic inflammation, infertility.
Immunology	Immuno-modulator, autoimmune disorders, adjuvant in treatments with radiation and in immunodeficiency.

Table 1. Indiciations of Ozone Therapy in General

4.1. Acute and chronic bacterial, viral and fungine infections

Intuitively, ozone therapy is very useful in both acute and chronic bacterial, viral and fungine infections because the generated ROS are the natural and most effective agents to which even antibiotic resistant pathogens do not resist. [3-61] Moreover, improvement of metabolism and immunological functions contribute to a favorable outcome. Abscesses, anal fissures, fistulae, bed sores, furunculosis, inveterate osteomyelitis, vulvovaginitis, necrotizing fasciitis and torpid ulcers of various origin have been shown to improve rapidly, particularly using the combination of O3-AHT with topical treatment using either direct O2_/O3 exposure or the cleansing and stimulating effect of ozonated water and oil. The activity of ozonated solutions in eliminating the infectivity and enhancing healing is almost unbelievable. However, in Western countries accustomed to the use of antibiotic creams (often with corticosteroids) there is no mental attitude to profitably use the inexpensive and most active ozonated oil. [62]

4.2. Ischemic diseases

Chronic limb ischemia (atherosclerosis, diabetes, Burger's disease) is most effectively treated at stage II-b with complete disappearance of pain and claudication. Moreover, since 1981, Rokitansky et al.[49] demonstrated that a cycle of O3-AHT (usually 14 treatments) led to a very good improvement in 70.6% and 53.8% of either stage III or stage IV (Fontaine) patients, respectively. Amputation of toes and limbs could be avoided in pre-terminal phases.

These results have been amply confirmed by Giunta et al.,[63] Mattassi et al. [64] and Tylicki et al.[65] Preterminal patients with chronic heart ischemia and no further susceptibility to conventional treatments have shown marked improvement after a cycle of 14 treatments of extracorporeal circulation of blood against O2_/O3.[66] A randomized controlled study is in progress for establishing the validity of this more invasive method than classical O3-AHT.

4.3. Age-related macular degeneration

A 6-year study in 90 patients with the 'dry' form of ARMD has been carried out performing a cycle of 13_/14 O3-AHT treatments. Mean distance best-corrected visual acuity was significantly improved in the treatment group of patients while in the control group, first treated with oxygenated autohaemotherapy, only a modest and not significant improvement in mean distance visual acuity was observed. No adverse effects have been noted and the patient's compliance has been excellent. [26] Owing to the constant increase of ARMD patients and the lack of an effective conventional treatment, this approach appears mandatory.

4.4. Orthopedic diseases

Until recently it was unthinkable that a mixture of O2_/O3 could be useful in orthopedics. Indeed lumbar disk herniation and osteoarthritis, although having different etiologies, have a common inflammatory background expressed by a localized chronic oxidative stress due to excessive production of ROS, release of proinflammatory cytokines and activation of cyclo-xygenases. Common sense would proscribe the use of ozone, a master generator of free radicals and, as it is well shown, [34 – 67] after pulmonary exposure, a superb inflammatory agent.

Contrary to all expectations, it is now well demonstrated [68] that combined intradiscal and periganglionic injection of medical ozone allows an excellent outcome in 70.3% of patients treated for disk herniation performed after conservative management failed to respond. In the same vein, it appears very surprising that the application of medical ozone in acute and chronic painful diseases of the joints allows rapid pain relief, disappearance of inflammation and improvement of mobility. Thousands of patients have been successfully treated and the lack of side effects is noteworthy. [69] These positive empiric observations need to be explained. Ozone is indeed a surprising gas that paradoxically, after prolonged administration at low concentrations, induces tolerance, a phenomenon termed 'hormesis' by Goldman [70] to indicate 'a beneficial effect of a low level exposure to an agent that is harmful at high levels'. Thus, at this stage, we use the definition of 'ozone paradox' for explaining these excellent therapeutic results. Immediately after O2_/O3 administration in the nucleus pulposus or into inflamed endoarticular cavities; a sort of oxidative shock seems to subvert all the traditional rules by inducing an antioxidative response due to several factors, among which is the cholinergic antiinflammatory pathway. [71] A detailed discussion is reported elsewhere. (3)

