

Biological and clinical effects of ozone. Has ozone therapy a future in medicine?

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(Accepted 14 May 1999)

Abstract: Although ozone therapy has been used as an alternative medical approach for four decades, it has encountered scepticism, if not outright objection, by orthodox medicine. This prejudice is not unjustified because ozone therapy often has been used without rational basis or appropriate controls. With the advent of precise medical ozone generators, it is now possible to evaluate some mechanisms of action and possible toxicity. In contrast with the respiratory tract, human blood exposed to appropriate ozone concentrations is able to tame its strong oxidant properties and neither acute nor chronic side effects have ensued in millions of patients treated with ozonated autohaemotherapy. This paper summarises studies aimed at clarifying biological effects, defining any possible damage, the therapeutic window, and suitable doses able to express therapeutic activity. Although an unfashionable and unpopular approach, it is hoped that orthodox medicine will help to critically assess the validity of ozone therapy.

Key words: Antioxidants. Cytokines. 2,3-diphosphoglycerate. Oxidative stress. Ozone. Reactive oxygen species.

Introduction

Ozone (O_3) is a strong oxidant; however, after being used as a potent disinfectant for almost a century, its usefulness in medicine remains controversial.^{1,2} The problem should not be neglected because every year many patients worldwide, including in the US, undergo some form of ozone therapy, and in 1995 the Office of Alternative Medicine of the National Institutes of Health (NIH, MD, USA) included ozone therapy among its pharmacological and biological approaches.

Unfortunately, there is no current constructive dialogue because proponents believe it represents a wonderful remedy and opponents say that O_3 is toxic and should not be used in medicine at all. It is obvious, however, that prejudice exists on both sides and this works against objective judgement. In fact, in my opinion, ozone therapy should only complement orthodox medicine, or substitute it in cases where no other effective therapy exists. On the other hand, although O_3 is very reactive,³ it is not necessarily toxic, like any other drug, when used properly.

The purpose of this paper is, firstly, to evaluate objectively the clinical application of O_3 , secondly, to show that its toxicity can be controlled — thus explaining the lack of adverse effects — and, finally, to point out that the efficacy of ozone therapy can be demonstrated only by randomised, double-blind clinical trials carried out in several medical centres.

The medical applications of ozone therapy

It appears correct to use ozone therapy when orthodox medicine fails to be effective, and the prejudicial view that this alternative therapy cures everything must be dispelled on the grounds that its versatility is simply due to blood cells having different biological functions.² Early work was carried out in Germany, and during the last six decades several methods for the application of O_3 in medical therapy have been developed on empirical bases.

The intravenous (IV) route was used mostly in the

past, and involved slow injection of a daily dose of up to 420 mL of O_2-O_3 over two weeks. This technique has been prohibited since 1984 because it caused lung embolism and other adverse effects, and produced doubtful therapeutic benefit. The rationale behind this technique was to consider the human body, composed of about 66% water, as a water sterilisation plant.

The intra-arterial route was used⁴ for slow injection of up to 20 mL of O_2-O_3 (O_3 concentration: 30 $\mu\text{g/mL}$) into the femoral artery in cases of chronic limb ischaemia. Although it was not as dangerous as the IV route, it still presented problems and has been abandoned in favour of autohaemotherapy (AHT). Currently, the direct injection of O_2-O_3 is still practised: via the subcutaneous route, to treat lipodistrophy; via the intramuscular route in the paravertebral muscles, to treat low back pain; via the intradiscal-intraforaminal route, to treat a herniated disc; and via the intraperiarticular route, to treat acute and chronic arthrosis.⁵

Payr⁶ and Aubourg⁷ were the first to introduce rectal insufflation of O_2-O_3 , now performed with a Teflon cannula (rubber is destroyed by O_3). This route has been used widely in human immunodeficiency virus (HIV) infection,⁸ ulcerative colitis and Crohn's disease, with apparently satisfactory results,⁹ using up to 800 mL of gas at a maximal O_3 concentration of 40 $\mu\text{g/mL}$, administered over a few minutes. In addition, low concentrations of O_3 (3–5 $\mu\text{g/mL}$) have been insufflated into the nasal, tubal, oral, vaginal, vesical, pleural and peritoneal cavities in cases of chronic bacterial and parasitic infection when they become resistant to conventional antibiotic therapy.⁴

All of these routes for O_3 administrations have been discussed extensively elsewhere,² but two brief observations should be emphasised. First, despite widely different applications, no toxic effects have been reported and only the respiratory tract appears extremely sensitive to O_3 (thus it should not be inhaled). Second, these procedures are empirical and difficult to standardise. Thus, the only approach amenable to scientific control is the exposure of a known amount of blood to a precise O_3 concentration in a known volume of gas.

