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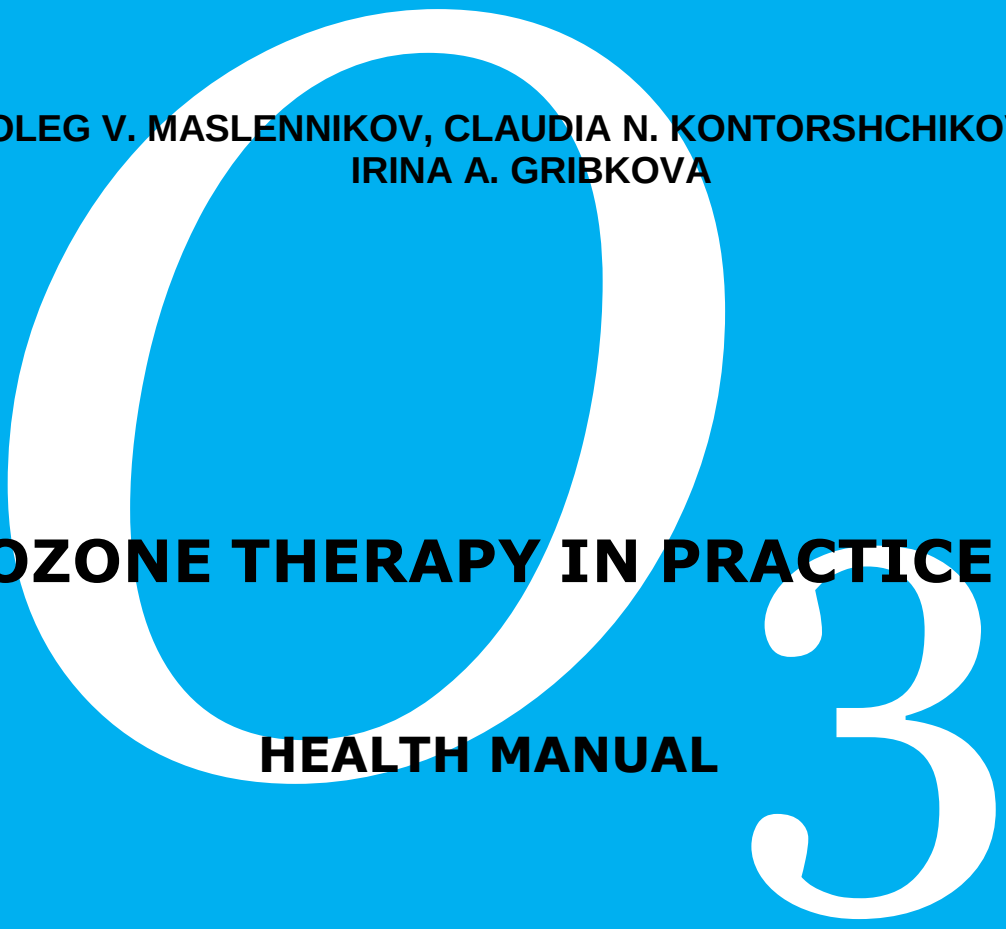

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OZONE THERAPY IN PRACTICE

HEALTH MANUAL

Nizhny Novgorod, Russia
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Ozone therapy in Practice. Health Manual. - Nizhny Novgorod

The Health Manual offers a variety of information on recognized methods and techniques for clinical application of ozone therapy in various pathologies. It briefs the readers on ozone properties and therapeutic effect of ozone-oxygen mixtures administered in different diseases, including the dosage and schemes of treatment that are used by Russian doctors.

Ozone therapy in Practice is a practical guide for doctors of various specialities, who use medical ozone in their practice, as well as for interns, residents in medicine and senior medical students

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INTRODUCTION

Recent years have been marked by increasing number of people susceptible to allergic diseases and weakened response to antibiotics. In these conditions and regarding the constantly growing prices for medicinal preparations the appearance of new non-medication methods can be only welcomed and appreciated.

Among these new methods ozone therapy has been gaining a justified recognition in many countries of the world thanks to ozone disinfective effect and its capacity to transport and release oxygen into tissues The properties of medical ozone have been used in therapy, surgery, obstetrics and gynecology, dermatology, stomatology, in infectious and veneral diseases.

Ozone therapy has found its way into medical practice in Germany, where it is successfully used. Germany was the first country to start manufacturing medical ozonators and to use ozone-oxygen mixtures in vascular surgery, stomatology and geriatrics. Italian ozone-therapists have focused their activity on medical cosmetology. Specialized ozone therapeutic clinics operate in Switzerland and other countries of West Europe. Cuba is well-known for its Ozone Research Center. Interesting and promising results with the help of medical ozone have been received in some clinics of the USA, Mexico, Brazil and Japan.

Ozone therapy was found to be efficient, easy to use, ensuring good tolerance and no side-effects.

According to the chosen therapeutic concentration ozone can produce its immune-modulating, anti-inflammatory, bactericidal, virucidal, fungicidal, analgesic and other effects.

Medical ozone proves to be of great therapeutic potential for in numerous cases it exceeds the resources of medication-based methods. The procedures of its application are simple, economically preferable and beneficial. However, medical communities and practical health service still prefer not to notice the available convincing facts and evidences that might bring it into wide practice. By now, there have been accumulated quite enough experimental and clinical findings that make it possible to present the routs of ozone therapy application for effective and safe management of patients with various pathologies.

FUNDAMENTALS OF OZONE THERAPY

OZONE AS IT IS

Ozone is known to be one of the most important gases in the stratosphere. It acts as a screen for ultraviolet radiation of short wavelength of 260-280 nm, thus protecting the living organisms on earth and absorbing infra-red radiation coming from the earth and, hence, preventing its cooling.

Protective ozone layer with its maximal width not exceeding 2-3 mm and ozone concentration of $1mg/m^3$ is found about 20-30km above the surface of the earth. Diminution of ozone concentration in ozonosphere results in its depletion and developing of ozone holes. At the same time in the lower atmosphere under the influence of ultraviolet rays and in the presence of atmospheric oxygen ozone is generated from different components of the smog. Ozone concentration is used to estimate the intensity of industrious smog and air pollution. In contrast to ozone, generated from medical oxygen, ozone in the polluted air together with toxic nitrogen oxides produces harmful effect on the epithelium of the respiratory track.

Ozone is highly reactive chemical element. It is constantly formed as a colourless gas at about 20-50 km above the surface of the earth under the influence of vacuum ultraviolet (UV) light from atmospheric oxygen:

$$3O_2 \rightarrow 2O_3$$

And on the contrary, a molecule of ozone can absorb a particle of UV so producing diatomic oxygen again. Ozone molecules dissociate into their constituent atoms These atoms combine with oxygen molecules to form ozone and as a result of this process ozone layer – ozonosphere is formed, which acts as a shield from the sun's ultraviolet radiation, maintaining the biological balance in biosphere. Insignificant amount of ozone due to turbulent flows reaches the lower layers of atmosphere so that it can be traced by a characteristic smell after a thunderstorm.

Ozone - O_3 is a reactive allotrope of oxygen. Oxygen can be present in one of its forms:

1. Monatomic oxygen – highly reactive and unstable form, for it has two free covalent bonds – (O).
2. Diatomic oxygen – the most abundant and stable form, for it has not no free bonds (O-O)
3. Ozone – a molecule formed from three atoms of oxygen, having a free bond resulting in its high reactivity.

Ozone appears to be a more powerful oxidant compared with oxygen for it can oxidize practically all organic and non-organic compounds.

Regarding bioorganic compounds, ozone was found to have a selective reactivity with double bonds compounds: amino acids, peptides, proteins, nucleic acids, and primarily, unsaturated fatty acids, making up the basis for lipid bio layer of cellular membranes and lipoprotein complexes of blood plasma. In biological media ozone reactions with unsaturated fatty acids are dominating and are accompanied by formation of various products, including ozonides (compounds, containing various number of oxygen atoms in a molecule).

DISCOVERY OF OZONE AND OZONE PROPERTIES

Ozone as a chemical element was discovered at the end of XVIII century. In 1785 Martinus Van Marum, a Dutch physicist, subjecting oxygen to electrical discharges noted some specific "odor of electrical matter". In 1848 C.Schonbein, a German physicist, having repeated the experiments, named the smelling gas Ozone (from the Greek "ozone"-odorant) and described some of its properties. He thought it to belong to the same class of bromine and chlorine and to have negative electric charge. Mariniak and Delarive showed that it is an allotropic form of oxygen and Mulliken and Dewar clarified its molecular structure) A century later, in 1953 Andrews reported on ozone being an allotropic form of oxygen. In 1957 Warner created tubes for magnetic induction, capable to produce ozone in large quantities and that launched an extensive research of ozone properties.

The use of ozone is based on its oxidative, disinfective and bactericidal properties. Ozone inactivates bacteria and fungi in a much less time compared with chlorine. It is also very effective in destroying viruses and carcinogenic agents, which in most cases are not killed by usual chemicals used for water purification. Being able to destruct smell-creating substances, ozone came to be used as deodorant agent.

For water purification ozone has been used for about 80 years. In Paris they use ozone-treated water. With its high oxidative potential ozone is used in textile and cellulose industry as a bleaching means. Ozone bactericidal properties are successfully used in breeding oysters and mollusks as well as in mineral water industry.

The history of medical ozone starts in the XX century. The pioneers to apply ozone in clinical practice were E. Payer, A. Fish and H. Wolf. Ozone as an antiseptic means had been known and used from the beginning of the XX century, however, extensive and systemic research in the field of ozone therapy, and primarily in Germany, started in mid 70s, when ozone-resistant polymer materials and convenient ozone generating equipment came into every day clinical practice.

The interest to ozone therapy was growing with the growing number of reports coming from different clinics the world over on biological ozone effect and its successful use in the treatment of various diseases.

At present time the International Ozone Association(I.O.A.), incorporated in the United States of America, holds its World Congresses every second year in various parts of the world. Within the (I.O.A.) framework physicians from different countries and of different specialities share their results and achievements through conferences, workshops, symposia proceedings and other public information media.

In Europe different companies manufacture different ozone generators meeting the needs of practical doctors.

Profound research work is carried on in Russia under supervision of two main schools of ozone therapy with their Research Centers and Clinics in Moscow and Nizhny Novgorod.

CLINICAL EFFECTS OF OZONE THERAPY

Ozone can produce different effect according to the chosen concentration and the way of its administration. In medical practice the most important are the following ones.

1. Bactericidal, fungicidal and virucidal

When applied externally in a form of gaseous mixture or in ozonated solution it is recommended to use high ozone concentrations which produce direct oxidative effect on the

microorganism membrane. Ozone can destroy practically all kinds of bacteria, viruses, fungi and protozoa. Gram-positive bacteria and capsular viruses having a lipid bio-layer are particularly sensitive to oxidation.

The use of therapeutic ozone concentrations provides bactericidal effect which indirectly activates the non-specific defense system (phagocytosis activation, enhanced synthesis of cytokines-interferons, interleukin tumor necrotic factor) as well as components of cellular and humoral immunity.

There have been reported evidences of partial oxidation of virus receptors that makes them incapable to virus binding.

Besides there was revealed inhibition of reverse trascriptase enzyme which promotes AIDS virus destruction.

2. Anti-inflammatory effect is revealed in ozone capacity to oxidize the compounds containing double bonds, the arachidonic acid (20:4) and its derivatives - prostaglandins, in particular. These biologically active substances participate in the development and sustaining the inflammatory process. Besides, ozone regulates metabolic reactions in tissues at the place of inflammation and resolves pH.

Ozone therapy efficiency in bronchial asthma can be partially explained by oxidation of double bonds in such pathological compounds as leukotriens also derived from arachidonic acid.

3. Ozone analgesic effect is provided by oxidation of the products of albuminolysis, the so-called algopeptides. They act on the nerve endings in the damaged tissue and determine the intensity of pain response. To add to that,

analgesic effect is also caused by normalization of antioxidant system and accordingly, by the decrease in the amount of toxic molecular products of lipid peroxidation on cellular membranes, that modify the function of membrane-inbuilt enzymes, which participate in ATP synthesis and in maintaining the vital activity of organs and tissues

4. Detoxication effect of ozone is revealed in correction and activation of metabolic processes in the hepatic and renal tissues, thus ensuring their main function of neutralization and evacuation of the toxic compounds from the organs.

5. Activation of oxygen-dependent processes. Ozone doses, however low they are, cause the increase in the content of free and dissolved blood oxygen with rapid intensification of enzymes that catalyze aerobic oxidation of carbohydrates, lipids and proteins with formation of ATP energy substrate. Of great importance is the mitochondrion activation of H-ATP-ase, responsible for conjugation of respiratory processes and oxidative phosphorylation, resulting in ATP synthesis.

6. Optimization of pro- and anti-oxidant systems is regarded as one of the main effects of systemic ozone therapy which is realized through its influence on cellular membranes and brining to balance the levels of lipid peroxidation products and of antioxidant defense system. In response to ozone there occurs the compensatory increase in the activity of antioxidant enzymes -superoxidismutase (SOD), catalase and glutatioperoxidase. Due to restored aerobic metabolic reactions there is the accumulation of NADH2 and NADPH2 which function as proton donors to restore the oxidized components of non-enzymic antioxidant system (glutathione, vitamin E, ascorbic acid, etc.). The use of exogenic antioxidants with preliminary calculated dose is obligatory when high ozone concentrations are used.

7. Ozone haemostatic effect depends on the dose. High concentrations administered for external use cause evident hyper coagulation effect, while parenteral administration of low concentrations is characterized by the decrease in thrombocytic and coagulative levels of hemostasis and increase in fibrinilytic activity.

8. Ozone immune-modulating effect is based on its interaction with lipid structures of cellular membranes and depends on the chosen dose. Low ozone concentrations promote the accumulation of ozonides on the membranes of phagocytic cells –monocytes and macrophages. Due to ozonides these cells stimulate the cytokines synthesis of different classes. Cytokines being biologically active peptides, contribute to the further activation of non-specific defense system (elevation of body temperature, generation of acute-phase peptides in the liver) and, apart from it, they activate cellular and humoral immunity. All together they facilitate the treatment of secondary immune-deficiency.

Ozone high concentrations produce aggravating effect on the processes of lipid peroxidation in cellular membrane of the same phagocytic cells with the accumulation of the toxic and hard products of lipid peroxidation (malon dealdehyde and Shiff bases), which inhibit cytokines synthesis and thus eliminate the activation of T-helpers lymphocytes, aimed at regulation of immune globulin generation by B-lymphocytes. This effect is used in the management of patients with auto-immune pathology (rheumatoid disease, disseminated sclerosis, sclerodermia) without the administration of drug treatment.

METHODS OF OZONE THERAPEUTIC EFFECT

Topical ozone therapy is the very fist method of applying ozone in the history of medical practice. It should be noted that high concentrations are used for disinfection, while low concentrations promote epithelialization and healing

External administration includes

- The use of ozonated antiseptic salines
- Application of ozonated ointments and ozonated vegetable oil
- Ozone aerated plastic bags under lowered pressure(Gas bags)
- Balneotherapy

Parenteral methods include:

- Major and Minor autohemotherapy with ozonized blood
- extracorporal plasma and lymph treatment
- subcutaneous ozone injections into biologically active points
- paravertebral intramuscular injections
- intravenous infusions of ozonated physiological solution

The method of intravenous infusions of ozonated physiological solution is the priority of the Nizhny Novgorod School of Ozone therapy. It was developed and clinically tested in the Central Research Laboratory of the Medical Academy of Nizhny Novgorod by Sergey P.Peretyagin and Claudia N. Kontorschikova. The method was recognized as safe and easy to use.

In 1997 Professor Sergey D. Razumovsky, a well-known chemist, completed the investigation of ozone effect on standard isotonic physiologic solution.

It was found that with the use of ozone neither chemical reactions take place in the solution, nor new compounds (type of chlorine active derivatives) are accumulated.

Enteral method of ozone oxygen mixture is recommended in gastro-intestinal pathology. It includes

- intake of ozonated distillated water per os;
- intestinal irrigation with ozonated distilled water
- rectal insufflations with ozone oxygen mixtures

In numerous experimental studies in vitro and in vivo we defined the optimal tachometric blood-gaseous ozone ratio, where powerful ozone oxidative effect is reduced to its minimum, while its metabolic effects are significant. (5 to 40mcg of Ozone per 1 liter of blood). The suggested dosage does not exceed the summary antioxidant potential of the body and its use does not cause any negative reactions (Patent*№ 2020945* of 1986. Authors: Claudia N. Kontorschikova, Sergey P. Peretyagin, Gennady A. Boyarinov)

GENERAL QUESTIONS

FORMS AND METHODS TO USE OZONATED MATERIALS

Ozone therapy is used in a form of parenteral and enteral administration of ozone/oxygen mixtures, aeration in closed volumes (gas bags) and applications with ozonated materials. Clinical trials reported very few cases of side effects or complications when the procedures were done properly. Of primary importance is the dose of the administered ozone, which must not exceed the potential of antioxidant enzymes. This requirement is to be observed to prevent the surplus of active forms of oxygen.

This book presents the schemes of treatment with the doses selected for the pathologies treated at the Medical Center of the Department for New Medical Technologies at the Medical Academy of Nizhny Novgorod. The doses were chosen on the basis of experimental and clinical trials done at the Academy and regarding the available published reports.

Ozonated Distilled Water

Water barbotage with ozone/oxygen mixture is done in glass bottles with the concentration of 5mg/l (O_3/O_2), duration depending on the volume of water to be ozonized(3 liters – 30 min., 5 liters – 46 min., 10 liters –60 min.). Ozonated water is widely used in surgery and gynecology for irrigations and lavage. In gastroenterology it is administered per os as a drinking water in esophagitis, gastritis and ulcers. In colitis it is used for clyster procedures. In stomatological practice ozonated water is administered in a form of gargarism (mouthwash) as a disinfection of oral cavity in paradontosis, stomatitis, contaminated wounds and suppuration of dental canals. In otolaryngology ozonated water is used for inhalations.

On being barbotaged ozonated water should be used within 30 minutes.

Ozonated Vegetable Oil

Ozone disinfectant properties are well revealed in the use of ozonated vegetable oil. Ozonated oil was found to have antiseptic activity several hundreds higher compared with ozonated saline. It is used for oral administration and in a form of ozonated applications.

For ozonation we use refined vegetable oil (sunflower, olive, maize etc.).The barbotage is done with different concentrations and duration:

- For oral administration 100ml of oil is ozonized for 10 min with ozone concentration of 20mg/l (O_3/O_2), 5 min –ozone concentration 40mg/l (O_3/O_2).
- For external use 100ml of oil is ozonized for 15 min with ozone concentration of 20mg/l (O_3/O_2), 30 min –ozone concentration 10mg/l (O_3/O_2).
- For external administration in mycosis 100ml of oil is ozonated for 15 min with ozone concentration of 24mg/l (O_3/O_2), 8 min –ozone concentration 50mg/l (O_3/O_2).

Ozonated oil should be kept in a dark glass bottle. According to the latest data it can preserve its properties for 4 month when kept in room temperature, when kept in refrigerator it can be used for 2 years.

When administered for oral use, it should be started with one teaspoonful 20-30min before meals 2-4 times a day, gradually increasing the dose to one tablespoonful 2-4 times a day.

Ozonated Saline for Intravenous Infusions

Ozonated saline is administered to solve different clinical tasks by using different concentrations ranging from 400 to 100000mcg/l of ozone/oxygen mixtures at the outlet of ozone generator.

To achieve general stimulating metabolic effect we use the method of simultaneous saturation and intravenous infusion of physiological saline. Ozone concentration is calculated by of 40mcg per kg of body weight, e.g. patient's weight is 80 kg, so ozone concentration is 40x80=3200mcg at the outlet of ozone generator.

Physiologic saline saturation can be achieved in the following way. Ozone/oxygen mixture is passed through a 200 ml flask for 10 minutes and then the saline is intravenously infused by drops for 15-30 minutes.

Introduction of Ozone/Oxygen Mixture In Gaseous Phase

This method provides analgetic, anti-inflammatory and stimulating effect.

Subcutaneous and intracutaneous injections are done into painful and acupuncture points, 1ml per each; for microinjections of focal lesions we use 5-10ml with ozone concentration - 10mg/l. In peri-articular microinjections we use 1-3ml with the concentration-10-15mg/l

Intramuscular injections are done with 10-20ml, concentration being 10-15 mg/l.

Intra-articular injections are done with the concentration- 15mg/l and the volume of

- 1-1,5ml for minor joints
- 5-7ml for middle joints
- 20ml for major joints

Rectal Insufflations With Ozone/Oxygen Mixture.

The procedures are done with Janet syringe or with a help of special poly-chlor-vinil tube with a patient lying on the left side with knees bent. Purgative enema is to be done two hours before the procedure. Rectal insufflations are done with ozone concentration in ozone/oxygen mixture of 10-60mg/l, the volume ranges from 150ml to 1000ml, depending on the pathology, its course and stage. For newborns the volume is 20-50ml, for children –50-100ml (H. Dorstewitz, 1990).

Intestinal insufflations can be administered, first of all, as anti-inflammatory and disinfectant remedy to restore the bacterial flora misbalanced by pathogenic microorganisms. Secondly, it can be administered as an alternative to major autohemotherapy, because ozone/oxygen mixture on being instantly absorbed produces general metabolic effect. This procedure can be turned to in those cases when intravenous injections are difficult to handle

The usual therapeutic dose to produce metabolic effect is 75mcg per 1kg of patient's weight, e.g. for a patient of 80kg the ozone dose is to be 75 x 80= 6000mcg. The course of treatment is to be started with a half-dose and minimal volume of ozone/oxygen mixture (150-200ml) which is gradually increased to the required one.

Vaginal Insufflations with Ozone/Oxygen Mixtures

Vaginal insufflations are done with ozone concentration 0f 2-2,5mg/l in ozone/oxygen mixtures with the gas rate – 0, 5-1l/min for 5-10 minutes. The procedures are done with

special nozzles put on the vaginal speculum. Vacuum suction is obligatory to prevent ozone inhalation effect on the patient and the doctor. (Гречканев Г.О. с соавт. 2000).

Minor Autohemotherapy with Ozone/Oxygen Mixtures

MAHT is used to produce a stimulating effect in conditions with immune deficiency. The procedure is simple and easy to perform. Venous blood (5-10ml) on being taken into a 20ml syringe with 10-15 ml of ozone/oxygen mixture, ozone concentration –10-40mg/l, and carefully mixed, is then injected intramuscularly.

Major Autohemotherapy with Ozone/Oxygen Mixtures

For Major Autohemotherapy we use a flask or a special plastic bag with anticoagulant and fill it with 50-150ml of venous blood taken from the patient. The blood upon being mixed with ozone/oxygen mixture, ozone concentration should not exceed 40mg/l (according to V.Bocci, higher ozone concentrations can lead to hemolysis) is returned to the patient via intravenous injection.

According to S.Rilling and R.Vieban instructions (1987), ozone in the doses of 6-10 mg produce immune-suppressive effect and it should be administered in cases of active rheumatism and rheumatic arthritis. In atherosclerotic diseases of cardio-vascular system, in contaminated surgery and in chronic diseases that require immune stimulating treatment the4 recommended ozone dose is 1-3mg, in rare cases 4mg (R.D.Rentschke, 1986).

Ozone doses of 8-9mg are administered in acute stage of infectious hepatitis, which are gradually reduced to2,0-0,8 mg with remittance of the exacerbation (H.Wolf, 1986).The same doses are used in herpetic infection.

Ozone Aerated Plastic Bag

The method has proved to be highly efficient in the treatment of trophic ulcers, purulent sluggish wounds, bed sores, painful cicartices, burns and defects caused by sequestrations of irradiated surficial and subcutaneous tumors

Before the procedure the affected leg is damped with water or saline and then a plastic bag is put on and hermetically sealed. The bag is filled with gas mixture until the excessive pressure is reached. Then a destructor is switched on. The aerating is done for 15-20 minutes.

In patients with vascular diseases when the skin surface is not affected ozone concentration is 6-8ml/l of ozone/oxygen mixture.

In cases of trophic ulcers or purulent wounds the affected area should be covered with a dressing soaked in saline or distilled water. The initial ozone concentration is 5-6mg/l until ulcer becomes detersive. At the stage of granulations the concentration is diminished to 1-1,2mg/l.

CONTRAINDICATIONS TO OZONE THERAPY

1. All cases with Blood Coagulation Failure
2. Bleeding Organs
3. Thrombocytopenia
4. Ozone Allergy

5. Hemorrhagic or Apoplectic Stroke

6. Ozone Intolerance

Note. Ozone in low concentrations is known to produce a moderate hypo-coagulation effect, so all the drugs decreasing blood coagulation (anticoagulants, aspirin, etc.) are to be discontinued during the course of ozone therapy. In women the treatment course is to be broken up for menstrual periods.

OZONE THERAPY IN DIFFERENT PATHOLOGIES

OZONE THERAPY IN SURGERY

Contaminated surgery was one of the first to recognize ozone therapy and it is contaminated surgery where it has been widely used.

General Peritonitis

Diffuse Peritonitis

In the treatment of general peritonitis, including the cases complicated by "intestinal insufficiency" ozone has been used for its powerful bactericidal properties concerning aerobic and anaerobic microorganisms. It was found to be effective in reduction of lipid peroxidation (LP) processes and of antioxidant defense system (AOS), in reparation and immune system stimulation, in intoxication control.

Medical ozone displays its best therapeutic capacities when used in combination with conventional treatment and included into a complex management of a patient

Routes

Ozone therapy during the operation

Intra-operational sanitation of abdominal cavity with ozonated physiological saline

Ozone therapy in postoperative period

Peritoneal lavage with ozonated physiological saline or programmed laparostomy

Intravenous infusions of ozonated physiological saline

Major autohemotherapy

Management

Ozone therapy during the operation

Intraoperational sanitation of abdominal cavity with ozonated physiological saline(volume not less than 5-7 liters, ozone concentration 4-5mg/l) is to be done for 20 minutes on having eliminated the source of peritonitis and small intestine decompression. Laparotomy is to be completed with drainage of the abdominal cavity and adjustment of silicone tubes for lavage to follow.

Ozone therapy in postoperative period

In postoperative period lavage procedures of the abdominal cavity are recommended. The first procedure is done 4-6 hours after the operation for 25-30 minutes with the use of ozonated saline via the tubes adjusted in the upper part of the abdominal cavity. The saline being continuously ozonated is introduced via intravenous infusion drip. The procedure is repeated twice – 4-6 and 8-12 hours later. On the average postoperative lavage is done within the period of 72 hours following the operation.

Programmed laparotomy proved to be an effective method in the treatment of peritonitis. On having the surgical intervention performed, the source of peritonitis eliminated, the sanitation of the abdominal cavity done and small intestine being decompressed (naso-intestinal tube), the abdominal cavity is not to be closed completely with the edges brought together over the

cellophane drape covering the intestines. During the first 24 hours after the operation peritoneal lavage is done every 8 hours with ozonated saline, ozone concentration being 5-6mg/l, the volume - not less then 5liters. The lavage is done till the flushing waters become clean. Prior to bracing of the abdominal wall, the abdominal cavity is filled with 0,5l of ozonated saline. Drainage is done via small pelvis. On the second postoperative day the procedure is done every 12 hours. On the third day the procedure is done only once followed by laparorhaphy.

Intravenous infusions are done with 200ml of ozonated saline once a day during the first two postoperative days, and then every second day. Major autohemotherapy is done within the first 12 hours after the operation and then every second day. The course consists of 2-3 procedures.

Such a course of complex schemes of treatment resulted in earlier control of endotoxicosis events, normalization of biochemical and immunological parameters and less cases of early postoperative complications (from 33% to 14%) with 15% decrease in lethal outcome (Векслер Н.Ю. с соавт. 2000,Семенов С.В. с соавт.,2000, Снигоренко с соавт. 2000)

Warning: Never use ozone/oxygen mixture in gaseous form to treat abdominal cavity, for it can lead to the development of peritoneal adhesions

The use of ozone does not exclude the whole complex of medical procedures to correct homeostasis disorders.

Localized Peritonitis

Routes

- Intra-operational sanitation of the abscess cavity with ozonated physiological saline
- Intravenous infusions of ozonated saline
- Minor autohemotherapy
- Major autohemotherapy

Management

Intra-operation sanitation of the abdominal cavity is done with 0,5-0,8liter of ozonated saline. The lavage is done only in the area of inflammatory process, followed by carefull suction of flushing waters. Biluminal drainage tube is brought to the peritonitis focus and taken out through a special micro-incision. Laparorrhaphy is done up to aponeurosis with retension sutures upon the skin. During the first post-operative day the lavage procedure is done 3 times with 30-50ml of ozonated saline, ozone concentration - 5-6mg/l. After a period of 15-20 minutes the remnants of the saline are either sucked out with a syringe or removed by the natural way.

Intravenous infusions of 200ml of ozonated saline are done one procedure for the first two days and then every second day. The course consists of 4-5 procedures. Two procedures of minor autohemotherapy are done every second day and major autohemotherapy – 1-2 procedures a week. In cases when peritonitis is progressing relaparotomy is performed, followed by sanitation and one of the recommended techniques of intra-operation and post-operation abdominal sanitation

Contaminated Wounds of Soft Tissues

Optimal results can be achieved in a complex treatment of contaminated wounds when alongside with major autohemotherapy or intravenous infusions of ozonated saline and minor autohemotherapy, the wound is ozonized with wet tampon and then the limb is put into a plastic bag (Родоман Г.В. с соавт., 2000).

Routes

- Major autohemotherapy
- Intravenous infusions with ozonated saline(or rectal insufflations with ozone/oxygen gaseous mixtures)
- Minor autohemotherapy
- Wound aeration with ozone/oxygen mixture in a plastic bag under high or low pressure
- Wound treatment with a stream of ozone/oxygen mixture under the sphere
- Stimulation of biological active points in wounds of low extremities
- Sterile dressings with ozonated oil

Management

In the course of treatment the phase of wound process should be taken into consideration. The treatment should be a complex one and include all forms of ozone therapy.

Wound aeration with ozone/oxygen mixture

The procedure starts with mechanical (sparing) wound cleansing from detritus, then a drape soaked in ozonated saline or distilled water is put into the wound. Aeration is performed either in a plastic bag or under a sphere for 20-30 minutes, ozone concentration 5-6 mg/l.

Wound aeration is to be done 2-3 times a day until the wound is cleansed from pyonecrotic discharge.On development of granulation tissue and epithelization the procedures are done every second day with ozone concentration of 2-2,5mg/l.When marginal epithelization appears**,** the concentration is diminished to 0,8-1,2mg/l

Major Autohemotherapy

The course consists of 5-6 procedures. The first 3 procedures are done every second day, the rest – every third day.

Intravenous infusions or rectal insufflations with ozonated saline

The procedures can be done as an alternative to major autohemotherapy. The course consists of 8-10 procedures, the first three are to be done daily, the rest – every second day.

Minor Autohemotherapy

The course consists of 3-5 procedures done every second day in combination with intravenous infusions or rectal insufflations with ozonated saline.

Dressings with Ozonated Oil

The dressings are to be applied as soon as wound epithelialization appears.

Stimulation of Biological Active Points

The procedures are recommended when wounds are on lower limbs by making subcutaneous injections of ozone/oxygen mixtures, volume-0,5-1,0ml., ozone concentration –10mg/l. The shin points are – BL-40, BL-57; foot points are- Liv-1, St-45, GB-44.

Osteomyelitis Of Long Tubular Bones

Ozone therapy proved to be effective in the treatment of osteomyelitis and purulent arthritis (Зайцев А.Б. 1998, 2000)

Routes

- Ozonated saline to soak the dressings (Ozonated dressings).
- Aeration with ozone/oxygen mixture in a plastic bag
- Intravenous infusions with ozonated saline
- Minor Autohemotherapy
- Major Autohemotherapy
- Intra-osseous infusions with ozonated saline

Management

The treatment includes all the routes of ozone therapy enlisted above.

Regarding the stage of the purulent process, ozonated dressings are to be changed once or twice every day. Plastic bags are to be put on for 20-30 minutes, ozone concentration-5-6mg/l. The procedures are done until the fistulas are closed and pyorrhea disappears.

Intravenous infusions with ozonated saline are to be done daily within the first three days and then - every second day (up to 10-12 procedures)

Minor Autohemotherapy is to be done every second day (up to 4-5 procedures).

Intra-osseous injections of ozonated saline are to be done daily within the first three days and then - every second day (up to 10-15 procedures).

Intravenous infusions with ozonated saline and minor autohemotherapy can be substituted by 6-8 procedures of major autohemotherapy done every second day.

Arthroempyesis

Routes

- Abarthrosis puncture washing with ozonated saline.
- Abarthrosis flushing drainage with ozonated saline.
- Intravenous infusions with ozonated saline
- Minor autohemotherapy
- Intra- and peri-articular injections of ozone/oxygen mixtures
- Major autohemotherapy

Management

Arthrocentesis is done with the evacuation of purulent materials and abarthrosis washing with ozonated saline is done till the washing waters become clean.

In cases when puncture washing appears to be ineffective, it is necessary to prepare the flushing drainage system to wash articular cavity. To do it micro-incisions are to be done along the lateral articular surface and micro-drainages are put into the articular cavity and anchored to the skin. One of the drainages is used for the continuous instillation of ozonated saline. The saline ozonation is done by a non-stop barbotage, with ozone concentration in the saline being 4-5mg/l.

Puncture washing and flushing drainage are to be done at least for two days, till the beginning of the inflammatory regress. Then the drainage system is removed, followed by intra- and peri-articular injections of ozone/oxygen mixtures (up to 4-5 procedures).

Alongside with the topical treatment, intravenous infusions with ozonated saline are done daily till the regress of the inflammation and then – every second day. The course of treatment consists of 10-12 procedures of intravenous infusions and 4-5procedure of minor autohemotherapy.

Intravenous infusions with ozonated saline and minor autohemotherapy can be substituted by 6-8 procedures of major autohemotherapy done every second day.

Note. Artificial ankylosis is obligatory until the pyo-inflammatory process subsides.

Trophic Ulcers. Decubitus Ulcers

Applications with ozonated oil are successfully used in the treatment of trophic ulcers of different etiology (Кузнецов Н.А. с соавт. 2000, Газин И.К. 2000, Горбунов С.Н. 2000).

Routes

- Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture
- Minor Autohemotherapy
- Major Autohemotherapy
- Aeration with ozone/oxygen mixture in a plastic bag under high or low gas pressure
- Ulcer treatment with the stream of ozone/oxygen gas mixture under the sphere
- Stimulation of biologically active points, when wound is located in the lower extremity.
- Microinjections with ozone/oxygen mixture along the ulcer ends

Management

The course of treatment should include all kinds of enlisted route procedures. The obligatory condition is to clean the ulcer surface completely from the incrustation.

Ulcer Aeration with Ozone/Oxygen Mixture

The procedure is to be done daily in a plastic bag or under the sphere for 20 minutes with ozone concentration of 6-7mg/l until the ulcer gets cleared off the purulent coat. With the development of granulation tissue and epithelization the procedures are done every second day with ozone concentration of 2-2,5mg/l, with development of marginal epithelization the concentration is diminished to 0,8-1,2mg/l.

The skin of the treated surface is to be damp. That is why it is to be either covered with wet napkin or wiped with a damp cloth. The ulcer surface is to be filled with gauze dressing soaked with ozonated saline.

Dressings with Ozonated Oil

The dressings are to be applied with the beginning of ulcer epithelization

Intravenous infusions with ozonated saline, minor autohemotherapy, major autohemotherapy

- Along with topical treatment intravenous infusions are done daily till the abatement of the inflammatory process and then every second day up to 12-15 procedures. Minor autohemotherapy are done up to 6-8 procedures. Intravenous infusions and minor autohemotherapy procedures can be substituted by 10-12 procedures of major autohemotherapy, the first four procedures are to be done every second day, the rest - two times a week.

Microinjections with ozone/oxygen mixture

Microinjections are done daily until the ulcer detersion.

Stimulation of biological active points

The procedures are done when ulcers are localized on lower limbs by subcutaneous injections of ozone/oxygen mixtures with volume of 0,5-1,0ml, and ozone concentration –10mg/l. The shin points are – BL-40, BL-57; foot points are- Liv-1, St-45, GB-44.

Osteoarthrosis

Intra-articular injections of ozone/oxygen mixtures proved to be very efficient in the treatment of osteoarthrosis.

Routes

- Intra-articular(intrasynovial) injections of ozone/oxygen mixtures
- Peri-articular injections of ozone/oxygen mixtures
- Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture
- Minor autohemotherapy

Management

- The course of treatment should combine all the above listed routes Intra-articular injections of ozone/oxygen mixtures are to be done every second day and alternated with intravenous infusions with ozonated saline (or rectal insufflations with ozone/oxygen mixture) and peri-articular injections. Minor autohemotherapy is to be done once a week. The early stages of osteoartrosis require a 2-3 week treatment course, while the late ones – 4 or 5 weeks.

Atherosclerosis Obliterans Of Peripheral Vessels

In instituting the course of ozone therapy we regarded the stages of chronic arterial insufficiency (CAI), which are used for differential treatment and regular medical check-up (В.Ф. Болгов с соавт.,2000):

CAI-1 stage – 1 and 2a stages of ischemia;

CAI-2 stage – 2b and 3a stages of ischemia;

CAI-3 stage - 3b and 4 stages of ischemia.

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy
- Minor Autohemotherapy
- Stimulation of biological active points in the lower extremities with ozone/oxygen injections
- Aeration with ozone/oxygen mixture in a plastic bag under excessive gas pressure

Management

The management depends on the stage of chronic arterial insufficiency.

Patients with the CAI-1 stage undergo through 10 procedures of Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture done every second day and 2-3 minor autohemotherapy procedures done every third day. This scheme can be substituted by 6-8 major autohemotherapy procedures. These patients are also administered 2 procedures of stimulation of biological active points

Patients with the CAI-2 stage undergo through 10 procedures of Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture done every second day and 3-4 minor autohemotherapy procedures done every second day. This scheme can be substituted by 6-8 major autohemotherapy procedures. The patients receive 4-5 procedures for stimulation of biological active points done every second day. The treatment is complemented with aeration with ozone/oxygen mixture in a plastic bag under excessive gas pressure. Each procedure (10 for the course) lasts for 20 minutes, ozone concentration being 5-6mg/l, and is done every second day.

Patients with the CAI-3 stage undergo through 10-12 procedures of intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture done every second day, 4 - 5 minor autohemotherapy procedures done every third day. This scheme can be substituted by 8 -10 major autohemotherapy procedures. The stimulation of biological active points includes 4 –5 procedures. Aeration with ozone/oxygen mixture in a plastic bag under excessive gas pressure is obligatory. The procedures are administered every second day to patients without any trophic skin changes in the foot and the shin. Daily procedures are administered in cases with trophic changes. The number of the procedures – from 10 to 20, ozone concentration being 5-6mg/l; duration – 20-30 minutes

The results received in 147 patients that underwent the course of treatment in our clinic are presented in the following table (Болгов В.Ф. с соавт.,2000).

Results of Ozone Therapy done in Patients with Obliterating Atherosclerosis in Lower Extremeties

The stage of Chronic Artherial Insufficiency	Number of patients	Results		
		Positive	Satisfactory	Unacceptable
CAI-1 stage	6	6	-	-
CAI-2 stage	109	106	2	1
CAI-3 stage	32	30	2	-
Results of Treatment (%)		96%	3%	1%

OZONE THERAPY IN INTERNAL DISEASES

Atherosclerosis And Ischemic Heart Disease (IHD)

Ozone has been found to produce hypolipidemic effect. According to different authors (Камышева Е.П. с соавт., 1998, Густов А.В. с соавт., 1999, Быков с соавт., 2000), after the course of ozone therapy patients with atherosclerosis had evident decrease in the levels of total cholesterin (6,4-18,4%), of lipoproteins of low density(7 - 28,7%), of triglycerides (10,5 - 17,2%) and increase of lipoproteins of high density(3,7 – 6,8%) levels.

The development of atherosclerosis is known to be caused not only by hypercholesterolaemia but also by disorders in regulation of free radical processes. Lipoproteins of low density undergo the process of oxidative modification in the liver and become more atherogenic. On being secreted into the blood, they start intensively accumulate, due to macrophages, in the endothelial cells in the damaged area and then get transformed into foam cells, which make the basis for atherosclerotic plaque.

Ozone therapy when using small doses of ozone increases LP processes and, what is more important, it activates antioxidant defense system, thus eliminating lipoprotein toxicity, decreasing their capacity to penetrate the vessel wall, making it more resistant. Hence, ozone therapy can be regarded as an antisclerotic method of treatment.

Ozone therapy proved to be effective in all IHD patients (stenocardia, cardiosclerosis, arrhythmias) at various stages of the disease from mild forms to severe ones). Its efficiency was found to be more pronounced in severe forms for it helps to control hypoxia in tissues which develops with the advance of heart insufficiency. In tissues with insufficient blood circulation the oxygen uptake by cells is done in much greater volume under ozone influence, This effect cannot be achieved with the help of medication orders. This statement seems to be extremely important for it explains the positive effect of the method.

Routes

- Intravenous infusions with 200ml of ozonated saline with ozone concentration of 20μg/kg of patient's weight (ozone concentration at the output from the generator)
- Rectal insufflations with ozone/oxygen mixture, ozone dose being 75μg/kg of patient's weight
- Major Autohemotherapy, ozone dose being 1-3 mg.

Management

In the course of treatment we use one of these routes The management is done according to the patient's condition, which is evaluated on the basis of the accepted functional classes (FC):

- 6-8 procedures for FC-I patients;
- 8-10 procedures for FC-II patients;
- 8-10 procedures for FC-III and FC-IV patients.

The first 2 procedures of intravenous infusions or rectal insufflations are to be done every day, the rest – every second day. The procedures (6-8) of major autohemotherapy are to be done twice a week.

Note. In cases when patients are on conventional treatment and start ozone therapy, coronaroactive preparations are not discontinued immediately. The dose is gradually diminished with the improvement of patient's condition.

The following table presents the results we received on having used ozone therapy for 142 patients with different FC of IHD.

The Results of Ozone Therapy in 142 Patients with IHD

Severity of the Disease	Number of Patients	Results of Treatment		
		Positive	satisfactory	unacceptable
I	3	3	-	-
II	82	74	6	2
III	50	45	4	1
IV	7	7	-	-
Results of Treatment (%)		91 %	7 %	2%

The improvement in the condition of patients with stenocardia was defined by the less episodes of heart strokes and nitroglycerinum intake. Angina attacks were completely regulated in 50% of patients; in 41% of patients the number of attacks was double decreased.

Hypertensive Disease

Ozone therapy is a pathogenic method for hypertension treatment. It corrects the decreased energy of cells, the main component of pathogenesis. No other available hypotensive preparations are known to have such properties. In the management of Hypertensive disease ozone therapy can be used as a monotherapy and in combination with other medications. As a monotherapy it is efficient in patients at the initial stage of the disease, in mild, labile hypertension. Positive results were received in 70% of patients.

Patients with steady hypertension are to combine ozone therapy with hypotensive preparations. According to our findings, conventional hypotensive preparations can be administered in lower doses when combined with ozone therapy. In combined treatment clinical manifestations of hypertensive disease, such as headaches, dizziness, heart pains – either disappeared within a shorter time period or abated.

Routes

- Intravenous infusions with 200ml of ozonated saline with ozone concentration of 20μg/kg of patient's weight (ozone concentration at the output from the generator)
- Rectal insufflations with ozone/oxygen mixture, ozone dose being 75μg/kg of patient's weight
- Major Autohemotherapy, ozone dose being 1-3 mg.

Management

In the course of treatment we use one of these routes. The treatment with intravenous infusions or rectal insufflations starts with 2-3 daily procedures, the rest 3-4 procedures are done every second day. With the decrease of arterial pressure the number of procedures is

diminished to two procedures a week, and then one procedure a week. The total number of procedures is 8-10. Major autohemotherapy is done twice week with a total number of procedures up to 6-8.

Diabetes Mellitus

Ozone therapy appears to be an effective method for DM treatment. The reason is in ozone mechanisms when it can perform a number of processes, which provide its positive effect.

First, ozone improves the penetration of cellular membranes for glucose. It is achieved by stimulating pentose-phosphate pathway and aerobic glycolisis that in case of DM are inhibited. It promotes hyperglycemia decrease due to better transport of glucose into tissues.

We observed a group of 70 patients with insulin-dependent and non-insulin-dependent diabetes. After the course of ozone therapy the average level of hyperglycemia had a 26% decrease.

Ozone activates glucose metabolism that results in increasing content of 2,3 diphosphoglycerate in erythrocytes which provides better oxygen supply into the tissues. Patients with diabetes mellitus have the so called glycosylated hemoglobin forming very strong bonds with oxygen, thus, inducing hypoxia and determining the severity of the disease. That is why hypoxia control with the help of ozone therapy is of the key importance in the course of treatment.

After the course of ozone therapy the patients had significant decrease in the levels of urine, cholesterol and fibrinogen.

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy
- Minor Autohemotherapy
- Subcutaneous microinjections with ozone/oxygen mixture
- Stimulation of biological active points with ozone/oxygen injections

Management

The basic treatment includes intravenous infusions of ozonated saline or rectal insufflations with ozone/oxygen mixtures which are done every second day (8-10 procedures). These procedures can be substituted with major autohemotherapy which is done twice a day up to 6-8 procedures for the course of treatment. Other procedures are administered according to the type of diabetes mellitus and the presence of complications.

In DM, Type-2 the treatment course also includes stimulation of biological active points with ozone/oxygen injections using the conventional schemes.

In signs of secondary immune deficiency (pustular inflammatory diseases) in addition to the basic course of treatment subcutaneous microinjections with ozone/oxygen mixture are done in the area of purulent foci. Minor autohemotherapy is done every second day up to 6-8 procedures for the course.