4.5. Dentistry

This is another medical specialty where ozone has been recently evaluated with exceedingly interesting results.[72] Primary root carious lesions are being treated with a novel ozone delivery system able to avoid any toxic risk for the mouth cavity and lungs. The tooth's lesion is exposed for 10_/20 sec to a sort of ozone 'hurricane' based on a gas flow of 615 ml/min of O2_/O3 at a low concentration (4 mg/ml), perfectly enclosed in a tightly fitting silicone cup enclosing the tooth. It is not surprising that all bacteria, particularly lactobacilli, are destroyed so that the ozone-sterilized dental surface becomes quickly remineralized, becoming hard and resistant to further bacterial attack. This new approach is simple, inexpensive and well tolerated, as opposed to the conventional and painful 'drilling and filling' management of primary root carious lesions. Dermatological, pulmonary, renal, hematological and neurodegenerative diseases Owing to the ability of ozone to activate a number of biological targets, ozone therapy could be proficiently used in some dermatological, pulmonary, renal, hematological and neurodegenerative diseases. However these pathologies so far have not been evaluated in a controlled fashion. Most of the patients with metastatic cancer resistant to radiotherapy and chemotherapy report a striking improvement of the quality of life with prolonged (twice weekly for months) O3-AHT treatments. [25 – 73] This is a constantly observed result, most probably due to a multifactorial neuroendocrine response.

5. Summary

Finally it must be emphasized that if ozone is judiciously used according to precisely defined guidelines, it causes neither acute, nor chronic side effects. After two decades of practical applications and the results observed in patients after conventional remedies have proved unsatisfactory. One has the feeling that, if ozone therapy could be accepted and used in all

hospitals, it would represent a small but important medical revolution able to cure or stabilize several diseases in many patients in both rich and poor countries.

6. Discussion and conclusions

There is no doubt that ozone can be toxic, and even today its hazardous employment by charlatans and unprepared physicians has contributed to a poor consideration of ozone therapy. That is one reason why the use of ozone is prohibited in some states of the USA and why this therapy is still regarded with skepticism by orthodox medicine even in Germany, where this approach was first conceived. Moreover, the following data tend to generalize that ozone is always toxic and should not be used in medicine:

1. Overwhelming evidence shows that the bronchial-pulmonary system is very sensitive to ozone and this gas should never be inhaled. [67]
2. This is very true because the respiratory tract lining fluid is constituted by a very thin, watery film containing a minimal amount of antioxidants that makes mucosal cells extremely vulnerable to oxidation. The opposite situation occurs for blood cells suspended in plasma, which has a potent antioxidant capacity (1.23_/1.83 mmol/l) able to tame any ozone dose within the therapeutic range (10_/80 mg/ml).
3. Saline-washed erythrocytes suspended in saline undergo extensive hemolysis after ozone exposure. [12]
4. Cells in culture, even if exposed to very low ozone concentrations for a long time, undergo apoptosis. [74]
5. One-hour exposure of saline-diluted blood to 5 mM of ozone induces genotoxic effects on leukocyte. [75]

But is ozone always toxic?

As a matter of fact millions of O3-AHT, even if performed in an empirical fashion during the past three decades, has neither yielded acute nor chronic toxic effects. According to Jacobs [76] this procedure has yielded the lowest number of medical complications.

However, four deaths have been recorded due to pulmonary embolism, which occurred during direct intravenous administration of O2_/O3, an application prohibited by the European Society of Ozonetherapy since 1983. Thus ozone seems like Janus and his two faces require an explanation. This is now reasonably clear. Since 1988 we have investigated the problem in a scientific way using precise ozone generators, which allow checking ozone concentration in real time by a photometer calibrated with the classical iodometric method. A review [61] and a critical book (3) have extensively clarified the issue but this does not seem sufficient to dispel the dogma that 'ozone is always toxic'. However, we now consider ozone as a real drug that must be used with caution after having carefully defined its therapeutic window. First, the ozone must be calibrated against the antioxidant capacity of the patient's blood in order to control the potential ozone toxicity

