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It is Time That Health Authorities Promote the Use of Oxygen-ozone Therapy as an Integrative Therapy of Orthodox Drugs

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Authors' contributions

This work was carried out in collaboration between both authors. Author VB wrote part of the manuscript. Author EB wrote part of the manuscript and managed the literature search. Both authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Ozone is a very reactive gas and is toxic for the respiratory system but, under precise rules, it can be therapeutically useful for human disease with a chronic oxidative stress. An unfavorable combination of factors such as a wrong dogma, the fact that ozone is one of the worst troposphere pollutants and past misuse of ozone have led to a poor consideration of ozone therapy. However, basic and clinical work, developed during the last two decades, clarified both the biochemical and molecular mechanisms of action of ozone in biology and medicine. A judicious dose of ozone dissolved in blood immediately triggers a cascade of well defined chemical compounds acting on multiple cellular targets according to well-known biochemical and molecular pathways. Ozone therapy is proving to be very useful in the dry form of age-related macular degeneration (AMD), cardiovascular diseases, chronic obstructive pulmonary disease (COPD), cerebral diseases and healing disorders, where conventional medicine appears insufficient and too expensive. It is time that World Health Authorities abandon prejudice and skepticism and start to take advantage of an integrative medical application able to help the majority of world population.

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1. INTRODUCTION

The title of this review intends to be provocative because oxygen-ozone therapy is thought to be a toxic therapy with little evidence to justify its use in medicine [1]. It will be demonstrated that this supposition is incorrect when ozone is used as a medical drug. There is no doubt that ozone, the third strongest oxidant in chemistry (E= + 2.076 V) is intrinsically toxic. However, as reported in recent papers, oxidants are not damaging only. For example a moderate concentration of oxidants produced during exercise has a beneficial effects for the development of training induced adaptation in endurance performance or for the induction of endogenous defense systems [2]. In the case of ozone, the respiratory system [3] and to a lesser extent the mucosae and the skin [4] should never be exposed to ozone and other pollutants because they are minimally protected by the antioxidants present in the aqueous-lipid film layer. The problem is that during Summer, in heavily polluted cities, the bronchial alveolar lining, daily exposed to air polluted with 90-100 ppbv ozone, continuously produces toxic compounds such as reactive oxygen species (ROS), lipid oxidation products (LOPs), proinflammatory cytokines and proteases, which not only damage the lungs but, after being absorbed by lymphatics and capillaries, enter into the circulation and damage vital organs [5]. Moreover either saline-washed erythrocytes suspended in saline [6] or cells in culture [7] even if exposed at very low ozone concentrations, undergo haemolysis or apoptosis, respectively because unprotected by natural antioxidants such as ascorbate, uric acid, albumin and alpha tocopherol [8-9]. These experimental data have been misleading because, while human plasma and blood cells are endowed with a potent antioxidant system [10], washed erythrocytes or cell cultured in antioxidant-poor-media are very sensitive to ozonation. This situation has enticed chemists and cell biologists to establish the dogma that ozone is cytotoxic and should be proscribed in medicine. Unfortunately in the 90s, the direct intravenous (IV) administration of oxygen ozone in HIV patients, in the wrong belief that ozone will destroy the virus like other pathogens in a water pipe, often caused deadly lung embolism and resulted in prohibiting ozone therapy in several states of the USA. Actually the small amount of ozone is not responsible because it dissolves rapidly in plasma while oxygen, owing to its low solubility, is the cause of lung embolism. Since 1982, the prohibited application has not been enough because inexpert physicians, by misusing ozone, have contributed further to defame ozone therapy. To be objective ozone has been compared to the Roman god Janus [11], because ozone likes to be a controversial molecule: toxic in the troposphere but very useful in the stratosphere to block UV rays and now widely used for water sterilization [12], food processing and veterinary medicine [13]. Today the concept of oxidative stress was updated to include the role of redox signaling and there were efforts of redefining oxidative stress; ozone can be used as a drug within a well defined therapeutic window, and the low increase in the hydrogen peroxide production is quenched by the multiform antioxidant system (enzymes plus hydrophilic and lypophilic compounds) present in blood and cells [8]. Thus, although the intrinsic toxicity of ozone must be born in mind, skepticism and prejudice appear futile particularly taking into account that only a few side effects have been recorded [14]. It is unfortunate that Vioxx, a well known orthodox drug, may have caused the death of as many as 55.000 people during four years only in the USA [15].