Note. Due to the fact that ozone has hypoglycemic effect the dose of glucose decreasing preparations must be corrected and glucose level in the blood must be under constant control through out the course of treatment.

Positive effect was received in 92% of insulin-dependent cases and 89% of non-insulin-dependent cases. It was expressed in decrease of hyperglycemia, thirst reduction, disappearance of polyuria, pruritus, weakness etc.(Масленников О.В. с соавт., 2001).

One of the main signs for successful treatment of diabetes mellitus is the achievement of compensation of the patients condition. After the course of ozone therapy the number of compensated patients increased (by 4 times) and decompensated patients decreased(by 5 times).

Patients who underwent the course of ozone therapy could take a 20-25% reduced dose of glucose-reducing preparations for the period of 3-6 months.

Ozone therapy proved to be effective in the treatment of complicated forms of diabetes mellitus. In patients with diabetic foot syndrome ozone therapy, combined with topical treatment of purulent necrotic focus and with total metabolic effect, allowed to half-shorten the period for wound detersion from pyo-necrotic mass and development of regenerative processes, to lessen the number of limb amputations and of fatal outcomes, to decrease invalidism (Мошуров И.П., Глянцев В.П., 1998, Беляев А.Н. с соавт. 2000, Газин И.К.,2000).

Clinical observations in the course of lung tuberculosis in patients with diabetes mellitus, who were on ozone therapy, showed ozone therapy to produce glucose-reducing effect and to influence on the course of specific process. The most impressive was elimination of adiaphoria to antituberculosis preparations. (Белянин И.И. 1997)

Chronic Bronchitis. Bronchial Asthma

Ozone immune modulating properties are of primary importance in the treatment of chronic bronchitis. Ozone therapy provides anti-infection immune response to viral-bacterial infection invading the human body. It is revealed in intensifying local and general immunity which is suppressed in chronic bronchitis.

The principles in the treatment of bronchial asthma are known to be the following: activation of immune system and elimination of the following factors - viral and bacterial infection, bronchoconstriction, allergic reactions, hypoxia.

Ozone therapy efficiency is explained by its ability to influence various aspects of pathological process. First of all, it is its capacity to cope with bronchospasm as a result of dilatation effect on the smooth muscle of NO-radical which is formed in the endothelial cells due to ozone.

Ozone capacity to cope with tissue hypoxia is also of great significance. Patients with bronchial asthma are known to suffer from hypoxia resulting from pulmonary insufficiency caused by bronchospasmus. Ozone provides blood saturation with oxygen by-passing the lungs and delivering it to tissues via erythrocytes, thus improving blood rheology and eliminating hypoxia.

Immune modulating ozone effect can be revealed in activation of cytokines (interferon, tumor necrosis factor, interleukins) production by lymphocytes and monocytes.

Stimulation of immune system helps to suppress the inflammatory process, decreasing the activity of effector cells and diminishing their release of biological active substances responsible for bronchospastic reactions.

Routes

- Intravenous infusions with ozonated saline

- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy
- Stimulation of biological active points with ozone/oxygen injections
- Inhalations with ozonated distilled water

Management

The treatment is a complex one, including all the above enumerated routes. The course starts with 2 intravenous infusions of ozonated saline or rectal insufflations done daily. On improving the condition the next 5-7 procedures are done every second day and then once or twice a week (up to 7-10 procedures for the whole course)

Acupuncture with ozone/oxygen mixture is done according to conventional accepted methods.

Inhalations with ozonated distilled water are done once or twice a day daily for a period of 10-15 days.

Major autohemotherapy is administered in cases when positive effect is not received after 7-10 days of treatment and it is done instead of intravenous infusions or rectal insufflations. Procedures are done every second day up to 4-8 for the course of treatment.

The results of ozone therapy in chronic bronchitis in our practice are the following: the condition improved in 79% of patients, 29% having significant improvement(complete elimination of such symptoms as cough, breathlessness, weakness, rales) and 21% having satisfactory improvement.

Ozone therapy in 42 patients with bronchial asthma followed in our clinic gave the following results. The majority of patients (83%) had a moderate course of the disease. All the patients were on broncholytic preparations, some of them taking steroid hormones. After the course of ozone therapy the valid improvement in the condition was noted in 86% of patients who had half fewer episodes of suffocation and could reduce he dose of conventional preparations. In 7% of patients we achieved a complete control of asthmatic attacks and discontinue the intake of medications. In the rest 7% of cases we could not achieve improvement in their condition.

Chronic Pyelonephritis

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy
- Minor Autohemotherapy

Management

The treatment combines minor autohemotherapy (6-8 injections) with intravenous infusions of ozonated saline or with rectal insufflations with ozone/oxygen mixture (8 - 10 procedures). During the first three days the procedures are to be done daily and then every second day. Major autohemotherapy (6-8 procedures) is to be done twice a week and can be used as an alternate method, substituting the first three.

Chronic Gastritis. Type "B"

Ozone therapy proved to be an effective remedy in the treatment of chronic gastritis. Ozone due to its properties produces therapeutic effect on all the main pathogenic mechanisms responsible for the development of the disease. It produces bactericidal effect on Helicobacter Pylori (up to 93,7%)

Its anti-inflammatory effect is achieved due to oxidation of arachidonic acid, known as a precursor for prostaglandin E that starts the inflammatory process. To add to that, ozone produces its immune-modulating, anti-aggregation and analgetic effect (Андосов С.В. с соавт., 2000).

Ozonated oil and ozonated water produce bactericidal effect and deliver active oxygen to tissue, shortening the healing processes.

Ozone has been found to produce changes in the local immunity, increasing the release of secretory IgA by lymphocytes and plasmatic cells, responsible for the immune defense of the surface cells of the stomach.

Routes

- Ozonated water.
- Ozonated oil
- Minor Autohemotherapy
- Stimulation of biological active points with ozone/oxygen injections

Management

The treatment consists of daily intake of ozonated water (100-150ml) 30-40 minutes before meals 1-3 times a day. Ozonated oil is to be taken 3 times a day 15 minutes following the intake of ozonated water, starting with 1 teaspoonful, gradually increasing the dose to tablespoonful, if patients tolerate it well. The water and oil are to be taken for 2 – 3 weeks.

Minor autohemotherapy is to be done according to the following scheme. The first 3 procedures are to be done daily, the next 3 – every second day, and the remaining procedures – twice a week. The course consists of 8-10 procedures.

Acupunctures with ozone/oxygen mixture are done according to the conventional methods.

Ozone therapy was instituted to 101 patients. The received results were assessed as "significant improvement" in 57%, "improvement" – in 40% and satisfactory in 3% of cases. Clinical results were confirmed by endoscopic findings that revealed significant decrease or disappearance of inflammation, signs of hyperaemia and mucous edema.

Ulcer

Multifunctional ozone effect in the treatment of peptic ulcer is revealed first of all in its anti-inflammatory and anti-helicobacterial action. It results in accelerating the epithelization processes, infiltrate elimination in mucous membrane within a shorter period, compared with the traditional therapy. V.Maximov (1998) considering different schemes of ulcer treatment comes to the conclusion, that ozone therapy can substitute antibiotics and metronidasol preparations, providing better results regarding the ulcer healing and the degree of HP eradication.

Ozone therapy was found to produce positive effect on general and local immunity.

Ulcer Of The Stomach

Routes

- Ozonated water.
- Ozonated oil
- Rectal insufflations with ozone/oxygen mixture
- Minor Autohemotherapy
- Intravenous infusions with ozonated saline
- Major Autohemotherapy
- Stimulation of biological active points with ozone/oxygen injections

Management

Ozonated water and ozonated oil are to be taken following the scheme of the chronic gastritis.

The treatment begins with rectal insufflations with ozone/oxygen mixture done every second day up to 5-6 procedures. Starting from the second week the treatment is complemented with Minor autohemotherapy alternately with rectal insufflations. During the 3-d and the 4-th weeks the same procedures are done every second or third day. In cases when rectal insufflations are unacceptable, they are substituted by intravenous infusions with ozonated saline up to 12-15 procedures done every second day or by major autohemotherapy done twice a week with 6-8 procedures for the course.

Microinjections into paravertebral points with ozone/oxygen mixture are to be done every second day on the Th 6 - Th 9 level (up to 5-8 procedures for the course)

Ozone acupuncture is done according to the conventional methods.

Ulcer Of The Duodenum

Routes

- Ozonated water.
- Ozonated oil
- Minor Autohemotherapy
- Major Autohemotherapy
- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Stimulation of biological active points with ozone/oxygen injections

Management

Ozonated water and ozonated oil are to be taken following the scheme of the chronic gastritis.

The course begins with minor autohemotherapy procedures. The first 3 procedures are to be done daily, then 2 or 3 procedures are to be done every second day, the remaining ones are to be done twice a week. The course includes 8-10 procedures.

Starting with the second week the treatment is complemented with intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture up to 3-4 procedures that are alternated with minor Autohemotherapy.

Intravenous infusions of ozonated saline and minor autohemotherapy can be substituted by major autohemotherapy (8-10 procedures), the first two procedures are to be done every second day and afterwards – twice a week.

Ozone acupuncture is done according to the conventional methods.

The treatment course lasts 3-4 weeks. After cicatrization, ozonated oil is kept on to be taken 1 spoonful before going to bed at night for a period of 1-1,5 month.

In our practice we used ozone therapy as a mono therapy in 69 patients at the stage of exacerbation, 10 suffering from ulcer of the stomach, 59 from duodenal ulcer.

The results of treatment were regarded as "significant improvement" in 8 patients with complete healing of the ulcerative defect and disappearance of all the symptoms. In 2 cases the results were assessed as "improvement of the condition", for the healing was not complete, though the symptoms disappeared completely.

The condition of patients treated for duodenal ulcer was regarded as "significant improvement" in 56% of cases, "improvement of the condition" – in 39%. "Partial improvement" was assessed in 5% of patients that had incomplete healing of the ulcer defect and some of the remaining symptoms.

Note. Ozone therapy can be used both as a mono therapy and in combination with medicinal forms. Follow-up results of ozone therapy in 290 patients with duodenal ulcer were analyzed by S.Karatayev et al.(1998). Within the period of 4 years the cases of the recurrence of the disease were noted 5 times less in the group of patients that were on combined treatment compared with those that underwent the conventional therapy.

Chronic Nonulcerative Colitis

Ozone therapy in the management of patients with chronic non-ulcerative colitis takes a special place regarding the following reasons.

First, the use of rectal insufflations with ozone /oxygen mixture produces both topical anti-inflammatory effect and general multi-faceted including anti-hypoxic, immune-modulating, etc due to ozone capacity to be quickly absorbed by blood.

Second, chronic colitis is often concomitant with dysbacteriosis. Ozone advantages become evident, for if does not produce any harmful effect on flora of the intestine, compared with antibacterial preparations such as antibiotics and sulfanilamides

When rectal insufflations are used ozone gets stuck to mucous membrane and interferes with the infectious process penetrating the microbial cells thus preventing their further reproduction Besides, ozone enforces phagocytosis, improving blood circulation and humoral immunity. It leads to refection of homeostasis, with the normalization of microbial balance and subsiding inflammatory signs.

Contrary to various antiseptics still causing some damaging effect in tissues, ozone does not produce any harmful or ulcerative effect and, more than that, it does not induce any resistance to ozone therapy.

Routes

- Rectal insufflations with ozone/oxygen mixture
- Minor Autohemotherapy
- Stimulation of biological active points in the lower extremities with ozone/oxygen injections

Management

During the exacerbation period ozone insufflations of gaseous mixture are done every second day with the dose of 100μg per kg of patient's weight for the first two weeks, then 2 times a week with the dose of 75μg/kg. The whole course consists of 10-15 procedures.

Minor autohemotherapy is done 1-2 times a week up to 4-6 injections for the course.

Microinjections with ozone/oxygen mixture into the paravertebral points at Th 10 - L5 level are to be done daily or every second day up to 5-7 procedures for the course. Acupuncture procedures with ozone/oxygen mixture are done according to the conventional methods.

In colitis of various etiology some authors (В.А.Максимов с совт. -1998) recommend the use of intra-intestinal gaseous ozone up to 200-500ml with the concentration 60 mg/l.

In the Russian Centre of Restorative Medicine and Spa-resort Treatment they recommend rectal insufflations with concentration in the range of 10-40μg/ml and volume of 50-300ml for patients with inflammatory intestinal diseases. In atonic intestine they recommend low concentrations, in spastic conditions - higher concentrations.

Chronic Hepatitis

Ozone therapy can be used as a mono-therapy in the management of patients with chronic hepatitis.

In the treatment of infectious hepatitis the major effect is achieved due to ozone antiviral property. Inactivation of viruses results from peroxide oxidative activity when virus cell receptors get destroyed and cannot penetrate the host cell. The failure in virus multiplication process is also caused by RNA virus breakdown due to ozone. According to A.Zmyzgova (А.В.Змызговой с соавт.-1998) 2-months ozone therapy courses reveals no viraemia signs in 66% of cases with chronic viral B-hepatitis, and in 60% of cases with chronic C-hepatitis.

Peroxides activate endogenous cellular metabolism in Kupffer's cells, responsible for phagocytosis. Ozone therapy activates both, cellular and humoral immunity. Ozone induces lymphocytes and monocytes to release cytokines and, primarily, interferon which is regarded as one of the most important endogenous defense factors, protecting the body from the viral infection.

Ozone effect in alcoholic hepatitis is explained by the fact that newly-formed peroxides act as a trigger for antioxidant mechanism to detoxicate the glutathione system, which prevents hepatic cell membrane from being damaged due to LP activation processes.

Routes

- Rectal insufflations with ozone/oxygen mixture
- Minor Autohemotherapy
- Intravenous infusions with ozonated saline
- Major Autohemotherapy

Management

Apart from the exacerbation period the most common procedures in chronic hepatitis are rectal insufflations with the dose of ozone of 75μg/kg done alternately with minor autohemotherapy. For the first two weeks rectal insufflations are done every second day and then twice a week up to 20-30 procedures for the course. Minor autohemotherapy is done 2 times a week.

The preference for rectal insufflations can be explained by findings published by H.G.Knoch (1987,1988). Ozone/oxygen mixture infused via the rectum is of particular significance in the treatment of different forms of hepatitis. Rapid gas absorption causes immediate rise in the partial oxygen pressure in portal vein, thus providing "the shortcut" for the oxygen to the liver and contributing to the efficiency of the treatment.

Rectal insufflations can be substituted with intravenous infusions of ozonated saline (the same number of procedures) or with major autohemotherapy.

- In cases with the exacerbation of the disease the preference is given to major autohemotherapy and intravenous infusions of ozonated saline. During the exacerbation period major mutohemotherapy is done daily with a high dose of 6-8mg up to 5-8 procedures and then 2-3 times a week till the exacerbation subsides. Then the doses is decreased to 1-1,5mg

Intravenous infusions of ozonated saline (ozone concentration produced by ozone generator is 2mg/l) are done daily during the period of 10-12 days, and then every second day until the exacerbation subsides. After that – 2 times a week.

During the exacerbation period rectal insufflations with ozone dose of 100μg/kg of patient's weight are done daily. When exacerbation subsides the procedures are carried according to the conventional protocol.

In the course of treatment the major autohemotherapy procedures can be alternated with intravenous infusions of ozonated saline and rectal insufflations with ozone/oxygen mixtures.

The course of treatment lasts from 3 to 6 months.

Hepatic cells affected by the virus are less resistant to peroxide action compared with healthy cells. These weakened cells on being exposed to high peroxide concentration get destroyed alongside with viruses and eliminated. This phenomenon is described by A. Bolcany (1989), who revealed the elevation of transaminase level after the very first procedures of ozone therapy and explained it by the destruction of hepatic cells due to viral invasion.

OZONE THERAPY IN GYNECOLOGY AND OBSTETRICS

GYNECOLOGY

Inflammatory Diseases Of The Organs In The True Pelvis (Adnexitis, Endometritis, Parametritis, Pelvioperitonitis)

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy, ozone dose being 1-3 mg.
- Intrauterine irrigations with ozonated distilled water

Management

The treatment consists of intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture. Both can be substituted with major autohemotherapy.

Daily infusions with 200- 400 ml of ozonated saline, ozone concentration in ozone/oxygen mixture at the output from ozone generator being 1200μg/l are to be done for 5 - 7 days.

Rectal insufflations with ozone/oxygen mixture are done according to the accepted method reckoned on the basis of 75μg/kg of patient's weight. The gas volume is from 300 to 600ml with ozone concentration of 10-40 mg/l.

Major autohemotherapy is done twice week with a total number of procedures up to 4-6.

Intrauterine irrigations with ozonated distilled water (400ml) with ozone concentration of 4-5 mg/l are done to provide entire contact with the site of inflammation and to exclude any damage to the mucous membrane in different forms of endometritis. On being ozonated the water via the biluminal catheter is introduced into the uterine cavity and then evacuated via the same catheter . The procedure can be repeated 3 times during one session, which is done once a day (Гречканев Г.О., Качалина Т.С., Качалина О.В., 2000).

In combination with basic anti-inflammatory therapy the described sanitation of the uterine cavity prevents the generalization of the inflammatory process, shortens the course of treatment and makes it possible to discontinue any other dialysis preparations.

Note. The use of ozone therapy allows to decrease the dose of medications with detoxicating, rheologic, antioxidant, immune-correcting, analgetic and sedative effect.

Profuse bleedings are regarded as the main contra-indication for ozone therapy. Smeared bloody discharge and predisposition to hemorrhage require more careful and accurate control.

In cases of surgical intervention ozone therapy can be used in aftercare course.

Inflammatory Diseases Of Genital Tracts

Colpitis, Bacterial Vaginosis

Routes

- Vaginal irrigations with ozonated saline
- Applications with ozonated oil
- Vaginal insufflations with ozone/oxygen mixture

Management

Vaginal irrigations with ozonated saline with the volume up to 1liter and ozone concentration of 6-10mg/l are to be done daily(8-10 procedures per course) and are to be complemented with applications with ozonated oil (1-2times a day).

These procedures can be substituted by vaginal insufflations with ozone/oxygen mixtures, that are to be done daily within 5-8 days. Using special nozzle to vaginal speculum ozone/oxygen mixture, ozone concentration being 1,5-2,5mg/l, is introduced into the vagina. Before the insufflation procedure the vagina is to be washed with distilled water for 5-10 minutes at the rate of 0,5-1l/min.

This method resulted in a steady improvement in all 50 patients with nonspecific colpitis, elimination of pathogenic and opportunistic microorganisms in bacteroscopy and restored the immunity balance in vaginal secretion. The use of the method made it possible to discontinue the medicinal therapy. (Качалина Т.С. с соавт.1998, Гречканев с соавт.,2000).

Kraurosis Vulvae

Routes

- Applications with ozonated oil

Management

Applications with ozonated oil are to be applied daily on the damaged surface within the period of 8-10 days

Note. The oil (100ml) is to be barbotaged with ozone/oxygen mixture for 20 minutes, ozone concentration-10mg/l.

OBSTETRICS

The use of ozone therapy produces positive effect on the clinical course in such conditions as the risk of miscarriage, gestosis, anaemia of pregnancy, intrauterine growth retardation and risk of complications in obesity. It is linked with the immune-correcting and antioxidant ozone effect. The improvement in oxygen supply, rheology and microcirculation contributes to hormone-producing function (Кулаков В.И. с совт.,2001, Миненков А.А. с соавт.,2001).

Miscarriage. Early Toxicosis

Routes

- Intravenous infusions with ozonated saline or
- Major Autohemotherapy, ozone dose being 0,4-0,5 mg.

Management

Daily instillation infusions of 400 ml of ozonated saline, ozone concentration in ozone/oxygen mixture being 400μg/l, are to be done for 5 days. Ozone therapy proves to be most effective in the end of the first and the beginning of the second trimesters of pregnancy.

Major autohemotherapy is done twice week with a total number of procedures up to 4-6.

Note. Ozone therapy is contraindicated in genital tract bleedings of different intensity and can be instituted only after its entire termination. The immune-correcting and antioxidant preparations, including vitamins, as well as sex hormones can be discontinued for the time of ozone therapy.

Gestational Toxicosis. Anaemia Of Pregnancy

Routes

- Intravenous infusions with ozonated saline or
- Major Autohemotherapy, ozone dose being 0,4-0,5 mg.

Management

Daily instillated infusions of 200 ml of ozonated saline, ozone concentration in ozone/oxygen mixture being 400μg/l, are to be done for 5 days. Ozone therapy proves to be most effective in mild and moderate gestosis.

Major autohemotherapy is done twice week with a total number of procedures up to 4-6.

Note. Ozone therapy is contraindicated in genital tract bleedings of different intensity and can be instituted only after its entire termination. The preparations with anti-oxidant, immune-correcting, sedative, rheologic and detoxicating effect can be discontinued for the time of ozone therapy.

Intrauterine Infection

The prevention and treatment of pregnant women that are in the risk group for fetal infection are to be done during the second trimester of pregnancy.

Routes

- Intravenous infusions of ozonated saline or
- Major Autohemotherapy, ozone dose being 0,4-0,5 mg.

Management

Daily instillation infusions of 200 ml of ozonated saline, ozone concentration in ozone/oxygen mixture being 800μg/l are to be done for 3 – 5 every second day. -6 procedures.

.Major autohemotherapy is done twice week with a total number of procedures up to 4-6.

Note. Antioxidants and immune-correcting preparations can be cancelled during the course of ozone therapy.

OZONE THERAPY IN DERMATOLOGY

The use of ozone therapy in the management of patients with various inflammatory skin diseases makes it possible to delimit the inflammation and to improve trophical processes. Out of 495 patients that were on ozone therapy a complete disappearance of clinical picture or significant improvement were observed in a patients with dermatosis and herpes(100%); pyodermia(95%); - eczema(75%); neurodermaitis(66%) and psoriasis(60%) (Криваткин С.Л, Криваткина Е.В., 1998).

Neurodermititis. Eczema

Ozone therapy is used in the treatment of some limited forms of neurodermititis

Routes

- Intravenous infusions of ozonized physiological saline or rectal insufflations with ozone/oxygen mixtures or major autohemotherapy
- Ozonized vegetable oil
- Aeration with ozone/oxygen mixture in a plastic bag

Management

The course consists of 10-12 procedures of intravenous infusions with ozonized saline or rectal insufflations with ozone/oxygen mixtures done every second day. Major autohemotherapy is done twice a week up to 5-6 procedures.

Ozonized vegetable oil is applied on the injured surface twice a day for 20 minutes until the eruption disappears.

The aeration course consists of 5-8 procedures, done every second day for 20 minutes with ozone concentration being 5-20mg/l

Positive result in the treatment of patients with eczema was noted in 86,8% of cases (complete clinical cure was achieved in 29,4% and significant improvement(70% eruption regress) –in 57,4% (Кошелева И.В., Иванов О.Л, 2000).

Acneiform Eruption

Routes

- Minor autohemotherapy
- Major autohemotherapy
- Ozonated vegetable oil

Management

In mild cases (isolated eruption) Minor Autohemotherapy is administered up to 8-10 procedures done every second day.

In severe cases (massive eruption) Major Autohemotherapy is indicated up to 8-10 procedures done twice a week.

Ozonized vegetable oil is to be applied on the injured surface twice a day for 20 minutes. Applications are to be done until the eruption disappears.

Furunculosis. Pyodermia

Routes

- Major Autohemotherapy
- Minor Autohemotherapy
- Intravenous infusions with ozonated saline
- Subcutaneous microinjections with ozone/oxygen mixture around the focus of inflammation

Management

The course of treatment begins with Major Autohemotherapy up to 5 procedures done every second day, followed by intravenous infusions with ozonized saline which are alternated with Minor Autohemotherapy (6-8 procedures). Microinjections around the focus of inflammation are to be done every day till the rupture of the furuncle. The ruptured furuncle is to be irrigated with ozonized saline

Herpes

Routes

- Major Autohemotherapy
- Minor Autohemotherapy
- Ozonated vegetable oil

Management

The course of treatment includes10-15 procedures of Minor Autohemotherapy done every second day and 4 procedures of Major Autohemotherapy done once a week. Ozonized oil is to be applied two times a day on the dry elements till the rupture of the papules.

Note. In some cases there can be the exacerbation of the process at the very beginning of treatment. The exacerbation is less pronounced and is soon eliminated.

Psoriasis

Routes

- Intravenous infusions of ozonated physiological saline or rectal insufflations with ozone/oxygen mixtures
- Major Autohemotherapy
- Minor Autohemotherapy
- Ozonized vegetable oil

Management

Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixtures are done every second day up to 10 procedures. Minor autohemotherapy includes 6 procedures done twice a week.

Instead of intravenous infusions with ozonized saline, rectal insufflations with ozone/oxygen mixtures and minor autohemotherapy and a course of major autohemotherapy can be done. In includes 8-10 procedures, the first two procedures are to be done every second day, the remaining procedures are done twice a week.

Ozonated vegetable oil is to be applied on the injured surface twice a day for 20 minutes within a month to follow.

Mycosis

Routes

- Ozonated vegetable oil
- Minor Autohemotherapy

Management

The treatment consists of ozonated tampons applied on the nail plates twice a day for 30-40 minutes for a period of 3-6 months for fingernails and of 6-9months for toe-nails (until the new nail plate grows)

A course of major autohemotherapy of 3-6 procedures is done every three months, the procedures are done every second day.

OZONE THERAPY IN NEUROLOGY

Chronical Forms Of Cerebrovascular Insufficiency (Discirculatory Encephalopathy)

Routes

- Intravenous infusions of ozonated saline.

- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy

Management

The course consists either of intravenous infusions of ozonated saline (the procedures can be substituted by rectal insufflations) or major autohemotherapy procedures.

Intravenous instillated infusions are to be done daily up to 8 –10 procedures.

Major Autohemotherapy procedures are to be done every second day up to 6 –8 procedures.

Rectal insufflations with ozone/oxygen mixture are to be done according to the scheme. The initial dose is 200ml, which is to be increased by adding 100ml more each day until the required dose (see Forms and Methods to Use Ozonated Materials).

The received results, that can testify to the efficiency of ozone therapy in this category of patients, are presented in the Table "The Results of Ozone Therapy in Patients with Discirculatory Encephalopathy"

The Results of Ozone Therapy in Patients with Discirculatory Encephalopathy

Severity of the Disease	Number of Patients	Results of Treatment		
		Positive	satisfactory	unacceptable
I	30	26	2	2
II	26	16	5	5
III	5	5	---	---
Results of Treatment (%)		78 %	11 %	11%

Neurologic Manifestations of Spinal Osteochondrosis

Ozone analgetic effect has been successfully used in treating patients with vertebrogenic pain syndrome due to algopeptides direct oxidation, suppression of ischemia radices and blocking prostaglandin synthesis. Subcutaneous injections with ozone/oxygen mixtures into trigger points in combination with minor autohemotherapy and intravenous infusions of ozonated saline provide positive result in the majority of patients with osteochondrosis of cervical, thoracic or lumbar spine.

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Minor Autohemotherapy
- Para vertebral injections of ozone/oxygen mixtures
- Subcutaneous injections of ozone/oxygen mixtures into trigger points and biological active points

Management

The course consists of two alternated schemes of treatment

1) Intravenous infusions with ozonated saline or rectal insufflations are to be complemented the same day with subcutaneous injections along the nuchal bones line

and with para-vertebral injections. Paravertebral injections are in the points of palpatory tenderness into the depth of 5 – 6 cm with the volume of 5 –10ml.

1. Minor Autohemotherapy procedures are done the same day with the subcutaneous injections of ozone/oxygen mixtures into biologically active points (V19 -V28, V40, V57, V60, VB30, VG4, E32, E44, RP9, F9). Subcutaneous injections into biologically active points are done into the depth of 1 –1,5cm with the volume of 1-2ml. The course consists of 8 -10 procedures.

Inflammmatory Brain Diseases (Menigitis, Encephalitis)

Routes

- Intravenous infusions with ozonated saline
- Minor Autohemotherapy
- Major Autohemotherapy -ozone dose –1000-1200μg/100ml of blood

Management

The treatment includes all the above listed procedures. Major Autohemotherapy is done every second day alternated with intravenous infusions of ozonated saline or minor autohemotherapy , upon the whole, 12-15 procedures.

Note: Ozone therapy is performed complementary to anti-inflammatory treatment.

Migraine, Cephalgia

Routes

- Intravenous infusions with ozonated saline, ozone concentration being 1200μg/l
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy
- Minor Autohemotherapy
- Subcutaneous injections with ozone/oxygen triggers and biologically active points

Management

The treatment consists of the above listed procedures.

Intravenous infusions with ozonated saline or rectal insufflations with ozone/oxygen mixture are to be done daily or every second day up to 8-10 procedures.Minor autohemotherapy is done twice a week, up to 3 – 4 procedures.

Intravenous infusions with ozonated saline, rectal insufflations with ozone/oxygen mixture and minor autohemotherapy can be substituted with major autohemotherapy up to 8 – 10 procedures, the first two done daily and then 2 –3 times a week.

Subcutaneous injections with ozone/oxygen triggers and biologically active points of neck and collar zone are to be done daily though out the course of treatment.

The use of ozone therapy in 132 patients with various cephalgias resulted in significant improvement in the patient's self-assessed condition, revealed in the headaches with less intensity and of different character, as well as prolonged painless periods in 83% of patients with migraines, in 73% of patients with stress headaches and in 69% of cases with vertebrogenic cervicocranialgias (Мочалов А.Д., Котов С.А., 2000).

Mono- And Poly-Neuropathias Of Ischemic And Compression Type

Ozone therapy, though it does not eliminate the causes of compression of the nerve trunk, it stimulates the regeneration of the damaged nerve by improving the hemorheology and microcirculation, decreasing hypoxia and activating oxygen metabolism in the ischemic nervous tissue with aerobic processes.

Routes

- Intravenous infusions with ozonated saline
- Rectal insufflations with ozone/oxygen mixture
- Major Autohemotherapy

Management

The course consists of any of the above listed routes up to 8 –10 procedures. Intravenous infusions with ozonated saline are done daily or alternately. Rectal insufflations with ozone/oxygen mixture are done accordingly, starting with 200ml, which is to be increased by adding 100ml more each day until the required dose (see Forms and Methods to Use Ozonated Materials).

Major Autohemotherapy is to be done every second day.

Positive clinical results due to ozone therapy have been obtained in 95% of cases with significant decrease of paresthesias improved sensitivity in enervation zone of the damaged nerve (Потехина Ю.П.,1997).

Cerebral Circulatory Embarrassment. Ischemic Insults

Energy metabolic imbalance and significant decrease in the contents of macro-energetic compounds are considered to be the primary cause responsible for the changes taking place in neurons in ischemic stroke. The use of ozone therapy in patients with brain infarction appears to be highly recommended due to ozone optimizing effect on oxygen-transport blood function, increased oxygen utilization by brain calls due to activation of glycolysis, Krebs' cycle, β-oxidation of fatty acids (Густов А.В. с соавт.,1999).

Routes

- Intravenous infusions with ozonated saline
- Major Autohemotherapy -ozone dose-1mg/100ml of blood.

Management

In the course of treatment one of the routes is to be chosen.

The first 3 – 4 intravenous infusions with ozonated saline are to be done daily, then every second day(3 –4 procedures) with the rest done twice a week. The course includes 10 – 12 procedures.

The first 2 major autohemotherapy procedures are done daily, the next 3 – every second day, the rest – twice a week, up to 8 –9 procedures for the course.

The patients with ischemic stroke showed positive changes in oxygen-transport blood system (43% increase in PO2 following the ozonated saline infusion and 26% increase after the course of ozone therapy), in blood coagulation system (10-15% decrease thrombocyte aggregation capacity with 8-10% fibrinolysis activation), the improvement in lipid spectrum

(10-12% decrease in the total cholesterol. 7-10% in β-lipoproteins, 12-15% decrease in atherogenic coefficient). These changes brought positive results in the acute stage of the disease in 79% of cases and in 69% of cases in recovery phase (Густов А.В. с соавт., 1999).

Note. Ozone therapy is contraindicated in hemorrhagic strokes and in combined ischemic and hemorrhagic insults. Ozone therapy should not be administered in cases of ischemic strokes with unconfirmed diagnosis

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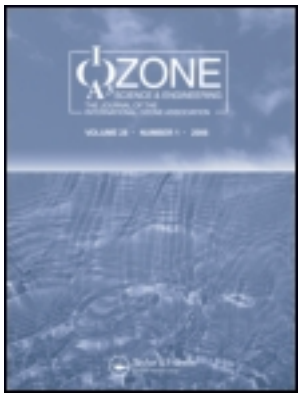

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Ozone in Medicine: The Low-Dose Ozone Concept—Guidelines and Treatment Strategies

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I. INTRODUCTION

Ozone in Medicine: The Low-Dose Ozone Concept—Guidelines and Treatment Strategies

Renate Viebahn-Hänsler,[1] Olga Sonia León Fernández,[2] and Ziad Fahmy[1]

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The low-dose ozone concept with its moderate oxidative stress represents an ideal hormesis strategy. Dose-response and concentration-effect relationships in the context with specific applications allow one to fix concentration ranges with therapeutical benefit. Based on the well-known reaction mechanisms of ozone, its biochemical and pharmacological effects, international guidelines have to be defined concerning physiological and ozone resistant materials, indications, applications and the effective concentration and dosage range in dependence on the specific indications. Following the international regulations of ozone concentrations outdoors and indoors, as working site concentrations by WHO (World Health Organization) and in conformance with the Medical Device Directives (MDD) for quality assurance and control, some European Medical Societies for the Use of Ozone have set up a draft for the essential requirements for the treatment procedures, including: (a) production of Medical Ozone; reactivity of O_3 and ozone-resistant materials; (b) ozone-free surroundings (WHO regulations) by the integration of effective catalytic systems; (c) ozone-resistant and physiologically indifferent materials used in disposables for MAH, intra-articular, intramuscular and other topical injections (for rectal insufflation, topical treatment as transcutanous gas bath and/or disinfection); (d) medical device directives for safety of the patient; (e) ozone measurement as requirement for concentrations and dosages; and, (f) concentration, dosages, and treatment frequency are listed in dependence on the indication and disease as well as on the underlying mechanism of action.

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Keywords Ozone, Ozone Therapy, Guidelines, Hormesis, Treatment Concepts, Concentration, Dose

INTRODUCTION

When used in specific diseases and conditions, medical ozone produces the same or similar therapy results worldwide. Improper application in the form of erratic methods and doses is the most frequent cause of ineffectiveness and adverse effects—and is always the cause of violent controversies.

For this reason, the medical societies for ozone application have set up treatment protocols as basis for standards and guidelines, revised and published as a result of the most recent research and 30 years of experience (Beck et al. 1998; Knoch et al. 2009). They have been used in the standardization of applications, indications, concentrations, doses, and frequency of treatment as based on the mechanism of action and the pharmacology of ozone.

1. In its pharmacological effect, medical ozone follows the principle of hormesis: low concentrations (or doses) show a high efficacy, which decreases with increasing concentration, finally reversing into a questionable and even toxic effect (Figure 1). The effect/concentration relationship for the systemic application of ozone—in the form of a standardized major ozone autohemotherapy and rectal ozone gas insufflation results in the following concentration ranges: concentrations of 10–40 μg ozone/ml ozone/oxygen mixture represent those levels that are physiologically effective and recommended for systemic application. In the high concentration range of 60–100 μg/ml the antibiotic effect of ozone has a wide range of applications in the treatment of infected wounds, diabetic foot, decubitus

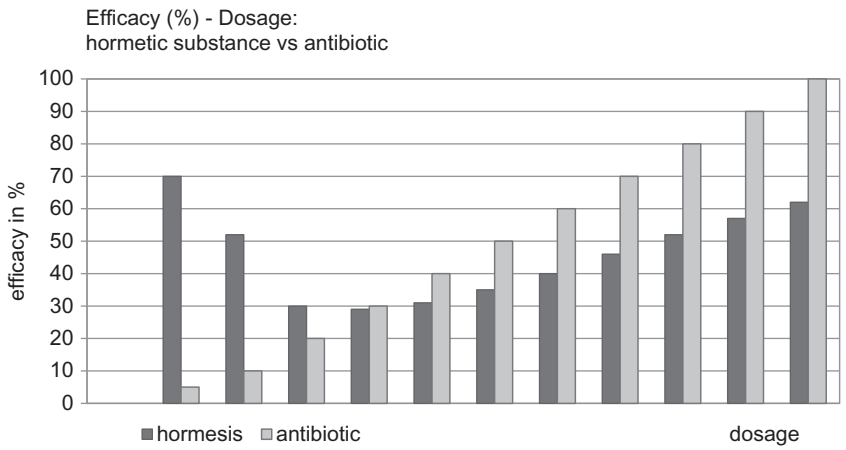

FIGURE 1. Dose-response relationship: hormetic substance vs. antibiotic (schematic): High efficacy at low doses and/or concentrations (e.g., ozone). The higher the dose, the more the efficacy decreases, thus becoming less effective in the middle-dose range and finally causing adverse or even toxic effects in the high-dose range. The efficacy of substances such as antibiotics is low at low doses and higher when the doses are increased.

ulcers and burns, but completely restricted to the topical forms of application.

2. Principally, ozone is applied complementary to a corresponding basic therapy. Diabetes, Type 2 diabetes, chronic inflammatory diseases such as inflammatory vascular disease, in particular diabetic angiopathia, chronic hepatitis forms, and chronic intestinal conditions belong to the classic indications within the low-dose ozone concept.

Chronic oxidative stress (pathologically increased values for malone dialdehyde MDA; hydrogen peroxide, H_2O_2; total hydroperoxide, TH, etc.) and an anti-oxidant deficit (disbalanced superoxide dismutase and subgroups of SOD as well as catalase CAT and others) are phenomena common to all the diseases listed. Here, in particular, low-dose ozone, as a hormetic substance, shows a regulatory effect on pathological processes.

OZONE PEROXIDE, THE PHARMACOLOGICALLY ACTIVE SUBSTANCE, AND ITS UNDERLYING MECHANISMS OF ACTION

Oxidative Stress *and* Ozone?

Does ozone treatment make sense at all if there is already an insufficiency of the cellular antioxidant system? Doesn't this increase H_2O_2, MDA, and TH (total hydroperoxides) as oxidative stressors? Thus reducing and/or destroying the antioxidant system which is already working beyond its capacity in these indications?

In a pharmacological context, we have been able to find clear answers to this and other questions, particularly from research over the last 10 years: in fact, we exert a regulatory influence on a disturbed oxidant/anti-oxidant balance (Figure 5a-b). Oxidative stress decreases in measurable form is shown by reduction of H_2O_2 (Figure 6), and a corresponding regulation of the antioxidants, as shown by SOD, GSH and G-6PDH (Figures 6 and 7). The oxidative stress markers MDA (Figures 8 and 9) and TH (Figures 10 and 11) decrease simultaneously.

"Ozone peroxides" assume the role of physiologically active ozone metabolites: Due to the selective reaction

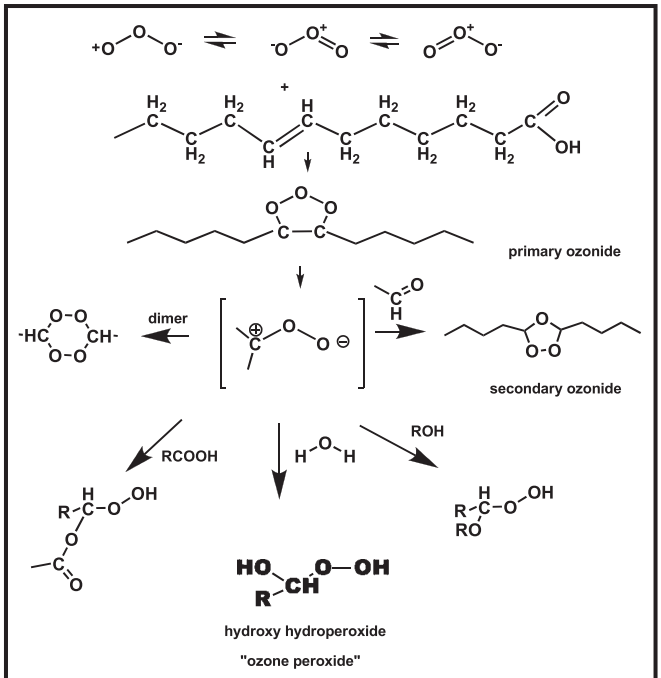

FIGURE 2. Ozone reaction with isolated double bonds, i.e., ozonolysis according to Crigée (1953, 1975). The hydroxyhydroperoxides, here "ozoneperoxides," are understood as the pharmacologically active substances.

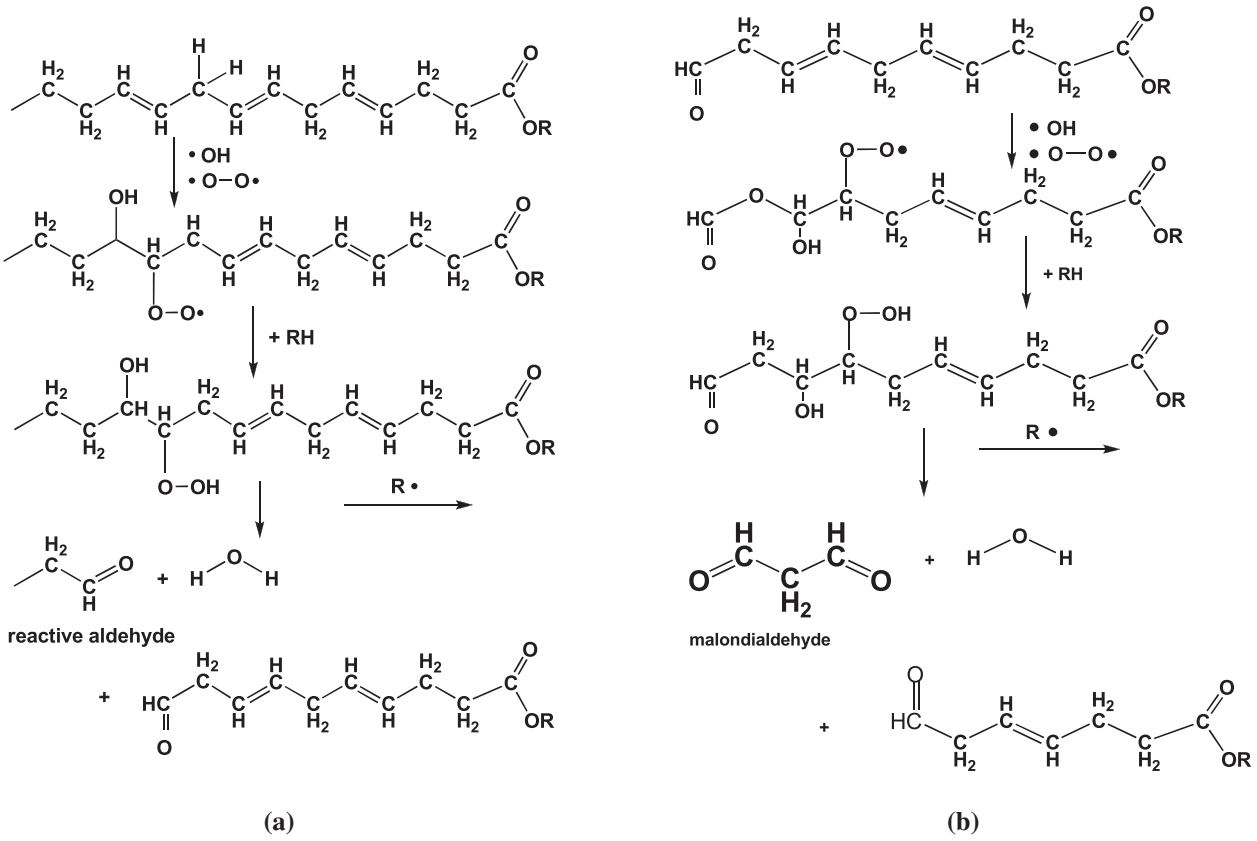

FIGURE 3. (a–b): Malone dialdehyde (MDA) formation by oxygen radicals + polyunsaturated fatty acids (PUFA).

behavior of ozone, the 1,3-dipolar (electrophilic) addition to the isolated C=C double bonds of essential fatty acids, according to classical ozonolysis as described by Criegée (1953, 1975), is the dominant reaction under physiological conditions with pH values $\leq$7,4.

Ozonolysis only takes fractions of a second, preferably forming short-chain hydroxy-hydroperoxides in an aqueous medium (Figure 2) here simply designated as "ozone peroxides," which are obviously responsible for the pharmacological effect in systemic ozone treatment.

Aldehydes, as secondary products, are only demonstrable in slight quantities, which means that malone dialdehyde MDA as measure for oxidative stress during extracorporeal blood treatment with ozone is negligible and stays within a physiological range (here see also: Bocci et al. 2005).

A short-term slight increase is possibly the result of oxygen (ozone/oxygen gas mixture) reacting with polyunsaturated fatty acids (PUFAs) in the form of autoxidation (Figure 3). These kinds of oxidations with free radicals such as OH, superoxide, peroxidic radicals, or oxygen itself, characterize also basic biological reactions for oxidative stress, which can be measured as an increase of the corresponding parameters in the blood (MDA, TH, H_2O_2 . . .). Note that this is not to be confused with ozonolysis: although ozone reacts in a highly selective way with isolated double bonds, this is not, or is far less, the case with polyunsaturated fatty acids, i.e., especially conjugated double bonds.

"Ozone Peroxides" as Second Messenger Molecules

As reactive oxygen compounds, membrane-associated "ozone peroxides," could act as second messengers via cysteine residues and/or reduction through glutathione GSH in a less aggressive way than the superoxide radicals ·O-O$^-$ and H_2O_2, and take over regulation of the anti-oxidants, i.e., without SOD and catalase demand as in the oxidative stress processes of relevant pathological conditions (Figures 4a–c).