Second, expert scientists in free radicals ought to distinguish the chronic intracellular oxidative stress typical of several pathologies by the transitory (5 min) calculated oxidative stress occurring when a precise volume of blood is exposed ex vivo to an equal volume of gas (O2_/O3) with well-defined ozone concentrations ranging from 20 up to 40 mg/ml per ml of blood. It needs to be emphasized that the exogenous oxidative stress caused by ozone in blood is due to the fact that ozone, once dissolved in the plasmatic water, instantaneously reacts with biomolecules and disappears but generates ROS, among which are H2O2 and LOPs. These are the effective ozone messengers that interact with a variety of cells and elicit the now-termed 'therapeutic shock' due to the multiform biological responses. That ozone acts as a real chemical drug is proved by the fact that the ozone messengers, to be effective, must reach a threshold because otherwise there are no biological effects and the therapeutic results, if any, are due to a placebo effect. Although we have proven that ozone therapy is not a nebulous approach and has been shown amenable to a precise scientific scrutiny, it is probable that much still remains to be uncovered.

Everyone knows that plasma and blood cells contain an almost redundant antioxidant system made up of hydro-liposoluble compounds and antioxidant enzymes. During aging or pathologic conditions, this is not sufficient to correct the intracellular oxidative stress, but normally it is adequate to tame ozone toxicity while allowing the generation of ROS and LOPs. Thus all data emphasizing ozone toxicity can be easily dismissed because the following is now well proven:

1. Blood is a much more ozone-resistant 'tissue' than the respiratory tract that, for anatomic, biochemical and metabolic reasons, is always at a loss when exposed to ozone, and therefore it is wrong to extrapolate ozone toxicity for the pulmonary system to blood.
2. Washed and saline-resuspended erythrocytes, fully depleted of the plasmatic antioxidants, are obviously very sensitive to ozonation, and all of these unnatural data have neither physiological nor practical significance.
3. The same occurs for cells cultured in antioxidant-poor media and exposed continuously for days to ozone. Surprisingly, cell biologists reported only the ozone concentration but have neither calculated nor taken into account the cumulative dose of ozone that after a long exposure kills the cells.
4. The conclusion is that, although ozone is potentially mutagenic, so far all experimental data performed in physiological conditions and clinical evidence have neithershown any cell damage nor adverse effects in patients. As a matter of fact, blood is exposed to ozone concentrations (0.21_/1.68 mM) lower than the mutagenic ones (1.5_/5.6 mM). The question of whether ozone is genotoxic and mutagenic is a critical one and has been extensively discussed elsewhere. (3) What has never been entirely appreciated is the fact that we can only use an ozone dose that does not overwhelm the antioxidant capacity of blood.

Hopefully this discussion should put an end to the confusion between the endogenously constant oxidative stress due to the oxygen and the transitory and occasional therapeutic 'shock' due to precise blood ozonation. A point that should not be overlooked is that ozone

messengers, by acting on different cells, elicit a variety of biological effects that cannot ever be dreamed of with the usual reductionist approach of using a drug for a single target. This consideration can explain the far superior therapeutic effect of parenteral and topical ozone therapy in advanced cases of chronic limb ischemia to the conventional infusion of prostanoids. Another relevant characteristic is that the judicious strategy 'start low, go slow' in using ozone is able to induce in patients the adaptation to the chronic oxidative stress (i.e. ozone) paradoxically upregulates the antioxidant defenses. The scientific evaluation of ozone therapy efficacy remains the crucial point: results accrued during the past 20 years show that is very useful in chronic limb ischemia, ARMD, chronic infectious diseases and, most surprising, in orthopedics and even in dentistry after conventional medicine has failed to provide a real advantage. There are no adverse effects and most of the patients report a feeling of wellness. The efficacy remains uncertain in other pathologies such as neurodegenerative, autoimmune diseases and cancer because clinical experience is fragmentary and anecdotal.

However, orthodox medicine remains skeptical because controlled clinical trials are few and are not considered satisfactory. Unfortunately our good will is not sufficient to overcome prejudice and lack of sponsors. It is distressing to realize that a wrong dogma, commercial interests and the disinterest of Health Authorities delay the application of a medical approach that could help billions of patients, particularly in poor countries. Finally, this paper may serve the purpose of opening a fruitful discussion on the beneficial versus the toxicological actions of ozone, and a referee has proposed that the debate may be hosted as a forum by Mediators of Inflammations.

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