2. THE BIOCHEMICAL, IMMUNOLOGICAL AND MOLECULAR EFFECTS OF OZONE

Since 1998, the problem has been investigated using precise ozone generators, which allow checking the ozone concentration and final dosage in real time by a photometer calibrated with the classical iodometric method [16-19]. These papers have reported the first comprehensive framework for understanding and recommending ozone therapy in diseases with a chronic oxidative stress. Ozone is considered a real drug and as such it must be used with caution after having defined its therapeutic window (from 10 μg/ml or 0.21 mM up to 40 ug /ml or 0.84 mM per ml of blood) in order to precisely calibrate the ozone dose against the antioxidant capacity of the patient's blood (1.28-1.83 mmol/L plasma in the European population) [20,21], thus controlling the potential ozone toxicity. Earlier and more recent clinical applications have demonstrated that the classical treatment, denominated ozonated

autohaemotherapy (AHT) consists of exposing a precise volume of blood to a precisely calibrated ozone dose for a few minutes followed by the reinfusion of activated blood in the donor. In this way, ozone activates several biochemical pathways without eliciting acute or chronic toxicity. The potent antioxidant capacity of blood tames the reactivity of a calculated ozone dose and readily reconstitutes the antioxidant titre [22,23]. The sweeping statement that ozone is always toxic is also inconsistent with the physiological knowledge that another two potentially toxic gaseous molecules: nitrogen monoxide (NO) and carbon monoxide (CO), can operate as crucial activators thanks to their minimal concentrations, short time of exposure and particular location [24]. On the other hand, during chronic inflammation typical of vascular diseases, COPD, type II diabetes, atherosclerosis, an excessive and constant release of ROS, NO and peroxynitrite are detrimental and aggravate the pathological state. During major ozonated autohaemotherapy we produce a precise and brief increase of hydrogen peroxide induced by physiological ozone concentrations, which is able to restore an adaptive response in the redox cell regulation [25]. Owing to the high solubility of ozone in the plasma (0.02 M),which is ten fold more than oxygen, it reacts immediately with the antioxidants of the plasma (uric acid, ascorbic acid, GSH, albumin (Cys 34) and with the available polyunsaturated fatty acid (PUFA) producing immediately two crucial messengers:

$$R\text{-}CH{=}CH\text{-} \; +O_3.+. \; H_2O\ldots..> \; H_2O_2 + 2 \; R\text{-}CHO$$

Within 1-2 min of reaction, ozone is totally consumed and therefore ozone acts as a pro drug. H_2O_2 is one of the reactive oxygen species (ROS) and aldehydes such as 4-hydroxy-2-nonenal (4-HNE) are the result of PUFA peroxidation. Normally the ozonated blood is infused back in the donor patient only after 5 minutes. Only the auto-transfusion of blood is allowed. From now on, only H_2O_2 and the peroxidation terminal compounds are responsible for the successive biochemical and molecular reactions happening in many different cells all over the body. It is unfortunate that many physicians in the world ignore this simple biochemistry and therefore continue to infuse ozonated blood in patients with bacterial or viral diseases thinking that gaseous ozone will destroy either bacterial or viral agents which are well protected by the plasma and cellular antioxidants. During the infusion, H_2O_2 being unionized enters all blood cells and activates the following reactions:

1) Erythrocytes. The chemical gradient between plasma and the intracellular concentration of H_2O_2 ranges between 3-5 μM equivalent to about 10% of its plasma concentration, which avoids any toxicity [26,27]. We have measured an increase of 2,3 diphosphoglycerate (2,3-DPG) which allows a shift to the right of the oxyhemoglobin dissociation curve [28,29], which, importantly, enhance the release of oxygen especially into ischemic tissues. H_2O_2 transitorily favors also an increase of ATP in erythrocytes.
2) Leukocytes: ozone therapy stimulates a modest increase of phagocytosis and H_2O_2 mildly activates the NF-kB which allows a transitory increase of synthesis of TNF alpha, IFN gamma, IL-2 and Il-8 from the ozonated lymphocytes. This change may improve the immune status during immune depression.
3) The ozonated platelets are modestly activated with demonstrated increase of PDGF-AB, TGF beta1 and IL-8 [30]. The release of growth factor may be useful in patients with chronic limb ischemia.

2.1 How is the Nrf2 Activated?

The transcription factor is normally inactive because bound to a large protein called Keap1 (Kelch-like ECH associated protein) which represses Nrf2. This protein has a molecular weight of 95-110 KDa [31] and it was first described by Moi et al. [32]. The Keap-1 forms an E3 ubiquitin-ligase complex with Cullin 3 and RING-box protein1. Under unstressed conditions, the complex Nrf2-Keap1-Cul3-RBX1 E3 ubiquitin ligase complex conjugates ubiquitin onto Nrf2 leading to its degradation by the proteasome every about 20 minutes. The repressor protein Keap 1 contains several cysteines (at position 151, 273, 288, 226, 613 and 434) [33]. Electrophiles such as 4-HNE and heavy metals can bind to several cysteine residues in Keap1 impairing its function and leading to Nrf2 derepression/ activation.

4-HNE produced during PUFA peroxidation is either metabolized or eliminated via bilirubin, or, if bound to cysteine circulates all over the body and particularly in chronically inflammed organs, can enter into cells undergoing chronic oxidative stress. 4-HNE, by its binding to either Cys 273 or Cys 288 of Keap 1 allows the release of Nrf2,

which then transfer into the cell nucleus and binds to the antioxidant response element (ARE) of gene promoters. This induces the expression of over 230 genes able to provide the synthesis of potent cellular antioxidant and detoxification systems. Chronic diseases such as cardiovascular diseases including chronic limb ischemia and brain stroke, type II diabetes, AR-macular degeneration, multiple sclerosis and probably Alzheimer's and Parkinson's disease, COPD and asthma and probably some autoimmune diseases undergo a drastic change because of the cell reactivation of phase II antioxidant and detoxification response including GSH synthesis, GSH-reductase, GSH - transferases, GSH-peroxidases, NAPDH-quinone reductase1 (NQO1), Heme–oxygenase 1, thioredoxin and thioredoxin reductase. The Nrf2-based down regulation of GH/IGF and insulin is also very useful in prolonging life and protecting from Type II diabetes. Usually the Nrf2 effect is rather short and in our experience declines during one day. Consequently two or most three AHTs per week are enough to modify the cell response against chronic oxidative stress and reduce its negative effect. It is now certain that the ozone stress is well tolerated either because the initial dose is minimal and implies a robust response against ROS and xenobiotics. It is now clear why the acute, although weak, ozone stress is well rewarded by the potent antioxidant defense of the body.

Contrary to skeptical expectations, it has been demonstrated that the judicious application of ozone in the atrophic form of ARMD, chronic vasculopathies, Type II diabetes, COPD, wound healing disorders, orthopedics and dentistry is yielding useful results that the medical community continue to ignore. The fact that ozone therapy can be used in different pathologies has been derided by skeptical scientists, who do not know that ozone, as soon as it is dissolved in the water of the plasma, reacts immediately with antioxidants and polyunsaturated fatty acids generating two messengers able to activate blood, endothelial and a variety of parenchyma cells, which, having different functions, are responsible for restoring homeostasis. This explains why ozone therapy, integrating the orthodox drugs of conventional medicine, can be applied in many but not all diseases and does not mean that it is a panacea because, in reality, it can be uniquely useful in only the most relevant diseases for perfecting the value of orthodox drugs. A few examples of this concept:

3. AGE-RELATED MACULAR DEGENERATION, DRY FORM

In the UK alone, there are about 200.000 patients affected by the dry form of ARMD, suitable to be treated with O_3-AHT [34]. Nonetheless, unaware or skeptical ophthalmologists can only prescribe lutein, zeaxantine and zinc that may not be harm, but are ineffective. Since 1994 we have treated almost 700 patients with the dry form (performing as many as 11.000 AHT) and 3/4 of them gained an improvement of 1-2 lines on the visual acuity chart. It is important starting the therapy at the earliest time as possible [35,36]. Usually 15-18 treatments, at an initial ozone concentration of 15 micrograms/ml of gas per ml of blood, slowly upgraded to 40 micrograms/ml (twice weekly), following by one monthly session as a maintenance therapy allow sufficient visual acuity for many years [36]. Neglecting that almost blind patients can regain autonomy, and the fact that it would have been unethical to run a parallel study using only oxygen, patients reported an improved quality of life and appreciate treatment so much by the indicated excellent compliance during the successive maintenance period. In this disease there is a progressive de generation and death of the fovea centralis photoreceptors and of the pigmented retinal epithelium (PRE) as a consequence of several derangements, one of which is age, a chronic hypoxia and an excess of oxidants. Although ozone therapy induces a pleiotropic response, the main advantage is due to an increased delivery of oxygen to the retina, which is the tissue with the greatest need. It is worth noting that the improved oxygenation worsen the exudative form, and in multigenic and progressive diseases, namely retinitis pigmentosa and recessive Stargardt's disease. The exudative form, characterized by an aberrant choroidal vascular growth beneath the retina and the PRE, is today treated with the intravitreal administration of angiostatic inhibitors. It may well be that in the future, the implantation of stem cells may be able to renew the retina but today this is not yet feasible.

4. CHRONIC VASCULAR DISEASES (THESE INCLUDES CHRONIC HEART DYSFUNCTION AND CHRONIC LIMB ISCHEMIA)

Ozone therapy, in comparison to pentoxyfylline and prostanoids (the gold standard of orthodox medicine) has been similarly proved to be more effective and atoxic in ischemic vascular

diseases [37]. This was a small trial when 28 patients were randomized to either receive their own ozonated blood or IV infusion of prostacyclin. Naturally all patients continued the conventional treatment with statins, antihypertensive and antiaggregant drugs. Ozone therapy was more effective than prostacyclin in terms of pain reduction, improvement of the quality of life but no significant differences in the vascularisation of the lower limbs before and after treatment were found in either group, most likely because only 14 treatments in seven weeks were performed. Another number of studies [38-49] have proven the validity of the concept in this complex pathology but it is a mistake to stop therapy too early in these patients because ozone therapy, as any other conventional drug, acts as a temporary biological modifier and therefore must be continued at a slower rhythm for life. A possibly improved schedule consists of two ozonated AHTs (225 ml blood + 25 ml sodium citrate solution, 3.8%), weekly for at least four months plus topical therapy with ozonated olive oil, when an initial dry gangrene or ulcers are present. In the world, there are millions of people affected by chronic limb and heart ischemia that altogether represent the first cause of death and have a huge socioeconomic relevance. Owing to the lack of financial support and total disinterest of pharmaceutical industries, that obviously envisage the very inexpensive ozone therapy as a competitor, it has not yet been possible to perform clinical studies on at least 1000 patients comparable to those (in particular regarding the statins, published by orthodox medicine. Peripheral arterial ischemia is due to many factors such as genetic predisposition, ageing, smoking, overfeeding, Type II diabetes, hyperlipidemia and sedentary life. To limit the damage all the numerous and useful conventional drugs ideally coupled to ozone therapy, which, by acting on different cells elicits a variety of biological effects able to improve the therapeutic results must be used. It can be missed the opportunity to state that ozone therapy is an integrative approach to be used either for improving the therapeutic response or when orthodox drugs are insufficient, to correct the chronic oxidative stress.

