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Does Ozone Therapy Normalize the Cellular Redox Balance?

Implications for the Therapy of Human Immunodeficiency Virus Infection and Several Other Diseases

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Abstract — The role of ozone on earth is controversial, as in the stratosphere it is protective against excessive ultra violet irradiation, and in the troposphere it is toxic for animals and plants. The effectiveness of ozone against pathogens is well recognized and ozone appears to be the best agent for sterilization of water. In spite of this, the use of ozone in medicine has been overlooked or despised, mostly because it has been either misused or used without appropriate controls. Studies carried out in our laboratory have revealed that ozone can display relevant biological effects and that, having defined its therapeutic index, can become an important and reliable drug for the treatment of several diseases. An exciting new aspect is that ozone, being a strong oxidizer, can stimulate the increase of cellular anti-oxidant enzymes, eventually inhibiting the oxidative stress.

Introduction

Of about 150 articles appearing yearly on ozone, 15% deal with the bothersome thinning of the ozone layer in the stratosphere while the bulk of articles address the important problem of ozone toxicity for the pulmonary system in the troposphere. The biomedical aspect of ozone is almost absent from the world literature because conventional medicine of the leading western countries disregards ozone therapy and considers it a matter for quacks. It is indeed unfortunate that the biomedical literature on ozone is so scarce and this is due to both poor quality and to a minimal number of biomedical papers on ozone. Recent media stories in the USA have aggravated the image of ozone therapy, often performed by incompetent and possibly unscrupulous therapists.

Since 1988 when I made the serendipitous observation that ozone can act as a cytokine inducer (1,2), my aim was to objectively evaluate the pros and cons of ozone therapy and to emphasize that skepticism and prejudice are the worst enemies of any scientific endeavour.

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Where do we stand today?

During the last 7 years, thanks to the work carried out in our laboratory (1,3–9), we have achieved important results as briefly follows. Firstly, ozone must be used with the same accuracy as any other drug: by knowing the ozone concentration (μg/ml) and the gas volume ($O_3 + O_2$ present as carrier) the dose delivered can be as precise as with insulin. At long last this has made it possible to perform therapy reproducibly, with the most effective and minimal toxic dose (8).

Secondly, it appeared crucial to define the therapeutic index when ozone is mixed with blood for carrying out ozonated autohemotherapy (AHT). Major AHT has usually been performed by collecting 100 ml of blood in citrate-phosphate-dextrose (CPD) and using a final ozone concentration well below 40 μg/ml/g of blood (10). Since Wehrly and Steinbart (11) described the procedure in 1954, relevant biochemical or immunological parameters have not been checked. AHT has been performed mostly in private medical practice millions of times reporting 'good' clinical results in viral diseases, cancer and vascular disorders without any appropriate controls (10) so that at least in part it could have acted as a placebo. By selecting several parameters, namely extent of hemolysis (4,5); formation of plasma lipid peroxides (9); level of intra-erythrocytic reduced glutathione (GSH) (5); production of cytokines (1,3–6) such as interferon (IFN) α, β and γ, interleukins (IL) 1, 2, 6, 7, 8, 10, tumor necrosis factor α (TNF), granulocyte-macrophage colony stimulating factor (GM-CSF) and transforming growth factors (TGF) βs (7) and morphological evaluation by electron-microscopy of the blood cell components (Bocci et al, manuscript in preparation), we have found that the optimal cytokine production is obtained by using an ozone concentration around 70 μg/ml/g of blood (8) that is almost double the dogmatic established one (10). Actually the optimal concentration ranges between 70 and 90 μg/ml (5,6) because soluble anti-oxidant compounds vary considerably among blood samples. However, the concentration of 70 μg/ml is the safest, because hemolysis does not exceed 2.5% (4,5), plasma lipid peroxides increase only 2–3-fold (9), intra-erythrocytic GSH levels fall transiently no more than 15% (5), production of cytokines is small but consistent (4,5,7) and there is no modification of leukocyte viability and morphologic aspect.

Thirdly, when ozonated autohemotherapy is intended to act as an immunoadjuvant agent, the substitution of CPD with heparin, by preserving the extracellular Ca^{2+} level, leads to improved cytokine production (4). However, heparin cannot be used in patients with dyscoagulation or under anticoagulant therapy.

Fourthly, in order to accelerate the immunological reconstitution, each autohemotherapy is carried out by collecting 250–300 ml of blood depending upon a body weight of 50–80 Kg. Indeed this volume of blood contains only about 0.1% of the entire blood mononuclear cell (BMC) pool (12) and therefore by repeating the procedure 50 times (twice weekly for 6 months) we can ozonate a blood volume of 15 L that comprises only 5% of the whole BMC population. Nonetheless this calculation does not take into account the important priming effect of cytokines released by activated cells in lymphoid microenvironments that, by acting in neighboring cells, can lead to a considerable amplification of the immunological effects.

Fifthly, AHT as it is now performed using our optimized procedure (6,8) does not yield any side-effects and actually procures in most of the patients a feeling of well-being that could be partly due to hormonal stimulation.