The merit of having proposed ozonated autohaemotherapy (O_3 AHT) goes to Wehrli and Steinbart,¹⁰ and particularly to Wolff,¹¹ who applied it in the late 1960s. Millions of sessions have been carried out since then, without immediate or late side effects. Several years ago, I began to consider the value of ozonising blood, and what type of reactions would take place.

During the past few years, the autohaemotherapeutic procedure has been standardised, and now comprises *ex vivo* sterile exposure of a known weight of blood (ranging from 200–300 g) to a predetermined O_3 dose (gas volume $\times$ O_3 concentration), with O_3 concentra-

tion determined precisely in real time by ultraviolet photometry. In the autotransfusion glass bottle, the blood phase is allowed to equilibrate completely with the gas (O_2-O_3) phase for five minutes while it is gently and continuously mixed to avoid foaming. It is then reinfused into the donor.

Depending upon their solubility coefficients, both gases dissolve partially in the plasma water; however, while O_2 is practically stable, and the PO_2 reaches a plateau value well above 100 mmHg within five minutes, O_3 reacts immediately with a range of substrates, including polyunsaturated fatty acids (PUFAs), antioxidant compounds and carbohydrates, such that Henry's law does not apply to O_3 . Using this procedure, most, if not all, of the O_3 dose reacts with plasma, and an effort is being made to inform practitioners that a shorter mixing time is suboptimal.

There are five main areas where O_3 AHT can be useful — in infectious diseases, vascular disorders, immune depression, degenerative disease, and orthopaedic pathology.

Infectious diseases

Exploiting the disinfectant activity of O_3 ,¹ and the activation of the immune system,² it is used as either ozonated bidistilled water or oil in the treatment of war wounds, anaerobic infection, trophic ulcers and burns.¹² Abscesses, anal fissures, bed sores, fistulae, fungal disease, furunculosis, gingivitis, inveterate osteomyelitis, peritonitis, sinusitis, stomatitis, vulvovaginitis and impaired wound healing improve because ozonated solutions have a cleansing effect and act as a powerful disinfectant to which even antibiotic-resistant or anaerobic bacteria succumb.^{1,6,7,13–19} It would appear that O_3 not only reduces infection due to its bactericidal activity but also stimulates the metabolism by improving oxygenation and reducing local inflammation. With the current increase in medical costs and antibiotic-resistant infection, O_3 therapy deserves attention because it does not produce resistance and is extremely cheap.

Vascular disorders

Improved delivery of O_2 and release of growth factors appear beneficial in reducing ischaemia and enhancing wound healing. Several observations^{20–24} have been reported for chronic lower limb ischaemia, severe Raynaud's syndrome, and cerebral and heart vascular disorders. In order to understand how vasodilation and increased O_2 delivery come about, a series of studies were undertaken to verify the biological effects induced by ozone in blood during the course of AHT.

It has been determined that erythrocytes increase their 2,3-diphosphoglycerate (2,3-DPG) content so

that the dissociation curve of oxyhaemoglobin (HbO_2) shifts to the right (to $\text{Hb} + \text{O}_2$) and enhances O_2 delivery to hypoxic tissues, and, more recently, a significant increase in intraerythrocytic adenosine triphosphate (ATP) and energy charge has been demonstrated (Bocci *et al.*, unpublished data).¹ Another relevant line of research aims to evaluate the *in vitro* response of human endothelial cells after exposure to ozonated plasma, simulating the *in vivo* situation during reinfusion of ozonated blood.

Endothelial cells consistently release higher amounts of nitric oxide (NO), which stimulates vasodilation, thus explaining the rapid disappearance of spontaneous pain in patients with ischaemic limbs. However, as yet it is unknown whether or not O_3 AHT is also able to induce neoangiogenesis in the ischaemic areas, a very important lead pursued at the same time by gene therapy.²⁵

The recent finding²⁶ that platelets in heparinised plasma release huge amounts of platelet-derived growth factor (PDGF) and transforming growth factor β 1 (TGF β 1) after ozonation explains, at least in part, the enhanced healing of torpid ulcers in patients¹ with limb ischaemia following O_3 AHT.