4.1 Type II Diabetes

A controlled and randomized clinical trial had been recently performed at the Institute of Angiology and Vascular Surgery at the University of Havana, Cuba, recruiting 101 patients with diabetic foot: 52 patients were treated 15 times in 20 days with ozone (local and rectal insufflation of the gas mixture including about 96% oxygen and about 4% ozone with a fixed ozone dose of 10 mg) whereas 49 patients were treated with systemic antibiotics and conventional topical treatment. Ozone therapy improved glycemic control, prevented oxidative stress, normalized levels of organic peroxides, increases the intraerythrocytic level of superoxide dismutase, enhanced the healing of the ulcers and significantly reduced number of amputations. Martinez Sanchez et al. [47] concluded that the medical ozone treatment could been an alternative therapy in the treatment of diabetes and its complications. This study reports exceptional data that should be controlled, possibly in several American or European countries as soon as possible. Only 15 treatments based on the rectal administration of ozone, a practical, cheap, but in comparison to O3-AHT an imprecise and biochemically less effective procedure, procure such exceptional improvements in advanced diabetes, World Health Authorities will become very negligent if they do not endorse this protocol and promote an evaluation of a least 10.000 patients. Recently, by considering the relevance of oxidative stress in diabetes [48], the association of antidiabetic drugs with AHT appears as the best combination probably valid in reducing the diabetic epidemic.

4.2 Lung Diseases

Lung diseases such as the chronic obstructive pulmonary disease (COPD) are the fourth cause of death and with emphysema and asthma represent invalidating disease mostly due to smoking and air pollution. By using corticosteroids, long acting beta2 agonists and antibiotics, orthodox medicine is certainly helpful [49,50] but, even the best pneumologist considers impossible to change the course of COPD. In a series of elderly patients, simultaneously affected by macular degeneration and either emphysema or COPD, we have observed a remarkable improvement by combining ozone therapy with the best orthodox treatment [51]. Besides typical analyses, already using the simple walking test, a striking improvement due to improved oxygenation and reduced chronic infections has been observed. Patients are enthusiastic for receiving a combination therapy. Ozone therapy is also effective in asthma: [52] have treated 113 patients with either ozonated AHT (applied at dose of 4 and 8 mg) or rectal insufflation of gas.

It appears that in Cuba, ozone therapy is used in all hospitals and, because of the large number of patients, they have found it very practical and quick to receive the gas via the rectal route. Using a fixed ozone concentration of 40 micrograms/ml of blood (8 mg dose), after completing a cycle of 15 treatments, they measured significant reduction of IgE, HLA-DR levels and as it was expected, an increased blood antioxidant capacity determined as an enhanced GSH levels and GSH peroxidase. They also noted a significant improvement of lung functions and symptoms. On the other hand, rectal insufflation of gas (10 mg for each treatment per 20 sessions) in one group of patients was found less effective indicating that the ozonated AHT was the most effective treatment.

4.3 Chronic Cerebral Diseases

A very novel development may be the autohemotherapy treatment of chronic cerebral diseases such as multiple sclerosis, Alzheimer and Parkinson diseases. The first is particularly appealing because affects young people [53].