Short-chain hydroxy-hydroperoxide with its low tendency to radical reactions could initiate the regulation of anti-oxidant protective mechanisms as redox signal e.g., via the nuclear factors NFkB in stress and inflammation processes via Nrf2 (Gough 2009; León et al. 1998; Viebahn-Hänsler 2006). Figures 5a–b present a schematic survey.

H_2O_2 formed from ozone peroxides, as proposed by other authors (e.g., Bocci et al. 2011), will probably not fulfill this function, as the H_2O_2 content is, as a rule, pathologically increased in the patients concerned; the corresponding

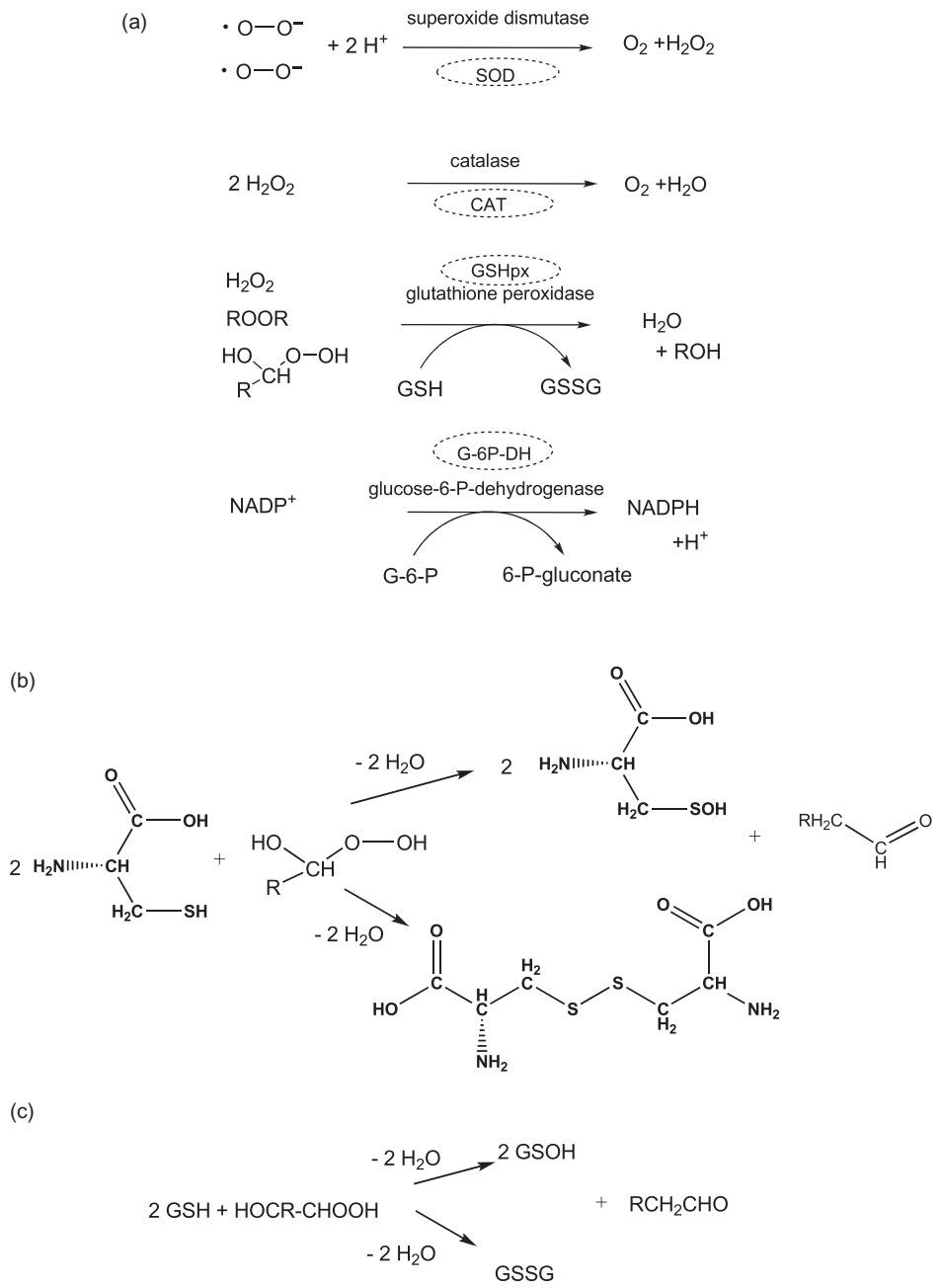

FIGURE 4. (a) The biological anti-oxidant system controlling the reactive oxygen species ROS, i.e., the oxidative stress situation. The "ozone peroxides" are controlled by the glutathione system (not by catalase); (b) Reaction of "ozone peroxides" with cysteine (residues); and, (c) glutathione.

signal transduction is insufficient, and there is a deficit in anti-oxidant enzymes.

THE LOW-DOSE OZONE CONCEPT

The effects of medical ozone as a hormetic substance can be assigned to those defined by hormesis (Rattan 2008). In living organisms, single or repetitive administration of otherwise potentially dangerous or toxic substances in small doses increases their homeodynamics (homeodynamic space), i.e., their self-regulatory capacity. Or, alternatively, moderate oxidative stress stimulates the protective mechanisms of cells and organs and is biologically useful.

Long-term ozone inhalation (e.g., for 8 h and using ozone concentrations up to 1 ppm in animal studies) produces oxidative distress, with a corresponding increase in the formation of reactive oxygen compounds, lipoperoxides,

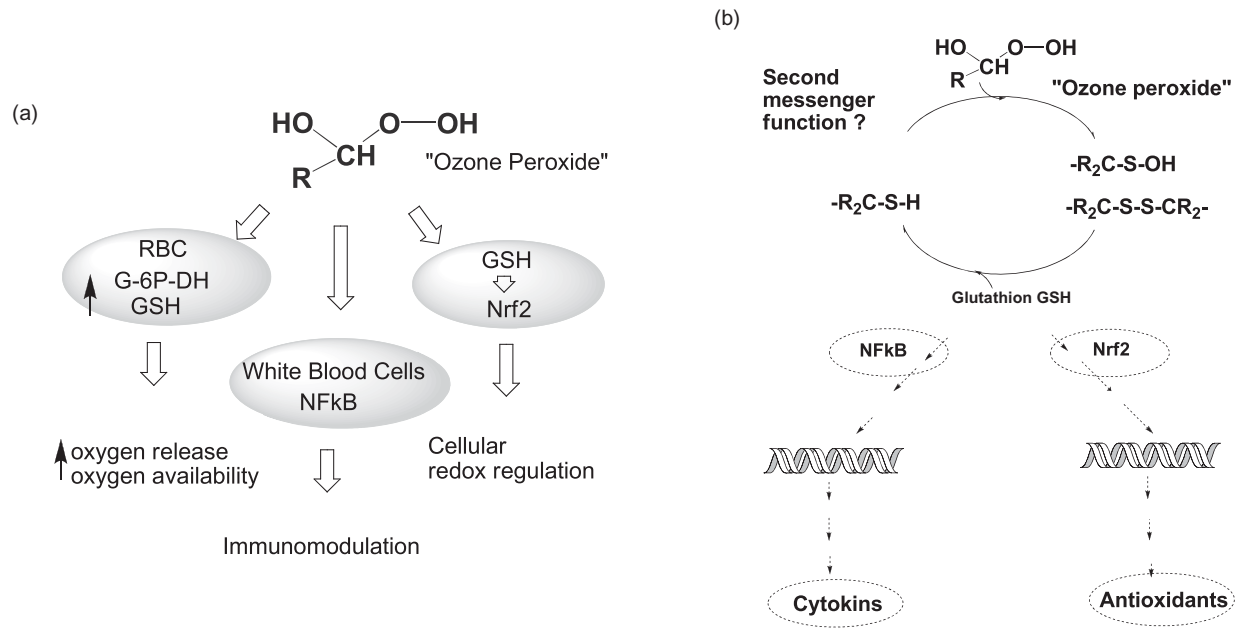

FIGURE 5. (a) Pharmacological effects of medical ozone via ozone-produced peroxides. The three effects: (1) improved oxygen release by the red blood cells (RBCs); (2) Immunomodulation through activation of the white blood cells (WBCs); and, (3) Regulation of cellular anti-oxidants. (b) "Ozone peroxide" and its second messenger function by signal transduction. Cysteine residue and glutathione oxidation processes induce the cytokine- or anti-oxidant regulation via Nrf2 or, NFkB, respectively.

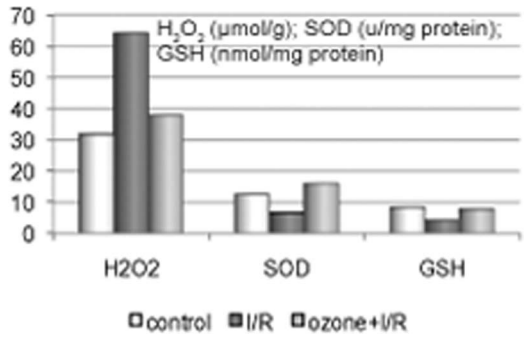

FIGURE 6. Protective effect of ozone on injury associated with hepatic ischemia reperfusion in an animal model. Pro-oxidant H_2O_2 and anti-oxidants SOD and GSH are regulated by pretreatment with ozone in the form of 10 daily rectal insufflations followed by 90 min of ischemia and 90 min of reperfusion (Peralta et al. 1999).

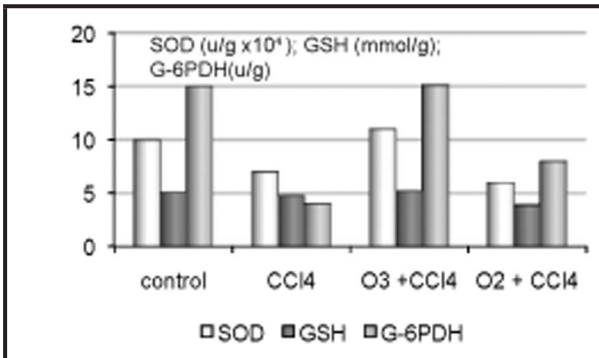

FIGURE 7. Protection against hepatocellular damage by free radicals induced by CCl4. Preventive ozone application (15 daily rectal insufflations; animal model) before administering CCl4. Liver seems to be completely protected in the ozone group: SOD, GSH and G-6PDH show physiological values, whereas the CCl_4- group as suppressed anti-oxidants (as well as the O_2-group; León et al. 1998).

cytokines, increasing infiltration of neutrophils and activated macrophages inducing and maintaining inflammatory processes. Biological response in the form of superoxide and OH radicals finally ends in a chronic inflammatory process with disfunction and downregulation of the cellular anti-oxidants.

Therapeutic usefulness is obtained via specific and well experienced forms of application, whereby airways are a taboo in ozone therapy. Single doses at low concentrations produce positive oxidative stress, i.e., equivalent to a single dose of "ozone peroxides," which result in a short-term effect on red and white blood cells involving induction of cell metabolism and bioregulation of enzymatic anti-oxidants. Table 1 shows the hormetic principle of ozone: toxicity versus therapeutical benefit in a shortened version.

Over the last 20 years, the low-dose ozone concept has developed into an established treatment method with a sound scientific basis. Its biochemical action and pharmacological

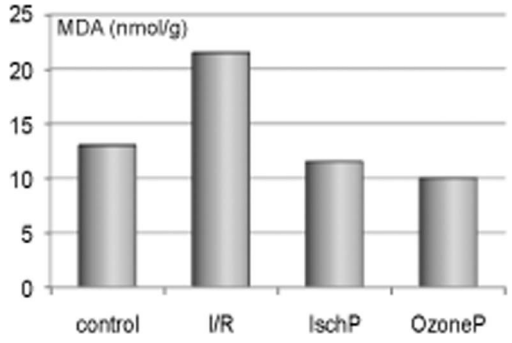

FIGURE 8. Protective effect of ozone in an ischemia/reperfusion animal model by pretreatment with ozone in the form of 15 daily rectal insufflations. Malondialdehyde MDA (as measure for oxidative stress) levels show a similar protection as ischemia preconditioning (Ajamieh et al. 2003).

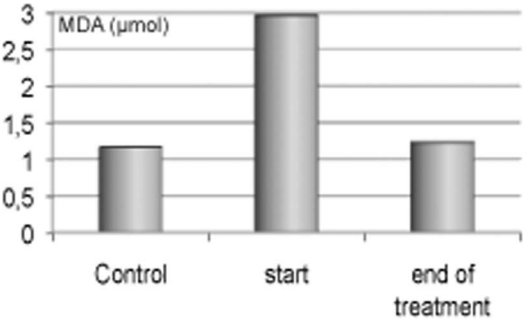

FIGURE 9. Malondialdehyde as plasma marker for oxidative stress in patients with disc hernia (acute) and after 20 days of paravertebral ozone injections (daily). Ozone reduces the oxidative stress, statistically high significance (León Fernández et al. 2012).

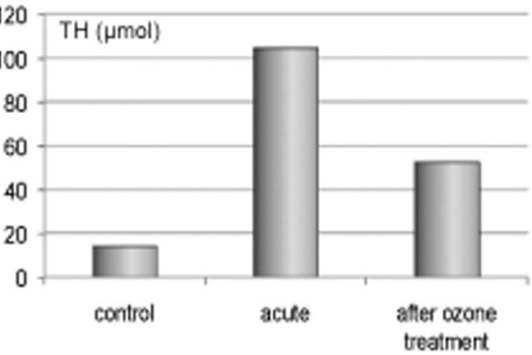

FIGURE 10. Total hydroperoxides TH as plasma marker for oxidative stress in patients with disc hernia (acute) and after 20 days of paravertebral ozone injections (daily). Ozone reduces the oxidative stress, statistically high significant (León Fernández et al. 2012).

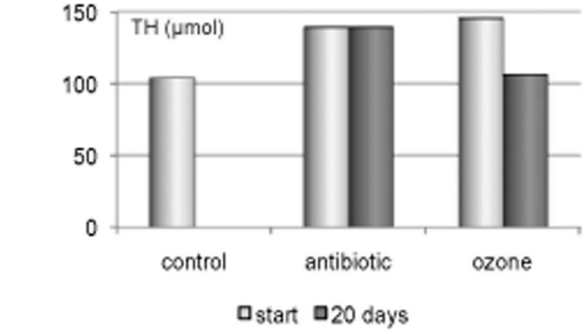

FIGURE 11. Total hydroperoxides as measure for oxidative stress in a diabetic study with 160 patients (60 healthy as control, 49 in the antibiotic group and 51 in the ozone group) decreasing significantly in the ozone group (Martinez et al. 2005).

properties have been well investigated, documented, and published in internationally recognized journals in the context of its classical indications. In the meantime, applied on a worldwide basis, ozone therapy continues to provide good and reproducible results, particularly in diabetic angiopathia, diabetic foot, chronic hepatitis, and chronic intestinal disorders.

However, application techniques and doses still vary to a certain extent; these now require recognized international standardization to establish the optimum forms of ozone application and the most effective concentration range in the relevant indications. This has been one of the major subjects dealt with by the "Medical Society for the Use of Ozone in Prevention and Therapy," in Germany, as one of 9 members (2011) of the European Cooperation of Medical Ozone Societies, over the last decade. These are based on scientific results and more than 30 years of experience (Beck et al. 1998; Knoch et al. 2009, Viebahn-Hänsler 2009).

Definition, Concentration Range, and Preparation

Medical ozone, an ozone/oxygen mixture consisting of purest O_2 and purest O_3, is produced from medical oxygen (in accordance with pharmaceutical legislation) using a medical ozone generator (Medical Devices Directives: 93/42EC or equivalent directives in non-European countries) in a concentration range between 1 and 100 µg/ml (mg/l). Therapeutically, and specific to each application, the concentration and dose ranges are listed in Table 2.

Data on the toxicity of ozone (mitotic index, cell aberrations, chromatic breaks) determined in (intraperitoneal) animal studies using mice show cytotoxicity at 2300 µg/kg, and a slight clastogenic activity at 1400 µg/kg mouse at 70 µg/ml. When administered intraperitoneally on 15 subsequent days, low concentrations of 4, 11, 20, and 35 µg O_3/ml, showed no toxicity, even at volumes of 80 ml/mouse (approx. 30 g body weight) (Fernández et al. 1989). This means that whether ozone is therapeutically useful or toxic is determined by both concentration and dose.

TABLE 1. Ozone and Hormesis: Toxicity Versus Therapeutical Benefit

Toxicity of Ozone in the Respiratory System via Inhalation	Therapeutic Effect in the Specific Forms of Medical Ozone Application
Long-Term Exposure (8 h, etc.) with ozone concentrations up to 1 ppm* (in animals)	Single Dose Low concentration
→ Oxidative DYS-Stress	Low dose → Positive stress
→ ROS (reactive oxygen species), LOP (lipid oxidation products) cytokines	→ Oxidative EU-Stress
→ Increasing infiltration of neutrophils, activation of macrophages	
→ Chronic inflammation	→ Induction of cell metabolism
→→→ Superoxide and OH-radicals.	
→ Disfunction of Anti-oxidants	
→ Downregulation of anti-oxidants	→ Regulation of anti-oxidants

* 1 ppm ≈ 2 mg/m^3

TABLE 2. Application-Relevant Concentration and Dosage Ranges in Ozone Therapy

Application	Ozone Concentration Range	Ozone Volume	Dosage/Ozone Amount Per Treatment
Systemic Treatment			
Major autohemotherapy (MAH)	10–30 μg/ml (max. 40 μg/ml)	50 ml	500–1,500 μg (max. 2000)
Rectal insufflation	10–25 μg/ml	max. 300 ml	3,000–7,500 μg
Minor autohemotherapy	10–20 μg/ml	10 ml	100–200 μg
Topical Treatment			
Wound cleansing	80–100 μg/ml		
Wound healing	10–25 μg/ml		
Injections in pain Syndrome	1–10 μg/ml	1 ml–20 ml	1–200 μg
In combination with local anesthetic	10–20 μg/ml	1 ml–20 ml	10–400 μg

Compared with this, in humans, the doses of 21.4 μg ozone/kg body weight applied (70 kg) in major autohemotherapy and 107 μg ozone/kg body weight in rectal insufflation are low, and can be administered over an extended period of time without any adverse effects. To make full use of the bioregulative function of ozone, low concentrations and small doses are required in the standardized applications (Figure 12). Figure 13 gives a concentration/effect relationship for systemic ozone applications.

Preparation and Measurement of Medical Ozone

Contrary to technical and smog ozone, the O_3 used in medicine is produced from pure medical oxygen via silent electrical discharge; it is not acceptable to use oxygen concentrators or oxygen/air mixtures due to their nitrogen component and the consequent possibility of nitrogen oxides being formed in the discharge tube.

As with other pharmaceuticals, medical ozone is a clearly defined molecule with a clearly defined range of action. With a half-life of 55 min in a 50 ml disposable injection syringe (completely siliconized and ozone resistant), medical ozone must be prepared on-site and made especially available for the type of application required.

As the concentration and decomposition rate of ozone is extremely dependent on different parameters such as temperature, pressure, volume flow rate, etc., medical ozone generators have to be equipped with a measurement device to ensure continuous concentration control.

Ozone produced in excess, either as part of the generator gas or after local application, must always be completely reduced back to oxygen to avoid odor and inconvenience to the respiratory tract; correspondingly, the system must be equipped with high-power catalysts (active carbon must not be used due to temperature and combustion hazard). The maximum workplace concentration is 120 μg/m^3 (or 180–240,

FIGURE 12. Major autohemotherapy. Standardized system acc. to the "Medical Device Directives" 93/42 EC. (1) Ozone syringe (Polypropylene); (2) Bacterial filter (0.2 μm); (3) Vacuum bottle (glass); (4) Transfusion set (polyethylene) (color figure available online).

respectively) (European Communities 2008); the maximum immission concentration is: 100 $\mu g/m^3$ (WHO 2006).

Measuring Ozone

Due to a powerful absorption band in the ultraviolet range (Hartley Band) with a maximum absorption at 253.7 nm, a photometric procedure is a method of choice at this wavelength for continuous ozone concentration measurement, and has become an international standard on which other measuring methods base their values and correspondingly apply for calibrating, as summarized by the International Ozone Association (Masschelein et al. 1998).

Quality Assurance

From a quality assurance and quality control point of view, the high reactivity of ozone with organic substances requires a careful selection of materials needed for the different types of medical equipment:

- Only special materials can be used in ozone generators, such as Teflon (PTFE), specially anodized aluminum, V_4A quality stainless steel (in long-term use, V_2A quality is subject to surface changes), glass, and ceramics.
- For application systems only "ozone-resistant" materials such as glass, polyethylene (PE), polypropylene (PP), and PTFE may be considered.
- Other plastics, especially for syringe pistons, must be silicone-coated.
- Medical plasma *flasks* as used for reinfusion should be made of *glass only*. Plasma bags or blood bags made of non-ozone-resistant, soft polyvinylchloride (PVC) must not be used. Reactions between ozone and these materials can occur producing xenobiotic and/or toxic substances, especially during O_3 blood treatment requiring up to 5 min to obtain the proper effect. The substances arising from a decomposition of the softening agents in the plastic, such as hydrogen peroxide or phthalic acid esters are not only able to distort the desired effects of ozone, but also damage the patient's health.
- For preparing and storing ozone-treated water, containers made exclusively of glass are to be used. These must have as small a volume (e.g., 250 ml) as possible; they should be completely filled and well sealed with O_3-resistant material.
- Use sterile, siliconized, 50-ml disposable syringes (measured ozone half-life: 55 min) to transport ozone/oxygen mixtures for use during home visits.

Ozone generators as well as all the disposables used for treatment have to fulfill the MDD (Medical Device Directives) in Europe (93/42 EC) and are labeled with a "CE" sign, including the number of the supervising authorities. Manufacturers must possess detailed and valid certificates.

THERAPEUTIC APPLICATIONS AND INDICATIONS

Major Autohemotherapy (MAH) with Ozone as Systemic Application

Writing guidelines for quality assurance and MAH procedures is only useful if, right from the beginning, incorrect applications are recognized as quality detraction factors that must be avoided at all cost. These are:

1. Application of intravenous injections and transfusions under pressure - (hazard of embolism!)
2. Application of O_3 gas with inadequate or unsuitable materials; e.g., use of non-ozone-resistant blood bags – this may result in the formation of xenobiotic substances
3. Withdrawal of an O_3/O_2 gas mixture via a direct and solid tube connection between the outlet valve of the ozone generator and the flask – (retrograde contamination with blood!), or
4. Repeated reuse of syringes without disinfecting, cleansing and sterilizing them as required – (infection hazard!).

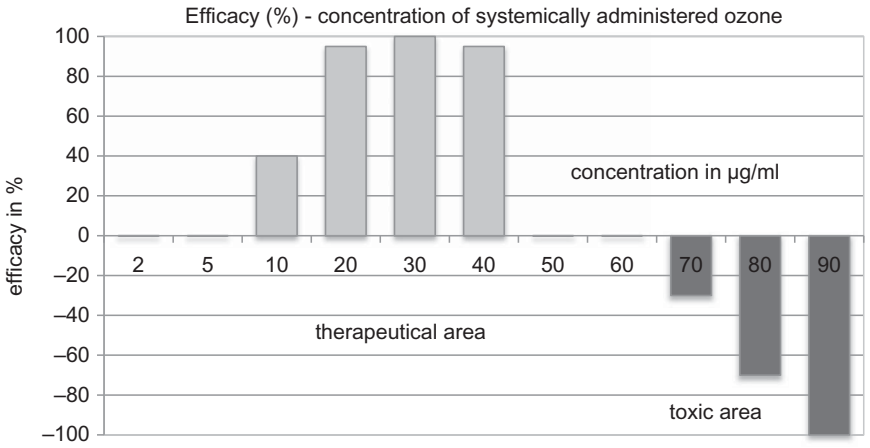

FIGURE 13. Efficacy concentration relationship of systemically administered medical ozone.

MAH Indications and Application Methods

MAH is reserved as a complementary medical concept for special indications. These are:

Arterial circulatory disturbances

- Peripheral arterial circulatory disturbance
- Cerebral circulatory disturbance (poststroke)
- Ocular circulatory disturbances (retinopathies)
- Inner ear circulatory disturbances (acute hearing loss (AHL), tinnitus)

Angiopathia

- Diabetic angiopathia in particular

Virus-caused diseases

- Hepatitis B and C
- Herpes simplex, herpes zoster

General immune deficiency

- As complementary therapy in general asthenia, geriatric and environmental medicine

Complementary concept in oncology

Chronic inflammatory processes in orthopedics and rheumatology

Contraindications

- Glucose-6-phosphate dehydrogenase deficiency (favism, acute hemolytic anemia)
- Hyperthyroidism if not controlled
- The first 3 months of pregnancy
- MAH is *not* indicated in leukemia

Procedure

In MAH, under strict aseptic conditions, 50–100 ml venous blood are withdrawn from the patient into a vacuum flask with sodium citrate as anticoagulant, where the medical ozone/oxygen gas mixture is added to it—extracorporeally in a closed, sterile system—before being re-infused via drip infusion (pressure-free).

The ozone/oxygen mixture must pass through the patient's blood evenly, preferably using what is called the microbubble system to produce a reaction surface as large as possible in the short reaction time (less than 1 s). Thus contact with ozone can take place with nearly all RBCs and nearly all WBCs making the ozone reaction as effective as possible, while the oxygen bubbles through, forming a layer of O_2 gas above the liquid level in the flask, see Figure 12 and Tables 3a–b.

Following contact with the blood outside the body, not one single ozone molecule nor one single oxygen molecule, enters the patient's vascular system. Only the products of a reaction between the ozone and the cellular components of the blood, i.e., activated RBCs and activated WBCs, are infused. In the presence of organic substances such as membrane lipids, the life of a highly reactive ozone molecule is extremely short (<1 sec), i.e., it is reduced prior to re-infusion.

Ozone Concentrations and Dosage in MAH

Based on the results of fundamental research over the last 18 years, the ozone concentrations and required total amounts determined in practice can be given in concrete terms. The concentrations used below are cited in the standard measuring unit of µg/ml (1 µg/ml = 1 mg/l = 1 g/m^3) under ambient conditions on-site /around the patient. Here, care must be taken whether we are discussing:

- µg ozone per ml ozone/oxygen mixture which is delivered by the ozone generator,
- µg ozone per ml blood, or
- the total quantity of ozone in µg per total quantity of blood, or the total quantity of ozone in µg per treatment.

TABLE 3a. Preparation and Performance of Major Autohemotherapy (MAH) Disposables

- Hand disinfectants on an alcohol basis
- Skin disinfectants on an alcohol basis or sterile alcohol
- swabs vacuum-packed in plastic foil
- Sterile cotton wool or gauze swabs
- Hypoallergic injection plaster
- Sterilized covering cloth
- 250 ml sterile vacuum gas flask with microbubble system, with sterile, pyrogen-free sodium citrate, without preserving agents
- Sterile, pyrogen-free transfusion unit with gravity drip chamber and tube clamp
- Sterile, pyrogen-free butterfly (cannula) set
- Sterile, pyrogen-free transfusion set with bacterial filter and tube clamp, for O3 administration in the 250 ml sterile vacuum gas flask
- Sterile, silicone-coated 50 ml disposable syringe, with preconnected bacterial filter
- Mobile (! Independent of patient) ozone supply unit (generator) equipped with a photometer for concentration measurement.

TABLE 3b. Performing MAH, Including Aseptic Procedures

- Disinfect both hands properly using 3–5 ml of a special hand disinfectant, observing the prescribed time to take effect of at least 30 s; in cases of possible contamination with stable viruses (HBV, HCV, HIV), this period should be 5 min. It is the aim of these elaborate precautions to encourage the wearing of protective (surgical) gloves, a preferable measure in all cases.
- Remove the protective cap of the 250-ml vacuum flask; the preferable method is to use both thumbs, pushing up and away from below. Disinfect the stopper with a skin disinfectant by spraying on, allow to dry (requires >1 min to take effect).
- Close clamps and introduce the cannula of the "germ stop" system through the cross marked on the stopper (microbubble system).
- Close clamp of the transfusion set; introduce it through the large circle marked on the stopper by piercing through.
- From the Teflon adapter on the generator, withdraw the O3/O2 mixture with a sterile, silicone-coated 50 ml disposable syringe with a preconnected bacterial filter (after previous loosening the piston of the syringe to overcome possible adhesion). The syringe is filled by the inherent pressure in the unit. Flush out the syringe as required with the gas once more. The remaining ozone is converted back to pure oxygen by the catalyst. In this way, neither the generator nor the syringe comes directly into contact with the patient. Always remember that **dry** ozone is **not** able to act as a microbiocide, inactivate viruses, or disinfect; so bacterial filter and syringe can only be used once.
- Connect the syringe filled with 50 ml gas mixture to the cone of the bacterial filter of the "germ stop" system
- Disinfect the patient's skin properly in the area around the infusion site (arm vein) via spraying on a skin disinfectant and distributing it with a sterilized cotton swab or gauze (in vacuum pack); allow to take effect for at least 1 min. From the butterfly cannula, withdraw approx. 50 ml patient's blood via the infusion system into the vacuum flask and fix butterfly with strip of plaster.
- Withdraw the O3/O2 mixture under vacuum from the disposable syringe via the bacterial filter of the "germ stop" system to ensure a smooth passage through the blood in the form of minute bubbles producing the desired immediate reaction between the ozone and blood cells. After passing through the blood, the remaining oxygen accumulates in the flask above the surface of the liquid.
- Carefully turn over the vacuum flask, remove the gas syringe for de-aeration and pressure-free re-transfusion of the ozone-treated blood, open and regulate the clamp in the transfusion set (60–90 drips/min).
- Remove the intravenous butterfly cannula, dab over the infusion point using a sterile cotton or gauze swab before covering it with a pressure dressing (hypoallergic injection plaster).

Ozone dosage covers a range between 500 μg and maximum 4000 μg ozone per treatment, using a quantity of blood between 50 and 100 ml. The sometimes recommended blood quantity of 200–300 ml is to be *rejected*, as this can present a risk from a hemodynamic viewpoint, especially in elderly or decompensated patients.

For blood treatment, concentrations of 80 μg ozone per ml whole blood and above are also to be *rejected*, on account of the increasing risk of hemolysis (up to 10% at 100 μg ozone per ml whole blood), a decrease in 2,3-diphosphoglycerate (2,3-DPG) and in anti-oxidants. Empirically, in major autohemotherapy (MAH), concentrations between 10 and

TABLE 4a. Major Autohemotherapy—Standard Procedure

Standard Procedure: 50ml of Blood + 50 ml Ozone Oxygene Mixture (or 100 ml of Ozone per 100 ml of Blood)			
Ozone concentration per ml of gas	10–20 μg/ml gas	30 μg/ml gas	Maximum 40 μg/ml gas
per ml blood = biologically relevant concentration	10–20 μg/ml blood	30 μg/ml blood	40 μg/ml blood
Total ozone amount per 50 (100) ml blood	500 –1,000 μg per treatment	1,500 μg per treatment	2,000 μg per treatment

40 μg ozone per ml blood have demonstrably shown themselves to activate cellular metabolism and have immunomodulatory effects as well as a regulatory effect on the intracellular anti-oxidants. The standard procedure for MAH is shown in Table 4a and the treatment protocols for the different indications in Table 4b.

Rectal Ozone/Oxygen Insufflation

This is one of the earliest forms of application in ozone therapy (Aubourg 1936). Based on animal investigations and a comprehensive proctologic study (Knoch et al. 1987), rectal insufflation with an O_3/O_2 gas mixture is increasingly being used as a systemic therapeutic form, and is already being viewed as an alternative to MAH; it is the method of choice in pediatrics.

Indications

Local

- Ulcerous colitis
- Proctitis, stages I and II
- Anal fistulae and fissures

Systemic

- Indications cited for MAH
- Hepatitis B and C
- For immunomodulation (complementary method in oncology)

Method

A rectal insufflation set consists of: An ozone supply container with lock valve, dosing bag with nonreturn valves, connecting tube with luer/luer lock or 50 ml silicone-coated disposable syringe, and rectal catheter.

Dosage

- Systemic: 10–25 μg ozone/ml oxygen gas mixture, volume 150–300 ml; for children: 10–20 μg/ml, volume 10–30 ml
- Local: in ulcerous colitis, high O_3/O_2 concentrations (70–80–100 μg/ml) and small volumes (50 ml) are applied; on cessation of hemorrhage, this is reduced to 30–20 μg/ml, followed by systemic efficacy: 10–20 μg/ml, 150–300 ml volume.

Rectal ozone application is simple, low-cost and practically free of adverse reactions when dosages are adhered to exactly.

As an adjuvant therapy in proctitis and proctocolitis, rectal insufflation is scientifically founded and to be recommended. Rectal O_3 insufflation is being increasingly used in pediatrics, sports medicine, geriatrics, and as a complementary method in oncology (Table 5).

Minor Autohemotherapy with Ozone

As a non-specific, immune stimulant therapy, comparable "autovaccination," not only ozone-specific.

Indications

- Acne vulgaris
- Allergies
- As an adjuvant in cancer therapy
- Immunoactivation

In minor autohemotherapy (MinAH), under aseptic conditions, 2–5 ml blood is removed intravenously and drawn into a sterile, pyrogen-free 30 ml disposable syringe (already containing the ozone-oxygen mixture), where it is mixed with 10 ml of an O_3/O_2 gas mixture, intensively shaken and slowly reinjected intramuscularly in the ventrogluteal region. Ozone concentration: 10–20 μg/ml, Table 6. (Caution: Never fill a syringe containing blood at the ozone generator!)

Topical Ozone Applications

In the local application of an O_3/O_2 gas mixture externally to skin or wounds—already practiced during the First World War—it was the disinfectant and deodorizing effect of ozone that stood in the foreground. It is now known that, with the topical application of O_3/O_2 gas mixtures, from ozone-treated water or ozone cream (ozonides) and beyond, a wound-healing effect is produced, which is being used to an increasingly successful extent.

TABLE 4b. Major Autohemotherapy MAH—Treatment Concept

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency	Number of Treatments
Arterial Circulatory Disorders					
Cerebral and peripheral stage II	15–20 μg/ml	50 ml	750–1,000 μg	2x per week	Series of 10 treatments, 2–3× per year
Stages III and IV	20–30 μg/ml	50 ml 100 ml	1,000–1,500 μg 2,000–3,000 μg per 100 ml of blood	Daily at first, later 2× per week	
Immune Regulation, Oxidative Stress Regulation					
Revitalization, general immune deficiency, infection prevention	20–25 μg/ml	50 ml	1,000–1,250 μg	2x per week	Series of 10 treatments, 2–3× per year
Geriatrics AMD	15–20 μg/ml	50 ml	750–1,000 μg	2x per week	Series of 10 treatments, 2× per year
Virus-Caused Diseases					
Hepatitis acute stage	30 (max. 40) μg/ml	50 ml 100 ml	1,500 (2.000) μg 3.000 (4,000) μg	Daily in the beginning, then 1× per week, 2× per month	As per control
Chronic form (B/C)	10–20 μg/ml	50 ml 100 ml	500–1,000 μg 1,000–2.000 μg	2× per week, then 1×per week, then 2× per month daily	6–12 months
Herpes zoster Acute	40 μg/ml	50 ml	2,000 μg		As per control
Postacute	20–30 μg/ml	50 ml	1,000–1,500 μg	2× per week	As per control
Complementary Oncology					
Prevention of side effects of chemotherapy and/or radiation	10–15 μg/ml	50 ml	500–750 μg	2–3× per week	If possible 10 treatments before starting chemo- or radiation therapy
During chemotherapy	10–15 μg/ml	50 ml			
Or as complement to a biological concept	10–15 μg/ml	50 m	500–750 μg	2× per week	As per control and in compliance with the patient
Inflammatory Processes					
Rheumatoid arthritis acute stage	30–35 μg/ml	50 ml (100 ml)	1.500–1.750 μg per 50 ml blood (3.000–3.500 μg per 100 ml of blood)	Daily	As per control
non acute stage	20–25 μg/ml	50 ml	1.000–1.250 μg	1x per week, then every 2nd week	In compliance with the patient
Angiopathia, diabetic angiopathia	20–25 μg/ml	50 ml	1.000–1.250 μg	2x per week, later 2x per month	In compliance with the patient

Indications

- External ulcers (ulcus cruris, decubitus ulcers)
- Burns, superinfected
- Skin lesions (wounds)
- Local infections (smear infections, herpes simplex, herpes zoster, mycosis)
- Eye injuries and infections.

Application forms

- Ozonized water (acute treatment: e.g., injuries, burns, ulcers, as intraoperative rinsing)
- Pressure-free application in ozone-resistant plastic bags, in the form of transcutaneous O_3 gas bath (e.g., ulcus cruris, immune vasculitis)

TABLE 5. Rectal Ozone Application—Treatment Concept

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency	No. of Treatments
Local Effect					
Ulcerate colitis	70–100 μg/ml	50 ml	3,500–5,000 μg	daily at first, later 1–2× per week	3–5, then like proctitis
Proctitis, particularly stage I	10–25 μg/ml	300 ml (150 ml)	3.000–7.500 μg (1.500–3.750 μg)	2–3× per week	4 weeks as percontrol
Anal fistulae	10–40 μg/ml	10–50 ml	Insufflation into the fistular passage	Daily at first, later 2×per week	As per control
Systemic Effect (Rectal Insufflation as Alternative to MAH)					
General immune activation, infection prevention, elderly	15–20 μg/ml	300 ml	4,500–6,000 μg	2× per week	Series of 10 treatments, 2–3× per year
Arterial circulatory disorders (stage II)	20 μg/ml	300 ml	6,000 μg	2× per week	Series of 10 treatments, 2× per year
Diabetic angiopathia	20–25 μg/ml	300 ml	6,000–7,500 μg	2× per week	In compliance with the patient
Complementary oncology					
Before chemo-/ radiation therapy	15 μg/ml	300 ml (150 ml)	4,500 μg 2,250 μg	daily	6–10 treatments
During and after chemotherapy or radiation	15 μg/ml	300 ml (150 ml)	4,500 μg 2,250 μg	2× per week	In compliance with the patient
Without chemotherapy or radiation	15 μg/ml	300 ml	4,500 μg	2× per week	In compliance with the patient
Virus-Caused Diseases and Inflammations					
Hepatitis, esp. chronic form (B/C)	25 μg/ml	300 ml	7,500 μg	Daily in the beginning, then 2× per week, 2× per month	As per control but mostly 6–12 months
Herpes zoster	25 μg/ml	300 ml	7,500 μg	2× per week	As per control
Rheumatoid arthritis	20–25 μg/ml	300 ml	6,000–7,500 μg	2× per week	In compliance with the patient

TABLE 6. Minor Autohemotherapy

Indication	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency
Acne, furunculosis	10–20 μg/ml	10 ml	100–200 μg	1× per week (max 2× per week
Allergies	20 μg/ml	10 ml	200 μg	1× per week
Additional Cancer therapy	10–20 μg/ml	10 ml	100–200 μg	1× per week

- Subatmospheric ozone gas application under an ozone-resistant suction cup according to Werkmeister (1995) (e.g., decubitus)
- O_3 gas application in the low-pressure plastic boot ("Rokitansky boot") (e.g., diabetic gangrene)
- Ozone cream (ozonides) for long-term treatment: e.g., lesions, burns

Ozonized Water

In topical applications, the use of ozonized water is now gaining in importance. Ozone is present in water in molecular form, i.e., as O_3, presenting a physical solution. When using bidistilled water (aqua bidestillata) and a high-quality ozone generator, a maximum saturation of approx. 20 μg ozone per ml of water at room temperature can be obtained. It reacts immediately on skin contact, contrary to ozonides (such as ozone cream), which have a long-term effect.

Indications

- Local infections
- Ulcus cruris
- Decubitus ulcers
- Mycosis, mycotic infections
- Herpes simplex and herpes zoster (also including subcutaneous ozone injections where required)
- Burns, also superinfected burns
- Intraoperative rinsing
- Eye injuries and infections
- Surgical scars (healing: primary or secondary)
- Edemas of traumatic or bacterial origin

Methods and dosage

For 5–15 min, allow an ozone/oxygen gas mixture at an O_3 concentration > 100 (10 min) or 60–80 μg/ml (15 min) to pass in the form of small bubbles through 1-l aqua bidestillata with a water column of approx. 40 cm. In bidistilled water, the half-life of ozone is approximately 10 h at room temperature, the concentration remaining approximately 18–24 μg/ml at 20 °C (68 °F). In the refrigerator, ozonized bidistilled water can be kept for several days.

Over dosage is not possible, as the dose is limited by the solubility of ozone in water, approximately 24 μg/ml for aqua bidestillata. Ozonized water is basically applied on account of its pain-relieving, disinfectant and anti-inflammatory effects, as well as its tissue-activating properties in acute and chronic injuries with and without infection. In these indications, it is being applied with increasing success. Here, especially, the elimination of perifocal edema is in the foreground. Ozonized water is also being used intraoperatively for rinsing (disinfection), as in hand surgery, in dental medicine and particularly in oral surgery. The healing time for primary scars is shortened and irritation-free. In a number of cases, long-term treatment can be continued using peroxidic oils (Table 7).

Ozone Cream (Ozonides and Peroxides)

As reaction products of O_3 and unsaturated fatty acids, ozone peroxides and ozonides also stimulate wound healing. This could be clearly demonstrated for burns and mechanical injuries in an animal study. Peroxidic oils are used in the long-term treatment of injuries, burns and local infections such as skin and nail mycosis, as well as in the follow-up treatment of ulcus cruris and decubitus ulcers.

Topical Treatment as Transcutanous Gas Immersion or "Bath" (Bagging)

Transcutaneous "ozone gas immersion" is a method of choice in extensive, deep topical infections. Here, after moistening the extremity to be treated or the area over the organ concerned, direct O_3 gas immersion is applied inside a sealed,

TABLE 7. Topical Applications

Indication	Ozone Concentration	Form Of Application	Treatment Time	Treatment Frequency
Decubitus ulcers	80–100 μg/ml in the beginning	Low pressure suction cup	2–10 min	Daily first, then 1–2× per week
After wound cleansing	20–30 μg/ml			
Diabetic gangrene	80–100 μg/ml in the beginning	Low pressure boot (or plastic bag)	10–20 min	Daily first, then 1–2× per week
After wound cleansing	20–30 μg/ml			
Ulcus cruris	80–100 μg/ml in the beginning	Plastic bag (not low pressure!)	10–20 min	Daily first, then 1–2× per week
Wound cleansing				
Wound healing	20–30 μg/ml	compresses + rinsing with ozone water	1–5 min	several times daily
Burns stage 1 and 2	20–30 μg/ml	Plastic bag, compresses + rinsing with ozone water	10–20 min 1–5 min	First 1–2× per day, several times per day

ozone-resistant plastic bag, or in a slight vacuum using a special, low-pressure "boot" or under a low-pressure cup.

Indications

- Skin lesions, burns, superinfected wounds (surgical scars), diabetic foot, phlegmons (erysipela)
- Large-surface, open and deep chronic ulcers, possibly infected, and decubitus ulcers.

In transcutaneous ozone gas immersions or low-pressure applications used in the treatment of infected ulcers, the O_3/O_2 mixture is initially applied at higher concentrations (70–100 μg/ml) after moistening of the area to be treated. Its microbicidal and virostatic effect already takes place at lower concentrations (< 40 μg/ml). Once wound healing has actually started, the concentration can again be reduced (< 20 μg/ml), thus making full use of the metabolically stimulant and immunomodulatory effect of ozone as the healing process continues.

Low-pressure ozone treatment is not a monotherapy, as the other forms of wound treatment must be continued at the same time, according to the condition of the wound and in conformity with current rules. The considerable local hyperemic effect due to the mildly subatmospheric conditions plus the properties of ozone contributes to the healing process (Table 7).

Intra-articular ozone injections

Intra-articular ozone injections in acute and chronic, painful joint conditions represents a complementary treatment method providing rapid pain relief, decongestion, a reduction in inflammation, and an improvement in motility. It involves mainly knee and shoulder joints presenting chronic pathological symptoms.

Indications

- Symptoms of the rheumatic and degenerative type, diseases and injuries of the joints (arthrosis, arthropathias)
- Active gonarthrosis, acute diseases of the shoulder joints involving partially restricted functional movement (shoulder stiffness)
- Chronic shoulder joint conditions with calcification and painfully restricted movements in the final stage.

Intra-articular ozone injection is being applied with increasing success, particularly in orthopedic clinics, where inflammatory and degenerative diseases of the bones and joints as well as posttraumatic conditions (i.e., following sport injuries) and surgery of the large joints are involved, cases in which additional MAH has a supportive and stabilizing function.

Performance

Prior to ozone application, the standard injection points can be infiltrated with a slow-acting local anesthetic. For intra-articular ozone injections, a volume of approx. 20 ml for shoulder and knee is used, with ozone concentrations of 10–20 μg/ml and 2–11 μg/ml for peri-articular and subcutaneous infiltrations (Table 8).

Intra-articular injection of an ozone/oxygen gas mixture must be considered as being an intervention in a sterile system (joint), to be carried out under especially strict aseptic precautionary measures (Table 9). In addition, the ozone unit must be cleaned every day after use as hygiene requires (see manufacturer's instructions), and additionally disinfected by wet wiping with corresponding agents (surface disinfectants) when contamination with blood has occurred.

Regular maintenance of the unit must also be counted as hygienic safety. The aseptic steps recommended agree, for the most part, with those issued by the "German Orthopedics and Traumatology Association," the Orthopedic Physicians' Professional Association, and the "Guidelines on Intra-articular Injection Procedures" published by the Hospital Hygiene Work Group. These guidelines continue to be the officially recognized standard for medical experts and law courts, and should carefully be adhered to.