In spite of these encouraging results, Kraft *et al.*²⁷ concluded that O_3 AHT is not a useful alternative to conventional treatments in patients with mild hypertension. This report is important because rarely has a randomised double-blind, placebo-controlled crossover study been performed to evaluate the efficacy of O_3 AHT. The treatment was able to reduce blood pressure significantly, but only for about four months — a result observed also in the treatment of age-related macular degeneration (ARMD). However, this is to be expected and can be minimised by extending the treatment, as happens with other forms of medication.

Another controversial issue is the treatment of retinitis pigmentosa with O_3 AHT, electric stimulation and ocular surgery. Berson *et al.*²⁸ correctly criticised this simultaneous use of various approaches because they have not produced a significant improvement, and it remains unclear whether or not any one of them is effective. Similarly, mixing heat, O_3 and ultraviolet (UV) irradiation in the VasoCareTM therapy^{24,29} makes the interpretation of biological and clinical studies very difficult. Moreover, retinitis pigmentosa is a genetic disorder and O_3 AHT may only procure a temporary improvement, at best — hardly encouraging when observed in only one patient.^{30,31}

Pathology linked to immune depression

Reactivation of a suppressed immune system represents a meaningful approach in various immunodeficiencies associated with chronic viral disease³² and cancer,³³ particularly after high-intensity chemother-

apy and radiotherapy. O_3 AHT may activate several mechanisms, previously discussed at length,^{32,33} leading to reactivation of immunological surveillance, with practically no side effects. While there are anecdotal reports³³ on the application of ozone therapy in cancer, no controlled clinical trials have been performed.

This approach is appealing in elderly patients where palliative monochemotherapy results in a poor quality of life and little therapeutic advantage. However, it is difficult to assess the number of autotransfusions needed to achieve reactivation of the immune system because it is unclear whether or not the mononuclear cells primed *ex vivo* trigger further activation of resting or suppressed immune cells once these reinfused leucocytes infiltrate lymphoid tissue. About 50 treatments (twice weekly for six months) may activate at least 3×10^{10} immune cells;³³ however, this is only a tentative estimate. Indeed, two clinical studies^{34,35} failed to prove the efficacy of O_3 in HIV infection, although, in the study by Garber *et al.*,³⁴ blood was badly mistreated by heat, UV irradiation and O_3 in unknown concentrations.

Degenerative diseases

Surprisingly, a brief calculated oxidative stress, such as that achieved with O_3 AHT, may correct a permanent imbalance caused by excessive and chronic oxidative injury. It has been shown that chronic exposure to increased O_3 tension and low O_3 levels induce tolerance in plants,^{36,37} bacteria,³⁸ mammalian cells,³⁹ rats^{40,41} and humans,⁴² and this property is largely caused by up-regulation of antioxidant enzymes. An improvement in antioxidant defence may be useful in conditions such as senile dementia, Parkinson's disease, optic nerve dysfunction, and maculopathies, in which the control of endogenous oxidation has gone awry owing to life-long oxidative damage.⁴³⁻⁴⁵

It is becoming clear that modest, repeated ozone treatment increases the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH Px). Investigations of O_3 AHT, carried out in patients with cardiac infarction,²² neurodegenerative disease,⁴⁶ HIV infection,⁴² and ARMD⁴⁷ have shown a marked increase in GSH Px, glucose-6-phosphate dehydrogenase (G-6-PD) and SOD in erythrocytes. These studies need to be extended because the possibility of inducing a state of oxidative stress adaptation is very interesting and has important therapeutic implications.

Orthopaedic pathology

Surprising results have been obtained in the treatment of acute and chronic arthropathies,⁵ and discal hernia,^{48,49} using small volumes of O_2 - O_3 and peri-intra-

articular and intradiscal insufflation, respectively. It appears that the treatment for arthropathy, although somewhat painful for a few seconds, has no adverse effects and, in the majority of patients, produces pain relief, decongestion, reabsorption of oedema and increased mobility.