A preliminary investigation of ozonated autohemotherapy in human immunodeficiency virus patients brings about an unexpected finding

Recently, Westendorp et al (13) showed that in T lymphocytes HIV-1 Tat protein suppresses the expression of Mn-dependent superoxide-dismutase (SOD) with the consequence of reducing intracellular GSH and lowering the ratio of GSH: GSSG. This remarkable finding implies that the Tat protein creates a pro-oxidative condition in the infected cells, favoring a constant activation of the cytoplasmic nuclear factor-kappa B (NF-KB), that, in turn, enhances HIV replication (14–16). It has already been found that dietary and pharmacological supplementation of reducing compounds such as n-acetyl-L-cysteine (NAC) (17), GSH (18), cystamine (19) and thiamine disulfide (20) can partially suppress HIV replication, although infected cells are somewhat unable to reconstitute the intracellular reservoir of anti-oxidant compounds.

When I put forward the proposal (21) for investigating the effect of AHT in HIV patients last year, some possible mechanisms of action summarized here in Table 1, were discussed in detail. Together with many critics of the therapeutic use of ozone, I was considering that this gas, being a strong oxidizer, would be the last thing to use for the therapy of HIV infection. However, an aspect easily overlooked is that ozonation implies an oxidative stress on the collected blood for only a few minutes at most (8,9). Indeed, in a few seconds ozone decomposes in plasma into a number of reactive oxygen intermediates (ROI), of which hydrogen peroxides and lipid peroxides can easily enter the cells from the plasma (14) and activate

Table 1 Mechanisms postulated to occur during major autohemotherapy in HIV infection due to ozone action

1. Oxidation of free viral particles in plasma and lysis of HIV infected cells
2. Continuous generation of new variant immunogens (autovaccination?)
3. Induction and release of cytokines
   a. IFNγ, IL-2, IL-12
   b. Production of cellular antiviral factor (CAF)?
   c. GM-CSF, erythropoietin, thrombopoietin?
4. Synthesis of neutralizing antibodies
5. Activation of cytotoxic T lymphocytes, natural killer cells and antibody-dependent cellular cytotoxicity
6. Improvement of cellular oxygenation and metabolism
   a. shift to the right of the HbO_2 dissociation curve
   b. vasodilation due to increased release of NO
   c. *upregulation of the enzymatic antioxidative system*
   d. *increase of intracellular GSH*
7. Hormonal stimulation

the NF-KB finally responsible for the induction of cytokines in normal cells. Infected cells, depleted of reducing compounds and protective enzymes, are probably unable to survive this extra-oxidative stress. On the other hand, ROI are rapidly scavenged by extracellular and intracellular anti-oxidant compounds and enzymes, namely superoxide dismutases (SODs), catalases and peroxidases, so that they are able to activate biochemical pathways with minimal cell damage. It needs to be emphasized again, that as is shown by the extent of hemolysis, this does not exceed 2.5% when blood is mixed with an ozone concentration of 70 μg/ml/g of blood (4,6), implying negligible cell damage. It has become clear that, unless we use this apparently high concentration, the resulting biological effects are minimal. On the whole, the data clarify the existence of a threshold concentration implying that too low a concentration is ineffective, while an excess of ozone, hence of ROI, by overwhelming the intracellular anti-oxidant capacity, can be toxic (8,21). It has also become evident that ozone and ROI are relatively unselective agents and that a considerable amount of the dose is quenched by the oxidation of ascorbic acid, uric acid and the like present in plasma. The sacrificial role of these compounds has been clearly shown in several important contributions (22–26). There is also a growing body of evidence (27–29), consistent with this reasoning and showing that ozone and ROI can induce production of cytokines in various cellular systems leading to overt toxicity and apoptosis when excessive ozone dosing is used.

On this basis, ozone yields only a very transient oxidative stress and indeed the HIV patients so far treated with AHT have improved their general condition, and have not shown any sign of enhanced HIV replication that could have been expected as a consequence of an oxidant burden (30). The unexpected finding has been that plasma lipid peroxide levels, usually high in symptomatic HIV patients, in spite of the fact that reinfusion of ozonated blood unavoidably contributes some newly generated peroxides (9), decrease during the course of the treatment, usually after 6–10 sessions. This finding has now been interpreted as an in vivo upregulation of anti-oxidant enzymes induced by repeated AHT. This hypothesis is supported by preliminary data obtained in two patients who underwent AHT 15 times during the last 2 months: while lipid peroxides increased during the first 7–12 days and began to decrease thereafter, SOD levels in plasma started to progressively increase after 10–15 days, reaching a plateau after 36 and 48 days. These results have relevant implications that need to be actively pursued not only in HIV patients, but in other diseases where oxidative damage may play a role.

With the benefit of hindsight it is not very surprising that over-oxygenated and ozonated blood can bring about an upregulation of the expression of anti-oxidant enzymes in order to minimize cell damage. During hyperbaric oxygen and/or ozone exposure, in an attempt to restore the homeostatic balance, animals (31–33) as well as plants (34) are able to increase the synthesis and/or the activity of probably all the defense enzymes so that they can survive in a hostile environment. On the other hand, during HIV infection, while the intracellular virus creates a hyperoxidative state to its own advantage, at the same time it inhibits the defense system and succeeds in killing the cell. In order to reverse the balance in favour of the host (Fig. 1) the chronic treatment with oxygenated-ozonated blood appears to be a practical, inexpensive, atoxic approach that is probably able to block the progress of the disease.