Subcutaneous and Intracutaneous Application

Indications

- Pain management
- Trigger points, acupuncture points
- Herpes zoster

TABLE 8. Intra-articular Injections—Treatment Concept

Indication	Form Of Application	Ozone Concentration	Ozone Volume	Ozone Amount	Treatment Frequency
Arthrosis	Intra-articular	7–20 μg/ml	1–20 ml	7–400 μg	1–2× per week
	Peri-articular	2–11 μg/ml	2–5 ml	4–55 μg	
Knee joints	Intra-articular	10–20 μg/ml	5–20 ml	50–400 μg	1–2× per week
Shoulder joints	Intra-articular	10–20 μg/ml	5–20 ml	50–400 μg	1–2× per week
Finger joints	Intra-articular	10–20 μg/ml	1–2 ml	10–40 μg	1–2× per week

TABLE 9. Aseptic Procedure for Intra-articular Ozone Injections (Beck et al. 1998)

For intra-articular injections, special aseptic precautionary measures are necessary to prevent infection! [see e.g., "German Orthopaedics and Traumatology Association" (Deutsche Gesellschaft für Orthopädie und Traumatologie) Guidelines] Caution: To avoid general or local infections and skin lesions at injection site and surrounding area:

- Hygienic hand disinfection using an RKI/DGHM listed (or equivalent) alcohol-based detergent. Always allow > 30 s for it to take effect. In cases of suspected HBV or HCV, etc., virus carriers, allow for 5 min before treating or, even better: use (sterile) disposable surgical gloves!
- Alternatively: after surgical hand disinfection, put on sterile surgical gloves, sterile protective clothing, and use sterile cloth covers around the injection site (e.g., when in contact with the patient's skin over the area under treatment).
- Disinfection of skin at treatment site using an RKI/DGHM listed (or equivalent) alcohol-based disinfectant: spray liberally over site and gently rub in with a sterile gauze swab. Allow > 1 min to take effect.
- From the Teflon valve of the supply unit, remove the prescribed quantity of medical ozone/oxygen gas using a sterile, silicone-coated 50 ml disposable syringe with a preconnected bacterial filter.
- Using a long, thin, sterile disposable cannula, e.g., 0.8 × 40 mm (size 2 metric) or 0.6 × 60 mm, inject the ozone/oxygen gas mixture.
- Immediately cover the injection site with a (sterile) quick-action wound dressing.

- Trigger points in combination with local anesthetics (neural therapy)
- Tonanalgetic systems

Ozone concentrations: 2–5 without local anesthetic, 10–15 μg/ml with local anesthesia.

Intradiscal injections

Intradiscal injections in herniated discs may only be performed when monitored by imaging systems as found in hospital departments where these are available (Alexandre et al. 2012, this issue).

FINAL COMMENTS

These guidelines for the use of medical ozone have been set up and evaluated on the basis of nearly 40 years experience and on relevant scientific literature as published in national and international journals and handbooks.

- The German "Medical Society for the Use of Ozone in Prevention and Therapy" was founded in 1971 as a nonprofit organization.
- Together with the sister societies in Switzerland, Austria and Italy the "European Cooperation of Medical Ozone Societies" EUROCOOP was founded in 2002.
- The 9 medical societies in 2011 consisted of the following countries: Switzerland, Austria, Germany, Spain, Romania, Egypt, Turkey, Japan, and Indonesia. Its aim has been to standardize ozone therapy to the greatest possible extent. In the same way, the member societies will aim at providing mutually valid training and development guidelines, making provisions to the fullest possible extent for all indications and application methods of ozone therapy. In the long run, a European Standard Qualification could become a uniform objective.
- Every 2nd year an International Ozone Congress is held, organized by one of the member societies.

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MADRID DECLARATION ON OZONE THERAPY

Approved at the "International Meeting of Ozone Therapy Schools" held at the Royal Academy of Medicine in Madrid on the 3rd and 4th of June, 2010, under the auspices of the Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO)

Taking into account that since the discovery of ozone by the German chemist Christian Friedrich Schönbein in 1840, its medical use has increased in different parts of the world; there is more interest from health professionals to know how it works and what are its benefits; the number of ozone therapists keeps growing all around the world; and an increasing number of patients are benefiting from it. However its consolidation has not been easy, resistance it still found within the medical community and its recognition in the legal field will require more and coordinated efforts.

Recalling that pre-clinical research and clinical trials on the use of ozone therapy have been carried out in Cuba, Germany, Italy, Russia and other countries, with considerable scientific rigor, obtaining results that support its practice using different medical protocols.

Bearing in mind that the preclinical studies, genotoxics, toxicology and clinical studies carried out, endorse the application and the innocuous character of this medical therapy using a fairly wide range of doses.

Emphasizing that research and clinical experience with medical ozone are making progress, despite the various obstacles they face, becoming a permanent challenge for researchers and for ozone therapy associations, mainly due to the lack of access to financial resources which they need in order to be able to continue with the scientific research that is required.

Stating that it is absolutely necessary to work with specific objectives, planning globally those necessary actions, so that ozone therapists working together will be forwarding with great precision and securely the practice of ozone therapy.

Recognizing that there is variance that the medical community wishes to standardize, and that progress already has been made, that it should be taken into account; it is necessary to continue with the development of medical definitions of procedures and protocols determining the best applications where it is necessary, as well as a code of good practice, in order to overcome more efficiently the possibility of malpractice.

Welcoming with great satisfaction that ozone therapy practice was regularized in Russia in 2007 by the Federal Service Public Health Control and Social Development, the first country in the world to do so; in Cuba in 2009, by the Ministry of Public Health; in Spain, by the Balearic Islands, and the Canary Islands (2007), Madrid (2009) and Galicia, Castilla-La Mancha, and Castilla y León (2010) Autonomous Communities; that in Italy significant advances have been done towards ozone therapy by the Regions of Lombardy (2003), Emilia-Romagna (2007) and Marche (2009), and favorable court decisions have been taken by the Administrative Court of Lazio (1996

and 2003).

The speakers at the "International Meeting of Ozone Therapy Schools" as well as the associations of ozone therapy present at the same have adopted the following

CONCLUSIONS

First. To approve the "**Therapeutic Ranges for the Use of Ozone**" detailed within the "Recommendations" section of this Declaration.

Second. To increase the exchange of knowledge, research, and experiences, both positive and negative that occur in the field of ozone therapy, in furtherance of increasing the knowledge of the huge benefits that this therapy has. To stimulate the publications of research results in specialized medicine journals.

Third. To encourage health researchers to increase their creative efforts, so that, ozone therapy continues to demonstrate its therapeutic benefits with safety and effectiveness under the development of controlled clinic trials.

Fourth. To stimulate the creation of Standardized Operative Procedures, according to good clinical practices for each procedure, taking into account knew developments, with the view to increase the quality and make homogeneous diverse treatments.

Fifth. To make systematic efforts to ensure that each scientific congress/meeting to be organized adopts conclusions that reflect the progress made and set achievable and realistic targets, sharing the findings and aims to encourage and promote research to deepen the understanding of ozone therapy. To work towards the harmonization and unification of criteria at the international level among different scientific societies.

Sixth. To encourage the different associations to work in their own countries where the ozone therapy has not yet been regularized to get it properly regularized and therefore to enjoy a legal status.

Seventh. To encourage the preparation of text books, the organization of theoretical courses and practical training on ozone therapy, so that those who practice it do so based on sound knowledge; this will necessarily be reflected on a more efficient medical health care which will benefit the patients.

The speakers at the "International Meeting of Ozone Therapy Schools" as well as the participants associations at the same have adopted the following

RECOMMENDATION

That the "**Therapeutic Ranges for the Use of Ozone**" as detailed in the annex to this "Madrid Declaration" and an integral part thereof, serve as a reference to ozone therapists in order for them to implement them carefully and systematically.

These **"Therapeutic Ranges for the Use of Ozone"** are the summary of scientific research in different countries and are the result of many years of experiential and

clinical practice.

The speakers at the "International Meeting of Ozone Therapy Schools" as well as the participants associations at the same

We express our most sincere recognition to **Dr. Velio Bocci**, Emeritus Professor of Physiology at the University of Siena, for the significant and important contributions he has made in favor of ozone therapy in the fields or research, teaching, information and patient care, to the point that within the ozone therapy history he must be considered as one of its most important pioneers.

Finally we express our gratitude to the **Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO)** for its initiative and implementation of this "International Meeting of Ozone Therapy Schools" warmly housed in the centenarian walls of the Royal National Academy of Medicine in Madrid.

Madrid, June 4, 2010

ANNEX TO THE MADRID DECLARATION ON OZONE THERAPY WHICH IS INTEGRAL PART THEREOF

Recommendation approved at the "International Meeting of Ozone Therapy Schools" held at the Royal Academy of Medicine in Madrid on the 3rd and 4th of June, 2010, under the auspices of the Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO)

THERAPEUTIC RANGES FOR THE USE OF OZONE

1. THERAPEUTIC BASIS

Ozone therapeutic indications are based on the knowledge that low physiological concentrations of ozone may play important roles within the cell. At molecular level, different mechanisms of action have been shown that support the clinical evidence for this therapy.

There are therapeutic, non effective, and toxic concentrations, of ozone. It has been proved that concentrations of 10 or 5 µg/ml and even smaller, have therapeutic effects with a wide security margin, so it is now accepted that the therapeutic concentrations range from 5 to 60 µg/ml. This range applies to local and systemic application techniques.

It should be emphasized that each route of application has a minimum and a maximum dosage as well as concentration and volume to manage.

All the therapeutic dosages are divided into three types, according to their mechanism of action:

a) Low doses: These doses have an immunomodulatory effect and are used in those diseases where there is suspicion that the immune system is compromised.

b) Medium doses: They are immunomodulatories and stimulate the antioxidant enzyme Defence System. They are most useful in chronic degenerative diseases such as diabetes, atherosclerosis, COPD, Parkinson syndrome, alzheimer, and senile dementia.

c) High doses: They are employed especially in ulcers or infected injuries. Also they are used to ozonize oil and water. The ozonization of oils never can be produced with a medical generator because it cannot be avoided that oil steam diffuse in the high-voltage pipes. ¡The result is the production of several very toxic substances! Except in the generators with valve that cuts the exit of ozone.

2. OZONE THERAPY BASIC PRINCIPLES

The three basic principles that must be taken into account before any ozone treatment process is implemented are the following:

a) Primum non nocere: Before anything else, not to do any harm.

b) Stagger the dose: Start always with low doses, and increase them gradually. The exception will be in infected ulcers or injuries, where the reverse will be applied (start with a high concentration, and diminish it according to the improvement in the patient's condition).

c) Apply the necessary concentration: Higher ozone concentrations are not necessarily better, in the same way that it occurs with all the medicines.

Should the redox balance not be known (antioxidants/pro-oxidants) and the patient is in an oxidative stress, an initial medium or high dose, may damage cellular antioxidant mechanisms and aggravate the clinical picture. It is therefore preferable to start with low doses and to phase in the increase according to patient response.

3. MAIN ROUTES OF APPLICATION

Medical ozone can be applied locally or parenterally. The various routes of application of ozone can be used alone or combined, in order to attain a synergistic effect.

3.1 RECOMMENDED ROUTES OF APPLICATION

The routes of application described below are safe and proven because they are the result of many years of experience and research.

We welcome the therapeutic range indicated by the guidelines of the Russian Ozone Therapy Association, published in its "Handbook of Ozone Therapy" (2008); the "Guidelines for the Use of Medical Ozone" published by the German Medical Society for the Use of Ozone in Prevention (2009); the guidelines published by the Ozone Research Centre, scientific unit of the Cuban National Centre for Scientific Research, in its book "Ozone Basics Aspects and Clinical Applications" (2008); and the significant contribution from Dr. Velio Bocci in "Has Oxygen-Ozone Therapy a Future in Medicine? (Rev. 2010) and sent by the author to this "International Meeting."

Routes of application	**LOW**	**LOW**	**LOW**
	Conc. µg/ml	Vol. ml.	Doses µg
RI*	10 20	100	1000 2000
MAHT**	10 20	50 100	500 2000
MiAHT***	5 10	5	25 50

Application Routes	**MEDIUM**	**MEDIUM**	**MEDIUM**
	Conc. µg/ml	Vol. ml.	Doses µg
RI*	20 30	100 150	2000 4500
MAHT**	20 30	50 100	1000 3000
MiAHT***	10 20	5	50 100

Application Routes	**HIGH**	**HIGH**	**HIGH**
	Conc. µg/ml	Vol. ml.	Doses µg
RI*	30 60*[a]	150 30-50	4500 18000-3000
MAHT**	35 60**[b]	50 100	1500 6000
MiAHT***	10 20	5	50 100

*** RI: Rectal insufflation.**
Bear in mind that major concentrations of 40 µg/ml can hurt the enterocite.
*[a] Exceptionally, in case of acute bleeding, begin with a high concentration (60 µg/ml / ml and 50 ml Vol.) Once the bleeding diminishes, reduce concentration.
** **MAHY:** Major Autohemotherapy
*** **MiAHT:** Minor Autohemotherapy
**[b] Although in general is preferred to employ concentrations around 40 µg/ml, in some cases it could be assessed the employment of until 60 µg/ml which has proved to be safe and with greater capacity of induction of citoquines.

3.1.1 Major Autohemotherapy (MAHT)

The rank of volumes to use varies between 50 ml and 100 ml. Blood volumes greater than 200 ml must be avoided to prevent any risk of hemodynamic disturbances, especially in elderly or unbalanced patients. The perfusion set to be used must be certified and never should be made of PVC or other materials that react to ozone.

Ozone concentrations of 80 µg/ml and above, should also be avoided because of the increased risk of haemolysis, reduction of 2, 3 DPG and a consequent inability of activating immunocompetent cells.

The number of treatment sessions and the ozone dosage administered will depend on the patient's general condition, age and main disease. As a general rule, every five sessions the dose of ozone is increased and it is given in cycles that vary between 15 and 20 sessions. From the clinical point of view the patient's improvement occurs between the fifth and tenth session, and it is considered that after the twelfth session the antioxidant defense mechanisms are already activated. The treatment is given in a cycle that is administered daily, from Monday to Friday and also could be administered two to three times a week.

3.1.2 Intramuscular, paravertebral and intrarticular injection

3.1.2.1 Paravertebral

The infiltration is made 2 cm lateral from the spine/column. The distribution of the needles is always bilateral, lateral or 2 cm. above and 2 cm below the hernia.

A depth from 2 to 4 cm should be considered when taking into account the patient's constitution and/or the area to be treated (smaller in thin patients and in dorsal region and greater in obese patients and lumbar region).

The treatment is done twice a week for the first two weeks and once clinical improvement is achieved, the treatments are spaced to once a week for four to six weeks and then one session every 15 days until one cycle of 20 sessions is completed, these can be shortened once the symptoms have disappeared. The recommended needle sizes for this procedure is 25 to 30 G x 1½". In some cases and with expert hands, longer needles may be used.

It is important that the physician examines adequately the muscles within the lumbo sacra region and the sacro iliac articulations to detect inflammation at this level or "trigger points" in that zone, above all in patients with discartrosis that do not respond adequately to the paravertebral infiltrations. If these points are detected they must be infiltrated.

Concentration [µg/ml] 10-20
Volume / ml 5-10
Dose / µg 50-400

3.1.2.2 Hernias

Cervical hernias
Concentration of 10 and 20 µg/ml, a volume of 5 ml is given.
Dorsal Hernias
Concentration of 10-20 µg/ml, a volume of 5 ml is given.
Lumbar Hernias
Concentration of 10-20 µg/ml, a volume of 5-10 ml is given.

3.1.2.3 Intraarticular treatment

Concentration: 5-10-20 µg/ml
Volume in function of the articulation size:

Fingers: 1-2 ml
Rest: 5 - 20 ml

3.1.2.4 Intradiscal Treatment
In general only one intradiscal infiltration should be performed, although it could be repeated within 2 - 4 weeks, under mobile radiologic arch or fluoroscopic control or CT. The patient has to be under sedation (no general anaesthesia) and with an antibiotic prophylactic therapy the same day of the procedure.

For lumbar discolisis a 5-10 ug/ml mixture of oxygen - ozone at a concentration of 25-30 ug/ml is used. For cervical discolosis 5 ml with the same concentration. The discolisis with ozone, although is effective after only one treatment, it requires specific infrastructure (for radiological control), anaesthetist and experienced personnel in the execution of the technique. Despite the fact that the paravertreval technique requires more sessions, it is equally effective and has a minimum level of risk.

3.1.2.5 Peridural treatment (translaminal)
An infiltration is performed in the peridural space, twice weekly previous identification of the peridural space. It uses a mixture of oxygen-ozone in a volume of 5 ml at a concentration of 20 ug/ml.

The translaminal peridural method or through the sacred hiatus route is an alternative to consider in the treatment of hernial disc with ozone therapy, despite being an indirect method in relation to the intradiscal method because:

- With this method, neither the operator is exposed to the risk of undergoing radiation nor the patient.
- Upon deposit of the gas in the peridural space at the level of the conflict zone disco-radicular, the same acts over both the disk and the damaged root.
- It is easy to perform, causing no neurological damage and incorporating the patient to his/her normal life soon.
- It requires few material resources and equipment which makes it a less expensive and effective method.
- It requires fewer sessions compared to the paravertebral method as an indirect method.
- It is very useful in the presence of multiple disc hernias.
- The success rate frequency is above 70%.
- It requires a minimum time to recover.
- It can be performed in patients with major associated diseases.

In any case, the three commented techniques require of strict asepsis and sterility measures and of an inform written consent.

3.1.3 Ozone Bag
Concentrations of 60 - 40 - 30 - 20 µg/ml, are used for periods of 20 to 30 minutes, depending on the stage and evolution of the lesion. It can use 60-70 µg/ml only in purulent infections. Once the infection is controlled and the healthy granulation tissue appears, the procedure is to reduce the concentration and to space the sessions in order to support the healing.

3.1.4 Subcutaneous application
The concentration of ozone used is 5 to 10 µg/ml in very small volumes of gas (1-2 ml) with a 30 G needle.

It is also efficient in the treatment of neuropathic pain. Can also be used for cosmetic purposes in cellulite, never using a volume larger than 100 ml per session.

3.1.5 Ozone Bell or Ventosa
Using concentrations ranging from 15 to 60 µg/ml, with a variation in the duration of the treatment between 15 to 20 minutes.

3.1.6 Insufflation in fistulas
Always the practitioner must be sure first that not communication with the respiratory tract exists. It is important to keep in mind the possible gas build-up in a closed cavity, blocked or cystic to avoid dangerous or painful increases in pressure, for example in cutaneous, perianales and surgical fistulas.

3.1.7 Ophthalmologic
In ophthalmological cases (queratitis, corneal ulcers, conjunctivitis and ocular burns), a special glass attachment adapted to the contour of the eye is used. Anesthetic eye drops are applied previously and a concentration of ozone between 20 and 30 µg/ml during 5 mn. Two to three applications per week can be made combined with subconjunctival application of ozone, at a concentration of 35 µg/ml with a volume of 1-2 ml.

3.1.8 Vaginal Insufflation
Ozone concentrations of 20-40 µg/ml and a volume between 1000-2000 ml at a continuous flow rate of 0,1 to 0,2 l/min for 10 min. are used. A vaginal wash with ozonized water must be carried out previously. For this application an ozone destructor device is required.

3.1.9 Insufflation vesicourethral
Insufflate between 50 and 100 ml of ozone into the bladder or urethra, according to the case to be treated. The recommended concentrations are from 10-15-20 and 25 µg/ml (increasing them progressively). The treatment could be combined with a pre-irrigation procedure with ozonized water.

3.1.10 Otic route
The external ear is moistened and then it is insufflated using a syringe or a special headset with an ozone destructor device. Check that the eardrum is intact. Concentrations between 20-30 µg/ml during 5 mn are used.

3.1.11 Intratonsilar route
It is a secure route in patients older than 12 years old, with the condition that they can actively cooperate when they are asked to hold their breath (apnea) meanwhile the medical ozone injection is applied. Concentrations of 15-20 µg/ml with a volume of 2.5 ml per point to infiltrate at the anterior and rear pillar of both tonsils are used. Four to five sessions are required.

3.1.12 Ozone micro doses in trigger points and acupuncture
As a general rule the trigger points are located in the muscles and often deeply, so the

application has to be intramuscular and the volume can be between 5-10 ml depending on the anatomical place, and, the concentration between 10 and 20 mcg/ml.

For acupuncture points or reflexology areas the application is intradermal and fluctuates between 0.1 to 0.3 ml and up to 1 ml (maximum) of the gas mixture of O_2-O_3 with concentrations below 30 µg/ml.

3.1.13 Topical application of water, oil and ozonized creams

It is applied on wounds, ulcers and several infected lesions at different concentrations**:** high, medium, and low, depending on what it is intended to achieve (to disinfect, to regenerate) and of the type of tissue where it will be applied.

3.1.14 Ozonized Saline Solution

The rank of concentrations of ozone used in the phase of gas (from the ozone equipment) is of 500 mcg/l to 5000 mcg/l.

The ozonization is carried out with very low ozone concentrations which are calculated according to the weight of the patient. The formula used is 25 mcg by 1 kg of patient's weight. For example: if the patient weighs 80 kg, it is multiplied as follows: 80 x 25 = 2000 mcg (2 mcg/ml or 2 mg/l).

This figure corresponds to the concentration generated by the equipment, which is very low and it does not reach the 2,0 mcg/ml. Under this method concentrations generated by the ozone equipment above 3,000 mcg/l are never used.

The procedure consists of:

- To bubble 200 ml of saline solution at 0,9% during 10 mn, time necessary to obtain an adequate saturation of the solution that goes from 20 µg/ml until 200 µg/ml of concentration.
- To initiate then the transfusion of the solution by drip to the patient during 25-30 mn, keeping a constant bubbling of ozone in the bottle, to maintain its concentration in the solution.
- To cut the bubbling and the transfusion at the 150 ml, leaving in the bottle 50 ml of solution as safety margin.
- Nowadays, an ozone equipment that maintains the ozone concentration in the solution without needing to maintain the bubbling during the transfusion is available.

3.1.15 Pediatrics dosages through rectal insufflation

Systemic application via, only by via rectal.

- The concentrations to be used depend on the grade of the oxidative stress of the patient and the pathology to be treated.
- The volume to be administered depends on the age of the patient.
- To perform the rectal insufflation a catheter is introduced 1-2 cm inside the anal sphincter.

3.1.15.1 Dosages for patients with an initial value of oxidative stress graded "0" or "1" (Light one)

Weeks of treatment	Concentration O_3 (µg/ml)
First	20
Second	25
Third	30
Fourth	35

3.1.15.2 Dosages for patients with an initial value of oxidative stress graded "2" or "3" (Moderated)

Weeks of treatment	Concentration O_3 (µg/ml)
First	15
Second	20
Third	25
Fourth	30

3.1.15.3 Dosages for patients with an initial value of oxidative stress graded "4" (Severe)

Weeks of treatment	Concentration O_3 (µg/ml)
First	10
Second	15
Third	20
Fourth	25

3.1.15.4 Volumes to be administered according to patient's age

Age of the patient	Volumes to be administered
28 days-11 months	15-20 cc
1 -3 years	20-35 cc
3-10 years	40-75 cc
11-15 years	75-120 cc

The dosage changes every five sessions. Cycles of 15-20 sessions are indicated every three months during the first year. Later the patient will be evaluated to determine frequency of the cycles for the second year.

3.1.16 Ranges of diseases for rectal insufflation and major autohemotherapy applications

3.1.16.1 LOW RANGE

- Biological regeneration
- Gout
- Fibromyalgia

3.1.16.2 LOW-MIDDLE RANGE

- Chronic kidney failure
- Cancer
- Nephropathies

3.1.16.3 MIDDLE RANGE

- Neurovegetatives illnesses: Alzheimer, parkinson, dementia syndromes.
- Pulmonary illnesses: Emphysema, COPED, acute respiratory distress syndrome.
- Ophthalmological illnesses: Retinosis pigmentarias, cataract, glaucoma, macular degeneration related to age.
- Hematology illnesses: Thalassaemia B, scklemia. • Vascular Illnesses: HTN, venous insufficiency, peripheral arterial illness, CVA, cardiacs ischemia, veined stasis.

3.1.16.4 MIDDLE-HIGH RANGE

- Viral Illnesses: Herpes simple, herpes zoster, AIDS, hepatitis A, B, C, papilloma human virus.
- Diabetes
- Cerebral palsy
- Dermatological illnesses
- Orthopedic illnesses
- Giardiasis
- Candidiasis and cryptosporidiosis.
- Allergic illnesses
- Chronic fatigue syndrome
- Lupus Erythematosus Systemic
- Rheumatoid arthritis
- Crohn’s illness
- Intestine inflammatory illnesses
- HIV/AIDS
- Multiple sclerosis

3.2 APPLICATION ROUTES NOT RECOMMENDED FOR NOT BEING SAFE

3.2.1 Direct intravenous injection of ozone

Its application is strongly discouraged due to the risk of air embolism which can occur

even in the case of using an slow infusion pump and volumes of 20 ml. The complications of stroke range from a simple axillary bubbling sensation, then cough, a feeling of retrosternal weight, dizziness, to changes in vision (ambioplia), hypotensive crisis, with signs of cerebral ischemia (paresis of the members) and death.

Furthermore, there is no justification to put the patient and the therapy at risk when there are methods that are safe, have been tested and are effective such as the major autohemotherapy, minor autohemotherapy and rectal insufflation.

3.2.2 Vitamins and ozone

During the treatment with ozone is necessary to suspend all the antioxidant supplements that contain vitamin C and vitamin E. The presence of these compounds in high concentrations in the blood, interferes with the ozone's action as an oxidant agent and therefore the good course of the therapy. It is important to communicate to the patient that s/he must not consume excessive quantities of foods very rich in these vitamins. In consequence, the vitamins or antioxidants should be given before or after the ozone therapy but never during the treatment.

3.3 APPLICATION ROUTE ON ANIMAL EXPERIMENTATION PHASE

Intraperitoneal

This route is still in the scientific experimental phase in animals, to which various tumor cell lines have been implanted, having found that ozone is more cytotoxic to tumor cells than many of the cytostatics used, without causing the adverse effects of the chemotherapy. The research into this matter is being undertaken by the Veterinary Services and Laboratory Animal Medicine of the Philipps-University of Marburg (Germany) by Medical Veterinarian Professor Siegfried Schulz.

It is exhorted that investigations in animals continue to be carried out.

Experimental studies for the treatment of cancer in human beings have not yielded convincing data so far.

In human beings has been used for peritonitis´ treatment applying a peritoneal wash with ozonized water using 200 to 300 ml in volume with a concentration between 10 and 20 µg/ml, through a silicone catheter fixed into the cavity.

3.4 APPLICATION ROUTE PROHIBITTED

Inhalation route

The inhalatory route is absolutely prohibited because of being highly toxic. The anatomical and biochemical characteristics of the lung make it extremely sensitive to oxidative damage by ozone.

3.5 APPLICATION ROUTE THAT HAS NOT RECEIVED TOTAL CONSENSUS

Ozonized Saline Solution

The Ukrainian and Russian schools utilize it as another form of systemic application of the ozone and its practice is well extended in those two countries. Its efficiency is

testified by the results of the scientific research submitted at the eight Practical Scientific Conferences that have taken place in Russia from 1992 to 2009.

Nevertheless this methodology still has not found the consensus between some schools and it is left to the criteria of the doctors whether or not to use this method.

3.6 ESSENTIAL REQUIREMENTS

The described routes of application require of technically qualified personnel to carry out any procedure, as well as a written informed consent, followed by strict measures of asepsis and sterility.

As with any another medical practice, all the material used in ozone therapy that be in contact with patient's tissue or fluids must be either disposable after only one use, or be sterilized (ex. surgical equipment), and before the administration of the ozone must pass an antimicrobial sterile filter <of 20 µm.

4. PATHOLOGIES MORE APPROPRIATE TO BE TREATED WITH OZONE THERAPY

The diseases sensible to the ozone treatment may be classified into three categories, based on the therapeutic success grade proved and obtained.

4.1 Diseases in the first category

These include among others:
a) Osteomyelitis, pleural emphysema, abscesses with fistula, infected wounds, bed sores, chronic ulcers, diabetic foot and burns.
b) Advanced ischemic diseases.
c) Related to age, macular degeneration (atrophic form) because the orthodox ophthalmology gives no significant treatment.
d) Orthopedic diseases and localized osteoarthritis.
e) Chronic fatigue syndrome and fibromyalgia.
f) Dental injury-related to primary cariogenic lesions, particularly in children.
g) Estomatology for chronic and recurrent infections in the oral cavity.
h) Acute and chronic infectious diseases, particularly those caused by bacteria resistant to antibiotics or to chemical treatments, viruses, fungi (hepatitis, HIV-AIDS, herpes and herpes zoster infection, papillomavirus infections, onichomicosis and candidiasis, giardiasis and cryptosporidiosis). Bartolinitis and vaginal candidiasis.

Although the ozone therapy represents a useful support for the treatment of these diseases, it is worth to underline that neither the ozone nor its metabolites, among them the H_2O_2, reach a germicide tisular concentration, because the free pathogens are protected by plasma antioxidants and intracellular viruses are unattainable.

For these pathologies the ozone therapy either used only as exclusive form or as a support for a specific treatment, according to the cases, becomes a medicine/treatment with a high therapeutic success.

4.2 Diseases in the second category

These include:
a) Cancer-related fatigue. The ozone therapy associated with orthodox treatments, may

accelerate and improve results. However, ozone therapy has so far not been able to show a therapeutic effect on cancer. For all these pathologies ozone treatment should be integrated with the conventional treatment, there is evidence of its utility**,** but more precise studies are required
b) Asthma.

4.3 Diseases in the third category
Among others include:
a) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Cohn's disease)
b) Senile dementia
c) Lungs diseases: emphysema, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and acute respiratory distress syndrome.
d) Skin diseases: Psoriasis and atopic dermatitis.
e) Cancer metastasis
f) Severe sepsis and multiple organ dysfunction.

In these cases the combination of orthodox treatments and ozone therapy, at least on theoretical grounds, show that it may be useful but there is no real clinical evidence. The anecdotal evidence suggests the existence of therapeutic effectiveness but, in many cases the efficacy has been achieved by using various types of therapy, therefore the results are not reliable. In some studies the combination of ozone therapy with another treatment has been evaluated, concluding that ozone therapy acts as complement.

5. GENERAL BASIS FOR TREATMENT

Not all patients respond equally to the small, controlled oxidative stress that is produced by the ozone therapy. Therefore, the ozone treatment should always be applied in a gradual and progressive manner, starting with low doses and increasing it gradually to avoid unnecessary risks, until a clinic diagnostic method for the oxidative stress is available, which allows to adjust the dose.

It is advisable to measure and classify the state of oxidative stress on the patient, using markers such as malon-aldehyde, catalase, superoxide dismutase, glutathione peroxidase and indicators of the total antioxidant activity in the medical cabinet.

If it is not possible to measure the oxidative stress degree of the patient by either of the established methods, it is very important that the physician value according to the clinical state of the same, if he is eligible or not to receive the treatment with ozone at that moment, or if it is necessary to improve his/her nutritious state first.

As with any medical treatment, patients may be divided into three types:
Normo-responders, hyper-responders and hypo-responders.

There are factors which can not be controlled, that depend of the patient's idiosyncrasy and the characteristics how the disease manifests itself.

Ozone therapy is a "medical act" and should be practiced by medical personnel and implemented with a scientific rigor, it can produce with a low frequency a minimum of adverse cases. Is for this reason that we consider that the regularization of the ozone therapy carried out by the authorities should include the following requirements, and in those cases where this has not been done the ozone therapists should apply them,

The medical centers where the ozone therapy is practiced should have the mandatory sanitary authorization for its functioning and should abide by the following requirements:

5.1 To have a qualified doctor with training and recognized experience in ozone therapy, this will be the persona responsible for the management of the treatment.

5.2 To use the appropriate equipment to generate and apply the ozone therapy, these should have also the required authorizations from the appropriate sanitary authorities. In the case of the European Community, should be marked with the CE. The equipment to generate ozone must be calibrated or revised periodically, according to the recommendation of the manufacturer, to avoid incorrect applications or concentrations.

5.3 To use medical oxygen provided by an authorized company.

5.4 To implement the various and appropriate protocols, according to the administration route chosen, in order to guarantee the quality in the treatment. The protocols should be appropriately validated and recognized by the scientific ozone therapy associations.

5.5 To establish an informed written consent, this should be signed by the patient and the medical doctor responsible for the implementation of the ozone therapy, leaving a copy in the clinical history of the patient.

5.6 To have an appropriate airing and ventilation system.

5.7 To have life saving drugs, ventilation support equipment or an Ambu balloon.

5.8 To take into account that the inter disk application of ozone should be done in a surgical room within a hospital centre or in an ambulatory unit for major surgery.

5.9 The key to the therapeutic success depends on diverse controllable factors that include the scientific preparation and technique of the ozonoterapist, the method that is employed, the quality of the ozone, the general application of the good clinical practices. The non controllable factors depend on the patient idiosyncrasy and in what is current state of the illness.

Madrid, June 4, 2010

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ITALY	
(Signed) **Professor Velio Bocci (MD).** Emeritus Professor of Physiology at the University of Siena.	(Signed) **Professor Lamberto Re** (MD). Professor, Clinical Pharmacology and Toxicology, University of Ancona.
(Signed) **Dr. Anna María Procopio (MD).** Paediatrician.	(Signed) **Professor Gregorio Martínez Sánchez** (Pharm. Dr., Senior Researcher). Scientific Director, Medinat srl. Ancona.
MEXICO	
(Signed) **B.S. Carla Núñez Lima** (Biochemistry). Culiacán, México	(Signed) **Dr. Froylán Alvarado Güémez**, MD. President of the Mexican Association of Ozone Therapy.
(Signed) **Dr. Jaime Rebeill Félix** (MD). Director, Pain and Spine Clinic, Hermosillo (Sonora), México.	
ROMANIA	
(Signed) **Dr. Tiron Stefan** (MD). President Founder, Scientific Romanian Association of Ozone Therapy.	
RUSSIA	
(Signed) **Professor Sergey Peretyagin** (PhD). Head of the Department of Experimental Medicine, Research Institute of Traumatology and Orthopedics, Nizhny Novgorod; President of the Russian Association of Ozone Therapy.	(Signed) **Professor Claudia N. Kontorschikova** (PhD) Head of Department of the Clinical Diagnostic Laboratory, Medical Academy, Nizhny Novgorod.
SPAIN	
(Signed) **Dr. Adriana Schwartz** (MD). Director, Clínica Fiorela, Madrid. President of Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO); President of the International Federation of Oxygen Ozone Therapy (FIOOT); Vice-President of the Asian-European Union Ozone Therapists.	(Signed) **Dr. Bernardino Clavo Varas** (MD). Specialist, Radiotherapy Oncology Department, Great Canary University Hospital Dr. Negrín.

(Signed) **Dr. Fernando Kirchner van Gelderen** (MD). Director, Gabinet Mèdic Maresme, Mataró (Barcelona).	
UKRAINE	
(Signed) **Dr. Sci. Eugeni I. Nazarov.** President of the Ukrainian Association Ozone Therapists, Executive President of the Asian-European Union Ozone Therapists.	
UNITED STATES	
(Signed) **Dr. Frank A. Shallenberger** (MD). Director, Center for Alternative Medicine, Anti-Aging, Nevada.	

Madrid, June 4, 2010

Translated from Spanish into English by Sara Esther Russy King (Nutritionist and Dietician), Roberto Quintero (Lawyer) and Fabricio Quintero Schwartz (English teacher).

Associations and Federations of Ozone Therapy that signed the "Madrid Declaration on Ozone Therapy" on June 4, 2010

Asian-European Union Ozone Therapists. Executive President: Dr. Sci. Eugeni I. Nazarov.
European Cooperation of the Medical Ozone Societies. General Secretary: Dr. Renate Viebahn-Haensler.
Inter American Society of Oxygen Ozone Therapy. President: Dr. Ana Elizabeth Rieck.
International Federation of Oxygen – Ozone Therapy (FIOOT). President: Dr. Adriana Schwartz.
German Medical Society for the Use of Ozone in Prevention and Therapy. General Secretary: Dr. Renate Viebahn-Haensler.
Mexican Association of Ozone Therapy. President: Dr. Froylán Alvarado Güémez.
Russian Association of Ozone Therapy. President: Professor Sergey Peretyagin.
Scientific Romanian Association of Ozone Therapy. President: Dr. Tiron Stefan.
Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO). President: Dr. Adriana Schwartz.
Ukrainian Association Ozone Therapists. President: Dr. Sci. Eugeni I. Nazarov.

Associations and Federations of Ozone Therapy that signed the "Madrid Declaration on Ozone Therapy" after June 4, 2010

Ecuadorian Society of Ozone Therapy. President: Dr. Danilo Ruiz Reyes.
Japanese Society of Oxidative Medicine. President: Dr. Takeo Watarai.

Madrid, July 30, 2010

A review

I have tried to highlight and draw into tighter focus those things that are particularly applicable to medical personnel whose exposure to ozone has been limited. T Lowe Tom@drsubi.com

Ozone Therapy and Its Scientific Foundations

November 18, 2012

Dr. Adriana Schwartz M.D. Secretary, International Scientific Committee of Ozonetherapy (ISCO3).

Dr. Gregorio Matínez Sánchez PhD. Pharmacy Doctor. Member, International Scientific Committee of Ozonetherapy (ISCO3).

For the full 44 page paper: click here

History: I leave it to those who have a desire to investigate this within the body of the paper Enough to say, 80 years of effective, safe history both in Europe and the US.

The directors of the most important hospitals in the U.S.A. published the book *Ozone and Its Therapeutic Action* in 1929, which lists 114 diseases and their treatment through the application of ozone.

Why is it not accepted in Western Medicine as an effective therapeutic agent? *In 1933 the American Medical Association (AMA), run at that time by Dr. Simmons, urged the United States Government to prohibit all therapies that were not medically authorized and duly registered, which caused the use of ozone to drop in that country. In this way, an exclusive benefit was granted to the monopoly of pharmaceutical companies.*

The main trouble for the wide-scale acceptance of ozone therapy is associated to a great extent with the obstacles that the large drug industry imposes, running media campaigns against its acceptance, to the point of reaching pure scientific ignorance. Unjustly and without scientific basis, it has been stated that ozone is toxic regardless of its use, forgetting that the effects of medical ozone, as for nearly all substances, depends on the dose; and that despite these false statements, ozone is considered one of the best disinfectants of drinking water, capable of avoiding infection outbreaks.

Current Situation: *At present there are more than 40 national and international associations that bring together the professionals that practice this therapy, indexed specialized journals, continuing training courses and congresses on the subject.* In the US it is AAOT.us American Academy Ozone Therapy

Despite the empiricism that preceded the practice of ozone therapy and the scarcity of funds available for research in this field, in recent years a growing number of books have been appearing (Annex I - 19 in the last 10 years) and research papers (Annex II - 91 in the last 3 years) that constitute scientific support for this therapeutic procedure.

Action mechanisms. General aspects

- **Improves the metabolism of oxygen**
- **Immunological Modulator**
- **Broad Spectrum Germicide**
- **Regulates oxidative Stress**
- **Intervenes in the release of Autocoids**
- **Metabolic regulator**

... hydrogen peroxide and other mediators have diffused it to the interior of the cells, activating different metabolic routes in erythrocytes, leucocytes and platelets, leading to numerous biological effects.13 The hydrogen peroxide then acts as a signaling molecule in the intracellular medium,14 a messenger that the therapeutic dose of ozone has been discharged.

Pg 12 D.1 Effect of ozone on the metabolism of oxygen
...explained from its promoting action of: 1) Changes in the rheological properties of the blood. 2) Increase in the speed of glycolysis of the erythrocyte.[3, 10]

Pg 14 D.2 Ozone as a modulator agent of the immune response
The immunological actions of ozone ....regulation is given because the ozone acts as an enhancer of the immunological system by activating the neutrophils and stimulating the synthesis of some cytokines.[20, 21]

Pg 16 D.3 Bactericide effect of ozone
The bactericide effect of ozone in the gram-positive flora of festering wounds and of trophic ulcers is made more effective when a high resistance of the microbes to the usual antibiotics is increasingly evident.

Pg 17 D.4 General actions
The general effects of ozone are:
1) disinfectant and direct trophic effects, when it is applied locally.
2) Antibacterial and systemic antiviral effect due to a discrete formation of peroxides.
3) It increases the deformity of the red blood cells with relative improvement of blood circulation.
4) It improves the delivery of oxygen to the tissues.
5) It improves the red blood cell metabolism, making the metabolism of glucose more efficient. 6) It improves the metabolism of the fatty acids for the activation of antioxidant enzymes in charge of eliminating peroxides and free radicals.

Pg 18 D.6 Action mechanism of ozone therapy on pain
...ozone has a dual action mechanism: analgesic and anti-inflammatory. These effects seem to be due to its way of acting on diverse targets:
1) Decrease the production of mediators of the inflammation.
2) The oxidation (inactivation) of metabolic mediators of pain.
3) It clearly improves local blood microcirculation,

Table 1. Principal therapeutic indications of ozone by specialization

Specialization	Pathology
Dermatology	Herpes Zoster and simplex, acne, eczema, lipodystrophy (cellulite), mycosis, psoriasis, atopic dermatitis, burns degrees with different areas, infective and prolonged wounds, post traumatic, postoperative, firearms osteomyelitis.
Internal Medicine	Hepatitis, diabetes, atherosclerosis, arterial hypertension, osteoarthritis, asthma, chronic bronchitis, gastritis, gastric ulcer, Crohn's disease, chronic constipation, hypothyroidism.
Nephrology / Dialysis	Adjuvant in the treatment of ischemic-metabolic pathologies.

Neurology	Migraines, depression, vasomotor cephalea, neuro-vascular disorders.
Dentistry	Treatment of cavities, disinfection of cavities during surgery and post-operatory period. Periodontitis, aphthas.
Orthopedic Rheumatology	Disc-radicular conflicts, disc herniation, articular rheumatism, lumbago, osteoarthritis, arthropathy, periarthritis, rheumatoid arthritis.
Angiology	Venous insufficiency, diabetic ulcer, arthropathy, coronaropathy, gangrene, postphlebitic ulcer, peripheral vasculopathy.
Gynecology	Bacterial infections by protozoa or mycosis, Bartholin's cyst, vaginitis, menopause, chronic pelvic inflammation, infertility.
Immunology	Immuno-modulator, autoimmune disorders, adjuvant in treatments with radiation and in immunodeficiency.

E.1 Adverse reactions
Ozone therapy, if it is applied respecting simple rules, does not have side effects (e.g. lung toxicity) and has very few contraindications. In Germany in 1988 more than a million autohemotransfusions with ozone were performed without the Department of Control of Adverse Effects Caused by Drugs registering a single adverse event.

3 pages of discussion regarding country by country statistics on its safety are available.

F. Contraindications for the use of ozone therapy
1) Patients that suffer a significant deficit of glucose 6 phosphate dehydrogenase (favism).
2) (imbalance) in patients with hyperthyroidism and thrombocytopenia.
3) Severe cardiovascular instability (recent myocardial infarction).
4) Convulsive states.
5) Hemorrhagic conditions (external or internal bleedings),[5] significant hypo-coagulation syndrome, blood diseases, haemophylia, thrombocytopenia, hemorrhagic vasculitis, acute, hemorrhagic insult,
6) pancreatitis, ... a few others are listed

H. Conclusion
There is much scientific evidence on the clinical use of ozone.... For the full 44 page paper: click here

Official medicine does not take into account the effectiveness of ozone therapy, principally because: 1) It is excessively centered on the molecular mechanisms of drug-receptor interaction and ignores the capacity of ozone as a pro-drug. 2) Most clinics are not aware that ozone can dramatically change the course of several diseases by means of the activation of multiple pathways. 3) The pharmaceutical industry has a good reason for ignoring ozone, since it costs almost nothing, is not patentable and does not produce wealth.

International Scientific Committee of Ozonetherapy
ISCO3

Ozone Therapy and Its Scientific Foundations

Approved by ISCO3 on November 18, 2012

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Ozone Therapy and Its Scientific Foundations

INDEX

Ozone Therapy and Its Scientific Foundations

Key words

ozone
ozone therapy
oxygen-ozone therapy
treatment with ozone

Abstract

In recent years, ozone therapy as an effective therapeutic method has become more developed and well known. Russia and Cuba have recognized it in their legislation; it is regulated in more than 76% of the Autonomous Regions of Spain; and in Italy four Regions have specified the criteria for practicing it, in addition to two favorable court decisions. Ozone therapy is characterized by the simplicity of its application, its great effectiveness, good tolerance, and by the virtual absence of side effects. This document, based on the latest books and scientific articles on the subject, updates the recent findings that justify, from the scientific point of view, the medical use of ozone. For many years the application of ozone in medical practice was not well accepted due to unfounded ideas about its toxicity in relation to the high concentrations used in industry. As with any healing technique, ozone therapy is dependent on the dosage. It is important to understand that in clinical practice the concentrations of ozone are lower than the toxic levels by several orders of magnitude. In this concentration range, the ozone acts as a therapeutic substance and presents immunomodulating, anti-inflammatory, bactericide, antiviral, fungicide, analgesic properties and others. There are an increasing number of scientific societies and clinical papers, including meta-analysis studies. At the same time efforts are being reinforced to regularize this medical practice.

A. Introduction. Historic background

Ozone therapy has been used for therapeutic purposes from the end of the 19th century, in different ways and with unexpected therapeutic results for some pathologies. Nevertheless there is still great prejudice in the general medical community to the use of this therapy. The objective of this paper is to analyze the background and principal findings that support the medical use of ozone from the scientific viewpoint.