Thus far we can only hypothesise⁵⁰ that O₃ therapy induces over-expression of antioxidant enzymes able to neutralise excessive reactive oxygen species (ROS) formation. Ozone may also induce either the release of cytokine antagonists and/or soluble receptors or immunosuppressive cytokines such as interleukin-10 (IL-10) and TGFβ1. After intradiscal injection, O₃ can accelerate the degradation of proteoglycans in the degenerate nucleus pulposus, leading to its reabsorption and the consequent reduction of herniated material responsible for radicular pain.⁵⁰

Finally, only experimental study will confirm the value of injecting O₂-O₃ (2 × 10 mL, O₃ concentration: 15–20 µg/mL) into the trigger points of the paravertebral muscles in patients with lower back pain syndrome.⁵¹ The mode of action remains uncertain but a plausible explanation⁵⁰ is that it represents a type of 'chemical acupuncture' in which the needle and O₃ may inhibit amyelic nociceptor fibres and activate the antinociceptive system, via the stimulation of inhibitory interneurons, and the release of enkephalins.

The analgesia produced permits muscle relaxation and vasodilation, and, hence, a reactivation of muscle metabolism by favouring the oxidation of lactate and neutralisation of acidosis, the increased synthesis of ATP, Ca²⁺ reuptake and reabsorption of oedema. The procedure is very easy and practically free of risk,⁵¹ and has become very popular in Italy. In those that show a response, approximately 10 treatments produce a marked improvement in 66% of patients.

Lower back pain syndrome affects approximately a third of the world's population, and this minimally invasive treatment is worth trying before surgical intervention.

Concern about ozone toxicity

Ozone is one of the most important components of photochemical smog⁵² and its toxicity is potentiated by other compounds such as CO, NO₂ and H₂SO₄.⁵³ Both acute and chronic exposure to these pollutants is harmful to the lungs because the thin layer of respiratory tract lining fluid does not possess sufficient neutralising activity to correct the acid pH, thereby blocking oxidants and producing cell damage.^{53–56} From a medical point of view, considerable effort is being made to minimise pulmonary toxicity,⁵⁷ but the best approach is to prevent O₃ inhalation.

An artificial situation was used to examine O₃ toxicity in human blood, when erythrocytes or other cells

were resuspended in a saline medium and exposed to O₃.^{58,59} In this nonphysiological environment, both the erythrocyte membrane and intracellular enzymes were oxidised; however, it would be wrong to conclude that O₃ is always toxic to blood. In contrast to the respiratory system,⁶⁰ blood is a fluid tissue, the components of which are in a highly dynamic state and have a considerable ability to rapidly renew antioxidants.^{61,62} Furthermore, both plasma and blood cells are endowed with a powerful defence system comprising hydrophilic, lipophilic antioxidants and proteinaceous metal chelators, which limit ROS production.

The antioxidant system is normally effective because it is highly integrated, and oxidative processes can eventually be blocked.⁶⁶ Through the activation of biochemical pathways, this system also can rapidly regenerate depleted levels of antioxidants such as α-tocopherol, ascorbic acid and reduced glutathione (GSH).^{61,62,65–67} Indeed, it is due to the antioxidant system that careful exposure of human blood to a gas mixture (approximately 97% O₂ and 3% O₃) is not harmful.

Pryor³ has shown that one facet of the reaction between an olefin and O₃ is the generation of H₂O₂ and aldehydes. Ueno *et al.*,⁶⁸ using the electron spin resonance (ESR) technique, recently confirmed that whole blood exposed to O₃ generates two radical species, probably deriving from PUFAs which are abundantly present in blood, particularly in lipoproteins, albumin and cell membranes. On the basis of the data, testing O₃ concentrations in the range 20–80 µg/mL gas per gram of blood (0.42–1.66 mmol/L), a great deal of the oxidant power of O₃ is quenched by PUFAs, antioxidants and albumin rich in -SH groups.

It has been established^{63,69} that, after oxidation, compounds such as uric acid and albumin act as 'sacrificial molecules' and are catabolised, while other compounds such as ascorbic acid, α-tocopherol and GSH are regenerated or resynthesised.^{61,62,67} The fact that a great deal of O₃ reactivity is exhausted by plasma components and does not harm blood cells is consistent with the data: using up to 80 µg/mL O₃ per gram of blood, methaemoglobin is undetectable and haemolysis is no higher than 1.0% and 1.5% when blood is anticoagulated with citrate phosphate dextrose and heparin, respectively.