How many diseases are due to oxidative stress?

ROI behave as friends and foes: they defend us

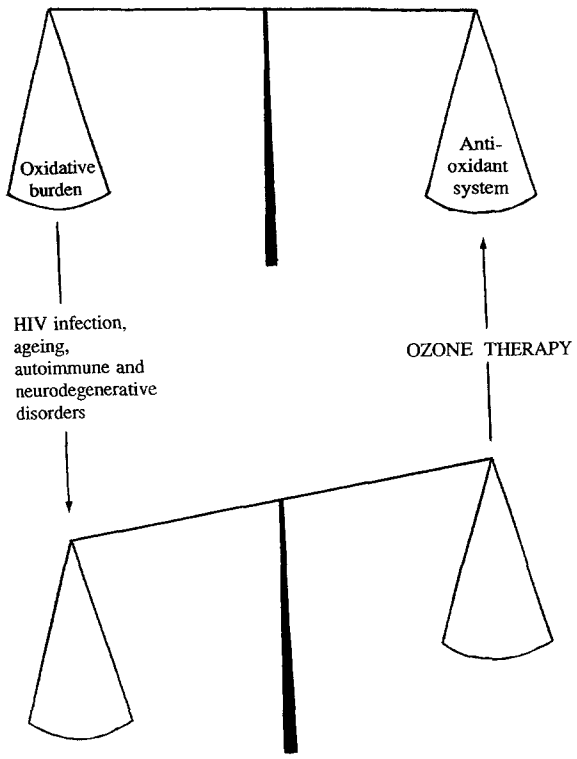

Fig. 1 The normal and pathological redox balance. The scheme suggests that ozone therapy, by upregulating the expression of anti-oxidant enzymes, may favour the normalization of the redox balance.

against pathogens and in small amounts perform vital cell functions. On the other hand if ROI are produced chronically in excess, they provoke tissue damage, particularly if cells are unable to upregulate the production of anti-oxidant enzymes or, owing to a genetic defect, produce an ineffective Cu/Zn SOD as happens in familial amyotrophic lateral sclerosis (35). However, a super-active mutant SOD may be equally damaging because excessive generation of H_2O_2 reacting with free iron, gives rise to highly toxic hydroxyl radicals.

Autoimmune diseases such as recurrent ulcerative gut disorders, rheumatoid arthritis and multiple sclerosis are at least in part due to an excessive localized generation of ROI as a consequence of an abnormal attack of dysregulated cytotoxic cells against self-antigens. Ageing may be accompanied by a more or less slow, pro-oxidant situation with a concomitant progressive decay of intracellular detoxification, so that the redox balance will tilt to the left (Fig. 1). The progress of Alzheimer's as well as Parkinson's disease, epilepsy, cerebral ischemia following stroke or trauma, age-related macular degeneration, cataract formation, vascular disorders with alternating elevation of ROI due to ischemia-reperfusion injury and neoplasia (36–38) may be arrested if ozone therapy can upregulate the endogenous synthesis of anti-oxidant enzymes and improve the metabolism. This therapeutic approach appears more rational than the pharmacological administration of heterologous SODs that may not even reach the sites where needed. On the other hand, a dietary supplementation of anti-oxidant compounds (26), given daily, may display a synergistic effect with ozone therapy. Finally I would like to mention that AHT seems to improve these pathological states (39), stressing the urgent need to carry out appropriate clinical studies for proving or disproving anecdotal reports.

Concluding remarks

The main object of this paper has been to describe an apparently paradoxical effect observed during autotransfusion of blood briefly exposed ex vivo to an appropriate concentration of oxygen-ozone. After reinfusion, ozonated blood components can trigger a variety of biological effects, of which the upregulation of SODs and probably of other anti-oxidant enzymes is the most recent and unexpected finding. Plasma levels of lipid peroxides that were abnormally high before ozone therapy progressively decrease thereafter. These results suggest that ozone therapy helps to normalize the redox balance altered in several pathological conditions due either to viruses, ageing or inflammatory-immune injuries.

It is regrettable that the biological and medical effects of ozone have been disregarded by the scientific community, because as the numerous mechanisms of action are being unraveled, it appears that ozone could become a useful drug – mostly because it exerts several biological effects that may act synergistically as hypothesized in Table 1. A further example is represented by the treatment of torpid ulcers in arteriopathies of the leg. The use of topic ozone as well as AHT is effective owing to a cleansing effect, a stimulation of bactericidal activity, an improved oxygen availability, local vasodilation, neoangiogenesis and last but not least, to the release of TGF-β. Thus the accelerated ulcer healing is due to the fact that ozone, by acting on different cell components such as erythrocytes, leukocytes, platelets and endothelial cells, elicits a multifactorial, well concerted response (40), without the need of evoking magic pathways.

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