The search for and locating of information included a review of books and scientific articles in the MEDLINE database (PubMed) and in the ISCO3 database (Zotero), between the years 2000 and 2012, for which the following descriptors were essentially used: ozone, ozone therapy, ozonetherapy, oxygen-ozone therapy and treatment with ozone. The sources of primary information (original articles) were located. The bibliographic search included scientific articles of reviews and of experimental results.

In the scientific literature, the first mention of ozone was made by the Dutch physicist Martin van Marum in 1785. During experiments with a powerful electrification installation he discovered that by passing an electric spark through the air a gaseous substance with a characteristic odor appeared, that has strong oxidizing properties. In 1840 the professor of the University of Basel, Christian Friedrich Schönbein, linked the information on the changes of the properties of oxygen with the formation of a particular gas that he called ozone (from the Greek word ozein, "to smell"). Schönbein detected for the first time the capacity of the ozone to bind with biological substrates in the double-bond positions.[1] The German chemist Christian Friedrich Schönbein is also known for the discovery of nitrocellulose.

In 1857 with the help of the "modern magnetic induction pipe" created by Werner von Siemens, the first technical ozonization device was constructed, which was used in a plant for the purification of drinking water. Since then, ozonization has allowed for the industrial production of hygienically pure drinking water suitable for human consumption. One hundred years later, Dr. Joachim Hansler constructed the first ozone generator that made possible the precise dosing of the ozone-oxygen mixture.[1] In Russia, the first studies on the biological effects of ozone (Ph.D. dissertation) have been implemented in the second half of the 19th century by Dr. Chemezov V.V. In 1876 he has published scientific work "On the action of ozone on animals". In 19th century Dr. Razenberg has used in Crimea ozone as chemical element in allergy treatment especially in respiratory diseases. He took out the patients in the open sea immediately after the storm, and they were breathing air full of ozone.

In 1885 the Florida Medical Association (United States of America) published the book *Ozone*, written by Dr. Charles J. Kenworth, where details were given on the use of ozone for therapeutic purposes. In October 1893, the first ozone water treatment system was installed in the Netherlands (Ousbaden), and there are currently more than 3,000 ozone water treatment plants. In September 1896 an O_3 generating system was patented by Nikola Tesla. In 1900 the *Tesla Ozone Company* was formed which began to sell ozone generating machines and ozonated olive oil for medical use.

In 1898 the Institute for Oxygen Therapy Healing was founded in Berlin by Thauerkauf and Luth. From that year, they began to experiment administering ozone through injections. In 1902 *A Dictionary of Practical Materia Medica*, compiled by J. H. Clarke, describes the successful use of ozonated water called *Oxygenium* in the treatment of anemia, cancer, diabetes, influenza, morphine poisoning, aphthas and whooping cough. That same year an article by Dr. Charles Linder appeared in a local Washington newspaper that described the use of O_3 injections in his usual practice.

In 1904 the book *The Medical Uses of Hydrozone (ozonated water) and Glycozone (ozonated olive oil)* by Charles March was published. March was a chemist from New York. On the book, that is preserved in the Library of Congress of the USA, a seal can be seen from the General Surgeons Association of that country, giving its approval. In 1911 *A Working Manual of High Frequency Currents* by Dr. Noble Eberhart of the Department of Physiology and Therapy of Loyola University in Chicago was published. In its Chapter 9, the use of ozone in the treatment of tuberculosis, anemia, chlorosis, whooping cough, tetanus, asthma, bronchitis, high fever, insomnia, pneumonia, diabetes, gout and syphilis was detailed. In 1913 the first German association of ozone therapy was created under the leadership of Dr. Eugene Blass and it was called *Eastern Association for Oxygen Therapy*.[2]

During the First World War (1914 – 1918), Dr. Albert Wolff of Berlin fostered the use of ozone for the treatment of wounds, trench foot (also known as immersion foot), gangrene and to mitigate the effects of poison gas. Ozone was also used for colon cancer, cervical cancer and pressure ulcers. At that time the use of rubber bags made the success of the treatment difficult.

In 1926, Dr. Otto Warburg of the Kaiser Institute of Berlin published that the cause of cancer is the lack of oxygen at the cellular level. This researcher received the Nobel Prize for Medicine in 1931. The directors of the most important hospitals in the U.S.A. published the book *Ozone and Its Therapeutic Action* in 1929, which lists 114 diseases and their treatment through the application of ozone.[3]

The Swiss dentist E. A. Fish (1899-1966) was the first to sense the enormous advantages of O_3 in local treatment. He started working with ozone and ozonated water before 1932 when he successfully treated gangrenous pulpitis with an injection of the gas. The patient he treated was Dr. Edwin Payr (1871-1946), who immediately understood the usefulness of ozone and was enthusiastic about its application in general surgery. In 1935 he published a 290-page article titled *Ozone Treatment in Surgery*, presenting it to the 59th Congress of the German Surgical Association. Between 1934 and 1938 Drs. Aubourg and Lacoste in France used ozone by rectal insufflation to treat problems of fistulas. In 1938 Paul Aubourg published an article on the successes achieved in the hospital of Beaujon (Cliché, Ile de France).

In 1933 the American Medical Association (AMA), run at that time by Dr. Simmons, urged the United States Government to prohibit all therapies that were not medically authorized and duly registered, which caused the use of ozone to drop in that country. In this way, an exclusive benefit was granted to the monopoly of pharmaceutical companies. The decision by Simmons produced unfavorable reactions in the heart of the AMA. Dr. Emanuel Josephson of New York wrote: *The methods which Simmons and his crew used in their battle for a monopoly of medical publications and of advertisements to the profession were often crude and illegitimate (….) The AMA has openly threatened firms advertising in media other than their own journals with the withdrawal of 'acceptance' of their products.* Dr. Josephson also described the behavior of Dr. Simmons inside the AMA as a *conspiracy in restraint of trade, and extortion,* adding that "*almost every branch of the Federal Government active in the field of medicine was completely dominated by the Association.*[2]

In 1951, Dr. William Turska wrote "*Oxidation*" which is a recommended reading even today. He was a pioneer in the injection of ozone in the portal vein to better reach the liver. His results were excellent. In 1950 Dr. W. Zable used it for the treatment of cancer and Drs. P. G. Seeger, A. Varro and H. Werkmeister followed his example. In 1952, the *National Cancer Institute* verified the findings of Dr. Otto Warburg with respect to which the cause of cancer could originate in the lack of tissue-level oxygen.

In 1953, Dr. Hans Wolff (1924-1980) created the first ozone therapy school, training many physicians; and in 1961 he introduced the techniques of major and minor autohemotherapy. In 1972 along with Dr. Joachim Haensler he created the *German Ozonotherapy Association*. In 1979 he published his book *Das Medizinische Ozon* [Ozone in Medicine] (Heidelberg, VFM Publications, 1979).

In 1957, Dr. Joachim Haensler (1908-1981) patented his ozone generator that has been the basis for the expansion of ozone therapy in Germany. Today more than 11,000 German healthcare professionals use ozone in their daily work. In Addition in 1957 at

the IV Congress of Physiotherapists Dr. I.A. Vetohin (Member of the Academy of Medical Sciences of Belarus) has demonstrated an experience of successful use of inhaled ozone therapy in otolaryngology, acute and chronic bronchitis, hypertension and allergic diseases.

In 1977, Dr. Renate Viebahn provided a technical description of the action of ozone in the body. Ten years later, in 1987, along with Dr. Siegfried Rilling, they published "*The Use of Ozone in Medicine*", which has become one of the leading books.

In 1979, Dr. George Freibott began to treat his first AIDS patient with ozone with hopeful results, followed by Dr. Horst Kieff who in 1980 reported on their results. The journal *Science* published the article: *Selective Inhibition of the Growth of Human Cancer Cells by Ozone*.[4]

At the end of 1978 scientific research conducted by Peretyagin S.P., Boyarinov G.A., Monachov A.N. from Russia, at experimental and clinical level was focus to validate the use of ozone therapy. This research was developed at the CSRL (Central Medical Research Laboratory) of Nizhny Novgorod State Medical Academy. Exploratory development demonstrated the benefits of myocardial protection using ozonized cardioplegic solutions. In April 1979 was assayed by first the ozonized cardioplegic solution into the coronary patient during surgery of congenital heart disease. In November of 1986, the first trial using ozonized extracorporeal circulation in patients during mitral valve replacement was conducted. Since then, CSRL became a world leader in this kind of ozone application.

The first Ozone Research Center in the world was founded in Cuba. In 1990 the successes in the treatment of Retinosis Pigmentaria, Glaucoma, Retinopathies and Conjunctivitis were published there by a group of researchers led by Dr. Silvia Menéndez, Dr. Frank Hernández, Dr. Orfilio Peláez and others.[5] In 1992, a group of Russian researchers reported their experiences treating large burns with baths of physiological saline at saturation limit first treated with bubbling ozone. Their results were amazing.

The first uses of ozone were based on its bactericide properties[6-8]. In 1993 Carpendale and Freeberg found important applications of O_3 in patients with HIV/AIDS, a study following the observations made in 1991 on dose-dependent viral inactivation (HIV-1 virus).[9] In 2002, the book "Oxygen-Ozone Therapy. A Critical Evaluation" appeared written by the professor from the University of Siena (Italy), Velio Bocci. The same author in 2005 published the book "*Ozone, a New Medical Drug*", which is a reference book for the practice of ozone therapy[10], followed by several others by the same author. The year 2008 was rich in publications of books of ozone therapy, among which are

found that of the Russian Oleg Maslennikov *et al*. "*Ozone Therapy in Practice: Health Manual. Ministry of Health Service of the Russian Federation"*, that of the Cuban Silvia Menéndez *et al. "Ozono Aspectos Básicos y Aplicaciones Clínicas"* [Ozone, Basic Aspects and Clinical Applications]; and that of the German Z. Fahmy, *"The Application of Ozone Therapy in Pain Management, Rheumatic and Orthopaedic Diseases."*The most complete work written in Spanish was published in 2011, the book "*Guía para el uso médico del ozono: fundamentos terapéuticos and indicaciones*" [Guide for the medical use of ozone: therapeutic basics and instructions] by Adriana Schwartz *et al.* published by the Asociación Española de Profesionales Medicos en Ozonoterapia, AEPROMO [Spanish Association of Medical Professionals in Ozone Therapy].[3]

B. Current situation of ozone therapy in the medical field

At present there are more than 40 national and international associations that bring together the professionals that practice this therapy, indexed specialized journals, continuing training courses and congresses on the subject. However, the generalized application of ozone therapy and its regularization by the authorities is a critical subject at present. Ozone therapy faces its introduction being blocked by the powerful pharmaceutical industry that would see the sale of its drugs diminished. In addition, accidents in its application could be generated by the sale of generator machines and devices for the therapy through the marketing of the products among healthcare professionals without complying with the established standards and/or without possessing adequate theoretical and practical preparation, which would damage the image of this therapy. Furthermore, their use by professionals not duly trained could lead to medical malpractice problems.

One of the most successful and recent attempts to unify the criteria regarding methods and standard procedures to follow was presented in the *Madrid Declaration on Ozone Therapy,*[11] signed in Madrid, Spain (June 4, 2010) during the International Meeting of Schools of Ozone Therapy, organized by AEPROMO – the Spanish Association of Medical Professionals in Ozone Therapy, in the Royal National Academy of Medicine. The Declaration has been signed by 26 national and international ozone therapy organizations and has been translated into ten languages. At present, the *Declaration* is the only truly global document existing on ozone therapy and its recommendations are broadly applied in different parts of the world. However, ozone therapy continues to face difficulties in obtaining wide acceptance in the medical world and its formal incorporation in the regulations of countries. Medical professionals and researchers continue in the battle for the application of this therapeutic method, seeking the benefit of the patients in the simplest and safest way.

It must be clear that for the practice of ozone therapy to be safe, one must: 1) Use an accurate generator. Within the European Union the generator must have the CE marking. 2) Handle precise and well defined doses, volumes and concentrations. The total dosage is calculated by multiplying the concentration by the volume. When the optimal dosage is known, a therapeutic effect is achieved without any toxicity. 3) Ensure that the doctor has good training in the therapy by recognized and competent entities. 4) Have from the health authorities the regulations that permit both the patient and the therapist to receive and work under safety rules. 5) Funds must be available for continued research.[3]

An analysis of the scientific works generated around the subject of the impact of ozone on human health showed how many of them are focused basically on the description of its toxic effects and its environmental impact (Fig. 1). To explain these effects, studies in great depth on its action mechanisms on the biological level have also increased. A rapid growth in the number of studies, whether basic or applied, related to the medical use of ozone can also be observed. Studies that include the explanation of the biochemical and pharmacological mechanisms by means of which the ozone exercises its effects.[2]

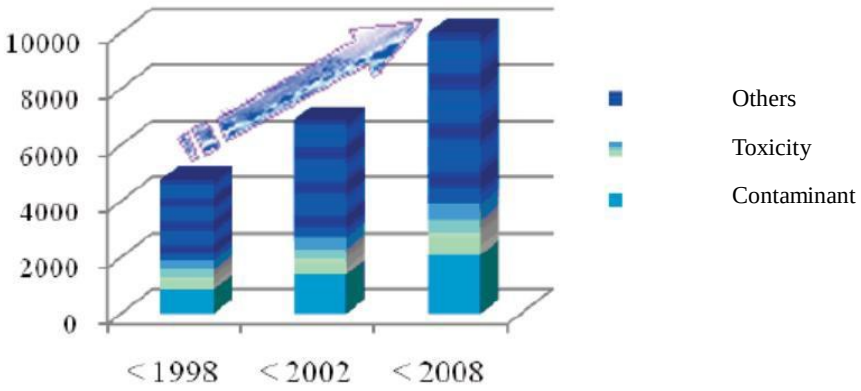

Figure 1. *Number of scientific papers referring to ozone studies. Data from the PubMed 2009 database.*[12]

The main trouble for the wide-scale acceptance of ozone therapy is associated to a great extent with the obstacles that the large drug industry imposes, running media campaigns against its acceptance, to the point of reaching pure scientific ignorance. Unjustly and without scientific basis, it has been stated that *ozone is toxic regardless of its use*, forgetting that the effects of medical ozone, as for nearly all substances, depends on the dose; and that despite these false statements, ozone is considered one of the best disinfectants of drinking water, capable of avoiding infection outbreaks. Used in appropriate concentrations, ozone can activate antioxidant mechanisms that protect the

organism from the effect of the free radicals, involved in aging and in a large number of pathologies.

Despite the empiricism that preceded the practice of ozone therapy and the scarcity of funds available for research in this field, in recent years a growing number of books has been appearing (Annex I) and research papers (Annex II) that constitute scientific support for this therapeutic procedure. According to the *Web of Science* database reviewed in 2009, the number of papers on the use of ozone in the medical field has increased notably. While in the 1974-1979 period only two articles appeared, between 2005 and 2008 this number had increased to 140 (Figure 2).[12]

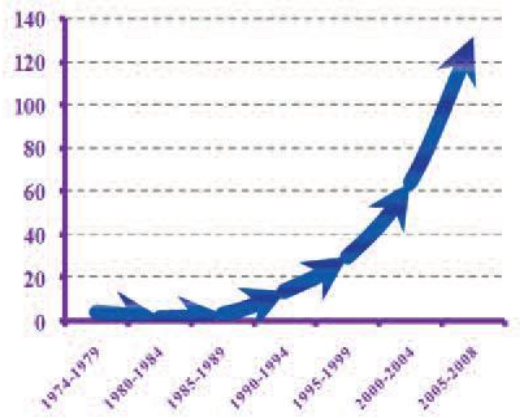

Figure 2. *Number of papers on the use of ozone in medicine (Web of Science).* [2]

C. Physico-chemical properties and action mechanism of the ozone

Ozone is the most important gas of the stratosphere, reaching its maximum concentration (above 1000 μg/m^3) at an altitude of 20-30 km. It is a gas of an unstable nature, sky blue in color, perceptible at concentrations between 98.16 μg/m^3 - 19.63 μg/m^3. It is composed of three oxygen atoms (it is an allotropic form of oxygen). It has a high speed of decomposition that varies on the order of 10^5-10^6 mol/s. Ozone is 1.6 times denser and 10 times more soluble in water (49.0 mL in 100 mL of water at 0ºC) than oxygen and, although ozone is not a radical molecule, it is the third strongest oxidant following fluoride and persulfate. Ozone is produced from three basic sources of energy: Chemical electrolysis, electrical discharges and UV light radiation. Ozone is an unstable gas that cannot be packaged or stored; hence it must be used immediately since it has a half-life of 40 min at 20 ºC. [3]

D. Action mechanisms. General aspects

The research conducted in the 19th century on ozone properties showed that it is capable of reacting with the majority of organic and inorganic substances up to its full oxidation, that is, until the formation of water, carbon oxides and higher oxides. In relation to its reactivity towards biological substances, the selective influence of ozone was established which has double and triple bonds. Among these are listed proteins, amino acids and unsaturated fatty acids, which form part of the composition of the lipoprotein complexes of plasma and of the double layers of the cellular membranes.

The reactions with these compounds are based on the biological effects of ozone therapy and they have significance in the pathogenesis of different diseases. Its action mechanisms are closely linked to the production of four fundamental species, by reacting with the membrane phospholipids: ozonides, aldehydes, peroxides, and hydrogen peroxide (H_2O_2). Their interaction will mostly be with substances with double bonds present in cells, fluids or tissues. They also interact with DNA molecules and cysteine residues of proteins. In adequate and controlled quantities, these derivatives of the reaction of the O_3 with the cellular double bonds carry out different biological and therapeutic functions; acting as second messengers, they activate enzymes, such as chemical and immune-response mediators, among others (Fig. 3).[3]

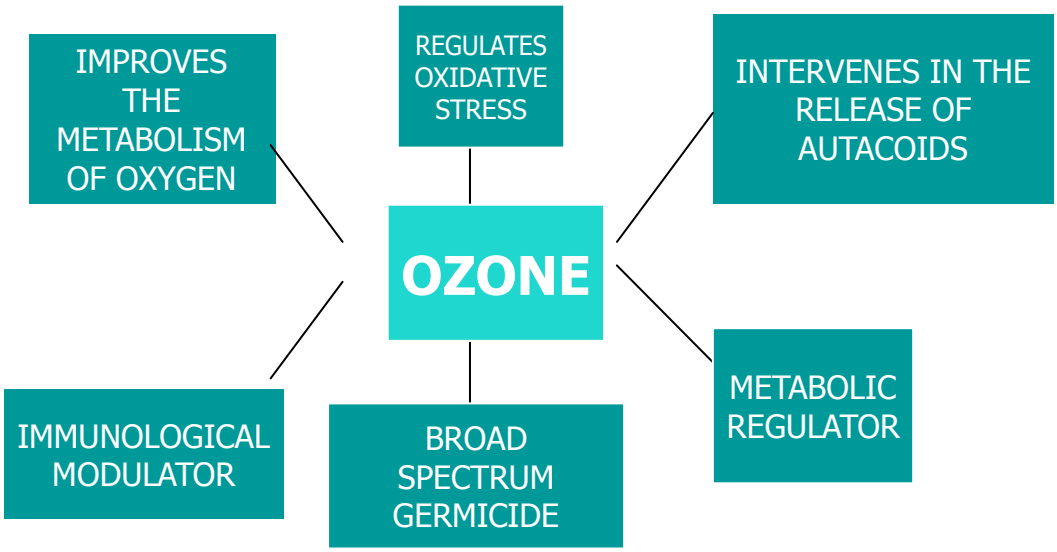

Figure 3. *Biological and therapeutic effects of ozone.*

When the ozone enters in contact with the biological fluids (blood, plasma, lymph, physiological saline serum, urine, etc.) it dissolves in the water present in these fluids and reacts in seconds. The hydrophilic and lipophilic antioxidants present in those organic liquids exhausts a considerable quantity of the ozone dose, but if the concentration applied is correct, it permits the formation of appropriate amounts of reactive oxygen species (ROS) and product of the lipoperoxidation (LPO). The formation of ROS in the plasma is extremely rapid (less than one minute) and is accompanied by a small transitory decrease, depending on the ozone, of the antioxidant capacity (which goes from 5% to 25%). This antioxidant capacity recovers its normal level at 15-20 min. But the hydrogen peroxide and other mediators have diffused it to the interior of the cells, activating different metabolic routes in erythrocytes, leucocytes and platelets, leading to numerous biological effects.[13] The hydrogen peroxide then acts as a signaling molecule in the intracellular medium,[14] a messenger that the therapeutic dose of ozone has been discharged.

D.1 Effect of ozone on the metabolism of oxygen

The effects of ozone on the metabolism of oxygen can be explained from its promoting action of: 1) Changes in the rheological properties of the blood. 2) Increase in the speed of glycolysis of the erythrocyte.[3, 10]

The rheological changes can be explained by its effects on a) the reversal of the erythrocytic aggregation of occlusive arterial diseases (it improves the transmembrane electrical charges and the values of tissue ATP). b) It increments the erythrocytic flexibility and plasticity. c) It favors tissue oxygen transport and delivery.

The effects on the deformation of the erythrocytes and on the metabolism of the erythrocyte are relevant in the actions of the ozone on the circulatory system. As a result it produces a net increase in the improvement of the transport of oxygen to the tissues. The most probable is that this effect takes place after one treatment cycle and acts through a mechanism not mediated by receptors. The net effect is similar to that which is achieved with physical training for which reason it is not appropriate to consider it as a doping practice.

The increase in the speed of the glycolysis of the erythrocyte is evidenced after one cycle of ozone therapy, by noting an increase in the Partial Oxygen Pressure (PO_2) in arterial blood and at the same time a decrease in the PO_2 in the venous blood. This occurs due to a slight decrease of the intracellular pH (Bohr effect) or an increase of the concentrations of 2.3-diphosphoglycerate.

By the ozone reacting immediately with the lipid bilayer, it generates short-chain peroxides, which penetrate to the erythrocyte and impact directly on its metabolism, deriving a functional sequence of small and controlled oxidative stress, which will finally determine the increase of the antioxidant systems.

The LPO during this period act as stress factors on the bone marrow, and these frequent stimulations produce adaptation of erythrogenesis to the ozone stress, with upwards regulation of antioxidant enzymes. The newly generated erythrocytes possess a G-6PD activity greater than that of the old ones, for which reason they have been referred to as *super-gifted erythrocytes*.[10] Consequently, a patient with chronic ischemia in a limb that is subjected to ozone therapy can improve thanks to the formation of cohorts of erythrocytes increasingly more capable of carrying oxygen to the ischemic tissues.

In the same way, 2.3 diphosphoglycerol (2.3 DPG), derived from the increase of the glycolytic process, is a direct inhibitor of the hemoglobin affinity for oxygen, facilitating the detachment of oxyhemoglobin from the latter:

HbO_2 + 2.3 DPG ➔ Hb + O_2

The repairing action of ozone has demonstrated being capable of recovering the internal wall of the small blood vessels, and evidence of this reality is the excellent results of a randomized clinical trial, published in the *European Journal of Pharmacology* (2005), where the recoveries of ulcers in diabetic patients are highly significant.[15] The beneficial effect of this gas on another element, nitric oxide, has also been shown; this element is crucial in maintaining optimal levels of vasodilation, and therefore, the blood flow throughout the entire body.[16]

Today we can ensure that with this therapy, of very low risk, the cellular damage due to the lack of oxygenation decreases substantially, regardless of the underlying disease. In addition, the products of the ozone decomposition behave like biological activators, which improves the level of energy and the capacity of our defense system, in benefit of diseases of allergic-autoimmune types such as psoriasis, asthma and rheumatoid arthritis.[17, 18]

It has been scientifically demonstrated that the controlled applications of medical ozone improve the cellular antioxidant machinery by having measured in the interior of the cells higher quantities of antioxidant agents, such as reduced glutathione or the superoxide dismutase.[19] As a direct consequence, the ozone acts as a real *cellular trash collector*, cleaning up the free radicals. In keeping with this concept, ozone therapy would have an anti-ageing effect on the cells. Aware that a greater quantity of

publications and research is necessary, papers of excellent rigor and quality can currently be consulted in journals as prestigious as *Nature*, *Transplant International*, *Shock*, *Free Radicals*, *Mediators of Inflammation*, *International Journal of Pharmacological Research*, *Liver International*, and the *Revista Española del Dolor* [Spanish Journal of Pain], among others (See Annex I and II), which scientifically endorse this therapy sufficiently.

D.2 Ozone as a modulator agent of the immune response

We know how complex the human immune system is, characterized by cellular or humoral responses, depending on what is required and on the pathology in question. All of them can be regulated by ozone. The questions would be: *in what way?*

Different research studies have demonstrated that ozone therapy has an immune-modulating action, through the synthesis or release of immune-stimulating or immune-suppressing cytokines. All of them are self-regulated with each other, for which reason the production of cytokines will not surpass values beyond what is necessary, once the counter-regulating elements are activated. Satisfactory results have been reported from applying ozone therapy to patients with conditions characterized by an exaggerated immunological response (the case of auto-immune diseases), as well as others with deficiency in their immunological functions.

The immunological actions of ozone on the blood is directed, fundamentally, to the monocytes and to the T lymphocytes, which once induced, release small quantities of practically all the cytokines, thus the release will occur in an endogenous and controlled manner. This regulation is given because the ozone acts as an enhancer of the immunological system by activating the neutrophils and stimulating the synthesis of some cytokines.[20, 21]

Certain transcription factors intervene in the regulation (i.e., NFK-β) which, as their name indicates, favor the transcription and transducing processes at the DNA level, acting as a promoter of this site (or series of nitrogenous bases) where the transcription of DNA to RNA occurs directly, in order to lead to the increase or suppression in the synthesis of a particular cytokine, either pro-inflammatory or anti-inflammatory (Fig. 4).[22]

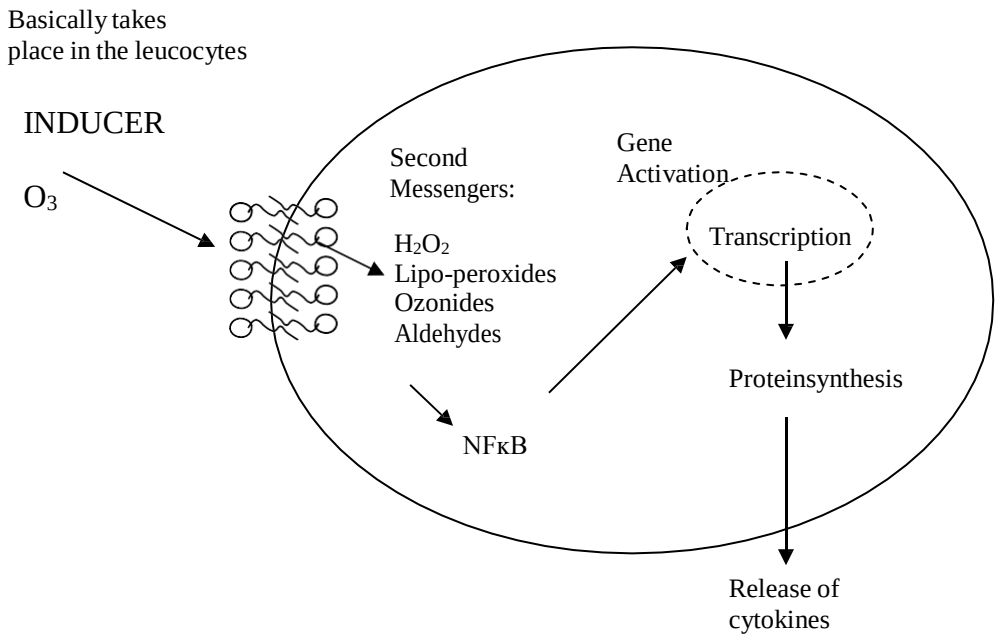

Figure 4. *Action mechanism of the ozone as regulator of the cytokine synthesis.*

Ozone acts through different action mechanisms. The optimization of the oxidant and antioxidant systems of the organism is one of the basic biological effects of the systemic interaction of ozone therapy, which is realized through the influence of the cellular membranes and consists of the normalization of the balance of the product levels of the peroxidation of the lipids and the antioxidant defense system. The hypothesis that an oxidant agent such as ozone can induce an antioxidant effect constitutes a great challenge for the researchers on the subject. In 1998 the first experimental papers appeared that elucidated the so-called oxidative pre-conditioning.[23]

The following year the effects of O_3 on neuromodulation were evaluated, finding that this gas is capable of inhibiting the release of neuromediators by an effect probably related to the modulation of the cytosolic calcium concentrations at the pre-synaptic level.[24] The clinical use of ozone was extended to different pathologies as its action mechanisms were elucidated, in particular their possibilities of activating endogenous antioxidant defense mechanisms. Its use in different pathologies linked to oxidative stress, of inflammatory and degenerative origin (autoimmune syndromes, rheumatoid arthritis, trauma, neuronal apoptosis, ageing, among others) became increasingly generalized. This pre-conditioning effect that ozone exercises is similar to that taking place with ischemic pre-conditioning.[25]

The fact that ozone at controlled doses can have antioxidant effects represents a therapeutic resource of great value in the treatment of multiple diseases that are manifested with a weakening of the endogenous antioxidant system. As a response to the introduction of the ozone in tissues and organs the compensatory increase occurs especially of the activity of the antioxidant enzymes such as: superoxide dismutase (SOD), catalase and glutathione peroxidase, which are broadly represented in the cardiac muscle, liver, erythrocytes and other tissues.

D.3 Bactericide effect of ozone

According to microbiological research data, the ozone is capable of killing all the known types of gram-positive and gram-negative bacteria, including the *Pseudomona aeruginosa* and *Eschericea coli*, both bacteria are tremendously resistant to antibiotics.

The local disinfectant, antiviral and antibacterial effects of ozone, therefore, are due to its germicide capacity, basically to its high oxidant capacity on the bacterial walls. This fact makes it a general broad spectrum germicide on which the classic mechanisms of microbial resistance do not act. Although at first it was thought that physiologically the generation of H_2O_2 was responsible for eliminating the microorganisms, new hypotheses have been presented based on which the concentrations of H_2O_2 are very low for achieving this effect. Such hypotheses indicate that H_2O_2 is only an intermediary in the formation of agents with greater oxidant power such as O_3.

The bactericide effect of ozone in the gram-positive flora of festering wounds and of trophic ulcers is made more effective when a high resistance of the microbes to the usual antibiotics is increasingly evident. This makes it a treatment of choice in these pathologies.

It is interesting to highlight that in 2003 it was discovered that ozone can be generated *in vivo* in activated neutrophils.[26] This discovery has a striking impact since it shows that this substance has a physiological role, not only as a bactericide agent but rather one that could form part of the physiological amplifying mechanisms of the inflammation and the activation of associated genes. Ozone *in vivo* is formed from singlet oxygen ($^1O_2$*), a reaction that is probably catalyzed by antibodies. The possibility is not ruled out of the existence of an endogenous enzyme (ozonase) that is capable of detoxifying the ozone. These subjects are at this moment being studied, the short half-life of ozone and the absorption of UV light at 260 nm (near to where they absorb proteins, nucleic acids and other oxidants such as H_2O_2 and HOCI) make this type of research difficult (Fig. 5).[27]

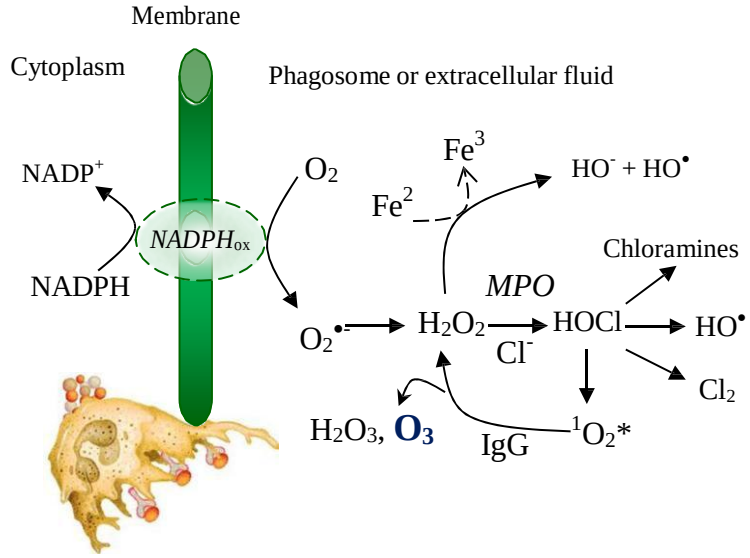

Figure 5

Schematic representation of the processes that lead in vivo to the formation of O_3 by activated neutrophils. $O_{2}^{\bullet-}$, superoxide anion radical; $^1O_2$, singlet oxygen; MPO, myeloperoxidase; HOCL, hypochlorous acid; HO*, hydroxyl radical; NADPHox, NADPH oxidase; IgG, immunoglobulin G; H_2O_2, hydrogen peroxide; H_2O_3,dihydrogen trioxide.*[2]

D.4 General actions

The general effects of ozone are: 1) disinfectant and direct trophic effects, when it is applied locally. 2) Antibacterial and systemic antiviral effect due to a discrete formation of peroxides. 3) It increases the deformity of the red blood cells with relative improvement of blood circulation. 4) It improves the delivery of oxygen to the tissues. 5) It improves the red blood cell metabolism, making the metabolism of glucose more efficient. 6) It improves the metabolism of the fatty acids for the activation of antioxidant enzymes in charge of eliminating peroxides and free radicals.

D.5 Effects of ozone on metabolism

The principal metabolic effects attributed to ozone are: 1) Increment of the use of glucose at the cellular level. 2) It improves the protein metabolism. 3) Direct effects on the unsaturated lipids, it oxidizes them and induces at the same time the repair mechanisms.

D.6 Action mechanism of ozone therapy on pain

Different data coming from the scientific research recognizes that ozone has a dual action mechanism: analgesic and anti-inflammatory. These effects seem to be due to its way of acting on diverse targets: 1) Decrease the production of mediators of the inflammation. 2) The oxidation (inactivation) of metabolic mediators of pain. 3) It clearly improves local blood microcirculation, with an improvement in the oxygen delivery to the tissues, essential for the regeneration of anatomic structures; the elimination of toxins and in general to the resolution of the physiological disturbance that generated the pain.

Figure 6 shows an interesting clinical observation: the application of ozone in an area where pain is experienced turns it red. The ozone has a *revealing* effect of the painful area. The possible explanation of this phenomenon could be the oxidation of specific mediators of the pain that the ozone could cause, but it is a subject that needs to be studied in greater depth. The anti-inflammatory effect of ozone is based on its capacity to oxidize compounds that contain double bonds, among them the arachidonic acid, and derived prostaglandins that participate in large concentrations in the development and in the maintenance of the inflammatory process.[28]

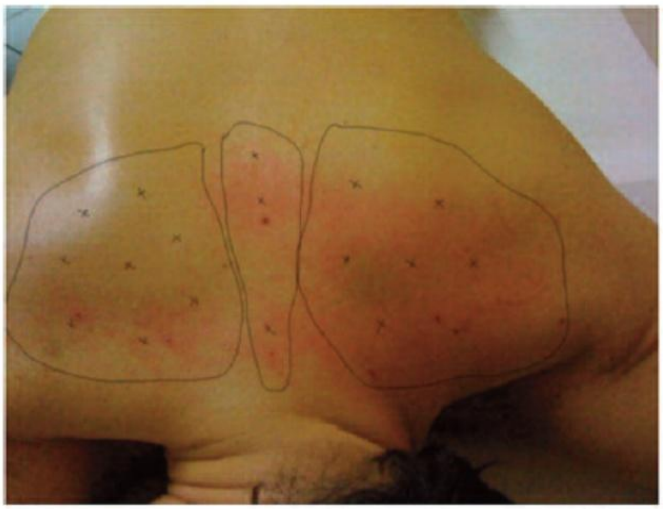

Figure 6
Photo of a patient with sub-scapular pain 1 min. after the injection (injection points marked with an X) with O_2/O_3 8 µg/mL, 2-3 mL. Note that in the area in which pain is reported (central and right of the photo) there is a rosy coloring (erythema) while in the area where there is no pain (left of the photo) the erythema does not appear.[28]

There is an additional mechanism with which the analgesic effects of ozone have been attempted to be explained. It is the reflex mechanism, already invoked for other techniques such as acupuncture. It is a mechanism by which a stimulus (in this case the puncture with the gas or the products formed by the ozone-pain mediator interaction) could activate endogenous analgesic mechanisms with the consequent increase in the concentration of endogenous endorphins (structures similar to morphine produced by our body) that has an analgesic effect. The treatment with ozone produces a short- and long-term effect (Fig. 7).

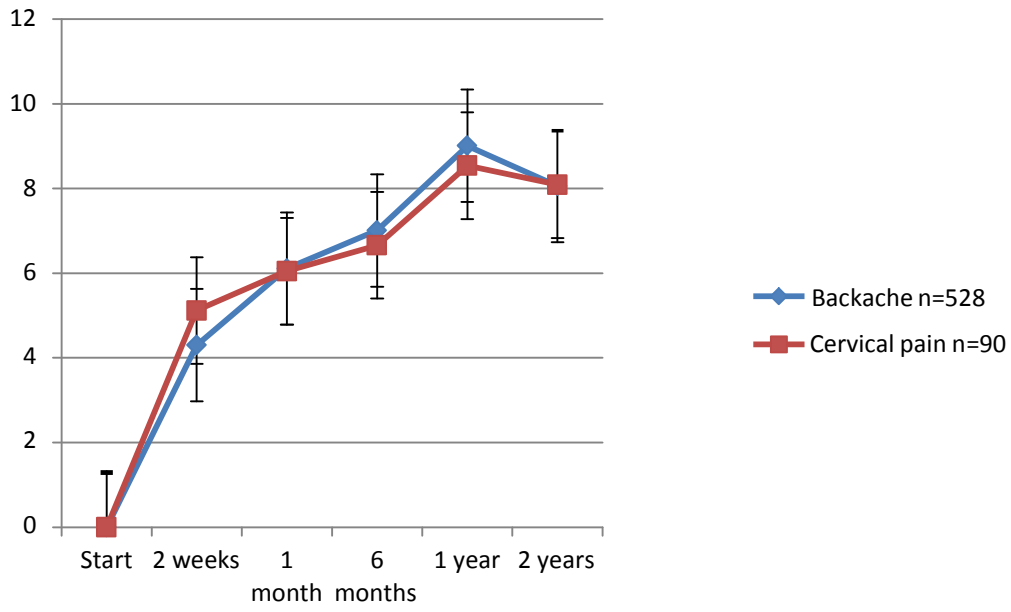

Figure 7.*Evolution of patients with back pain and cervical pain treated with ozone with the i.m. paravertebral technique in time. The scale of 1-10 indicates the perception of pain by the patient (0 means intense pain, 8-10 minimum pain).*[12]

Disc herniation. The pulposus nucleus of the herniated disc contains very high values of phospholipase A_2 which can initiate the inflammatory cascade and other inflammatory mediators such as prostaglandins, leukotrienes, bradykinin and histamine. When an annular crack is produced in the disk, which is the first phase of disc degeneration, these substances are released by the nucleus and may cause radiculitis, although there is no radicular compression.

The use of ozone therapy in the treatment of pain that disc herniation generates has been shown for more than 30 years of research on the subject. Thus, for example in some regions of Italy, such as Lombardy and Sicily the treatment has been included in the group of medical procedures that is covered by the Health Service.

Why does ozone work in disc herniation? A water-resistant effect is produced in herniated discs. The nucleus pulposus of the hernias contain a large part of water and mucopolysaccharides. The O_3 causes lysis or dispersion of the water and oxidation of the mucopolysaccharides that make up the nucleus, and upon being released, entails a desiccation of the disc, eliminating the pressure on the root, and therefore, the pain. In addition it promotes the healing of this nucleus since it does not have edematous (water retention) characteristics. With this, the hernia decreases in size and can even disappear. In addition, ozone therapy acts in this case eliminating the inflammatory factor because it fosters the elimination of the mediator substances of the pain and in particular several mediators that in this specific case amplify the painful sensation. In the case of intradiscal injection, there is reliable proof that the ozone reacts with complex macromolecular components, such as the proteoglycans and the glycosaminoglycans.[29, 30]

The reaction entails the oxidation of these substrates and the degradation of intra- and intermolecular chains, which leads to the disintegration of the tridimensional structure. The collapse releases the trapped water, which, after being reabsorbed, permits reducing the intradiscal pressure and the consequent disappearance of the pain, by decreasing the compression on the nerve root. Since the ozone is also released along the route of the injection (intraforaminal), the final therapeutic effect is due to the combination of vascular and biochemical effects (improved oxygenation, correction of the local acidosis, and the disappearance of the venous and lymphatic stasis).

Thus, the application of intradiscal and paravertebral ozone works on different levels:

a. Inhibition of prostaglandin E_2 and phospholipase A_2 (similar to steroids) and other cytokines pro-inflamamtories (IL 1, 2, 8. 12, 15, interferon α).

b. Increase in the release of immunosuppressive cytokines (IL10, factor B1): analgesic and anti-inflammatory.

c. Increase of local microcirculation, reduces venous stasis: analgesic effect, because the nerve root is very sensitive to hypoxia.

d. Presentation of a direct effect on the mucopolysaccharides and proteoglycans from the pulposus nucleus, which is called ozonolysis, producing a chemical discolysis with water loss and dehydration.

e. Subsequently, there is a matrix degeneration, which is replaced by collagen fibers in approximately five weeks, and by the formation of new blood cells: reducing the volume of the disc.

In summary, there is a dual ozone action mechanism in the Radicular Compression Syndrome: on the one hand, the dehydration of the material disc that would reduce the compressive mechanical factors on the root and the other, interrupting the inflammatory process with immediate installation of an analgesic effect.

Two recent meta-analysis about ozone treatments for herniated lumbar discs have been published in journals with high impact factors. They have demonstrated that ozone therapy is more efficient than control group with low index of adverse events and also enormous advantages in regards to cost. [31, 32]

For paravertebral ozone therapy level of evidence was II-1 and the grading of recommendation was I-B. For intradiscal ozone therapy level of evidence was II-3 and the grading of recommendation was I-C[32].

We may conclude that the paravertebral infiltration or percutaneous discolysis with ozone has been demonstrated effective and safe in the treatment of lumbar pain. Pain and function outcomes are similar to the outcomes for lumbar discs treated with surgical discectomy[31], but with a much lower index of adverse events.

E. Clinical indications of ozone and adverse reactions

The principal clinical indications of ozone are shown in Table 1. A broader explanation of the dosage and the general procedures is found in the Madrid Declaration on Ozone Therapy.[11]

Table 1. Principal therapeutic indications of ozone by specialization

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

E.1 Adverse reactions

Ozone therapy, if it is applied respecting simple rules, does not have side effects (e.g. lung toxicity) and has very few contraindications. Numerous clinical experiments indicate that the adverse reactions to the treatments with the ozone/oxygen mixture are rare and in the majority of the cases they are related to errors in the administering technique. In Germany in 1988 more than a million autohemotransfusions with ozone were performed without the Department of Control of Adverse Effects Caused by Drugs registering a single adverse event. In this analysis it is necessary to distinguish between the effects that can originate from the application of an incorrect technique and those that can be caused by ozone *per se*.

Adverse effects produced by ozone therapy: Ozone is not a drug and as such it does not cause side effects, does not cause allergic reactions and in general no interactions with other drugs have been described. The administering of the ozone in general is well tolerated by the patients; only when excessive doses are used may the patient feel a sensation of heaviness. This discomfort occurs in few patients; it is of short duration and is resolved spontaneously. Only in exceptional cases the painful stimulus induced by the needle puncture or the perception by the patient of his own blood can induce in the patient a vagal crisis (bradycardia, low pressure and sweating) which in general is transitory and does not need pharmacological treatment. Despite this, in every clinic where ozone therapy is practiced there must be a first aid kit and every provision made to act in these cases, although they are of rare occurrence.

The use of plastic bags that are not resistant to ozone can also lead to discomfort such as headaches. If the proper material is not used, the ozone reacts with the plastic material and introduces toxic compounds in the blood that are responsible for the adverse effects described.[33] In summary, the side effects are related to high doses of ozone, inappropriate use of materials, incorrect insertion of the needle, or to subjective factors of each patient. All can be minimized by the therapist if he knows the origin of these secondary effects.

It is important to clarify that ozone as well as oxygen do not cause embolism, due to the fact that the blood is thirsty for them and dissolves them very quickly. The few cases of embolism and death during the practice of ozone therapy have been due to various factors. The direct inoculation of the gas in the blood stream by a physical effect; the coagulation mechanisms are activated by introducing a great volume of gas. In other cases, embolism has been produced when equipment is used that generates medical ozone from the atmospheric air or when the ozone was injected in cavities or within the bone marrow. In all cases, the accidents were produced by iatrogenesis, that is, caused by the irresponsibility of the healthcare personnel that used inappropriate methods or low-quality ozone for the therapy.

When the application of ozone requires using large volume of gas, for example in lipodystrophy, it is important to make sure by different techniques, such as echocardiography, of the absence of aneurisms in the interatrial septum that are frequently associated with PFO (Patent foramen ovale) which, in turn, is the cause of embolism.

Complications related to the technique: are basically due to the trauma that is caused by the introduction of the needle into the anatomical structures during the penetration. The complications can be: hematomas (due to puncture of a blood vessel or extravasation of blood), pain or paresthesias with radicular distribution to the lower arch (due to contact

of the needle with the nerve root) and discitis (inflammation of the inter-vertebral disk) in the case of the intradiscal technique.