Comparatively, only slightly lower values are obtained when mixing blood with O₂ under the same conditions, probably because old erythrocytes are sensitive to mechanical stress.⁷⁰ However, when blood is exposed to O₃ concentrations between 100 and 250 µg/mL (2.1–5.2 mmol/L) per gram of blood, haemolysis increases progressively up to approximately 34%.² As an index of peroxidation, thiobarbituric acid reactive substances increase progressively with the O₃ dose, and at 80 µg/mL may become six- to eightfold

higher than base values. Interestingly, TBARS have been detected only in plasma and are unmeasurable in isolated erythrocytes after ozonation of whole blood, suggesting that these cells have not reacted with O_3 because they are shielded by albumin molecules.⁷¹

Evaluation of total antioxidant status^{72,73} and protein thiol groups^{73,74} in plasma showed, at worst, a mean decrease of 20% and 18%, respectively. Intra-erythrocytic GSH decreased by no more than 15% and 12% when blood was exposed to either O_2-O_3 (80 $\mu\text{g/mL}$) or O_3 alone.⁷⁵ Erythrocyte GSH reductase, GSH-Px, SOD, CAT and G-6-PD levels did not vary during blood ozonation, even when blood reinfusion was delayed for 35 min.² That an O_3 concentration as high as 80 $\mu\text{g/mL}$ per gram of blood could exert such a modest and reversible depletion of antioxidants without cell damage was an unexpected finding.

The evaluation of biochemical parameters in blood exposed, almost stoichiometrically, to increasing O_3 concentration ($\mu\text{g/mL}$ per gram of blood) has been instrumental in determining biological effect and/or possible toxicity, and it is evident that for decades ozone therapy failed to progress because it was used by untrained practitioners who were unable to critically analyse the results.

Three interesting points have emerged with ozonation of blood: (i) H_2O_2 is generated and although not a free radical it has been included among ROS and has a relatively long half-life;^{2,3} (ii) H_2O_2 levels depend upon O_3 concentration and result from a dynamic equilibrium during its formation, diffusion into intracellular water and degradation; and (iii) intracytoplasmic H_2O_2 , although transient, can activate biochemical and immunological pathways.^{2,76-78} After ozonation (80 $\mu\text{g/mL}$) of human plasma, levels of H_2O_2 up to 28 $\mu\text{mol/L}$ have been measured consistently that decline with an average half-life of 2.5 min.⁷⁹ In contrast, H_2O_2 disappears very rapidly in whole blood, even in the presence of CAT inhibitors, owing to a powerful combination of antioxidants.

These considerations help to put the problem of O_3 toxicity into the right perspective. We must bear in mind that endogenous ROS are produced throughout life, in several different cell types, during mitochondrial electron transport, metabolism of peroxisomal fatty acids, cytochrome P-450 reactions in the presence of xenobiotics, and in the respiratory burst activity of phagocytes. Therefore, vital cell structures are literally besieged by the continual production of ROS, and sooner or later, despite the presence of the antioxidant system, a shift in favour of oxidation appears unavoidable. Therefore, as O_3 is one of the strongest oxidants, it seems foolish to propose its use as a therapeutic modality. However, the concept that the drug has an intrinsic toxicity is accepted and the beneficial effects must be carefully weighed against toxicity. Moreover,

it is now clear that ROS from O_3 are generated and mostly quenched in the plasma; therefore, this exogenous oxidant is not as dangerous as endogenous ones. A second substantial difference is that in using an O_3 dose no higher than 80 $\mu\text{g/mL}$ per gram of blood, the calculated oxidative stress induced is transient. During a whole therapeutic cycle that may last up to six months (two AHT sessions weekly), up to 14.4 kg of blood are exposed briefly to approximately 0.9 g O_3 , and Halliwell⁸⁰ has estimated that a 70 kg human at rest produces no less than 5 g $\cdot O_2$ per day.

The problem of mutagenicity has been discussed extensively elsewhere.² With regard to ozonated blood, no risks have been demonstrated, provided that O_3 concentration is no higher than 80 $\mu\text{g/mL}$ per gram of blood. There is no evidence that O_3 AHT produces acute or chronic side effects, even after 60 sessions, and most patients report a feeling of well-being.^{2,42} As yet, we do not know if this is because of improved oxygenation and metabolism, real hormonal responses evoked during the reinfusion of ozonated blood, or a psychological factor.

In Germany, Jacobs⁸¹ analysed side effects occurring in over five million ozone therapy sessions in 384 775 patients. Technical errors accounted for minor problems (blood extravasation from the venous access, transient tremor of the lips, occasional nausea) in a minute percentage (0.0007%) of patients — one of the lowest in alternative medicine. However, four deaths occurred as a result of lung embolism following direct intravenous injection of O_2-O_3 — a technique now prohibited. Because of blatant malpractice, two deaths due to lung embolism were registered in Italy in 1997 and 1998.

