Chapter 5

General Aspects of Ozone Therapy

Ruhi Cakir

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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.

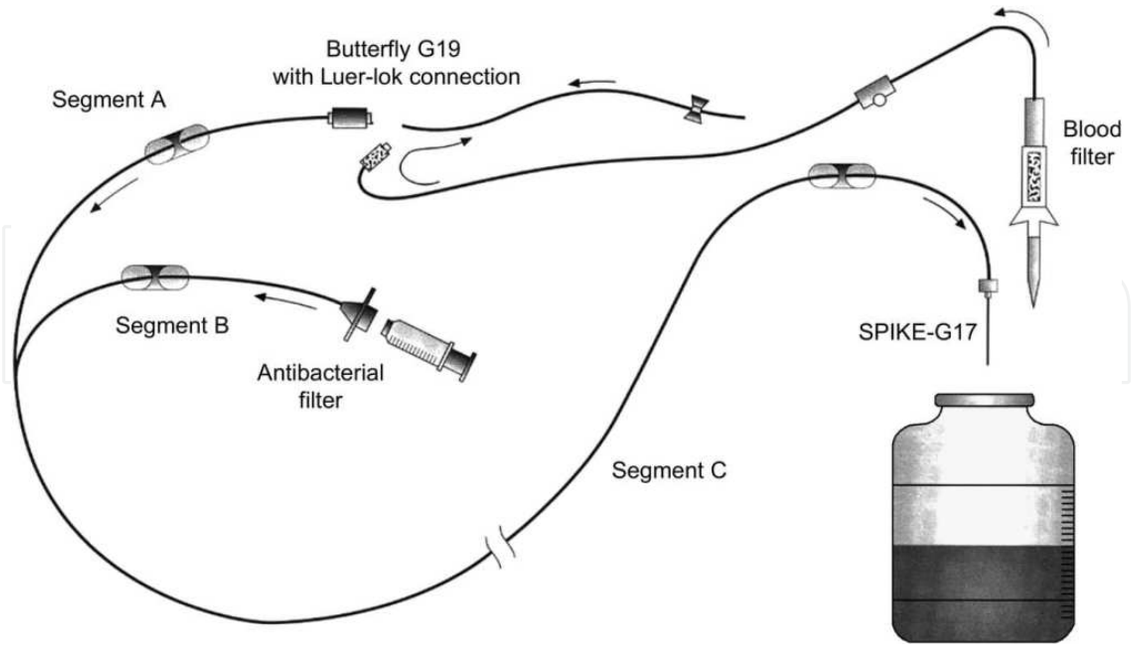

Figure 1. Schematic drawing of the components necessary to perform major autohematherapy

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.

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

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)

$$O_3 + \text{biomolecules} \rightarrow O_2 + O, \tag{2}$$

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:

$$R-CH=CH-R + O_3 + H_2O \rightarrow R-CH=O + R-CH=O + H_2O_2 \tag{3}$$

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

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.

Author details

Ruhi Cakir

Mediozon Clinics, Istanbul, Turkey

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