In all the cases, the complications can be minimized and their origin is in the use of an incorrect technique. Therefore, an expert ozone therapist has less probabilities of incurring in this type of error. Isolated cases of expulsion of the disc from the vertebral canal have been described in the scientific literature, when the CT-guided intra-discal ozone therapy technique has been performed. Even in these cases an excessive dose of ozone must have been used and the clinical protocols that are in effect for this method must not have been respected.

It is worthwhile to compare the safety of ozone therapy with that which is originated by other factors. For example, by comparing the data from the Center of Disease Control of Atlanta, Georgia (USA.) and the reports of adverse reactions described for ozone therapy of Germany, where it is estimated that around 10 million treatments have been performed in the last 40 years, we can appreciate how low the risk of the use of ozone is.

The studies that best show the complications of ozone therapy are found in the thesis by Jacobs (1986): *Typical Complications in Ozone-Oxygen Therapy*. The reasons for the research were the demands being brought by the community of German ozone therapists coming from the physicians that received data from the non-professional press on cases of complications with this new therapeutic method. Starting with this situation, the community of ozone therapists conducted in 1980 (at its own initiative) the analysis of the frequency and the qualitative composition of the complications, in comparison with the results of ozone therapy.[3]

In German-speaking territories 2,819 surveys were distributed to all the ozone therapists, of which 644 responses were received. In them, 159 physicians documented 336 cases of complications in 384,775 patients that received this treatment, to which 5,578,238 sessions of ozone therapy were applied. Considering all the cases of complications, they were produced in 6 out of every 100,000 sessions, which meant one complication for each 16,667 cases of ozone application. The cases were analyzed to determine in which the cause of the complication had been the ozone itself; as well as the cases related to its incorrect usage, when the supposed interrelation between the complication and the influence of the ozone had been completely excluded. It was observed that, of the 336 cases of complications, in only 40 of them (16%) the presumed cause had been the ozone, dealing with allergic reactions, phenomena of hypoglycemia and skin conditions in the area of administration. In the remaining 84% of the cases, the complications were not related to the actions of the ozone, but rather they were the result of incorrect anticoagulant treatments; or of other types of improper

treatment, such as the use of medication, wrong techniques of administering the ozone and non-sterile handling.

Starting with the 40 cases mentioned above, the calculation of the so-called pure complications coefficient was 0.7 cases in 100,000 sessions of ozone therapy. The author concluded that in comparison with the secondary effects of other types of treatments, this is an insignificant quantity. He cited the data of the representative of the company Sandoz, which shows that of the total number of patients hospitalized in the entire world, intolerance to the medicinal preparations varies between 6.4% and 25%.

If we make a comparative analysis regarding the safety of this therapeutic method, we must necessarily refer to the world plan coordinated by the World Health Organization (WHO) to take on the possible and different causes that may generate the deficiency existing on the subject of hospital safety. This plan indicates that in the industrialized countries, the nosocomial infections complicate between 5% and 10% of the admissions in intensive care of the hospitals. In the developed countries, the intrahospital operations produce greater complications, disability and prolonged hospitalization in 3% to 16%. Each year at least seven million incapacitating complications are produced, which include a million deaths.[34] Furthermore, the WHO indicates that out of every ten patients, approximately one is injured while receiving hospital care.[34]

In Spain, of 5,624 hospitalized patients analyzed in the National Adverse Effects Study, adverse effects were detected in 1,063 (18.9%). The health care was responsible in 9.3% of the cases, while the hospital care was responsible in 8.4%. Medication was the cause of adverse effects in 37.4% of the cases, nosocomial infections of any type in 25.3% and 25.0% were related to technical problems during a procedure.[35] Approximately 15% of the patients on whom a central venous catheterization was performed suffered complications, which put in danger the safety of the patient and increases the stays and the costs linked to the hospitalization, indicates a study fostered by the Spanish Society of Intensive Medicine. Critical and Coronary Units (SEMICYUC).[36, 37]

Despite the efforts that the hospitals are making to reinforce hospital safety, the figures continue being important. The health care infection rate, which was 6.5% in the years 2003 and 2004 in Spain, increased in 2007 to 7.0%.[38] The French newspaper *Le Monde* indicated that on average "900 medical accidents occur each day in the French hospitals and clinics, of which 400 would be avoidable", citing a study of the French Health Ministry.[39] The most delicate issue is rooted in the fact that the study recognizes that the comparative results of 2009 with respect to the previous survey of 2004 were similar and, therefore, the improvements were minimal.[40]

In the Spanish intensive care units, the most frequent adverse effects produced directly

or that *are associated with greater morbidity and mortality among the critical patients* are: 1) pneumonia related to mechanical ventilation (N-VM), 2) urinary infections related to urethral catheterization (IU-SU), 3) primary bacteremias and those related with vascular catheters (BP-C), 4) secondary bacteremias (BS).[36]

The described situation entails a serious economic hemorrhage. Hospital errors –indicates the WHO- have cost some countries between 6 billion to 29 billion U.S. dollars for additional hospital costs, infections acquired in the hospitals, economic loss to the patients, and court processes. The WHO warns that the safety of the patient is today a global problem that affects all the countries, regardless of their economic development.[34] The terrifying level of the cited figures which are devastating in themselves in terms of diseases and human lives, along with the economic drain that it involves, has led the WHO to say that up to 50% of the complications and the deaths could be avoided if the basic required care is observed.[34]

It is worthwhile to add what was stated by the Nobel Laureate in Medicine, Richard J. Roberts, when he affirmed that the *drug companies often are not as interested in healing you as in getting your money, so that investigation, suddenly, is diverted to the discovery of drugs that do not heal completely, but chronify the disease and make you experience an improvement that disappears when you stop taking the drug (…) It is usual that pharmaceutical companies are interested in research that doesn't cure but only make illnesses chronic with more profitable drugs that the ones that would completely cure once and forever.*[41]

The above is confirmed when in Spain the adverse effects could have been avoided in 42.8% of the cases.[35] The promotion of the campaign of *the practice of clean hands in the hospital centers as a hygienic measure to prevent nosocomial infections, something that, although of common sense, only is complied with in 50% of the centres.*[42] In view of the situation of the safety of the medical treatments that we have indicated, we can conclude that the use of intra-hospital ozone would effectively help to reduce the nosocomial infections, thanks to their potent bactericide properties.

On the other hand, around 55 thousand people in the United States and an undetermined number of Europeans may have died for taking VIOXX, one of the selective inhibitors of COX-2.[42] On the contrary, how many deaths from ozone have been recorded? None and the accidents recorded were exclusively of malpractice, and not for the effects of the ozone itself. We ask: Why require from ozone therapy safety measures over and above the existing ones described previously? Provided its dosage is correct, the ozone can produce a multitude of useful biological reactions, and possibly invert the chronic oxidative stress of age, chronic infections, diabetes, atherosclerosis, ischemic, degenerative and inflammatory processes. The therapeutic ozone act is interpreted as a

non-toxic but real *shock therapy*, capable of restoring homeostasis.[44, 45] Therefore, provided its dosage is correct, ozone will be beneficial.

It is clear to the international medical ozone community that ozone must be used in a controlled manner, like any other medication, and there must be good academic training in the application of the therapy. During recent decades, great efforts have been made and continue to be made to examine ozone therapy in a more scientific manner. We have at our disposal textbooks and the *Revista Española de Ozonoterapia*, ISSN 2174-3215, indexed in Lantidex and Dialnet. This journal is governed under the international publication standards. Each year international congresses are organized and in the case of Spain, AEPROMO has achieved that all its congresses have been recognized as *events of health interest*.

F. Contraindications for the use of ozone therapy

The contraindications for the use of ozone are basically those due to ethical or specific deontological situations: 1) Patients that suffer a significant deficit of glucose 6 phosphate dehydrogenase (favism). These persons should not receive this treatment, since an oxidation of the red blood cells could occur, causing hemolysis, due to their not having the protective systems against oxidation. 2) In some abnormal situations (imbalance) in patients with hyperthyroidism and thrombocytopenia. 3) Severe cardiovascular instability (recent myocardial infarction). 4) Convulsive states. 5) Hemorrhagic conditions (external or internal bleedings),[5] significant hypo-coagulation syndrome, blood diseases, haemophylia, thrombocytopenia, hemorrhagic vasculitis, acute myocardium infarctus, hemorrhagic insult, 6) pancreatitis, 7) tyreotoxicosis, 8) individual ozone intolerance, 9) Acute alcohol intoxication, 10) Convulsions in anamnesis.

Ozone therapy is not a panacea; it has precise indications in which great therapeutic success is attained, others in which its success is medium and others in which it is not useful.

G. Who can perform ozone therapy?

Ozone therapy must be performed by a physician or by a dentist; or by a veterinarian in the case of animals. The basic reason is that this type of treatment, if used improperly, exposes the patient to serious risks. For example, if the injections are performed at an improper site or with improper technic (e.g. directly in the veins), with non-sterile needles, at an excessive dosage, among others, they can lead to serious adverse effects

such as the risk of infections or collapse due to excessive vasodilatation. Directly application of O_3 in the veins was performed in the 1970' but is currently banned by the most ozone-therapist associations. The effect of the mayor authohemotherapy is more effective with practically no side effects. In contrast, direct application of O_3 in veins can induce emboli, tachycardia, anxiety and sweating that can persist up to 48 h. In fact, today application of O_3 can be considered a *mala praxis*.

Due to the scarcity of controls on this therapy and the non-existence of clear rules in this regard in the majority of the countries where it is practiced, upon occasion ozone therapy has been practiced by personnel that do not know with precision the correct protocols to follow and therefore they have exceeded the dose, the number of injections, and the frequency with which the treatment is repeated. They have used machines not suitable for generating quality medical ozone, failed to obtain satisfactory results or even worse, they have caused damage to the patients. This is the reason why the international medical ozone community is acting to advocate for the establishment of controls and regulation of the therapy through the corresponding health institutions, duly advised by the existing scientific associations of ozone therapy or even better, by the *International Scientific Committee of Ozone Therapy* (ISCO3) directly. The ISCO3 is a committee formed by 21 prominent worldwide experts in ozone therapy, who in this position do not represent any association or any commercial company.

H. Conclusion

There is much scientific evidence on the clinical use of ozone. The physiological formation of a mediator similar to ozone during inflammation is an indicator of its potential as a new biomolecule. This discovery implies additional efforts to clarify the hypotheses about its action mechanism and to advance towards the execution of more in-depth randomized and standardized clinical studies. Furthermore, the action mechanisms for the ozone on the biomolecules of the blood, with the consequent generation of various messengers responsible for its biological effects, have been clear since 2002. Official medicine does not take into account the effectiveness of ozone therapy, principally because: 1) It is excessively centered on the molecular mechanisms of drug-receptor interaction and ignores the capacity of ozone as a pro-drug. 2) Most clinics are not aware that ozone can dramatically change the course of several diseases by means of the activation of multiple pathways. 3) The pharmaceutical industry has a good reason for ignoring ozone, since it costs almost nothing, is not patentable and does not produce wealth. The lack of sponsors is also a constant obstacle since it makes it impossible to find grants for controlled, multi-center and randomized studies comparable to those that the pharmaceutical companies finance. The clinical trials, the number of books and articles on basic and applied subject relating to ozone therapy

grow daily. The professionals who practice ozone therapy must know all the steps to carry out the clinical trials to make this discipline stronger and more credible and to support it with scientific rigor from the clinical point of view. The battle for the regularization of this medical practice in the different countries where it is being practiced must also continue.

Annex I. List of books on the subject of ozone therapy

(Chronological order, last ten years)

Lamberto Re, Gregorio Martínez Sánchez. *Emerging therapies: ozone. What the patient should know and how the doctor must act*. Aracne Editrice, Roma 2012. ISBN: 978-88-548-4672-2.

Adriana Schwartz *et al*. *Guía para el uso médico del ozono: fundamentos terapéuticos e indicaciones*. Asociación Española de Profesionales Médicos en Ozonoterapia, AEPROMO. Madrid, 2011. 315 p. + XVIII + 11 p. láminas de color. ISBN: 978-84-615-2244-6.

Lamberto Re, Gregorio Martínez Sánchez. *Terapias emergentes: ozono. ¿Qué debe saber el paciente y cómo el médico debe actuar?* Aracne Editrice, Roma 2011. ISBN: 978-88-548-3706-5.

Lamberto Re, Gregorio Martínez Sánchez. *Terapie emergenti: ozono. Cosa il paziente deve sapere e come il medico deve agire*. Aracne Editrice, Roma 2011.ISBN: 978-88-548-3445-3.

Bocci, Velio.*Oxygen-Ozone Therapy: A Critical Evaluation*. 472 pp. Publisher: Springer; 1st Edition. (December 6, 2010). Language: English. ISBN-10: 9048160081. ISBN-13: 978-9048160082.

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Menéndez Cepero, Silvia *et al*. *Ozono Apectos Básicos y Aplicaciones Clínicas* Centro de Investigaciones del Ozono, La Habana, Cuba. Ed. CENIC ISBN 959-7145-06-5. (2008)

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Bocci, Velio.*Ozone: A new medical drug*.Springer, Netherlands 2005. ISBN 1-4020-3139 -4 (HB) and ISBN 1- 4020-3140-8 (e-book).
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Viebahn-Hänsler, Renate & Andrew Lee. *The Use of Ozone in Medicine: A. Practical Handbook* (4th revised ed.) 2002. 148 pages.

Annex II. List of the most important scientific articles on the subject of ozone therapy

Last five years, only on clinical applications. According to the database of the ISCO3 (https://www.zotero.org/groups/isco3_ozone(items) and PubMed, up to March 2012.

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REVIEW Open Access

Ozone acting on human blood yields a hormetic dose-response relationship

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Abstract

The aim of this paper is to analyze why ozone can be medically useful when it dissolves in blood or in other biological fluids. In reviewing a number of clinical studies performed in Peripheral Arterial Diseases (PAD) during the last decades, it has been possible to confirm the long-held view that the inverted U-shaped curve, typical of the hormesis concept, is suitable to represent the therapeutic activity exerted by the so-called ozonated autohemotherapy. The quantitative and qualitative aspects of human blood ozonation have been also critically reviewed in regard to the biological, therapeutic and safety of ozone. It is hoped that this gas, although toxic for the pulmonary system during prolonged inhalation, will be soon recognized as a useful agent in oxidative-stress related diseases, joining other medical gases recently thought to be of therapeutic importance. Finally, the elucidation of the mechanisms of action of ozone as well as the obtained results in PAD may encourage clinical scientists to evaluate ozone therapy in vascular diseases in comparison to the current therapies.

Introduction

Ozone is a double-faceted gas. It has a crucial protective relevance in partially blocking mutagenic and carcinogenic UV radiations emitted by the sun (wavelengths of 100-280 nm) in the stratosphere [1], while its increasing concentration in the troposphere causes severe pulmonary damage and increased mortality [2,3]. In spite of this drawback, there are growing experimental and clinical evidences about the medical use of ozone [4-11]. Since XVI Century, Paracelsus had ingeniously guessed that "all things are poison and nothing is without poison and only the right dose differentiates a poison from a remedy". In 2005, Timbrell reiterated the concept in his book: "The poison paradox; chemicals as friends and foes" [12]. During the Earth evolution, harnessing oxygen by metazoans has allowed a fantastic biodiversity and growth but it has also created a slow acting "poison". It is reasonable to believe that the antioxidant system slowly evolved and specialized during the last two billion years for counteracting the daily formation (3-5 g in humans) of anion superoxide in the mitochondria and the release of H_2O_2 by ubiquitous NADPH oxidases. However, there is a general consensus that the physiological production of H_2O_2 is essential for life. Olivieri *et al.* [13] and Wolff [14] were the first to describe the effect of either low concentrations of radioactive thymidine or of a very low dose of radiation inducing an adaptive response in human cells in comparison to a high dose. Goldman [15] introduced the term "hormesis" to mean "the beneficial effect of a low level exposure to an agent that is harmful at high levels". It goes to the merit of Calabrese [16-19] to have experimentally controlled this concept and to have presented a number of examples of stimulatory responses following stimuli below the toxicological threshold. Until 2002 ozone therapy was pharmacologically conceived as a therapy where low ozone doses were stimulatory, while high doses were inhibitory. This conception, reflecting the classical idea that a low antigen dose is stimulatory, where an antigen overdose is inhibitory, was vague and unsuitable because ozone acts in a complex way and a high dose can still be effective but accompanied by side-effects. Indeed, one of us in 2002 amply delineated the sequence of biochemical reactions elicited *ex vivo* after the addition of a certain volume of O_2-O_3 gas mixture to an equal volume of human blood [20]. First of all, mixing blood with an oxidant implies a calculated and precise oxidative stress, i.e. a homeostatic change with

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production of highly reactive messengers. The oxidative stress, like many others, induces a biological response leading to an adaptive phenomenon. The teleological significance of this response is universal, from bacteria to plants and Mammals, and small repetitive stresses induce an extremely useful adaption response represented by the revival of critical defense mechanisms [20-22]. At the same time, Calabrese and Baldwin described the "overcompensation stimulation hormesis" (OCSH) as the result of a compensatory biological process following an initial disruption in homeostasis [17]. After a reviewer's information also Re later on had expressed this possibility [23]. Ozone presents some subtle differences that will be explained by clarifying the biochemical reactions occurring between the organic compounds of plasma and this gas.

Ozone is a Strong Oxidant Gas

The three oxygen atoms in gas-phase ozone form an isosceles triangle with a distance among the equal sides of 1.26 Å, and exist in several mesomeric states in dynamic equilibrium [24]. In terms of oxidation potential (E°), ozone (2.07 V) is the third after fluorine (3.06 V) and hydroxyl radical (2.80 V). Other pertinent oxidants are: hydrogen peroxide (1.77 V), hypochlorous acid (1.49 V) and chlorine (1.36 V). Ozone has a paired number of electrons in the external orbit and, although it is not a radical molecule, it is far more reactive than oxygen and readily generates some of the ROS produced by oxygen. Ozone is very unstable and at 20 °C, with a half-life of about 40 min, it decomposes according to the exothermic reaction:

$$3O_2 + 68,400 \rightleftharpoons 2O_3$$

Such an aspect has generated the idea that ozone will donate its energy to the organism by reacting with specific body compartments [20]. However, after having ascertained the complexity of the mechanism of action, the conclusion is that ozone dissolved in the water of plasma acts as a pro-drug, generating chemical messengers which will accelerate transfer of electrons and the overall metabolism. It goes to the merit of Hans Wolff (1927-1980), a German physician, to have developed the O_3-AHT by insufflating *ex vivo* a gas mixture composed of medical oxygen (95%) and ozone (5%) into the blood contained in a dispensable ozone-resistant and sterile glass bottle [25].

Which are the Blood Components Reacting with Ozone?

For almost three decades ozone therapy was used only in Germany by practitioners who, by using empirical procedures, elicited skepticism and prejudice in academic clinical scientists. Only during the last fifteen years, by using modern ozone generators able to photometrically (253.7 nm) measure the ozone concentration in a specified gas volume, in real time, and in a precise manner (hence the precise ozone dose per ml of blood), it has been possible to accurately study the reactions of ozone with human blood. It has been clarified that ozone toxicity depends upon its dose and, more important, that judicious ozone dosages can be neutralized by biological defenses [4,20-22,26]. Blood contains some 55% of plasma and about 45% of cells, the bulk of which is represented by erythrocytes. The composition of plasma is complex but, simply said, it contains: about 92% of water; dissolved ions such as HCO_3^- and PO_4^{3-} regulate the pH within the range of 7.3-7.4; both hydrophilic (glucose, uric acid, ascorbic acid, cysteine and other amino acids) and lipophilic (bilirubin, vitamin E, carotenoids, lycopene) molecules; about 5 mg lipids (triglycerides, cholesterol, phospholipids and lipoproteins); proteins, among which albumin (4.5 g/dl), fibrinogen as well as globulins, among which either transferrin or ceruloplasmin binds either Fe^{2+} or Cu^+, respectively, coagulation factors and hormones. Among the plasma main functions, one is the antioxidant activity performed by a variety of molecules such as uric acid (4.0-7.0 mg/dl, 400 μM), ascorbic acid (Aa) (0.4 - 1.5 mg/dl, 22,7-85 μ; M), GSH (0.5-1.0 μM), the mentioned lipophilic compounds as well as albumin. In detail, erythrocytes have a great reservoir of GSH (about 1 mmol/l), thioredoxin with two available cysteine, and potent antioxidant enzymes (catalase, GSH-Rd, GSH-Px, GSH-Tr, and SOD). They can quickly wipe out great amounts of oxidants such as $^{\cdot}OH$, H_2O_2, OCl^-, $ONOO^-$ and, at the same time, recycle protons back to oxidised compounds by using protons donated by NADPH continuously regenerated by the activity of G6PD via the pentose phosphate pathway. It must be noted that most of these antioxidants work in concert accelerating the reduction of noxious oxidants (Figure 1). Albumin on its own is the most important because it holds nucleophilic residues, such as one free Cys34 as well as multiple Lys199 and His146 [27,28].

The Biochemical Reactions of Ozone with Blood

During the most precise and safe methodological *ex vivo* O_3-AHT approach, oxygen-ozone mixture dissolves into the water of plasma. Oxygen has a low solubility, but the pO_2 slowly raises up to about 400 mmHg [29]. Hemoglobin become fully oxygenated (Hb_4O_8) but this is hardly relevant because, during the infusion period, it mixes with venous blood which has a pO_2 of about 40 mmHg. On the other hand, ozone behaves quite differently because, by immediately reacting with ions and biomolecules, it does not follow the classical Henry's law in terms of linear solubility variation with pressure. First of all ozone is about tenfold more soluble than

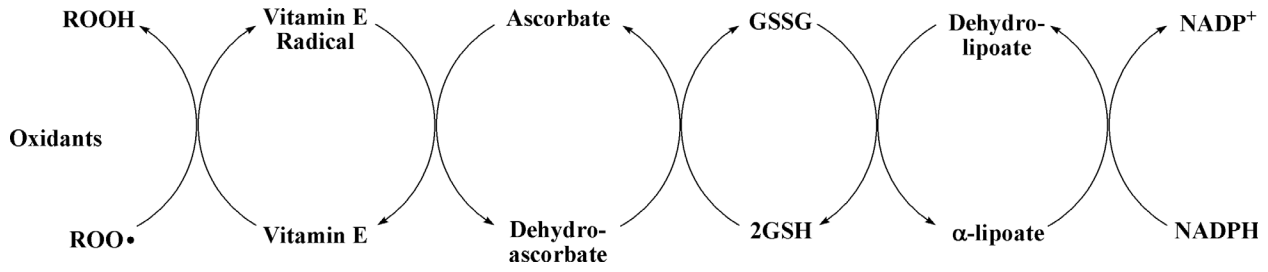

Figure 1 Cellular responses to oxidant exposure. ROOH and ROO• indicate lipohydroperoxide and its oxygen centered organic radicals formed by radical reactions with cellular components, respectively. GSH and GSSG represent the sulfhydryl/disulfide pair of glutathione species. Nicotinamide adenine dinucleotide phosphate, NADP(H), is the primary electron source, regenerated by the cellular reduction systems.

oxygen and, as ozone dissolves in the plasmatic water, it instantaneously reacts with hydrophilic antioxidants: by using an ozone concentration of 40 μg/ml, corresponding to 0.84 μ;mol/ml per ml of blood, within five min an average of 78% of Aa has been oxidized to dehydroascorbate and about 20% of uric acid has been oxidized to allantoin [30]. Only about 10% of alpha tocopherol has formed an alpha tocopheryl radical. At the same time the remaining ozone performs the peroxidation of available unsaturated fatty acids, which represent an elective substrate and are mostly albumin-bound. Peroxidation of n-6 PUFA leads to the formation of H_2O_2 and 4-hydroxy-2E-nonenal (4-HNE) [31], while n-3 PUFA leads to the formation of 4-hydroxy-2E-hexenal (4-HHE) [32,33]:

$$- R - CH = CH - R + H_2O + O_3 \rightleftharpoons 2RHCO + H_2O_2$$

As all of these reactions happen in a few seconds, ozone, until present in the gas phase, continues to dissolve in the plasmatic water and instantly reacts. Within the canonical 5 min, ozone is fully extinct with both a rather small depletion of hydrosoluble antioxidants and the simultaneous plasmatic increase of ROS and LOP. The ozonated blood is then infused into the donor patient.

What is the Significance and Fate of These Ozone Messengers?

First of all the brief life-span of H_2O_2 will be discussed. During the 5 min of mixing blood with the gas *ex vivo*, H_2O_2 will dynamically increase its concentration: rapid at first and progressively slowing down as ozone is being depleted. With the therapeutically high ozone concentration of 80 μg/ml per ml blood, the H_2O_2 concentration measured in plasma after 2.5 min is at most 40 μM because the rate of synthesis is equilibrated by multiple degradation routes. Some H_2O_2 is reduced by free soluble antioxidants including traces of catalase and GSH-Px. As the hemolysis is negligible (<0.5%), free Fe^{2+} or Cu^+ are not present and it is unlikely that hydroxyl ions are ever formed by either the Fenton-Jackson or the Haber-Weiss reactions. As H_2O_2 is unionized, it freely diffuse into all blood cells although the bulk is mopped up by erythrocytes. The establishment of a dynamic, yet transitory, H_2O_2 gradient between the plasma and the cytoplasmatic water of blood cells makes this oxidant a very early effector. Its final intracellular concentration may be not higher than 10%, hence 3-4 μmoles, as it has been demonstrated in other studies [34-39]. The smartness of this system is that the H_2O_2 concentration, though small, is enough to trigger several crucial biochemical reactions without toxicity because the internal cell environment contains a wealth of GSH, thioredoxin, catalase and GSH-Px, which do not allow a dangerous increase. In spite of a threshold of only a few micromoles, it has a critical relevance and means that an ozone amount below 0.42 μmol for each ml volume of the gas mixture (medical grade O_2 ≥95% and O_3 ≤5%) reacting in a 1:1 ratio with autologous blood may be ineffective, resulting in a therapeutic failure of O_3-AHT. It is also necessary to remind that the ozonation process greatly differs whether it occurs either in plasma or in blood. In plasma, TAS levels was, as expected, ozone-dose dependent and decreased between 46 and 63% in relation to ozone concentrations of either 0.84 μmol/ml or 1.68 μmol/ml per ml of plasma, respectively. On the other hand, in blood taken from the same donors, after being treated with the same ozone concentrations, TAS only decreased from 11 to 33% in the first minute after ozonation, respectively. Moreover, it was surprising to determine that they both recovered and returned to the original value within 20 min, indicating the capacity of blood cells to quickly regenerate dehydroascorbate and GSH disulfide [34]. It has been also brilliantly demonstrated that, thanks to erythrocytes, dehydroascorbate was recycled back to Aa within 3 min [40]. On the same way, only about 20% of the intraerythrocytic GSH had been oxidized to GSSG within one min after ozonation and promptly reduced to normal after 20 min [41]. Aa, alpha-tocopherol, GSH and lipoic acid undergo an orderly reduction by a cooperative

sequence of electron donation continuously supplied by NADPH-reducing equivalents to GSH-Rd and thioredoxin reductase [42] (Figure 1). These data, by showing that the therapeutic ozonation only temporarily and reversibly modifies the cellular redox homeostasis were reassuring regarding the safety of ozone as a medical drug. In summary, the initial disruption of homeostasis due to ozone oxidation is followed by the rapid reestablishment of homeostasis with two main advantages: the first being the value of triggering several biochemical reactions in blood cells and the second mediated by LOP compounds, the induction of an adaptive process due to the up-regulation of the antioxidant enzymes. This is in line with the temporal sequence of the OCSH dose-response relationship.

What is the Action of Ozone in the Blood Cells?

- Erythrocytes

Probably the activation of phosphofructokinase accelerates glycolysis with a demonstrated increase of ATP and 2,3-DPG [4,20]. Functionally, the oxyhemoglobin sigmoid curve shifts to the right owing to the Bohr effect, i.e. a small pH reduction (about 7.25) and a slight increase of 2,3-DPG. This metabolite increases only in patients who have a very low level but it remains to be clarified how the phosphoglyceromutase is activated. The shift to the right is advantageous for improving tissue oxygenation as the chemical bonding of oxygen to hemoglobin is attenuated, facilitating oxygen extraction from ischemic tissues. Rokitansky *et al.*, had previously shown that the pO_2 was lowered to 20-25 mm Hg in the femoral vein of PAD's patient throughout O_3-AHT sessions [43]. It seems obvious that erythrocytes ozonated *ex vivo* may be modified only for a brief period. Only repeated therapeutic sessions may allow to LOP compounds to reach the bone-marrow and activate a subtle development at the erythropoietic level, favouring the formation of new erythrocytes with improved biochemical characteristics, which provisionally were named "supergifted erythrocytes" [20]. If this hypothesis is correct, every day, during prolonged ozonetherapy, the bone marrow may release a cohort (about 0.9% of the pool) of new erythrocytes with improved biochemical characteristics. In fact, the therapeutic advantage does not abruptly stop with the cessation of the therapy but rather persists for 2-3 months, probably in relation to the life-span of the circulating supergifted erythrocytes [26]. It is interesting that during prolonged ozonetherapy, by isolating through a sedimentation gradient the small portion of very young erythrocytes, it has been demonstrated that they have a significant higher content of G6PD [44]. Such a result strengthens the postulation that only a cycle of more than 15 treatments (not less than 3 liters of ozonated blood) could improve an ischemic pathology.

- Leukocytes

Human neutrophils are able to generate an ozone-like molecule [45] and volatile compounds [46] as a part of their phagocyte activity. Neutrophil phagocytic activity has been found enhanced during ozonetherapy [47]. Moreover, H_2O_2 activates a tyrosin-kinase with subsequent phosphorylation of IkB, one of the trimeric components at rest of the ubiquitous transcription factor denominated NF-kB [48,49]. The phosphorylated IkB detaches from the trimer and it is broken down in the proteasome. The remaining eterodimer p50-p65 is transferred into the nucleus, where it can activate about 100 genes up-regulating the synthesis of acute-phase proteins, several proinflammatory cytokines (IFN-γ, TNF-α, IL-8) and even HIV proteins [50]. There is no doubt that H_2O_2 is the trigger as the activation is related to a cysteine oxidation that can be prevented by an excess of thiol. Although ozone is a very modest inducer of some cytokines [50], the consequent immunomodulatory effect may be useful in immune-depressed patients after chemotherapy, or in chronic infectious diseases. It must be clear that ozone in itself cannot exist in the circulation and moreover, due to the potent antioxidant capacity of plasma, it is unable to kill any pathogens *in vivo* whereas an activated immune system may be helpful [51].

- Platelets

During O_3-AHT, the detection of PDGF-B, TGF-β_1, IL-8 and EGF released in heparinized plasma in ozone-dose dependent quantities was not surprising because platelets are exquisitely sensitive to a progressive acute oxidative stress [20,52]. The increased level of these growth factors in the circulation may have the beneficial effect of enhancing the healing of foot-related problems from diabetes or PAD.

The pleiotropic LOP activities

As shown in Figure 2, LOP production follows peroxidation of PUFA present in the plasma: they are heterogeneous and can be classified as lipoperoxide radicals, alkoxyl radicals, lipohydroperoxides, F_2-isoprostanes, as well as aldehydes like acrolein, MDA and terminal hydroxyl alkenals, among which 4-HNE and 4-HHE. As free radicals and aldehydes are intrinsically deleterious, only precise and appropriate ozone doses must be used in order to generate them in very low concentrations. Among the aldehydes, 4-HNE is quantitatively the most important. It is an amphipathic molecule and it has a brief-half-life in saline solution. On the other hand it reacts with a variety of compounds such as albumin, enzymes, GSH, carnosine, and phospholipids [31,53]. There is no receptor for 4-HNE but it has been reported that, in concentration above 1 μM *in vitro*, after binding

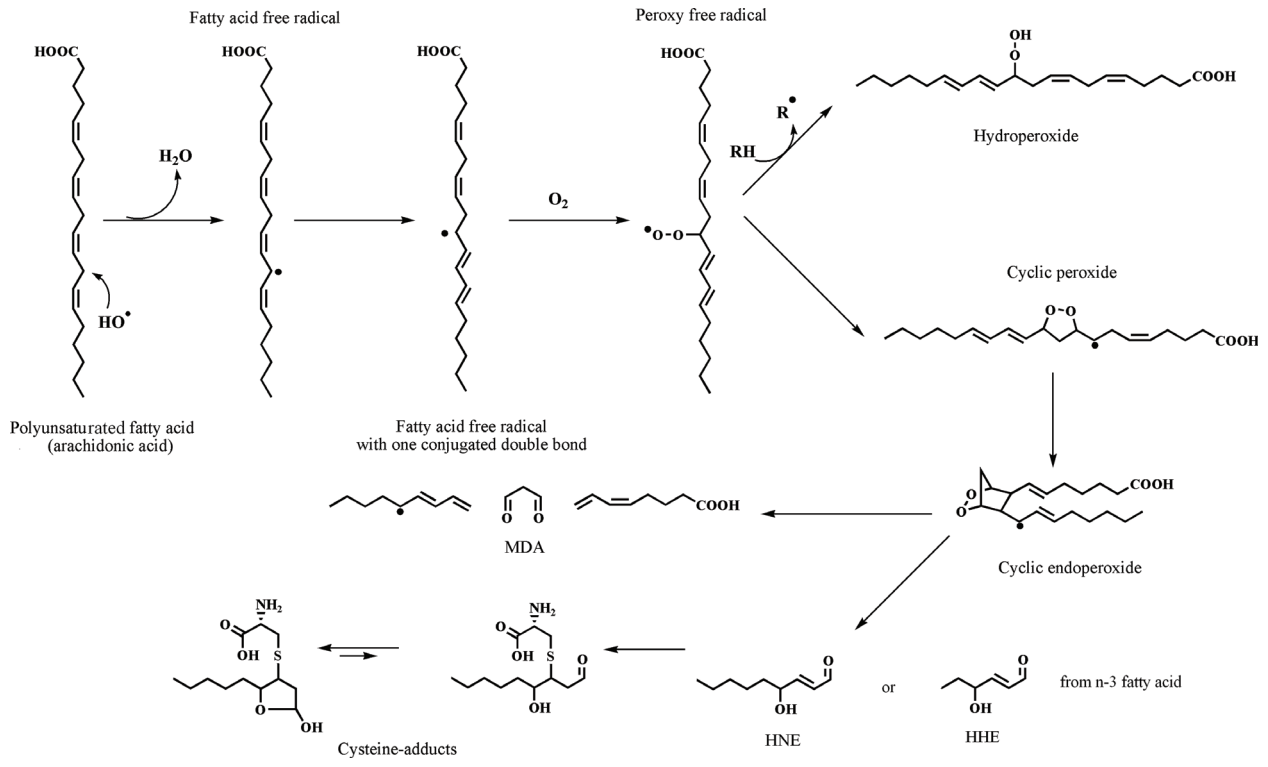

Figure 2 Generic scheme of polyunsaturated fatty acids peroxidation. Arachidonic acid reactions have been detailed, but similar pathways are applicable to other polyenoic fatty acids. MDA: malondialdehyde. HHE: 4-hydroxy-2E-hexenal. HNE: 4-hydroxy-2E-nonenal.

to more than 70 biochemical targets, it exerts some deleterious activity [31]. On the other hand, during the rapid reaction of ozone with blood, the generated hydroxy-alkenals, will form adducts both with GSH or with the abundant albumin molecules. This possibility is supported by findings which have shown that human albumin, rich in accessible nucleophilic residues, can quench up to nine 4-HNE molecules, the first being Cys34, followed by Lys199 and His146 [27,28]. Interestingly, when samples of ozonated human plasma were incubated at 37 °C for 9 hours, 4-HNE, most likely bound to albumin, remained stable [54]. These data clarify why a judicious *ex vivo* ozonation of blood does not harm the vascular system during the infusion into the donor. Aerobic organisms, in order to tolerate the continuous generation of aldehydic compounds have developed detoxifying systems as follows: the first is the ***dilution*** of these products in both the plasma and the extracellular fluid involving a volume of about 11 L in humans. The second is the ***detoxification*** operated by aldehyde dehydrogenase, aldose reductase and GSH-Tr [55,56] and the third is the ***excretion*** via bile and urine excretion [57-59]. The relevance of these catabolic pathways was appreciated when the half-life of infused alkenals present in ozonated blood in a patient was less than 5 min [60]. The interesting aspect is that albumin can transport 4-HNE in all body tissues, from liver to endocrine glands and the CNS. 4-HNE-Cys adducts, released at many sites, inform a variety of cells of a transient, acute oxidative stress and represent an important biochemical trigger. At submicromolar or picomolar levels, 4-HNE can act as a signaling molecule capable of activating the synthesis of γ-glutamate cysteine ligase, γ -glutamyl transferase, γ -glutamyl transpeptidase, HSP-70, HO-1, and antioxidant enzymes such as SOD, GSH-Px, catalase and last but not least, G6PDH, a critical electron-donor enzyme during erythropoiesis in the bone marrow. There is a wide consensus on the relevance of the induction of protective molecules during small but repeated oxidative stress [20,61-65]. In other words, the concept that a precisely controlled oxidative stress can strengthen the antioxidant defenses is well accepted today. Once again, the low level of stress by enhancing the fitness of the defense system, is consistent with the hormetic concept. Moreover at the time of ozonated blood infusion, 4-HNE-Cys adduct can also act on the vast expanse of endothelial cells and enhance the production of NO [35]. Such a crucial mediator on its own or as a nitrosothiol, with a trace of CO released with bilirubin *via* HO-1 activity, allows vasodilation, thus improving tissue oxygenation in ischemic tissues [66]. H_2S is another potentially toxic molecule that,

when released in trace amounts, it becomes an important physiological vasodilator like NO and CO [67,68]. Moreover, as it happens for the mentioned physiological traces of other gases, the small amount of ozone necessary to trigger useful biological effects is in line with the concept of the hormesis theory [69].

Another interesting aspect observed in about 2/3 of patients is a sense of wellness and physical energy throughout the ozonetherapy [70]. It is not yet known whether these feelings are due to the power of the generated ozone messengers which can modify or improve the hormonal secretion. On the other hand, the feeling of euphoria may be due to improved oxygenation or/and enhanced secretion of growth hormone, ACTH-cortisol and dehydroepiandrosterone [26,71]. Furthermore, when LOP reach the hypothalamic area they may improve the release of serotonin and endorphins, as it was observed after intense dynamic exercise [72]. Experience acquired after thousands O_3-AHT has clarified that there is neither objective nor subjective toxicity, or to use Calabrese's acronyms, there is no observable adverse effects (NOAEL). Moreover, neither structural nor enzymatic damages have been observed in blood components after ozonation of blood within the therapeutic window [73,74]. On the other hand, patients with more advanced disease during the initial session especially if performed with a high ozone dosage, frequently report to feel very tired and sleepy. This is the lowest observed adverse effect level (LOAEL) that has been observed in about 10% of PAD's patients with stage III and IV of the Leriche-Fontaine's classification. Such a knowledge compels to begin always with low ozone dosage and carefully observe the patient's response.

Which is the Most Suitable Term for Describing the Dose-Response Relationship Between Ozone and Blood?

Ozone is a toxic gas and it cannot be compared to either any usual immunological stimulus or to stable chemical compounds: firstly, nobody has ever described a cell receptor for ozone, and secondly the biochemical reactions with blood components generate various messengers with quite different half-lives, finalities and fate. Moreover, not only biological but also clinical responses have to be taken into account when using ozonetherapy in quite different pathologies such as cardiovascular, or autoimmune or orthopedic diseases. The hormetic dose response appears to be useful for describing the dual pharmacological response elicited by ozone, basically acting as a pro-drug. The most common form of the hormetic dose response curve, depicting low dose stimulatory and high dose inhibitory and toxic responses is the ß- or inverted U-shaped curve shown in Figure 3, panel a. However, the graphic illustration of the hormetic dose-response relationship between ozone and blood needs an explanation because it slightly differs from graphs presented on the effect of other stressors (Figure 3, panel b) [26,75-78]. It has been found that an ozone dose of only 10 μ;g/ml (0.21 μmol/ml) per ml of blood is fully neutralized by both uric acid and Aa, especially when the TAS of individual blood is between 1.5-1.9 mM [79]. It follows that the minimal reaction, if any, with PUFA will not generate enough messengers as

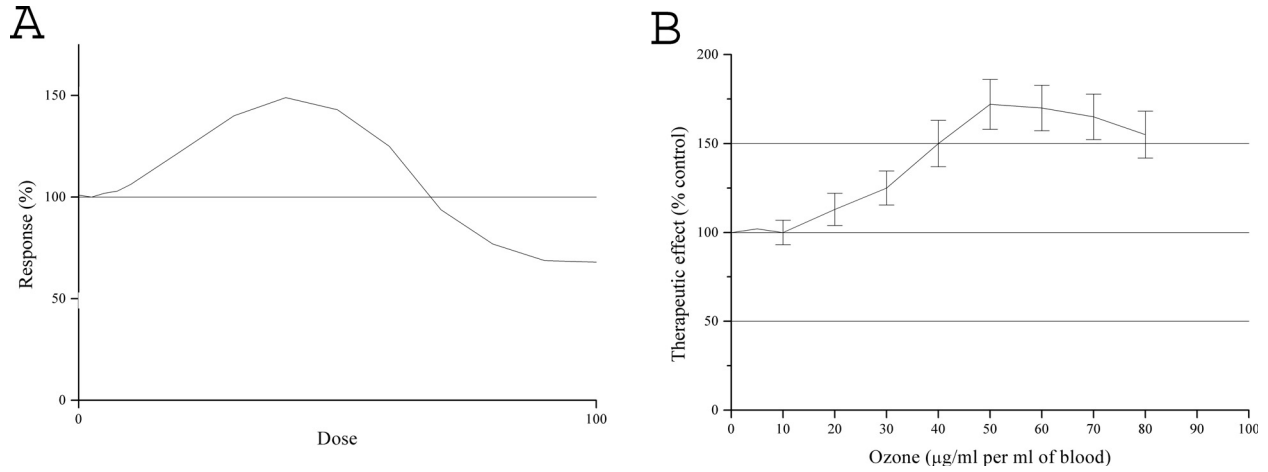

Figure 3 The hypothetical inverted U-shaped curve describing an ideal dose-response relationship (panel A). The inverted U-shaped curve drawn on the basis of the therapeutic effect in PAD's patients by using an ozone concentration range between 15 and 80 μg/ml of gas per ml of blood. During a course of 15-20 sessions, the initial ozone concentration of 10 μg/ml has been slowly upgraded to the concentration of 80 μg/ml (panel B). The end-points that have been considered to determine the therapeutic effects are: claudication; ankle-brachial index; disappearance of pain; healing of skin ulcers.

ROS and LOP to trigger biological effects. In this case the small ozone dose is totally consumed by available free antioxidants and the ozonated blood will not display therapeutic activity. Gaseous ozone doses between 20 and 80 μ;g/ml (0.42-1.68 μmol/ml) per ml of blood are well calibrated against blood's TAS and both biological and therapeutic effects will ensue. A recent metabonomic study has shown that the blood antioxidant capacity is almost exhausted when the ozone dose has been raised to 160 μ;g/ml per ml of blood [74]. In simple words, too little ozone, unable to modify the homeostatic equilibrium, is unable to elicit the hormetic response. On the basis of the last observation, it would be most interesting to analyze the response in normal volunteers.

Ozone Therapy in Oxidative-Stress Related Diseases

The metabolic syndrome is recognized as one of the most serious disease in Western countries caused by a number of metabolic alterations such as type-2 diabetes, hypercholesterolaemia, atherosclerosis and renal dysfunction with the common denominator represented by a chronic oxidative stress. Diabetic patients, particularly those with foot ulcers, are critical and today they still have a gloomy prognosis. This is because they need a multiform therapy aiming to eliminate the peripheral ischemia, the neuropathy and the infected skin lesions. The range of ozone concentrations between 15 and 35-50 μ;g/ml is safe also in individuals with a low TAS level and it appears to be particularly effective in PAD [43,80-85]. Several clinical studies performed in different hospitals seem to establish the validity of the inverted U-shaped curve in this frequent pathology (Figure 3, panel B). In line with "the concept of a beneficial effect within the context of a dose-response study is difficult to determine due to considerable biological complexity and the fact that beneficial effects are often seen with reference to a specific and relative setting" [17], a word of caution is necessary. This is especially true when ozone therapy is performed in different patients within the variety of three PAD's II, III and IV stages, according to the Leriche-Fontaine classification [86]. First of all it is necessary to trust the precision of ozone's dosages used by different clinicians and secondly, ozone activity cannot be compared with that expressed by a single compound (see, eg, Arsenic [76], and homocysteine [77]) in cultured cells. As it has been clarified, the real ozone messengers are H_2O_2 as a ROS and a variety of alkenals as LOP. These messengers act on different cells, have a quite different lifetime and alkenals are intrinsically toxic. Furthermore, each patient has his own medical history and his own psycho-physical reactivity. Consequently, ozone dosages between 0.42-0.84 μ;mol/ml generate less alkenals than dosages in the range 0.84-1.68 μ;mol/ml, and therefore patients with a low antioxidant capacity become more susceptible to a side effect like deep fatigue after the therapy session. Attention must be also paid to the type of pharmacological response achieved in different pathologies as either muscular-orthopedic or autoimmune diseases. So far, in the latter it remains unknown the ozone dosage, if any, able to increase the T-cell regulatory levels and activity. Consequently, at this stage the U-shaped curve remains meaningful only for PAD and only future trials will be able to define the ozone behavior in either stroke or chronic heart disease. Martinez-Sanchez *et al.* have also reported that the theoretical U-shaped curve fits the ozone therapy results [87]. Blood ozonation, even if performed within the therapeutic range and for a few minutes, represents always a calibrated acute oxidative stress. In order to never harm the patient, the strategy: "start low-go slow" is a golden rule to induce a valid adaptation to the far more dangerous chronic oxidative stress, typical of inflammatory and degenerative diseases [88]. Such an aspect implies that the final therapeutic effect is due to an average of progressively increasing ozone dosages.