Which are the effector molecules and which are the targets?

Theories that O_3 is capable of transferring a vital energy to the blood remain in the realm of fiction. Fig. 1 indicates how O_3 works. The sudden increase of intracytoplasmic H_2O_2 appears crucial for the activation of the hexose monophosphate shunt, with important implication for the function of erythrocytes and O_2 delivery.² Moreover, in line with current thinking,⁸²⁻⁸⁴ a sudden surge of intracytoplasmic H_2O_2 is responsible for the activation of the nuclear transcription factor (NF κ B), first identified by Sen and Baltimore.⁸⁵

In resting lymphocytes, the heterodimer (comprising one 50 kDa [p50] and one 65 kDa [p65] polypeptide) is complexed with the inhibitor protein moieties I- κ B. In simple terms, a protein kinase, activated in the presence of H_2O_2 , phosphorylates the I- κ B subunit and releases the heterodimer to move into the nucleus where, after binding to DNA control elements, it activates gene expression and cytokine synthesis. The

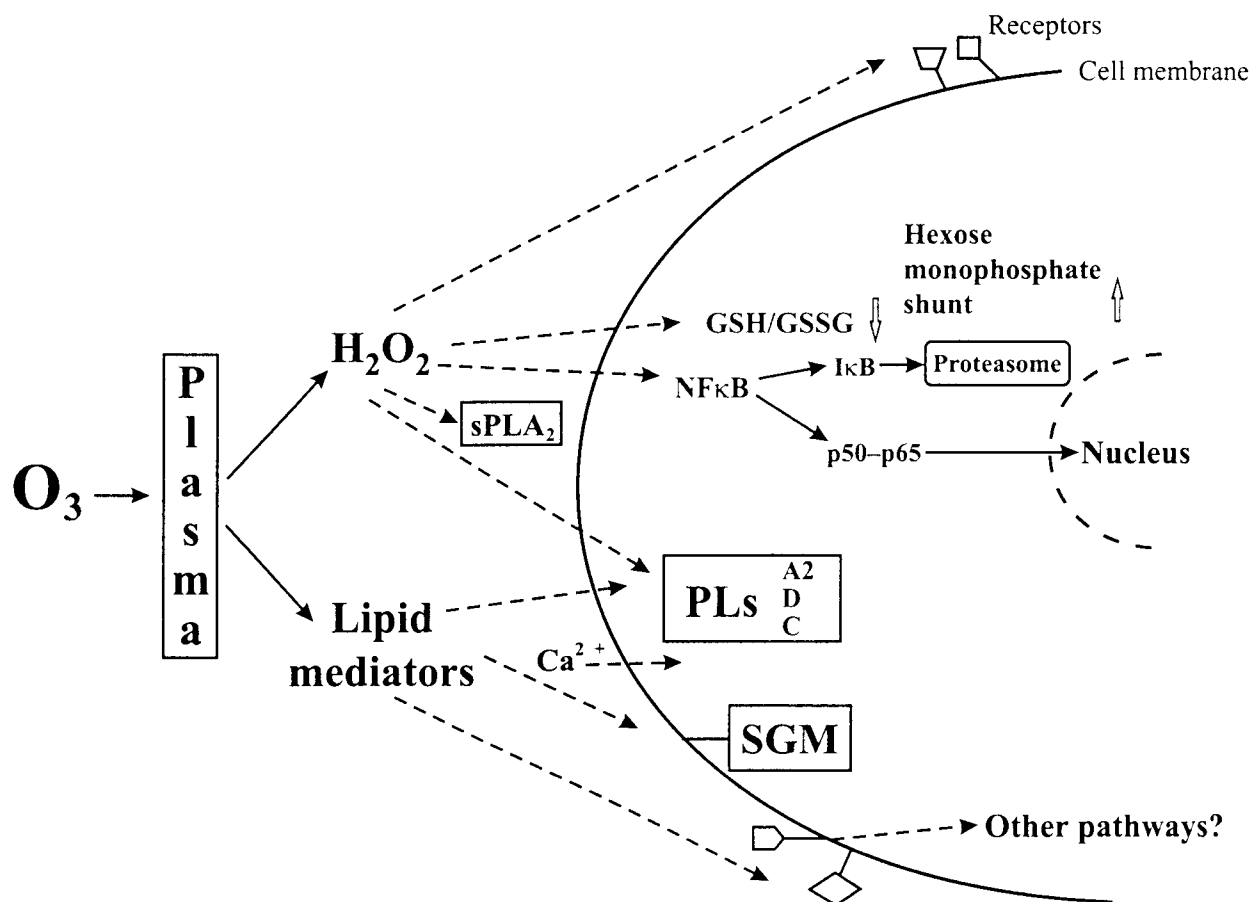


Fig. 1. Hypothetical sites and mechanisms of action of mediators generated from the reaction between O_3 and plasma. The aldehyde 4-hydroxynonenal favours the influx of Ca^{2+} , which in turn enhances intracellular events. GSSG, oxidised glutathione; NF κ B: nuclear factor κ B; PLs, phospholipases; SGM, sphingomyelinase; sPLA $_2$, soluble phospholipase A $_2$.

transient rise of intracytoplasmic H_2O_2 prompts a few considerations. First, O_3 concentration must be adequate to allow sufficient H_2O_2 generation to activate transducer molecules and to counteract the simultaneous degradation. Second, H_2O_2 concentration must reach a critical threshold; below this no stimulation will occur, but, if the concentration is excessive, oxidative damage may result. Therefore, a therapeutic window must be identified.

In the European working population, the mean total antioxidant status is between 1.28 and 1.83 mmol/L plasma,⁸⁶ and, owing to the individual variability of the antioxidant system, useful O_3 concentrations range from 20–80 μ g/mL (0.42–1.66 mmol/L) per gram of blood. This is in perfect agreement with Ueno *et al.*,⁶⁸ who, using another method, found that 'less than 100 μ g/mL O_3 was completely consumed by 1 mL of blood by 30 sec mixing.' Although not a practical proposition, it would be ideal to evaluate the optimal O_3 concentration for each patient. Below 20 μ g/mL

most of the oxidant power of O_3 is quenched by natural antioxidants and, therefore, precise measurement of O_3 concentration is crucially important in avoiding either a placebo or toxic effect.