4.4 Chronical Skin and Mucosal Infections

There is consensus on the value of ozone as the best topical disinfectant because bacteria, viruses, fungi and protozoa, free in water, are readily oxidized [12]. However the elimination of pathogens in plasma or cell by ozone is practically impossible because ozone reacts immediately with blood ex vivo and it is absent and moreover pathogens are not oxidized because of the presence of soluble antioxidants such as albumin, ascorbic and uric acids. This is easily explained because intracellular viruses or parasites are protected by the antioxidant system and that is why, in contrast with unrealistic claims, we found only a marginal benefit in HIV patients treated only with prolonged ozone therapy. In the 90s, it was claimed to “cure” HIV by the direct intravenous injection of gas (O_2 plus O_3) but I am convinced that only oxygen embolism and adverse effects were procured to desperate patients. Ozone can be usefully used as a topical treatment either as a gas mixture (about 2-3% ozone and 97-98% oxygen), which must be well contained in an ozone-resistant bag, or as ozonated water or, even better as ozonated sesame or sunflower oil for the treatment of wounds, bacterial, viral and fungi infections, burns, abscesses, anal fissures, fistulae, and inveterate osteomyelitis, gingivitis and vulvovaginitis. Topical therapy is most effective when combined with AHT owing to oxygenation of hypoxic tissues. Radiodermitis [54] and wound healing are markedly improved because ozonated solutions display a cleansing effect, act an unsurpassed disinfectant and stimulate tissue reconstruction far better than expensive and poorly effective growth factors. In 1996, 6.5 million people in the USA suffered from diabetic ulcers with an annual cost of about $21 billion. Wound healing disorders due to chronic ulcers and putrid wounds are one of the most distressing and difficult medical problems caused by ischemia, diabetes, immunosuppression and undernutrition. During the last decade both patients and medical personnel, who have used ozone have found it very beneficial. With the current increase in medical costs, ozone therapy deserve attention because it reduces hospital assistance and is extremely cheap. In agreement with the concept of the hormetic dose response, small ozone doses, or in other words, small repeated oxidative stresses induce stimulation, while high ones cause inhibition. In comparison with the in conclusive usefulness of oral antioxidants, experimental and clinical data have already shown that the cautious and prolonged use of ozone therapy can arrest or delay the progression of these diseases and improve the quality of life.

5. DENTISTRY

Just for emphasizing the versatility of ozone, recently ozone has been found very useful in dentistry for eliminating infections and blocking primary root carious lesions [55]. Prof. E. Lynch [56] has edited a book of 300 pages entitled “Ozone: the revolution in dentistry” (Quintessence publ, 2004)

6. ORTHOPAEDICS

Last but not least, the application of ozone in low back ache that sooner or later affects 80% of people has been found very effective. The application can be either direct (intradiscal) [57,58] or indirect via intramuscular administration into the paravertebral muscles corresponding to the metamers of the lesion. It is almost unbelievable that very small ozone dosages exert a multiplicity of effects such as the activation of the antinociceptive system and an antinflammatory action due to LOPs with the consequent inhibition of cycloxygenase 2 [59]. There cannot be a more conservative and cheaper treatment for hernial disc!

7. CONCLUSIONS

Among complementary medical approaches, ozone therapy is known all over the world but it is not yet practiced correctly everywhere because of the incomplete knowledge by improvised ozone therapists. However, on the basis of basic and small clinical studies performed in he last two decades, it has become clear that ozone, in very small dosages, behaves as a real drug and the biochemical and molecular mechanism of action are well within orthodox medicine. Ozone is an extremely versatile drug and the therapeutic range has been precisely defined to exclude acute and chronic toxicity. It is remarkable that the majority of patients report a feeling of wellness using prolonged ozone therapy. Particularly in chronic skin infections, which torture and depress millions of patients, the use of parenteral and topical ozone applications is far more effective than conventional medications for enhancing healing, simply because ozone disinfects, oxygenates and stimulates cell proliferation. The critical point remains an exhaustive clinical evaluation of ozone therapy in the outlined diseases. Orthodox medicine remains skeptical because multi-center and extensive clinical trials are not yet available owing to the lack of sponsors. Ethical Committees are reluctant to give permission for clinical trials because they prefer pharmaceuticals able to pay huge sums for insurance and all clinical tests. Unfortunately our good will is not sufficient to overcome prejudice and lack of sponsors. The few and small National Associations of ozone therapy, in comparison to the Pharmaceutical Industries, which register an annual business of many billion dollars, have no financial power and are unable to programme expensive clinical studies. One relevant possibility is that World Health authorities, always concerned with the increasing medical costs, could start evaluating ozone therapy and recognize its validity. It is distressing to realize that commercial interests and wrong ideas can prevent the correct and extensive use of ozone in medicine. A fairly recent Editorial published in The Lancet addressed the problem of the "catastrophic failures of public health". We believe that the implementation of ozone therapy in all public hospitals, particularly on behalf of patients with little medical assistance, could be a first important step in this direction.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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