The gas mixture medical grade oxygen-ozone can be proficiently used for the ozonation of blood because this incomparable liquid tissue contains an imposing array of antioxidants, which are able to tame not only its oxidant power but also its messengers (ROS and LOP) generated by the reactions with blood components. Therefore, if ozone is judiciously used within the established therapeutic window (0.42-1.68 μmol/ml per ml of autologous blood) in PAD, it can exert better therapeutic effects than the current therapy by prostacyclin analogue. Moreover, regarding the accompanying foot-related problems, both some ozone derivatives like ozonated water and different gradation of standardized ozonated vegetable oils will be used until complete healing [89,90]. As stroke, heart infarction and PAD are cumulatively the first cause of death and disability, if it will become possible to use ozone therapy in the public hospitals of the developed Countries, it may be possible to enter a phase where ozone will become an extensive remedy. Moreover, there is no doubt that either infective or autoimmune glomerulo-nephritis as well as end stages of renal failure associated with hemodialysis are characterized, to a different extent, by an imbalance between pro- and antioxidative mechanisms [91]. Moreover the kidney does not have the regenerative ability of liver and this is one of the reasons for explaining why too often "nephropaties lack a specific treatment and progress relentlessly to end-stage renal disease" [92]. Another important reason is that till today a valid strategy to reduce oxidative stress in renal diseases is not available.

Ozone therapy, not only may correct a chronic oxidative stress, but it may also stimulate untapped resources able to afford some improvement [9,93]. It appears therefore reasonable to suggest the combination of conventional treatments with mild O_3-AHT in any initial nephropathy for preventing the risk of progression towards a chronic disease.

In several Countries, among others Cuba, Russia, and Ukraine, treatments by ozone are already a reality, although different administration modalities, such as the infusion of ozonated saline and of the rectal insufflations of ozone, are in current use because inexpensive and applicable to thousands of patients every day [94]. Nevertheless, it is hoped that adequate ozone-based therapeutic treatments for patients affected by oxidative-stress related diseases could be implemented in every public hospital.

Conclusions

During the last two decades the paradoxical behaviour of ozone has been clarified: when it is chronically inhaled, it is highly toxic for the pulmonary system because the enormous alveolar surface, unprotected by sufficient antioxidants, is exposed to the cumulative ozone dose, which causes a chronic inflammation. This is not surprising because even for oxygen [95], as well as for glucose and uric acid levels a modification of the physiological concentrations is deleterious.

On the basis of the mechanisms of action, ozone therapy appears to be a safe, economical, effective treatment for patients with cardiovascular disorders based on the following biological responses [26]:

a) it improves blood circulation and oxygen delivery to ischemic tissue owing to the concerted effect of NO and CO and an increase of intraerythrocytic 2,3-DPG level;

b) by improving oxygen delivery, it enhances the general metabolism;

c) it upregulates the cellular antioxidant enzymes and induces HO-1 and HSP-70;

d) it induces a mild activation of the immune system and enhances the release of growth factors from platelets;

e) it procures a surprising wellness in most of the patients, probably by stimulating the neuro-endocrine system. However, ozone dosages must be calibrated against the antioxidant capacity of the patient's plasma, or otherwise the "start low-go slow" strategy must be used evaluating the subjective feeling of the patient after each session.

It remains to be clarified whether some messengers present in the ozonated blood are able to stimulate the release of staminal cells in the patient's bone marrow.

The evaluation of results obtained in several clinical trials performed in PAD has allowed to establish that the dose-response relationship in PAD can be depicted as an inverted U-shaped hormetic model with a brief, initial lack of effect due to the potency of blood antioxidants. A mild acute oxidative stress induced by ozone in blood *ex vivo*, as several other mild stresses due to either heat or cold exposure, a transient ischemia, other chemicals and physical exercise are able to induce a sort of "preconditioning response" often leading to both a repair and an increased defense capacity well within the "overcompensation stimulation hormesis". This new achievement, added to an increasing wide consensus in carefully using gases as NO, CO, H_2S, N_2O and H_2 as real medical drugs [68], suggests that also ozone may be soon included into this category. One of the basic functions of ozone, after dissolving in the water of plasma is to accelerate the exchange of protons and electrons or, in simple words, to reactivate the metabolism all over the body. In this way, crucial biological functions gone astray can recover indicating that ozone operated as both a biological response modifier and an antioxidant inducer.

It is hoped that this paper will elicit the interest of clinical scientists in evaluating ozone therapy in vascular, renal and diabetic diseases, thus translating the laboratory results to the patient's bed.

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Abbreviations

2,3-DPG: 2,3-diphosphoglycerate; 4-HHE: 4-hydroxy-2E-hexenal; 4-HNE: 4-hydroxy-2E-nonenal; Aa: ascorbic acid; ACTH: adrenocorticotropic hormone; ATP: adenosine triphosphate; CNS: central nervous system; EGF: epidermal growth factor; G6PD: glucose-6-phosphate dehydrogenase; GSH: glutathione; GSH-Rd: glutathione reductase; GSH-Px: glutathione peroxidase; GSH-Tr: glutathione transferase; GSSG: oxidized glutathione; HIV: human immunodeficiency virus; HO-1: heme-oxygenase-I; HSP-70: heat shock proteins (70 kDa); IFN-γ: interferon γ; IkB: inhibitor of NF-kB; LOAEL: lowest observed adverse effect level; LOP: lipid oxidation products; IL-8: interleukin 8; MDA: malondialdehyde; NADPH: nicotinamide adenine dinucleotide phosphate; NF-kB: nuclear factor kappa-light-chain-enhancer of activated B cells; NOAEL: no observable adverse effect level; OCSH: overcompensation stimulation hormesis; PaO_2: partial pressure of arterial oxygen; PO_2: partial pressure of oxygen; O_3-AHT: ozonated autohemotherapy; PAD: peripheral arterial diseases; PDGF-B: platelet-derived growth factor, subunit B; PUFA: polyunsaturated fatty acids; ROS: reactive oxygen species; SOD: superoxide dismutase; TAS: total antioxidant status; TGF-β_1: transforming growth factor β_1; TNF-α: tumor necrosis factor.

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Authors' contributions
VAB and VT conceived, outlined the direction of, provided information to shape the manuscript content and discussion, gathered references, and drafted the manuscript. IZ refined the search for information, gathered references, and generated the figures. All authors have read and approved the final manuscript.

Competing interests
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The Ozone Paradox: Ozone Is a Strong Oxidant as Well as a Medical Drug

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Abstract: After five decades characterized by empiricism and several pitfalls, some of the basic mechanisms of action of ozone in pulmonary toxicology and in medicine have been clarified. The present knowledge allows to understand the prolonged inhalation of ozone can be very deleterious first for the lungs and successively for the whole organism. On the other hand, a small ozone dose well calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms and reactivate the antioxidant system. In detail, firstly ex vivo and second during the infusion of ozonated blood into the donor, the ozone therapy approach involves blood cells and the endothelium, which by transferring the ozone messengers to billions of cells will generate a therapeutic effect. Thus, in spite of a common prejudice, single ozone doses can be therapeutically used in selected human diseases without any toxicity or side effects. Moreover, the versatility and amplitude of beneficial effect of ozone applications have become evident in orthopedics, cutaneous, and mucosal infections as well as in dentistry.
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Key words: oxidative stress; antioxidants; oxidative preconditioning; ozone; ozonated autohemotherapy

1. INTRODUCTION

A. A Brief Historical Review

Christian Friedrich Schönbein, in 1839, noticed the emergence of a pungent gas with an "electric smell." According to the Greek language, he called it "ozone" and presented a lecture entitled "On the smell at the positive electrode during electrolysis of water" at the Basel Natural Science Society.[1,2] In nature ozone is continuously produced in the stratosphere (at 25–30 km from the Earth surface) by UV radiation (<183 nm) by splitting an atmospheric oxygen molecules into two highly reactive oxygen atoms, in agreement with the Chapman theory. By an endothermic reaction, each of these atoms combines to intact oxygen to form the triatomic ozone.

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It is also produced during the electric discharge of lightning, which catalyzes the formation of ozone from atmospheric oxygen. Ozone has a molecular weight of 48 and it is a bluish gas with a pungent odor and a solubility in water, about ten-fold higher than oxygen (49 mL in 100 mL, 0.02 M, at 0°C), even though an ample variability is present in the literature.[3] While it rapidly dissolves in pure water and obeys Henry's law, in biological water ozone instantly reacts with inorganic and organic molecules dissolved in water generating a variety of free radicals. Ozone as a gas spontaneously decomposes with a half-life of 40 min, at 20°C. This means that ozone is a metastable gas with a temperature-dependent half-life, but it can be stored in liquid form at a temperature below −111.9°C with a specific weight of 1.571 g/mL. Methods for generating ozone are based on UV radiation, corona discharge, and an electrochemical process. Industrial ozone is produced from air but medical ozone must be generated ex tempore only by using medical oxygen because otherwise the simultaneous generation of nitric dioxide (NO_2) will be very toxic.[4] The most recent medical ozone generator can control the electric voltage from 5 kV up to about 14 kV, the space between the electrodes able to modulate a gradual increase in ozone concentration and the flow of pure oxygen usually regulated between 1 and 10 L/min. The final ozone concentration is inversely proportional to the oxygen flow, hence, per unit time, the higher the oxygen flow, the lower the ozone concentration. In the final oxygen–ozone mixture, the maximum ozone concentration can be only 5%.

2. BEHAVIOR OF OZONE

A. Ozone as an Oxidant

Ozone has a cyclical structure assessed by the absorption at 253.7 nm with a distance among oxygen atoms of 1.26 Å and exists in several mesomeric states in dynamic equilibrium[5] (Fig. 1). Among oxidant agents, it is the third strongest ($E^\circ = +2.076$ V), after fluorine and persulphate. Molecular oxygen, by containing two unpaired electrons, is a diradical but it has not the reactivity of ozone and, by a stepwise reduction with four electrons, forms water. On the other hand, ozone having a paired number of electrons in the external orbit is not a radical molecule, but it is far more reactive than oxygen and generates some of the radical oxygen species (ROS) produced by oxygen during mitochondrial respiration. Phagocytes reacting with pathogens[6–8] produce anion superoxide (O_2^-), H_2O_2, and hypochlorous acid (HClO) catalyzed by mieloperoxidase. Wentworth et al.[9,10] have postulated that in atherosclerotic patients human endothelium cells may produce ozone, but their findings remain still doubtful.[11] Moreover, H_2O_2 is produced by almost all cells by the nicotinamide adenine dinucleotide phosphate (NADPH)-oxydase isoenzymes, indicating the relevance of ROS in the normal organism. Interestingly, ozone, in the presence of inorganic and/or organic compounds immediately reacts and generates a great variety of oxidized molecules, disappearing in a matter of seconds.[12]

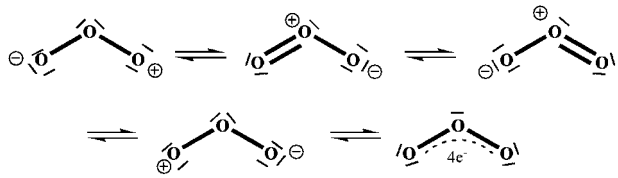


Figure 1. Structure and mesomeric states of ozone.

B. Ozone as UV screen

In the stratospheric layer, ozone has an average concentration of 10 parts per million (ppm) and it has the important role to absorb most of the UV radiations, particularly bands B (from 280 to 320 nm) and C (from 100 to 280 nm), which are mutagenic and can enhance skin carcinogenesis.[13] Unfortunately, during the last decades, short-sighted human activities, by releasing chlorofluorocarbons in the atmosphere, have led to a decreased ozone concentration, particularly in the Antarctic, which will take several decades to be restored.

C. Ozone as an Air Pollutant

On the other hand, the tropospheric amount of ozone ought to be about 1 $\mu g/m^3$ (0.001 ppm), ten times lower than our odor perception threshold for ozone about 20 $\mu g/m^3$ (0.02 ppm). However during the last decades, in large cities, ozone levels in summer time can increase up to dangerous levels ranging from 200 to 900 $\mu g/m^3$. Moreover, additional anthropogenic emissions of NO, NO_2, methane, CO, sulphuric compound, and fine particulates have enhanced the toxicity not only for the respiratory tract but also for the eyes and the skin.

The US Clean Air Act has set an ozone level of 120 $\mu g/m^3$ as an 8 hr mean concentration to protect the health of workers.[14] Evaluation of recent studies[15–18] allows establishing an average environmental ozone concentration of 90 ± 10 $\mu g/m^3$. However, ozone concentration in urban air can exceed 0.8 ppm in high pollution conditions.[19,20] For 8 hr at rest (a tidal volume of about 10 L/min and a retention of inspired ozone of no less than 80%), the ozone dose amounts to 0.70–0.77 mg daily. This is likely the minimal ozone intake because physical activity increases the volume of inhaled air, and, at peak time, the ozone levels can easily augment to 500–900 $\mu g/m^3$, reducing pulmonary functions and markedly enhancing the risk of cardiovascular deaths.[15,17,18]

Ozone levels of 500 $\mu g/m^3$ may not seem too high but one must consider that any single air inhalation implies an ozone dose that immediately reacts with the airway surface fluid and immediately at the epithelial lining fluid (ELF) generates the ROS and lipid oxidation products (LOP) minimally quenched by the scarce antioxidant present in a liquid film of about 0.1 μm.[21] As a consequence, the whole respiratory tract against the continuous inhalation of ozone-contaminated air opposes only the ELF's volume of about 20–40 mL,[22] which is negligible when compared to a plasma volume of about 2700 mL. Thus, throughout the day we must consider, neither simply the ozone concentration nor a single respiratory act, but the ozone cumulative dose that can easily sum up to 1–2 g ozone in 5 months. While ozone vanishes within the ELF,[23] the generated ROS, LOP, and nitrating species[24–28] damage the epithelial lining. The phosphorylation of a protein kinase, by activating the nuclear factor-κB (NF-κB), allows the synthesis and release of a number of cytokines such as TNFα, IL-1, IL-8, IFNγ, and TGFβ1. Moreover, this situation starts a vicious circle because the increased inflow of neutrophils and activated macrophages into the alveolar space worsens and perpetuates the production of more ROS including HClO,[8,26] tachykinins, proteases, alkenals, and F_2-isoprostanes[25,29] able to self-maintain a chronic inflammation. ROS have a very brief half-life and damage mostly the pulmonary microenvironment while alkenals and proinflamatory cytokines are absorbed by the human large expanse (about 70 m^2) of the bronchial–alveolar space. Recent studies[25,30,31] have detected 4-hydroxynonenal (4-HNE), isoprostanes, H_2O_2, and malondialdehyde (MDA) in the bronchoalveolar lavage fluid. The interesting study by Last et al.[32] has clearly shown that mice exposed to 1 ppm for 8 hr during three consecutive nights lose about 14% of their original body weight, decrease their food consumption by 42%, and enter into a cachectic state. Another important aspect of the pulmonary ozone toxicity is its reverberation on the whole organism, especially on the vascular system, heart, liver, brain, and kidneys. The pharmaco-toxicological behavior of both LOP compounds, ceramide signaling, and proinflammatory cytokines is characterized by a continuous absorption from the pulmonary area into the blood and, even

though the half-life of these compounds is brief,[28,33–37] the constant endogenous synthesis insures a constant toxicity explaining the increased morbidity and mortality of population inhaling polluted air for several months of the year.

D. Ozone as a Biological Cytotoxic Agent

Either normal or neoplastic cells in culture are very sensitive to a constant exposure of ozone even if the gas has a very low concentration.[38–40] This observation is correct but it has led to the misleading conclusion that ozone is always cytotoxic. Indeed, we know too well that cells culture studies are mostly performed with air–CO_2 at pH 7.3 but with a pO_2 of 160 mmHg, i.e. more than double of cells in vivo. Even more important is the fact that culture media have a significantly lower level of antioxidants than plasma, particularly of albumin.[41–45] Indeed, the usual fetal calf serum is added at a 5–10% concentration that is equivalent to hardly 50% of the albumin present in the extracellular fluid. Among antioxidants, albumin with its available –SH reducing group is one of the most protective compounds.[46] Moreover, antioxidant components are not dinamically replenished in vitro while cells remain exposed to a constant ozone concentration. Obviously ozone dissolves in the fluid every second, exhausts the scarce antioxidants, and generates toxic compounds that cannot undergo either dilution with extracellular fluid or excretion. This unfavorable situation has been demonstrated when thiobarbituric acid reactive substances (TBARS), incubated in vitro at 37°C and pH 7.3 in human ozonated plasma remain at a constant level for 9 hr.[47] On the other hand TBARS present in ozonated blood declined very rapidly with a half-life of 4.2 ± 1.7 min[48,49] after intravenous infusion in patients with age-related macular degeneration (ARMD) demonstrating the relevance of critical pharmacological properties to be extensively discussed in Section 4A. Moreover, the damaging effect of ozone on saline washed erythrocytes, totally deprived of the plasma protection, has noticeably contributed to consider ozone as a deleterious gas.

3. MAY OZONE BE USED AS A MEDICAL DRUG?

At first sight, the strong oxidizing properties of ozone discard the possibility that this gas may display some therapeutic effects. However, even today some ozonetherapists advance the whimsical idea that ozone, by decomposing in the blood, gifts the body its intrinsic energy accumulated during its synthesis, as shown

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

On the 19th century, ozone had been already identified as a potent bactericidal gas and it was used during World War I for treating German soldiers affected by gaseous gangrene due to *Clostridium* anaerobic infections. In two pioneristic studies, Stoker[50,51] reported the first 21 medical cases successfully treated with ozone at the Queen Alexandria Military Hospital. It remains uncertain how a Swiss dentist, E.A. Fisch (1899–1966)[52] had the first idea to use ozone as either a gas or ozonated water in his practice. By a twist of fate, a surgeon, Dr. E Payr (1871–1946) had to be treated for a gangrenous pulpite and remained astonished by the result achieved with local ozone treatment. He enthusiastically extended its application to general surgery and at the 59th Congress of the German Surgical Society (Berlin, 1935) reported "which other disinfectant would be tolerated better than ozone? The positive results in 75% of patients, the simplicity, the hygienic conditions and the safety of the method are some of the many advantages".[53] In 1936, a French physician, Dr. P. Aubourg successfully treated chronic colitis and rectal fistulae by the direct insufflation of oxygen–ozone mixture into the rectum. It seems that Dr. Payr was the first to inject a small volume of the O_2–O_3 gas

mixture directly into the human cubital vein, giving rise to a procedure that in the 90s, adopted by charlatans, became so dangerous to be prohibited. After the invention of the first medical ozone generator by the physicist Joachim Hansler (1908–1981), the physician Hans Wolff (1927–1980) deserves the credit for having developed the ozonated autohemotherapy (O_3-AHT) by insufflating ex vivo the gas into the blood contained in a dispensable ozone-resistant glass bottle. For almost three decades ozone therapy was used in Germany but the lack of scientific and clinical studies arose scepticism and prejudice still common today. Lacking the knowledge of the complexity of biological mechanisms, a distinguished chemist wrote that "ozone is toxic, no matter how you deal with it and should not be used in medicine" (personal communication to V.B.).[54] This negative concept may only be changed by valid scientific and clinical data. It is worthwhile to mention what Timbrell[55] wrote in his book "*The poison paradox; chemicals as friends and foes*." The essential facts are that first it is the dose that makes a chemical toxic, and second and more important, toxicity results from the interaction between chemical and biological defenses. Indeed the subtlety and complexity of biological systems may defy the concept that ozone is always toxic. Interestingly, Paracelsus (1495–1541) did not know biochemistry but guessed that "all things are poison and nothing is without poison, only the dose permits something not to be poisonous."[56]

4. BIOLOGICAL MECHANISMS ELICITED BY OZONE IN HUMAN BLOOD

As it was mentioned, ozone as a gas equilibrates in 5 min in pure water and, in a closed glass bottles, its concentration (about 25% of the ozone concentration in the gas mixture) remains fairly stable for many hours. However, in a physiological environment, it immediately reacts with antioxidants, polyunsaturated fatty acids (PUFA), proteins, carbohydrates and, if in excess, with DNA and RNA.[57,58] Thus, ozone leads to the formation of ROS, LOP, and a variable percentage of oxidized antioxidants.[59,60]

A. Reactions with Plasma Components

Blood is an ideal tissue because it is composed of about 55% plasma and cells, especially erythrocytes, able to cooperate for taming the oxidant properties of ozone. The plasma has a wealth of hydrophilic reductants, such as ascorbic acid (~50 μM), uric acid (~400 μM), and a little amount of reduced glutathione (GSH). These compounds have been measured before and after ozonation.[61–63] Plasma contains albumin (~45 mg/mL) that by virtue of a wealth of –SH groups, is one of the most important antioxidants also because the plasma pool contains about 112 g of albumin.[46] Moreover, the presence of proteins such as transferrin and ceruloplasmin quenches oxidizing reactions by chelating transition metals (mainly Fe^{2+} and Cu^{+}). Presence of traces of these metals must be avoided because either in the presence of hydrogen peroxide, via the Fenton's reaction, or in the presence of anion superoxide (O_2^-) via the Haber–Weiss reaction, they will catalyze the formation of the most reactive hydroxyl radical $^{\cdot}OH$.

$$Fe^{2+} + H_2O_2 \rightleftharpoons Fe^{3+} {}^{\cdot}OH + OH^-$$

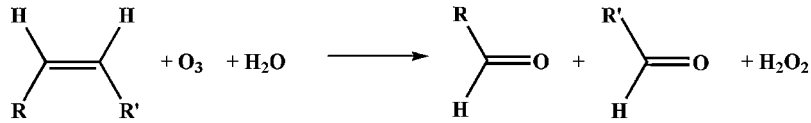
$$O_2^- + H_2O_2 \rightleftharpoons {}^{\cdot}OH + OH^- + O_2$$

Although $^{\cdot}OH$ has a half-life of 1×10^{-9} sec, it reacts with any other molecule and produces another radical. Blood cells contain not only the bulk of GSH (1–5 mM) but also thioredoxin and several lipophilic compounds such as α-tocopherol, retinol, lycopene, ubiquinol, and α-lipoic acid, which are able to cooperatively reduce oxidized compounds, thus restoring the initial antioxidant status. Moreover, blood cells contain a variety of en-

zymes (SOD, catalase, GSPase, GSH-redox system), which cooperate either simultaneously or in a sequential way to restore the redox system. The work performed during the last 18 years in our lab has clarified the most important compounds generated ex vivo during the initial reaction of ozone with some plasma components and how these compounds activate some biochemical pathways in cells revealed by therapeutic effects after the transfusion of ozonated blood in the donor.

The biochemical effects displayed by ozone when it comes in contact with blood components will be briefly reviewed.[47,63] After having performed thousands of treatments, the standard procedure is to add 200 mL of a gas mixture composed of medical oxygen (>95%) with ozone (<5%) to 180 mL of blood after the previous addition of 20 mL of 3.8% sodium citrate at room temperature. The blood–gas volumes are gently mixed in a sterile glass bottle by rotation, avoiding gas bubbling. Within 5 min, about 1.5 mL of O_2 and 2.4 mL of O_3 dissolve in the blood water but their fate is quite different. Oxygen physically diffuses into erythrocytes and fully saturates hemoglobin (Hb_4O_8) but in spite of the pO_2 as high as 450 mmHg, the therapeutic value of oxygenation is irrelevant because the successive infusion of oxygenated–ozonated blood (about 15 mL/min) hardly modify the pO_2 (~40 mmHg) of about 5 L/min of the simultaneous venous blood inflow to the heart. On the contrary, ozone dissolves more readily in plasma water than oxygen, and instantaneously reacts with hydrosoluble antioxidants and with readily available PUFA bound to albumin.

Several years ago, by using a reliable ozone generator able to deliver precise ozone concentrations, the first aim was to define if indeed ozone was always deleterious or if a range of ozone therapeutic concentrations could be determined. The range was determined between 10 μg/mL gas (0.21 μmol/mL) and 80 μg/mL gas (1.68 μmol/mL) per mL of anticoagulated blood, corresponding to total ozone doses comprises between 1 and 8 mg for 100 mL blood, respectively. It was crucial to precisely calibrate the ozone dose (gas volume × ozone concentration) against the individual variable antioxidant capacity of the patient's blood, thereby on one hand avoiding ozone toxicity and, on the other hand, allowing the activation of several biochemical pathways on blood cells. It was proven that during the slow mixing of the blood with the gas phase, all the ozone is consumed in less than 5 min. Several studies[47,51,59,63–65] have clarified that some albumin and uric acid behave as sacrificial molecules whereas several antioxidants after oxidation are rapidly reduced by an efficient recycling system.[66,67] Some ozone reacts with PUFA as follows



leading to the simultaneous formation of 1 mol of H_2O_2 (included among ROS) and 2 mol of LOP.[23,68,69]

The fundamental ROS molecule is H_2O_2, which is not ionized but is an oxidant able to act as an ozone messenger responsible for eliciting several biological and therapeutic effects.[70–75]

As it was mentioned, the old concept that H_2O_2 is always harmful has been widely revised because, in physiological amounts, it acts as a regulator of signal transduction and represents a crucial mediator of host defense and immune responses.[74,76–80] While exposure to oxygen is ineffective, ozone causes the generation of H_2O_2 and of the chemiluminescent reaction in both physiological saline and plasma.[47,81] However, while in saline there is a consistent and prolonged increase in H_2O_2, in the ozonated plasma both chemiluminescence and H_2O_2 increase immediately but decay very rapidly with a half-life of less than 2 min

suggesting that both antioxidants and traces of enzymes rapidly reduce H_2O_2 to water.[47] In ozonated blood the reduction of H_2O_2 is so fast that it has been experimentally impossible to measure it. H_2O_2 is able to easily pass through the cell membrane, but the intracellular concentration increases only 1/10 of the extracellular one.[72,74,78] Its relative stability allows measuring it in plasma; in normotensive subjects its concentration is of 2.5 μM.[70,71] In this case the intracellular concentration of H_2O_2 will be at the most of 0.25 μM, while the maximal intracellular concentration that can be generated for signaling purposes during the ozonation process may reach 0.5–0.7 μM.[47] It appears ubiquitous as it has been detected in urine and in exhaled air.[71] Depending upon its local concentration and cell-type, H_2O_2 can either induce proliferation or cell death.[78,80,82,83] It can regulate vascular tone by causing constrictions of vascular beds or vasodilatation although it remains uncertain if it acts as an endothelium-derived hyperpolarizing factor.[84]

A very enlightening finding was achieved by evaluating the variation of the total antioxidant status (TAS) as measured by the Rice-Evans and Miller's method[85] in plasma after ozonation and 1 min rapid mixing of the liquid–gas phases of either fresh blood or the respective plasma withdrawn from the same ten donor.

Figure 2 shows that, after ozonation of plasma with either a medium or a high ozone concentration (0.84 μmol/mL or 1.68 μmol/mL of gas per mL of plasma, respectively), TAS

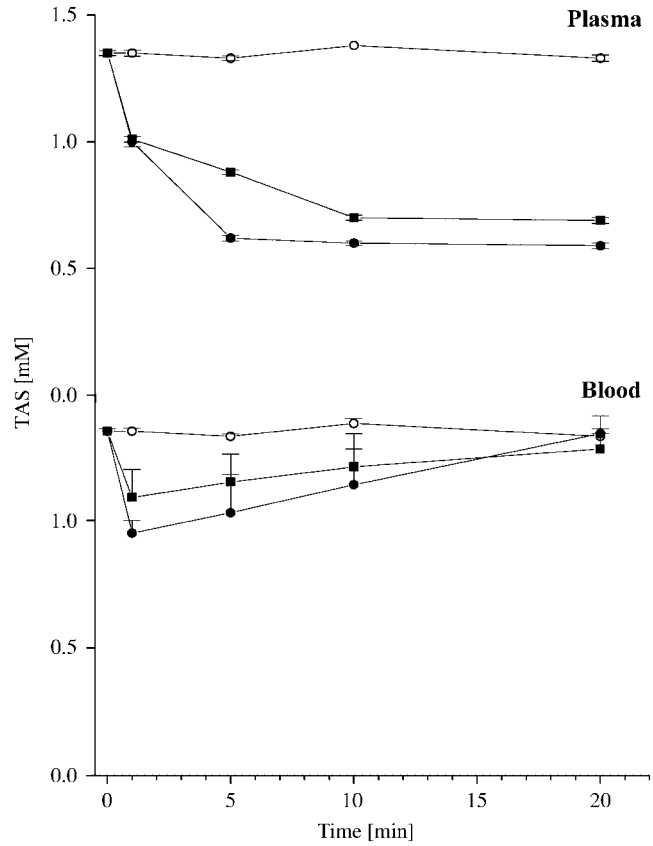


Figure 2. Kinetics of TAS levels in plasma (top) and in blood (bottom) samples from donors ($n = 10$; mean+SD; unpublished results). Plasma and blood samples were exposed for 1 min either O_2 (control,) or O_2–O_3 with ozone concentrations of 40 (■) and 80 (●) μg/mL.

level progressively decreases at first and then remain stable after 20 min.[47] The decrease was ozone-dose dependent and varied between 46 and 63%, respectively. Conversely, TAS levels in blood treated with the same ozone concentrations only decreased from 11 to 33%, respectively, in the first minute after ozonation. Then they recovered and returned to the original value within 20 min, irrespective of the two ozone concentrations, indicating the great capacity of blood to regenerate oxidized antioxidants, namely, dehydroascorbate and GSH disulfide (GSSG). Indeed, Mendiratta et al.[66,67] have found that dehydroascorbate can be recycled back to ascorbic acid within 3 min. Similarly, only about 20% of the intraerythrocytic GSH has been found oxidized to GSSG within 1 min after ozonation, but promptly reduced to normal after 20 min.[86] These data were enlightening and showed that the therapeutic ozonation modifies only temporarily and reversibly the cellular redox homeostasis. There is now full agreement that ascorbic acid, α-tocopherol, GSH, and lipoic acid, after oxidation, undergo an orderly reduction by a well-coordinated sequence of electron donations.[87]

LOP production follows peroxidation of PUFA present in the plasma: they are heterogeneous and can be classified as lipoperoxides (LOO), alkoxyl radicals (LO), lipohydroperoxides (LOOH), F_2-isoprostanes, and alkenals, among which 4-hydroxynonenal (4-HNE), acrolein and MDA. As free radicals and aldehydes are intrinsically deleterious, only precise and appropriate ozone doses must be used in order to generate them in very low concentrations. Figure 3 comparatively shows the modifications of plasma levels of TBARS, hemolysis, TAS, and protein thiols in a typical experiment when 13 human blood samples were exposed to air, O_2, or either 40 or 80 μg/mL ozone concentrations. Plasma TBARS in vitro are far more stable than ROS,[47] but, upon blood reinfusion, they have a brief half-life owing to a marked dilution in body fluids, excretion (via urine and bile), metabolism by glutathione-S-transferases (GST) and aldehyde dehydrogenase (ALDH).

Among the aldehydes, 4-HNE is quantitatively the most important. It is an amphipathic molecule and reacts with a variety of compounds such as albumin, enzymes, GSH, carnosine, and phospholipids.[88,89] There is no receptor for 4-HNE but Poli et al.[89] have reported that, after binding to more than 70 biochemical targets, it exerts some deleterious activity. Luckily, intracellular concentrations of GSH are high enough to frequently prevent or remove 4-HNE from adducts with enzymes. Owing to the unexpected stability of 4-HNE when samples of ozonated human plasma were incubated at 37°C for 9 hr, it was postulated that ozone, for its high solubility in the plasmatic water, steric reasons, and the abundance of albumin molecules prefers to target their bound PUFA. The scheme presented in Figure 4 envisages the events occurring in the plasma phase. It appears reasonable that during the rapid reaction of ozone with albumin PUFA in water, the suddenly generated aldehydes, mainly 4-HNE, will immediately form adducts with contiguous albumin molecules. This hypothesis is now well supported by recent findings,[90–92] which have shown that human albumin, rich in accessible nucleophilic residues, can quench up to 11 different 4-HNE molecules, the first being with Cys34, followed by Lys199 and His146. These important data clarify why ex vivo ozonation of blood does not harm the vascular system during the infusion of ozonated blood. The albumin-4-HNE adducts, not only are rapidly diluted in the blood pool but, being transferred into the extravascular pool, represent only a small aliquot of the whole albumin pool, containing as much as about 310 g protein. On this basis, it would be worthwhile exploring whether either the 4-HNE-modified albumin has an abnormal fate or how the aldehyde is released into other cell compartments, thus becoming able to trigger biochemical mechanisms. 4-HNE is the major product of peroxidation of n-6-PUFA, its concentration in normal plasma varies from 0.07 to 0.15 μM and increases with aging.[93,94] Needless to say that a constant increase in peroxidation as it happens after ischemia-reperfusion, CCl_4 intoxication,

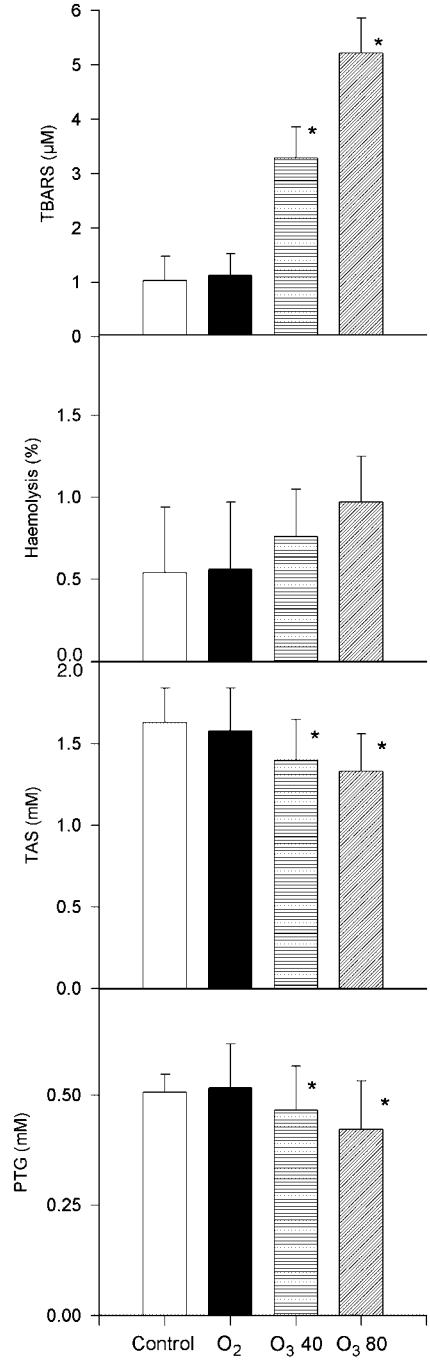


Figure 3. Thirteen human blood samples were exposed to air (control), or O_2, or O_2–O_3 with ozone concentrations of 40 and 80 μg/mL for 1 min. While TBARS, TAS, and PTG levels vary significantly ($p < 0.01$) after ozone exposure, there is a negligible increase in hemolysis. (Bocci V. How does ozone act? *Oxygen–ozone therapy. A critical evaluation*, chap. 13. Figure 40. Kluwer Academic Publishers; 2002. p 114. With kind permission from Springer Science+Business Media, formerly Kluwer Academic Publishers.)

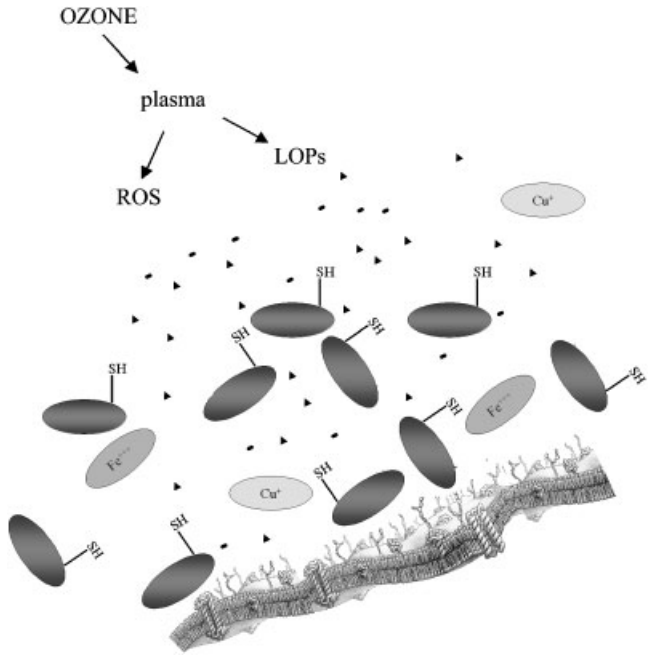


Figure 4. The scheme helps to imagine the multiplicity of substrate reacting with ozone dissolved in plasmatic water. Small circles, triangles, and squares symbolyze hydrosoluble antioxidants present in 100 mL of human blood (uric acid 4.5 mg/dL, ascorbic acid 1.5 mg/dL, glucose 80 mg/dL, etc…). Large albumin molecules (4,000 mg/dL) exposing –SH groups form a cloud over the cell membrane and protect it. Molecules such as transferrin and ceruloplasmin bind Fe^{3+} and Cu^{+} and prevent formation of $OH^{\cdot}$. The exogenous addition of 4–8 mg of ozone to 100 mL of blood is transitory and controlled by antioxidants. In contrast, the endogenous production of ROS is continuous and barely quenched by intracellular antioxidants.

ADP-iron overload, and chronic inflammation typical of some infections disease, diabetes, atherosclerosis, cancer, and degenerative pathologies causes a marked increase in 4-HNE levels, especially in the affected tissues. However, aerobic organisms, for accommodating the toxicity of aldehydic compounds, have simultaneously developed detoxifying systems[37,95–99] and their evaluation is relevant because the infusion of the ozonated blood into the donor patient implies an amount of an albumin-4-HNE adduct.

The following three processes schematically indicated in Figure 5 clarifies why 4-HNE is not a risk:

(1) *Dilution*: The highest concentration of 4-HNE measured after exposing 180 mL of human blood to the highest ozone amount (16 mg) is less than 1 mM in the plasma. During the 20 min intravenous infusion, the aldehyde will be promptly diluted in a total plasma-extracellular fluid volume of about 11 L, causing a transitory increase in the plasma level up to about 0.1 μM.

(2) *Detoxification*: Metabolism of 4-HNE is extremely fast either because small amounts of aldehydes interact with billions of cells endowed with several detoxifying enzymes such as ALDH, aldose reductase, and GST or the formation of an adduct with GSH.[36,37,98–100] Several authors[96,101,102] have determined a metabolic rate so high to conclude that "even with very high lipid peroxidation rates, 4-HNE cannot accumulate in an unlimited way".[89] These data are in agreement with our results in six patients when we could assess a half-life of infused TBARS of 4.2 ± 1.7 min.[48,49] On the contrary when the same preparation in ozonated plasma was incubated (at +37°C, pH 7.3) in acellular medium, TBARS levels hardly declined during the next 9 hr.[47]

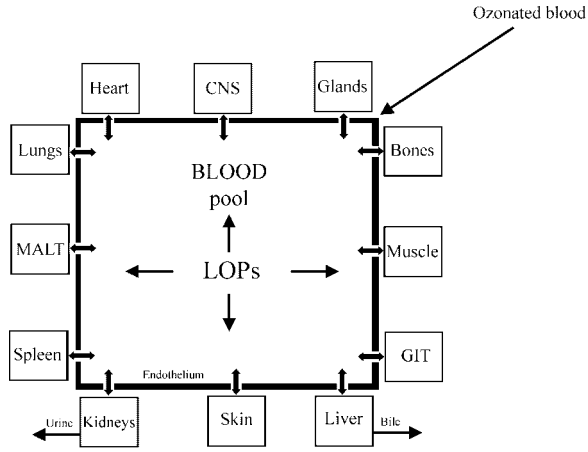


Figure 5. The multivariate biological response of the organism to ozonated blood can be envisaged by considering that ozonated blood cells and the generated LOP interact with a number of organs. Some of these represent real targets (liver in chronic hepatitis, vascular system for vasculopathies), while other organs are probably involved in restoring normal homeostasis. Gastrointestinal tract (GIT); mucosal associated lymphoid tissue (MALT).

(3) *Excretion*: Partially metabolized LOP are eliminated into both bile after hepatic detoxification and urine after renal excretion. In the rat, 4-HNE was detected in the urine as mercapturic acid conjugates.[35,98,103,104]

In normal conditions, owing to the efficiency of these processes, only submicromolar concentrations of LOP can reach organs such as bone marrow, endocrine glands, and even hypothalamic areas deprived of the blood–brain barrier where, via a variety of kinases and even a possible receptor for F_2-isoprostanes, may act as a signaling event of an ongoing acute oxidative stress[105–110] (Fig. 5). As a first conclusion it is clear that the ozonation process either happening in blood ex vivo or in an intramuscular site represents an acute, albeit small, oxidative stress. However, this process is acceptable only if the ozone is precisely calibrated against the antioxidant capacity of either blood or the injected tissue. Moreover, the ozone dose must never lower the antioxidant capacity more than 30% with a process lasting only a few minutes during which ozone reacts and disappears after leaving its messengers. Thus, the process of blood ozonation ex vivo has been characterized by the formation of ROS and LOP mainly acting in two phases. Among ROS, H_2O_2 is the earliest messenger rising and disappearing within 1 min in the plasma, while LOP during drug infusion in the donor reach the vascular systems, act on endothelial cells, and eventually reach parenchymal cells. Their pharmacodynamics minimize their potential toxicity thus making LOP as late and effective messengers.

B. The Effect of Ozone Messengers Onto Blood Cells

There are two questions to be clarified: first, does ozone directly activate the cells? Our methodological approach and experimental results exclude this possibility because when blood is gently mixed ex vivo with O_2–O_3, ozone dissolves rapidly in the water of plasma and there it immediately reacts with antioxidants and PUFA. Blood cell membrane phospholipids surrounded by a cloud of albumin molecules do not come in contact with ozone molecules because the calculated ozone dose is rapidly exausted (Fig. 4). This dangerous interference has been excluded by either a negligible hemolysis, or a change of the hematocrit value, or leakage of K^+ and lactate dehydrogenase, or a change of osmotic fragility, or of electrophoretic mobility, or increased methemoglobin.[47,54,65,111,112] Levels (mg/dL) of fibrinogen, cholesterol, triglycerids, HDL, and LDL in plasma are not modified even using the excessive

ozone concentration of 160 μg/mL per mL of blood.[112] Equally important is the stability of enzymes such as SOD, GSH-Pase, GSH-RD, and G6PDH in the erythrocytes.[112] Moreover, Shinriki et al.[65] after isolating the erythrocytic membranes after blood ozonation within the therapeutic range did neither detect a decrease in αα-tocopherol nor an increase in MDA.

It is unfortunate that in the past other authors[57,68,113–117] have reported that erythrocytes isolated from plasma, after three washings with saline and suspension in protein-free saline, undergo structural changes and intense hemolysis when exposed to ozone. These misleading and unphysiological data have greatly contributed to emphasize the ozone cytotoxicity, which obviously was enhanced by removing plasma antioxidants.[116] Moreover, the critical protective effect of plasma antioxidants has been emphasized in two recent studies.[118,119] These results were particularly evident on saline-washed blood mononuclear cells (BMC) with a marked decrease in mitochondrial functions.[118] Our thinking is well supported by other data[47,120,121] as well as recent results (Fig. 6) obtained after excessive ozonation of samples of normal human blood either collected in heparin or in sodium citrate. Interestingly, heparinized samples were far more susceptible to ozone most likely because of the remaining physiological Ca^{2+} level: in fact, a further addition of 2.5–5 mM Ca^{2+} enhanced the hemolysis up to 40%.

Second, how ozone messengers activate blood cells? Initially, the sudden formation of an H_2O_2 gradient between the ozonated plasma and the intracellular fluid causes the rapid passage of about 10% H_2O_2 into the blood cell cytoplasms and represents the triggering stimulus: depending upon the cell type, different biochemical pathways can be concurrently activated in erythrocytes, leukocytes, and platelets resulting in numerous biological effects. The rapid reduction of H_2O_2 to water is operated by the high concentration of intracellular GSH, CAT, and GSPase but, nonetheless, H_2O_2 must be above the threshold concentration for activating several biochemical pathways as follows.