In reacting with PUFAs,^{3,87} O_3 generates an array of lipid oxidation products (LOPs) including hydroperoxides, isoprostanes,⁸⁸ and terminal products such as malondialdehyde and 4-hydroxynonenal. These terminal products are of particular interest because, depending upon final concentration (>10 μ mol/L or <1 μ mol/L), they may be either harmful⁸⁹ or act as a physiological messenger.^{90,91} Owing to the wealth and heterogeneity of PUFAs, several types of LOPs may be generated and their biological activities, including potential toxicity, are unknown areas that need to be explored *in vivo*.

Phospholipases and sphingomyelinase (Fig. 1) are likely to be activated by LOPs, and this may lead to an amplification of some biological processes such as enhanced Ca^{2+} flux and further production of active

LOPs. Furthermore, LOPs have a short half-life but, upon reinfusion of ozonated blood, may reach specific sensors situated in critical organs such as bone marrow, spleen, liver and other sectors of the immune system. If true, LOPs may be responsible for transmitting⁹² peroxidative stress information and possibly inducing up-regulation of antioxidant enzymes, hence the tolerance to O₃. This interesting phenomenon, termed oxidative stress adaptation,^{2,42} has an important practical implication in the sense that judicious use of O₃AHT may reverse chronic oxidative stress syndrome in patients with chronic viral infections,⁹³ cancer^{43,94} and neurodegenerative diseases.⁹⁵

Administration of various antioxidants may only partly correct chronic oxidative stress, and the exciting possibility is to reverse it by inducing O₃ tolerance. This is a complex phenomenon, including a number of events represented by changes in gene expression,³⁸ followed by synthesis of heat shock proteins (HSPs) involved in adaptation to stress. When exposed to low levels of a toxic agent, living organisms may respond by apoptosis or become resistant by activating inducible genes. The latter is carried out by synthesising a variety of HSPs, several antioxidant enzymes, haem oxygenase and DNA repair enzymes. In this way, organisms may readjust the redox balance.⁴² Obviously, the calculated therapeutic stress of ozone therapy must continue, but its length of effectiveness remains to be determined during long follow-up of patients.

Leucocytes appear to be primed and able to release small amounts of cytokines, such as interferon β and γ , and several ILs.^{73,75-79,96,97} On reaching the lymphoid microenvironments, cytokines and LOPs may modulate immune cells and possibly correct an immune deficiency. However, a great deal of experimental study remains to be done to assess the full extent of immunological activation and the role of O₃AHT in the treatment of chronic viral infection.

It would appear that O₃AHT simultaneously triggers a range of biological effects that work together in hypoxic tissues, where, for example, vasodilation, enhanced O₂ delivery and release of growth factors accelerate healing of chronic ulcers.⁹⁸

Finally, the critical problem of the O₃ dose in different pathological conditions needs to be addressed.

The problem of ozone dose and the application of the 'start low, go slow' principle

The question of O₃ dose is one of the most frequently debated and it appears useful to give a guideline. On the basis of present knowledge, the therapeutic window range is 20–80 $\mu\text{g/mL}$ of O₃ per gram of blood. Within this range, toxicity is minimal or absent, even

if the total antioxidant status of plasma is as low as 1.2 mmol/L. As yet, it is impossible to provide specific doses for individual pathologies because ample and controlled clinical studies have yet to be performed. However, on the basis of biochemical and empirical results, it is possible to make suggestions (Table 1).

Table 1. Ozone doses used in autohaemotherapy

Pathology	O ₃ doses ($\mu\text{g/mL}$ per gram of blood)	
	Initial	Final
Vascular disease	20	40
Degenerative disease	20	40
Infectious disease	25	70
Respiratory disease	20	40
Autoimmune disease	20	?
Metastatic cancer	25	80

In order to avoid toxicity and allow oxidative stress adaptation, the safest strategy starts with very low doses, increasing in single steps of 5 $\mu\text{g/mL}$ per gram of blood to the highest level. As treatments are performed on a day-hospital basis, O₃AHT twice weekly is practical and sufficient to achieve a clinical response. If necessary, this can be increased up to four times weekly, allowing adaptation during the first three weeks.

In elderly patients who are undernourished or not on a proper diet, a multivitamin complex can be administered orally on the day before O₃AHT. Normally, a daily dose of vitamin C (0.5 g), supplemented with *N*-acetylcysteine (0.6 g) as a precursor of GSH is optimal. Larger amounts may prove useless or even have a detrimental effect.⁹⁹

Conclusions

The complementary approach of ozone therapy has been used for at least four decades, but objective discussion between those for and against it has yet to begin. After previous attempts,^{2,100,101} it is hoped that this review will succeed in opening constructive dialogue.

There is no doubt about the potential toxicity of O₃ and the harmful effects of acute and chronic exposure on the respiratory tract. Moreover, direct IV administration of O₃ should *never* be performed, as it is both irrational and dangerous. On the other hand, experimental data have shown that transient *ex vivo* exposure of blood to controlled and appropriate O₃ concentrations is not toxic to humans.

Recent advances in the understanding of signal transduction have shown that minute amounts of oxidants, gaseous regulators and even 4-hydroxynonenal

act as cell messengers, and therefore the appropriate use of O_3 in medicine no longer appears irrational.

I have come to regard O_3 as a real drug and, as such, it must be used with the utmost care — too little may be useless and too much can be harmful. The relevance of a placebo effect, either due to O_2 or too little O_3 , also needs to be clarified. Equally important is the definition of its clinical efficacy and when ozone therapy can be used. A typical example is the treatment of chronic hepatitis in elderly patients who are intolerant of interferon α — a costly substance which often produces side effects. This last point needs to be considered in relation to other pathological conditions, notwithstanding the necessity for careful monitoring of possible, as yet undetected, problems.

Although much remains to be done, ozone therapy is now amenable to scientific scrutiny and attention should be concentrated on the following points:

- accurate dosimetry of O_3 concentration;
- standardisation of the procedure for O_3 AHT;
- further understanding of biological effects, particularly oxidative stress adaptation;
- evaluation of any possible long-term toxicity;
- definition of optimal O_3 dose in different pathological conditions;
- randomised, double-blind clinical studies using either O_2 - O_3 or O_2 alone versus conventional treatments, with assessment of long-term follow up; and
- evaluation of novel approaches and routes of O_3 administration.

In the age of molecular medicine it is a real 'act of faith' to believe that ozone therapy might be a valid therapeutic option, but the history of medicine teaches us that we should not disregard any possibility. While strongly disapproving of the misuse of O_3 and the deplorable exploitation of patients, to remain sceptical and inert does nothing to help solve this problem.

This work has been partly supported by local (60%) and national (40%) MURST funds. The help of Mrs Helen Carter and Mrs Patrizia Marrocchesi in preparing the manuscript has been invaluable.

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