The mass of erythrocytes mops up the bulk of H_2O_2: GSH is promptly oxidized to GSSG and the cell, extremely sensitive to the reduction of the GSH/GSSG ratio, immediately corrects the unbalance by either extruding GSSG, or reducing it with GSH-Rd at the expenses of ascorbate or of the reduced NADPH, which serves as a crucial electron donor. Next, the oxidized NADP is promptly reduced after the activation of the pentose phosphate

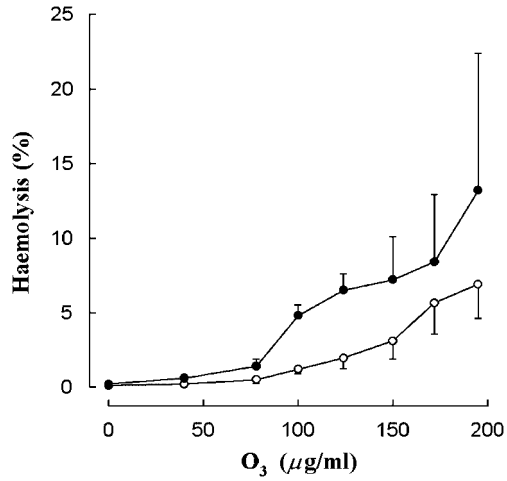


Figure 6. Kinetics of hemolysis in relation to ozone concentration (μg/mL per mL of blood). Blood of five donors was treated with CPD () or with 30 U/mL heparin (●) (mean+SD). (Bocci V. What happens in the intracellular environment after blood ozonation? *Oxygen–ozone therapy. A critical evaluation*, chap. 14. Figure 43. Kluwer Academic Publishers; 2002. p 123. With kind permission from Springer Science+Business Media, formerly Kluwer Academic Publishers).

pathway, of which glucose-6-phosphate dehydrogenase (G6PDH) is the key enzyme. In patients with ARMD, after 13 O_3-AHT, a small increase in ATP formation has been determined but whether this is due to the activation of the pentose cycle or to an increase in phosphofructokinase activity or to both remains to be clarified. The reinfused erythrocytes, for a brief period, enhance the delivery of oxygen into ischemic tissues because of a shift to the right of the oxygen–hemoglobin dissociation curve, due either to a slight decrease in intracellular pH (Bohr effect) or/and an increase in 2,3-diphosphoglycerate (2,3-DPG) levels as shown in Figure 7 (unpublished data). Obviously, an increase in this metabolite has a great significance because it enhances a shift to the right of the oxygenated hemoglobin, hence an increase oxygen delivery to hypoxic tissues. However, Figure 7 shows that the increase has been noted only in three patients where the initial levels were rather low. Thus, this observation needs to be explored in a large number of patients and it will be also necessary to clarify the activation of 2,3-bisphosphoglycerate mutase. Needless to say that one auto-hemotherapeutic treatment has a minimal effect and we need to ozonate at least 3–4 L of blood within a period of 30–60 days.

In another small group of five ARMD's patients after 15–17 O_3-AHT, an increase in some antioxidant enzymes has been determined (Fig. 8). This result has been reported also by other authors[122,123] and it is likely that LOP act as repeated stimuli on the endothelium and bone marrow and cause the adaptation to the ozone stress during erythrogenesis. Whether the enzymatic levels remain sustained for several months during the maintenance therapy need to be evaluated.

Another relevant finding was that in four patients with ARMD, after a cycle of 13 O_3-AHT treatments (in which ca. 3.8 L of blood were ozonated within 7 weeks), isopycnic centrifugation of blood separated old (heavy) and young (light) erythrocytes (RBC), which showed a marked increase in G6PDH in the young erythrocytic fraction generated during the course of ozone therapy (Table I). Whether the enzymatic levels remain sustained with time need to be evaluated. G6PDH activity, expressed as nmol/hr/mg hemoglobin, in total red blood cells was either 357 ± 91 or 406 ± 40, before and after the ozone therapy, respectively. While the enzymatic increase in the

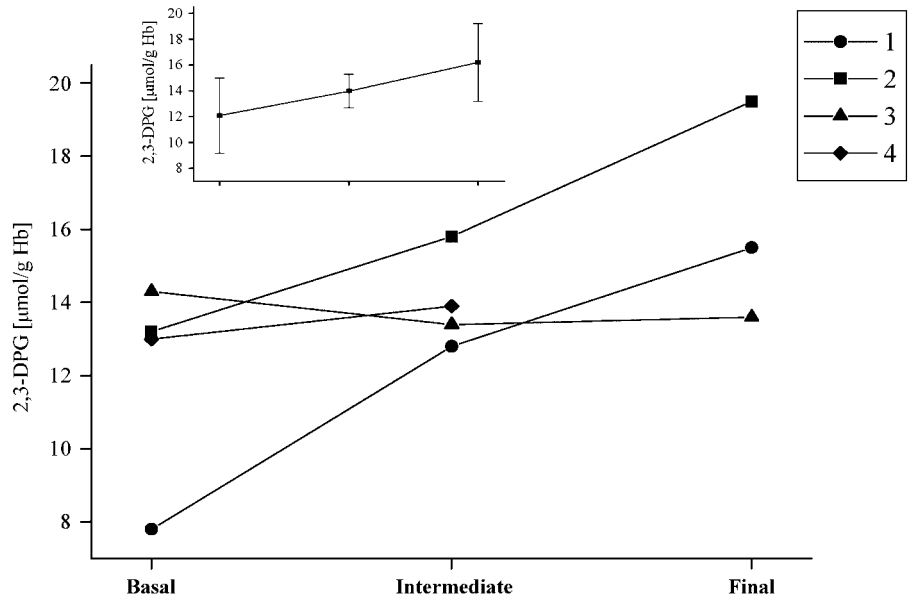


Figure 7. 2,3-DPG level variations in four patients performed before treatment (Basal), after 6–7 treatments (Intermediate), and at the end of treatments (Final). Insert shows the statistical dispersion (mean ± SD) of the data (unpublished results).

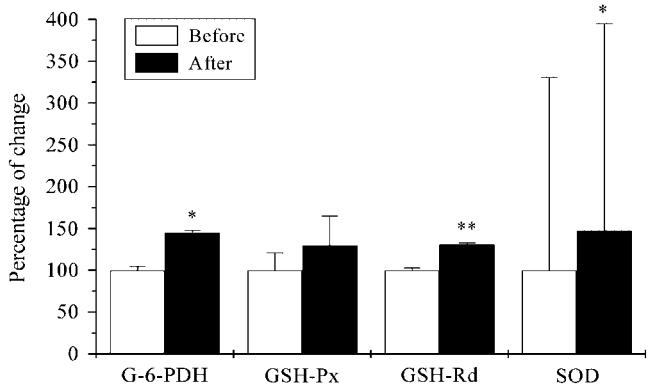


Figure 8. Increase in antioxidant enzymes in ARMD patients after 20–24 O_3-AHTs performed during 7–8 weeks ($n = 10$, mean +SD; unpublished results).

Table I. Evaluation of G6PDH Activity in Total, Young and Old Red Blood Cells (RBC) in Blood Samples from Four Patients With Age-Related Macular Degeneration Before and After an Ozone Therapy Cycle of 13 Treatments (Unpublished Results)

	G6PDH activity[a]		
	Total RBC	Young RBC	Old RBC
Before treatment ($n = 4$)	356.8 ± 90.7	550.3 ± 157.5	310.7 ± 127.3
After treatment ($n = 4$)	406.2 ± 40.4	784.2 ± 181.9	438.8 ± 86.7

[a]G6PDH activity expressed as nmol/hr/mg hemoglobin in whole erythrocyte population and in young and old fractions before and after 13 O_2/O_3 treatments. Results represent mean value $\pm$ SD.

whole erythrocyte population was understandably small, it was found markedly enhanced from 550 ± 157 to 748 ± 182 in very young (light) erythrocytes before and after ozone therapy, respectively. In the so-called old erythrocytes, which practically include the bulk of cells (20–120 days old), G6PDH obviously increased only from 310 ± 127 up to 435 ± 87 nmol/hr/mgHb. It is necessary to mention that the percentage of either young or old erythrocytes remained practically constant throughout the treatments (unpublished data). As a consequence, a patient with chronic limb ischemia (Phase II) undergoing ozone therapy shows a clinical improvement due to the formation of successive cohorts of erythrocytes progressively more capable of delivering oxygen to his ischemic tissues.

Although ozone is one of the most potent disinfectants, it has been shown[124,125] that ozone cannot inactivate bacteria, viruses, and fungi in vivo because, paradoxically, the pathogens are well protected, particularly inside the cells, by the powerful antioxidant system. Thus, the favorable effect of ozone therapy in some infectious diseases has been interpreted as due to ozone acting as a mild enhancer of the immune system, by activating neutrophils and stimulating the synthesis of some cytokines.[64,76,77,79,86,126,127] Once again the crucial messenger is H_2O_2 that after entering into the cytoplasm of BMC, by oxidizing selected cysteines, activates a tyrosine kinase, able to phosphorylate the transcription factor NF-κB. The release of an heterodimer, via effector genes, causes the synthesis of several proteins, among which, the acute-phase reactants, adhesion molecules, and numerous pro-inflammatory cytokines. This process, checked by a phosphatase or inhibited by cytoplasmic antioxidants, is very transitory. The release of several cytokines from ozonated blood upon in

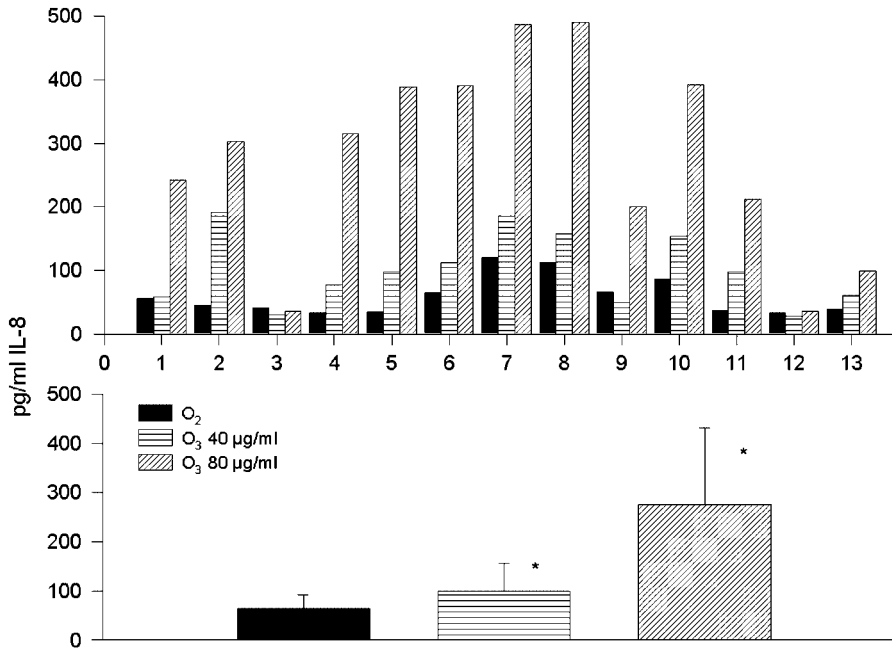


Figure 9. Effect of 1 min exposure of either O_2 or O_3 (40 and 80 µg/mL) on the production of IL-8 after 8 hr incubation of 13 blood samples. Average values are reported in the lower panel after subtraction of control values. *Significant difference ($p < 0.01$) compared with samples treated with O_2. The variable production of IL-8 among donors is noteworthy, particularly the lack of production of donors no. 3 and 12 likely due to a high TAS level. (Bocci V. What happens in the intracellular environment after blood ozonation? *Oxygen–ozone therapy. A critical evaluation*, chap. 14. Figure 53. Kluwer Academic Publishers; 2002. p 134. With kind permission from Springer Science+Business Media, formerly Kluwer Academic Publishers).

vitro incubation has been measured since 1990.[128] Once the ozonated leukocytes return into the circulation, they home in lymphoid microenvironments and successively release cytokines acting in a paracrine fashion on neighboring cells with a possible reactivation of a depressed immune system. This process, described as the physiological cytokine response,[129] is a part of the innate immune system and helps us to survive in a hostile environment. One of our most interesting result has consisted in observing the variable individual production of IL-8 by blood donors in 13 blood ozonated samples.[130] Figure 9 shows that the different release of IL-8 by medium and high ozone concentrations indicates the presence of high, medium, and no responders. The result was interpreted as due to both genetic factors and variable levels of plasma antioxidants.

During ozonation of blood, particularly if it is anticoagulated with heparin, an ozone-dose-dependent increase in activation of platelets has been noted[131,132] with a consequent release of typical growth factors, which will enhance the healing of chronic ulcers in ischemic patients (Fig. 10). Whenever possible, albeit with caution, the use of heparin as an anticoagulant is preferable to sodium citrate because, by not chelating plasmatic Ca^{2+}, reinforces biochemical and electric events.

Finally, during the reinfusion of the ozonated blood into the donor, the vast expanse of the endothelial cells is activated by albumin-LOP resulting in an increased production of NO, plasma *S*-nitrosothiols, and *S*-nitrosohemoglobin.[133–136] Figure 11 shows the in vitro production of nitrite by human vascular endothelial cells after addition of human ozonated serum. Production of NO· was markedly enhanced by the addition of L-arginine (20 µM) and was potentiated by O_3, while it was inhibited in the presence of the NO· inhibitor *N*-ω-nitro-L-arginine-methyl ester (L-NAME). While NO has a half-life of less than 1 sec, protein-

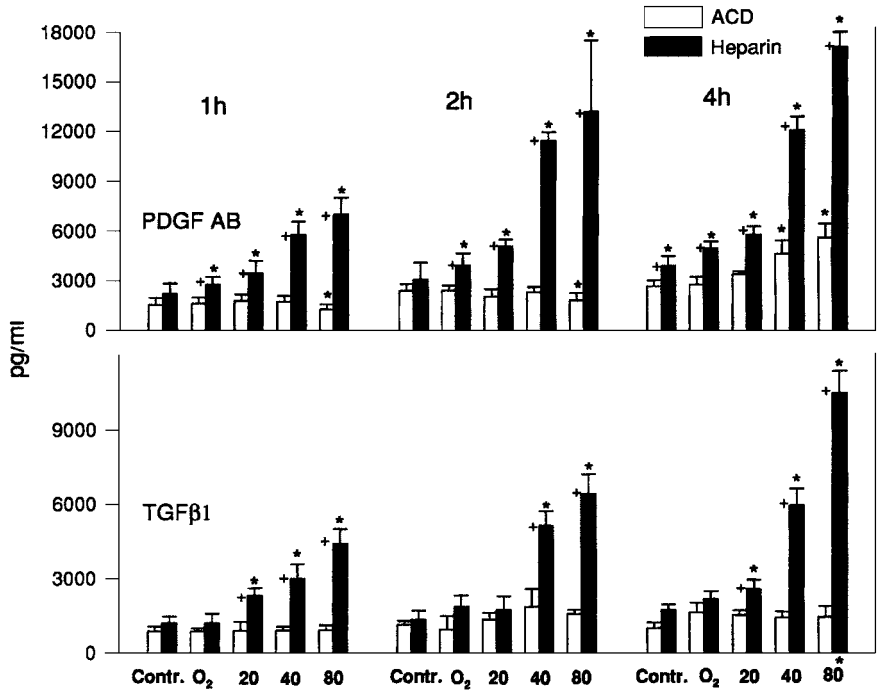


Figure 10. Release of factors from human platelets during 1, 2, and 4 hr incubation. The same PRP samples collected either in heparin or ACD were not exposed (control), or exposed to O_2 alone or to O_2–O_3 at concentrations of 20, 40, and 80 μg/mL for 30 sec before incubation. Statistical significance is indicated by (*) for intergroup analysis and (+) for intragroup analysis. (Bocci V. What happens in the intracellular environment after blood ozonation? *Oxygen–ozone therapy. A critical evaluation*, chap. 14. Figure 65. Kluwer Academic Publishers; 2002. p 158. With kind permission from Springer Science+Business Media, formerly Kluwer Academic Publishers).

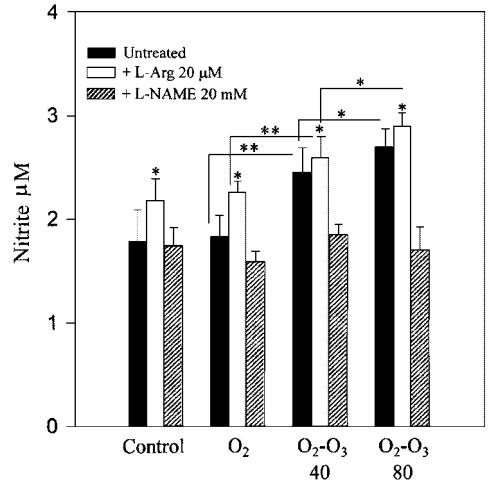


Figure 11. Production of nitrite by HUVECs, measured after 24 hr incubation, after addition of normal human serum either oxygenated or ozonated (at 40 and 80 μg/mL). Effects of addition of L-arginine and L-NAME. The data are presented as the mean+SD of six different experiments. (Bocci V. What happens in the intracellular environment after blood ozonation? *Oxygen–ozone therapy. A critical evaluation*, chap. 14. Figure 68. Kluwer Academic Publishers; 2002. p 165. With kind permission from Springer Science+Business Media, formerly Kluwer Academic Publishers).

bound NO can exert vasodilatation also at distant ischemic vascular sites with relevant therapeutic effect. There is little doubt that the therapeutic advantage observed in many patients with peripheral obstructive arterial disease (POAD) is due to multiple factors such as an increased release of oxygen due to vasodilation by trace amounts of NO and CO, and an increased availability of growth factors from platelets.

All of these data emphasize that submicromolar LOP levels can be stimulatory and beneficial,[137] while it is well established that micromolar levels can be toxic.[89] This conclusion reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result: too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue), so that they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity. There is no doubt that the process of blood ozonation must be precisely controlled with a calculated ozone dosage: at this condition it is not deleterious and actually capable of eliciting a multitude of useful biological responses and, possibly, reversing a chronic oxidative stress due to ageing, chronic infections, and the several diseases grouped within the metabolic syndrome. Indeed the ozonotherapeutic act has been interpreted as a safe "therapeutic shock" able to restore homeostasis.[138] These aspects are critical and imply two drawbacks: first, if the ozone generator is not well calibrated or periodically checked, it may release erroneous and dangerous ozone amounts and, second, if the ozonetherapist does not fully understand the ozonation process, he may do some mistakes and jeopardize the approach. Other aspects regarding the future of ozone therapy will be evaluated in Section 9.

5. IS OZONE ABLE TO INDUCE AN ADAPTATION TO CHRONIC OXIDATIVE STRESS?

That ozone, one of the most potent oxidizer, may induce an antioxidant response capable of reversing a chronic oxidative stress at first sight seems a paradoxical concept. However, this concept has become common in the animal and vegetal kingdoms.[147–150] Any change of the external or internal environment disturbs cell homeostasis, but if the stress is tolerable, or carefully calibrated in intensity, the cell or the organism can adapt to it and survive. If it is excessive or the cell is already damaged, the cell programmes its own death. Stresses include hyperthermia, hyperoxia, ischemia, hypoglycemia, pH modifications, radiation, very likely mental and hormonal derangement, and chronic infections, which imply an excessive ROS and LOP production. Obviously, ozone has to be included and the phenomenon of ozone tolerance is now well known. The concept of "ischemic preconditioning" for the heart, which after undergoing a brief, nonlethal period of ischemia can become resistant to infarction from a subsequent ischemic insult was pioneered by Murry et al.[151] "Oxidative preconditioning" has been also well demonstrated.[152–157] Therefore, it is of interest that small amounts of ROS and LOP can elicit the upregulation of antioxidant enzymes on the basis of the phenomenon described under the term of "hormesis."[158–162] On the basis of this phenomenon that says "the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response,"[159,160,163] it has been postulated that LOP, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress.[54] The bone marrow is particularly relevant because it can upregulate antioxidant enzymes during erythrogenesis and may allow the release of staminal cells for possibly regenerating infarcted organs.

The oxidative preconditioning or, as we prefer, the adaptation to the chronic oxidative stress has been now demonstrated experimentally.[40,45,48] The increased synthesis of enzymes such as SOD, GSPase, GSH-Rd, and CAT has been repeatedly determined in experimental

animals and in patients (reviewed in 57). Iles and Liu[164] have demonstrated the 4-HNE, by inducing the expression of γ-glutamate cysteine ligase, causes an intracellular increase in GSH, which plays a key role in antioxidant defence. Furthermore LOP induce oxidative stress proteins, one of which is heme-oxygenase I (HO-1 or HSP-32) that, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin.[165–171] Bilirubin is a significant lipophilic antioxidant and a trace of CO cooperates with NO in regulating vasodilation by activating cyclic GMP. Fe^{2+} is promptly chelated by the upregulated synthesis of ferritin.[172] The induction of HO-1 after an oxidative stress has been described in thousands of papers as one of the most important antioxidant defence and protective enzyme. Both mild ozone inhalation and ozonated plasma induce HSP-70.[170,173] When ozone is judiciously used in small doses, can become a useful drug able to correct an otherwise irreversible state of oxidative stress. There are serious pathologies such as chronic infections, neurodegenerative, and autoimmune diseases in which a vicious imbalance between overproduced oxidants and depleted antioxidant defenses become established and lead to death. How modern medicine correct this imbalance? Several therapeutic approaches among which administration of antioxidants with addition of *N*-acetylcysteine have been often reported[174–176] but they are only partly successful.

The ozone treatment is now envisaged as a transitory and miniaturized oxidative stress resulting in a sort of therapeutic "shock" for the ailing organism. Ozone acting as a prodrug, realizes this shock because generates a number of messengers able to reach all cells in the organism (Fig. 5).

Submicromolar levels of LOP act as key mediators and in still responsive cells may activate a sequence of biochemical mechanisms able to reactivate gene expression leading to a renewed synthesis of HSP and antioxidant enzymes. If the disease has gone too far, cells become anergic and are unable to respond to the treatment. Indeed, we have observed that after intensive chemotherapy, preterminal cancer patients do not improve with ozone therapy. That is also the reason why we always start using low ozone concentrations just above the threshold level to better achieve the ozone tolerance and in-line with the old concept "start low, go slow." Moreover, the stimulation of the endocrine and central nervous systems may help to understand why most of the reactive patients during prolonged ozone therapy report a feeling of euphoria and wellness probably due to an improved metabolism as well as to an enhanced hormonal or neurotransmitters release.

6. WHICH ARE THE ROUTES OF OZONE ADMINISTRATION?

Table II shows that ozone can be administered with great flexibility but it should never be injected intravenously as a gas because of the risk of provoking oxygen embolism, given the fact that the gas mixture contains always no less than 95% oxygen. So far the most advanced and reliable approach has been the O_3-AHT because, on the basis of the patient's body weight, a predetermined volume of blood (200–250 mL) to which has been added either sodium citrate 3.8% (1+9 mL blood) or heparin (20 IU/mL of blood) can be exposed to an equal volume of gas (O_2–O_3) in a stoichiometric fashion, with the ozone concentration precisely determined by using an ozone-resistant, disposable 500 mL glass bottle *under vacuum.*

This simple, inexpensive (all the necessary disposable material costs about 12 US$) procedure has already yielded therapeutic results in vascular diseases superior to those achieved by conventional medicine (discussed in Section 7A). Moreover, the therapeutic modalities, until now restricted to major AHT and to the empirical and imprecise rectal insufflation of gas,[139,177,178] have been extended: they include the quasi-total body exposure

Table II. Routes of Ozone Administration

Parenteral	Topical or locoregional
Intra-arterial (IA)[a]	
Intramuscular (IM)	Nasal[b]
Subcutaneous (SC)	Tubal[b]
Intraperitoneal (Ipe)	Auricular
Intrapleural (IPL)	Oral[b]
Intra-articular (IPL)	Vaginal
(a) Periarticular	Urethral and intrabladder
(b) Myofascial	Rectal
Intradiscal (ID)	Cutaneous
Intraforaminal (IF)	Dental
Intralesional (Iles)[c]	

[a]No longer used for limb ischemia. Hepatic metastasis could be embolized via the hepatic artery.
[b]To be performed during 30–40 sec apnea.
[c]Intratumoral or via a fistula.

to O_2–O_3[140,179] and the extracorporeal blood circulation against O_2–O_3.[141] The latter procedure is rather invasive because blood collected from a vein circulates through an ozone-resistant gas exchanger[180,181] and, with the help of a peristaltic pump, returns to the circulation via a contralateral vein. On the other hand, the partial cutaneous exposure to oxygen–ozone does not need any venous puncture and, owing to the vast expanse of the skin, allows a generalized and beneficial effect. Clearly, today we can select the most suitable method for different pathologies, their stage, and the patient's condition. A discussion on its own is needed for the minor AHT, which basically consists of withdrawing 5 mL of blood to be immediately and vigorously mixed for 1 min with an equal volume of O_2–O_3 at an ozone concentration ranging between 80 and 100 μg/mL of gas per mL of blood already extensively described.[142] The slightly oxidized blood, including the foam, is promptly injected into the gluteus muscle without the need of any anesthetic. As an unspecific immunomodulatory approach, it has been widely used during the last two decades for successfully treating herpetic infections.[143]

The slight hemolysis (~2%) is purposefully required because the heme released in the gluteal muscle will stimulate the synthesis of HO-1.[165,171]

7. *WHICH DISEASES ARE SUITABLY TREATED WITH OZONE THERAPY*

On the basis of the mechanisms of action, ozone therapy can induce the following biological responses: (a) it improves blood circulation and oxygen delivery to ischemic tissue owing to the concerted effect of NO and CO and an increase in intraerythrocytic 2,3-DPG level; (b) by improving oxygen delivery, it enhances the general metabolism; (c) it upregulates the cellular antioxidant enzymes and induces HO-1 and HSP-70; (d) it induces a mild activation of the immune system and enhances the release of growth factors; (e) it has an excellent disinfectant activity when topically used, while this is negligible in the circulation owing to blood antioxidant capacity; (f) it does not procure acute or late side effects;[182] (g) it procures a surprising wellness probably by stimulating the neuro-endocrine system. It does seem that ozone, by acting on many targets, can indirectly help in recovering functional activities gone astray because of a chronic disease and, if this interpretation is correct, ozone therapy acts as a biological response modifier. Although ozone therapy is now used in many countries, it is

mostly used by private physicians and the performance of large clinical trials has been severely hampered by lack of sponsors, disinterest of pharmaceutical as well as health authorities, and prejudice by clinical scientists. However, a number of studies have been performed with the following results:

A. Peripheral Obstructive Arterial Diseases

Even a modest obstruction of limb arteries due to atherosclerosis, diabetes, or Buerger's disease (thromboangiitis obliterans) leads to a progressive reduction of blood flow to the feet. Tissue ischemia and any minor trauma facilitate the formation of an ulcer, which will not heal because oxygen, nutrients, and growth factors indispensable for the repair process are lacking. This pathology is the best suited to be treated with O_3-AHT. According to Fontaine-Leriche classification, patient at either stage II (intermittent claudication and transitory pain), or stage III (continuous pain, cyanosis, and possibly initial ulcers) achieve the best results. Stage IV includes incipient necrosis of toes and unbearable pain leads to surgical amputation that can be avoided with O_3-AHT in about 50% of cases.[183–185] In comparison to pentoxyfilline and prostanoids (the gold standard of orthodox treatment), O_3-AHT has proved more effective and without side effects in ischemic vascular disease. In a small trial, 28 patients were randomized to either receive their own ozonated blood or an IV infusion of prostacyclin.[186] All patients continued conventional treatment with statins, antihypertensive, and antiplatelet aggregation drugs. Ozone therapy proved more effective than prostacyclin in terms of pain reduction and improvement in the quality of life, but no significant difference was seen in vascularization of the lower limbs in either group, most likely due to the short duration of treatment (14 treatments in 7 weeks). More prolonged treatments lead to a satisfactory healing of ulcers.[187] Previous studies[122,188–194] have shown the validity of O_3-AHT in this complex pathology, but it is a mistake to stop therapy too early in these patients because O_3-AHT, as with other conventional drugs, must be continued, albeit less frequently, for life. An improved schedule on a trial in progress consists of two O_3-AHT (225 mL blood plus 25 mL 3.8% sodium citrate solution), given weekly for at least 4 months. Topical therapy performed with ozonated olive oil is extremely useful when initial dry gangrene or ulcers are present. The frequency of O_3-AHT depends upon the stage of the disease and regarding the III and IV stages it can be done every day in the attempt to prevent amputation. How well O_3-AHT works it appears evident by the fact that the nocturnal excruciating pain disappears after the first two to three treatments, indicating the improvement of blood flow in the ischemic tissue and the lack of "stealing" blood away from underperfused muscle.

On January 2008, the Lancet published a double-blind, placebo controlled study (ACCLAIM trial) in 2,426 patients with New York Heart Association (NYHA) functional classes II–IV chronic heart failure (CHF).[195] Beside standard medication, the experimental group during a period of some 24 weeks, underwent about 25 intragluteal injections each patient receiving 10 mL of its own blood heavily oxidized with ozone associated with UV irradiation and heating at 42.5°C. It is unbelivable that 10 mL of blood were oxidized with as many as 75 mg of ozone, a dose that kills all cells and denature plasma proteins. This procedure, which is a sort of minor O_3-AHT,[196] had been invented with the aim to produce immunosuppressive compounds able to counteract the pathophysiological mechanisms responsible for the progression of CHF. Results have been disappointing because no difference in the composite endpoint of death for cardiovascular reasons between the control and the experimental group were noted. A few researchers[197–200] have criticized the approach that had been also a failure in the previous Simpadico trial in patients with chronic limb ischemia.[201] Actually this trial was stopped because of the risk of inducing neoplasia. This

approach has been discussed here because, being based on an irrational concept, may undermine the progress of the real O_3-AHT that utilizes the minimal amount of ozone just sufficient for triggering useful biological activities.

Millions of people suffer from chronic limb, brain, and heart ischemia, which represent the major cause of death worldwide. This has a huge socio-economic impact, particularly in the developing world. If only orthodox medicine will accept O_3-AHT as an adjunct to standard medication, a great leap forward will be noted.

B. Age-Related Macular Degeneration

In the UK alone, some 200,000 patients affected by the "dry" (atrophic) form of ARMD are suitable for treatment with O_3-AHT,[202] but all over the world there are about 30 million people searching for a therapy. Nonetheless, ophthalmologists can only prescribe antioxidants and zinc, which are minimally effective.[203,204] Since 1995, almost 1,000 patients with the dry form of ARMD have been treated with O_3-AHT at our polyclinic and three-quarters have shown an improvement of one to two lines on the visual acuity chart.[144,205]

Usually 15–18 treatments, at an initial ozone concentration of 20 μg/mL of gas per mL blood, slowly upgraded to 60 μg/mL (twice weekly), followed by two monthly session as a maintenance therapy, allows to maintain the improvement. Although uncontrolled, this study emphasizes that O_3-AHT is the only treatment able to dramatically improve the patient's quality of life. In this disease there is progressive degeneration and death of the fovea centralis photoreceptors and of the pigmented retinal epithelium (PRE) as a consequence of several factors, one of which is chronic hypoxia. Although O_3-AHT induces a pleiotropic response, the main advantage is due to an increased delivery of oxygen to the retina, which is the bodily tissue with the highest oxygen consumption. It is worth noting that O_3-AHT is useless, even harmful, in the exudative form of ARMD and in multigenic and progressive disorders (e.g., retinitis pigmentosa and recessive Stargardt's disease).[206] The exudative form, characterized by an aberrant choroidal vascular growth and a vascular hyperpermeability beneath the retina and the PRE, is caused by worsened ischemia, which negatively stimulates the release of the vascular endothelial growth factor. It must be emphasized that O_3-AHT (in the dry form) not only improves visual activity but at least, in part, renders the patient capable of autonomous life.

C. Chronic Infectious Diseases

Ozone is regarded as the best topical disinfectant because bacteria, viruses, fungi, and protozoa, when free in water, are readily oxidized.[207,208] Disappointingly, destruction of free pathogens in plasma by ozone either ex vivo or in vivo is greatly hampered by soluble antioxidants such as albumin, ascorbic acid, and uric acid and they are virtually unassailable when there are intracellular located.[124,125] However, ozone therapy still deserves attention because, by improving metabolism and operating as a mild cytokine inducer,[64] it can have a beneficial influence on infectious diseases. Thus, there remains a place for the application of O_3-AHT as an adjuvant in chronic viral infections (e.g., HIV, HCV, HSV), in combination with highly active anti-retroviral therapy (HAART), pegylated interferon-α plus either lamivudine or ribavirin and the acyclovir.

On the other hand, bacterial septicaemia must be treated with the most suitable antibiotics to prevent toxaemia and multisystem organ dysfunction. Particularly important is the topical application of either (i) ozone as a gas mixture (about 4% ozone and 96% oxygen);[209,210] or (ii) as ozonated water; or (iii) ozonated oils (where ozone is firmly stabilized as a triozonide)[208,211–214] for the treatment of bacterial, viral, and fungal infections, aphthous ulcers, burns, abscesses, and osteomyelitis. Topical therapy is most effective when combined

with O_3-AHT owing to the improved oxygenation of hypoxic tissues. Radiodermatitis[215] and wound healing have been enhanced because ozonated solutions display a cleansing effect, act as a disinfectant, and stimulate tissue reconstruction. A recent review reports that the high rates of diabetes in many parts of the world make foot ulcers a major and increasing public-health problem. Foot ulcers cause substantial morbidity, impair quality of life, engender high treatment costs (about US$17,500–27,987) and are the most important risk factor for lower-extremity amputation.[216] Although the constant use of rectal–colon insufflation of O_2–O_3 is not the optimal approach, it seems to improve the prognosis of diabetes by combining topical therapy with ozonated oil and O_3-AHT.[217] This study needs to be confirmed. Ozonated olive oil is an amazing preparation because combines antibacterial activity with healing properties due to the slow release of oxygen in hypoxic tissues and the stimulation of fibroblasts proliferation.[212,213] Chronic ulcers and/or putrid wounds are one of the most distressing and difficult medical problems with which to deal and are caused by ischemia, diabetes, immunosuppression, and malnutrition. During the past decade the use of ozone derivatives in such cases has proved very beneficial,[143] but so far official medicine has not yet discovered this excellent preparation far more effective than ointments containing often ineffective antibiotics and corticosteroids, which delays healing. With the current increase in health-care costs, O_3-AHT and ozonated oils deserve attention because they reduce hospital assistance and are inexpensive.

D. Pulmonary Diseases

Lung diseases, such as chronic obstructive pulmonary disease (COPD), will soon become the fourth most common cause of death, which, with emphysema and asthma, make significant incapacity. Using corticosteroids, long-acting β_2-agonists, and antibiotics, orthodox medicine has certainly proved helpful,[218] but it cannot change the course of COPD. However, in a series of elderly patients affected by macular degeneration and either emphysema or COPD, a remarkable improvement has been observed by combining ozone therapy[219] (using the schedule adopted for vasculopathies) with the best conventional treatments. It is unfortunate that so far a randomized study evaluating orthodox therapy with or without O_3-AHT has not been performed.

E. The versatility of Ozone Application in Orthopaedics and Dentistry

The application of ozone in low back pain has proved very effective. It can be administered directly (intradiscal),[220–224] or indirectly, via intramuscular administration into the paravertebral muscles. This latter type of administration has been assimilated to a "chemical acupuncture."[145] During the last 6 years, more than 30,000 patients with hernial disc have been treated in Italy with a success rate varying from 62 to 80%. The value of this approach, minimally invasive and without risk, has been already recognized in several countries, from China to Spain and South America. As shown also in another study on pain-related disorders due to sport injury (232 subjects) and inflammatory disorders (770 subjects)[225] it appears that ozone exerts a multiplicity of effects, such as the activation of the anti-nociceptive system, and it has anti-inflammatory action due to lipid peroxidation products, with the consequent inhibition of cyclooxygenase-2 (COX-2).[226,227]

Finally, ozone has proved very useful in dentistry for eliminating infections and blocking primary root carious lesions.[228,229] The interested reader will appreciate the notable book "*Ozone: the revolution in dentistry*."[230] After almost 80 years the intuition of Dr. Fisch could not receive a more enthusiastic appreciation by Prof. Lynch.

8. IS OZONE THERAPY A BAD COPY OF HYPERBARIC OXYGEN THERAPY?

It is often thought that ozone therapy tries to simulate the advantages of the much better known hyperbaric oxygen therapy (HOT)[231–233] and therefore it seems useful to clarify that these two approaches are both theoretically and practically different.

In the former, the drug is represented by ozone and, while we have described its initial reaction and the cascade of active messengers, it has also been pointed out that oxygenation of blood is not its primary intent. Conversely, by breathing almost pure oxygen at 2.6 bar into the hyperbaric chamber, the volume of dissolved oxygen in the plasma increases up to about 5 mL/dL, that is enough to satisfy ischemic tissues even if the absence of fully oxygenated hemoglobin. HOT is only transitorily effective because after 2 hr of therapy, hypoxia resumes in ischemic tissues and therefore the therapeutic effect is temporary. However, HOT has an exclusive role in CO-poisoning, air embolism, decompression sickness, and perhaps clostridial myonecrosis while ozone therapy is far more effective and practical to perform in POAD, heart ischemia, ARMD, diabetic foot, chronic ulcers, and bedsores. Thus, both approaches are relevant but each one has its selected field of application and the difference should be understood for the sake of the patient.[146]

9. CONCLUSIONS

The history of medicine remind us that in the past the application of several important approaches has been delayed owing to prejudice, lack of knowledge, or of sponsors and often by commercial competition. Ozone is inexpensive and therefore ozone therapy does not make an exception in spite of the fact that all chemical, biochemical, physiological, and pharmacological mechanisms elicited by ozone as primum movens are in the realm of orthodox medicine. One wonders if now with the advent of molecular medicine and gene therapy, ozone therapy is obsolete or worthwhile being pursued. Our many treated patients answer for us by saying that it is very beneficial. The compliance is excellent and the patients, as soon as the therapeutic effect declines, ask for a new cycle, showing the benefit and lack of side effects. It has been unfortunate that, in the past, the direct intravenous injection of the gas, now prohibited, the use of primordial ozone generators and misuse of ozone by incompetent quacks has generated serious doubts about its validity. Moreover, pulmonary toxicity due to prolonged inhalation of polluted air and many nonphysiological studies, performed in saline washed erythrocytes unprotected by the potent plasma antioxidants, have generated the dogma that ozone is always toxic and should not be used in medicine. This concept cannot be generalized because it does not take into account the profound difference between the endogenous chronic oxidative stress, due to aging or to a chronic disease, and the calculated, extremely brief, and well-calibrated oxidative stress induced on blood by using a precise and small ozone dose. When the appropriate ozone dose reacts with biomolecules it yields a number of compounds that in spite of their intrinsic toxicity, thanks for their pharmacodynamic, stimulate important biochemical pathways. Indeed, the medical effect depends upon a critical balance between an appropriate small dose of ozone and an almost infinite reacting variables such as the multiplicity of antioxidants, the life-time of ROS and LOP, their in vivo pharmacokinetic, and most important the variability of the biological response depending upon on enzyme reactivity and the stage of the disease.

Since the discovery of NO as a physiological messenger, other gaseous molecules such as CO, H_2S, and H_2,[234–236] in spite of being known as potentially toxic molecules, if used

judiciously are now considered as possible therapeutic agents. Any drug, depending upon its dosage can be either therapeutic or toxic. A striking example is represented by a vital compound such as glucose, its normal concentration in the plasma ranges between 0.7 and 1.0 mg/mL. However, when this concentration falls below 0.4 mg/mL, the consequent hypoglycemic coma can be deadly. On the other hand, if the glucose concentration remains constantly above 1.3 mg/mL, it induces the metabolic syndrome, which is well exemplified by the current diabetic epidemic. Finally, oxygen at 21% concentration in air (and an arterial pO_2 of about 99 mmHg) allows us to live for almost 80 years but it is deadly if we breathe pure oxygen for a few days. Thus, while a further discussion regarding ozone toxicity in medicine appears futile, it is important to examine if, indeed ozone therapy will be able to acquire a right place among the medical armamentarium. In the last decade, ozone therapy has attracted great attention in less-developed countries, while it remains partly prohibited in USA and poorly regarded in other developed countries. What can be done to change this severe outlook? Today we have a comprehensive framework for understanding the biochemical mechanisms and the biological effects of ozone and we have at least in part the capability of recommending ozone therapy in selected diseases either as a first choice or even better in combination with orthodox therapy. Thus, first, we must continue to organize specialized courses for physicians for avoiding conceptual or technical pitfalls. Second, while it is important to continue specific biologic studies, it is imperative to perform controlled and extensive clinical trials to prove beyond any doubt the value of ozone therapy at least in vascular diseases. Unless this is done, there is no future for ozone therapy within official medicine. The stumbling block is represented by lack of sponsors, disinterest of the pharmaceutical industry, and negligence of health authorities. As ozone therapy is a very cheap treatment, especially if it will be performed in all hospitals on a daily basis, it will markedly reduce both medical cost and invalidity. Almost needless to say that ozone therapy, like orthodox medicine, cannot "cure" several human diseases such as ARMD, atherosclerosis, and metabolic diseases. However, the maintenance therapy associated with conventional medication could improve the life of many patients. By considering the huge cost of reliable controlled and randomized clinical trials, unless health authorities give a financial support, ozone therapy will remain in limbo and in the hands of private physicians who can only report anecdotal and yet useless data. Only scientifically well-demonstrated therapeutic advantages will be able to dissipate prejudice and allow oxygen–ozone therapy to become a world wide useful medicinal treatment.

10. ABBREVIATIONS

4-HNE	4-hydroxynonenal
ALDH	aldehyde dehydrogenase
ARMD	age-related macular degeneration
ASF	airway surface fluid
ATP	adenosine triphosphate
BMC	blood mononuclear cells
CAT	catalase
CCl_4	carbon tetrachloride
CGMP	cyclic guanosine monophosphate
CHF	chronic heart failure
CNS	central nervous system
CO	carbon monoxide

COPD	chronic obstructive pulmonary disease
COX-2	cyclooxygenase-2
DPG	2,3-diphosphoglycerate
ELF	epithelial lining fluid
G6PHD	glucose-6-phosphate dehydrogenase
GSH	glutathione
GSH-Rd	glutathione reductase
GSPase	glutathione peroxidase
GSSG	oxidized gluthathione
GST	glutathione-*S*-transferases
HAART	highly active anti-retroviral therapy
HClO	hypochloric acid
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HO-1	heme oxygenase-1
HOT	hyperbaric oxygen therapy
H_2O_2	hydrogen peroxide
HSP	heat stress proteins
HSV	herpes simplex viruses
HUVEC	human vascular endothelial cells
IFNγ	interferon gamma
IL-1	interleukin-1
IL-8	interleukin-8
LDH	lactate dehydrogenase
L-NAME	*n*-omega-nitro-L-arginine methyl ester
LOP	lipid oxidation products
MA	mercapturic acid
MDA	malondialdehyde
NADPH	nicotinamide adenine dinucleotide phosphate
NF-κB	nuclear factor-κB
NO	nitric oxide
N_2O	nitric dioxide
O_2^-	anion superoxide
$^{\cdot}OH$	hydroxyl radical
O_3-AHT	ozonated autohemotherapy
PDGF	platelet-derived growth factor
POAD	peripheral obstructive arterial disease
ppm	parts per million
PUFA	polyunsaturated fatty acids
RBC	red blood cells
ROS	reactive oxygen species
PRE	pigmented retinal epithelium
SOD	superoxide dismutase
TAS	total antioxidant status
TBARS	thiobarbituric acid reactive substances
TGFβ1	transforming growth factor β1
TNFα	tumor necrosis factor alpha
Trx	thioredoxin
UV	ultraviolet radiation
VEGF	vascular endothelial growth factor

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BIOMEDICAL ESSAY

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

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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 used[4] for slow injection of up to 20 mL of O_2–O_3 (O_3 concentration: 30 μg/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]

Payr[6] and Aubourg[7] 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 μg/mL, administered over a few minutes. In addition, low concentrations of O_3 (3–5 μg/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_3AHT) 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 × O_3 concentration), with O_3 concentration 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_3AHT 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 Hb + 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). 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_3AHT 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 finding[26] that platelets in heparinised plasma release huge amounts of platelet-derived growth factor (PDGF) and transforming growth factor β1 (TGF β1) after ozonation explains, at least in part, the enhanced healing of torpid ulcers in patients with limb ischaemia following O_3AHT.

In spite of these encouraging results, Kraft *et al.*[27] concluded that O_3AHT 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_3AHT. 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_3AHT, 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 VasoCare™ therapy[24,29] makes the interpretation of biological and clinical studies very difficult. Moreover, retinitis pigmentosa is a genetic disorder and O_3AHT 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 disease[32] and cancer,[33] particularly after high-intensity chemotherapy and radiotherapy. O_3AHT 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 reports[33] 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_3AHT, may correct a permanent imbalance caused by excessive and chronic oxidative injury. It has been shown that chronic exposure to increased O_2 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 upregulation 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 lifelong 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_3AHT, carried out in patients with cardiac infarction,[22] neurodegenerative disease,[46] HIV infection,[42] and ARMD[47] 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 hypothesise[50] that O_3 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, O_3 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 O_2–O_3 (2×10 mL, O_3 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 explanation[50] is that it represents a type of 'chemical acupuncture' in which the needle and O_3 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, Ca^{2+} 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 smog[52] and its toxicity is potentiated by other compounds such as CO, NO_2 and H_2SO_4.[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 O_3 inhalation.

An artificial situation was used to examine O_3 toxicity in human blood, when erythrocytes or other cells were resuspended in a saline medium and exposed to O_3.[58,59] In this nonphysiological environment, both the erythrocyte membrane and intracellular enzymes were oxidised; however, it would be wrong to conclude that O_3 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% O_2 and 3% O_3) is not harmful.

Pryor[3] has shown that one facet of the reaction between an olefin and O_3 is the generation of H_2O_2 and aldehydes. Ueno *et al.*,[68] using the electron spin resonance (ESR) technique, recently confirmed that whole blood exposed to O_3 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 O_3 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 O_3 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 O_3 reactivity is exhausted by plasma components and does not harm blood cells is consistent with the data: using up to 80 μg/mL O_3 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 O_2 under the same conditions, probably because old erythrocytes are sensitive to mechanical stress.[70] However, when blood is exposed to O_3 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 O_3 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 μg/mL) or O_2 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 μg/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 (μg/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 μg/mL) of human plasma, levels of H_2O_2 up to 28 μmol/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 μg/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 Halliwell[80] has estimated that a 70 kg human at rest produces no less than 5 g $^{\bullet}O_2$ 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 μg/mL per gram of blood. There is no evidence that O_3AHT 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, Jacobs[81] 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 (NFκB), 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-κB. In simple terms, a protein kinase, activated in the presence of H_2O_2, phosphorylates the I-κB 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

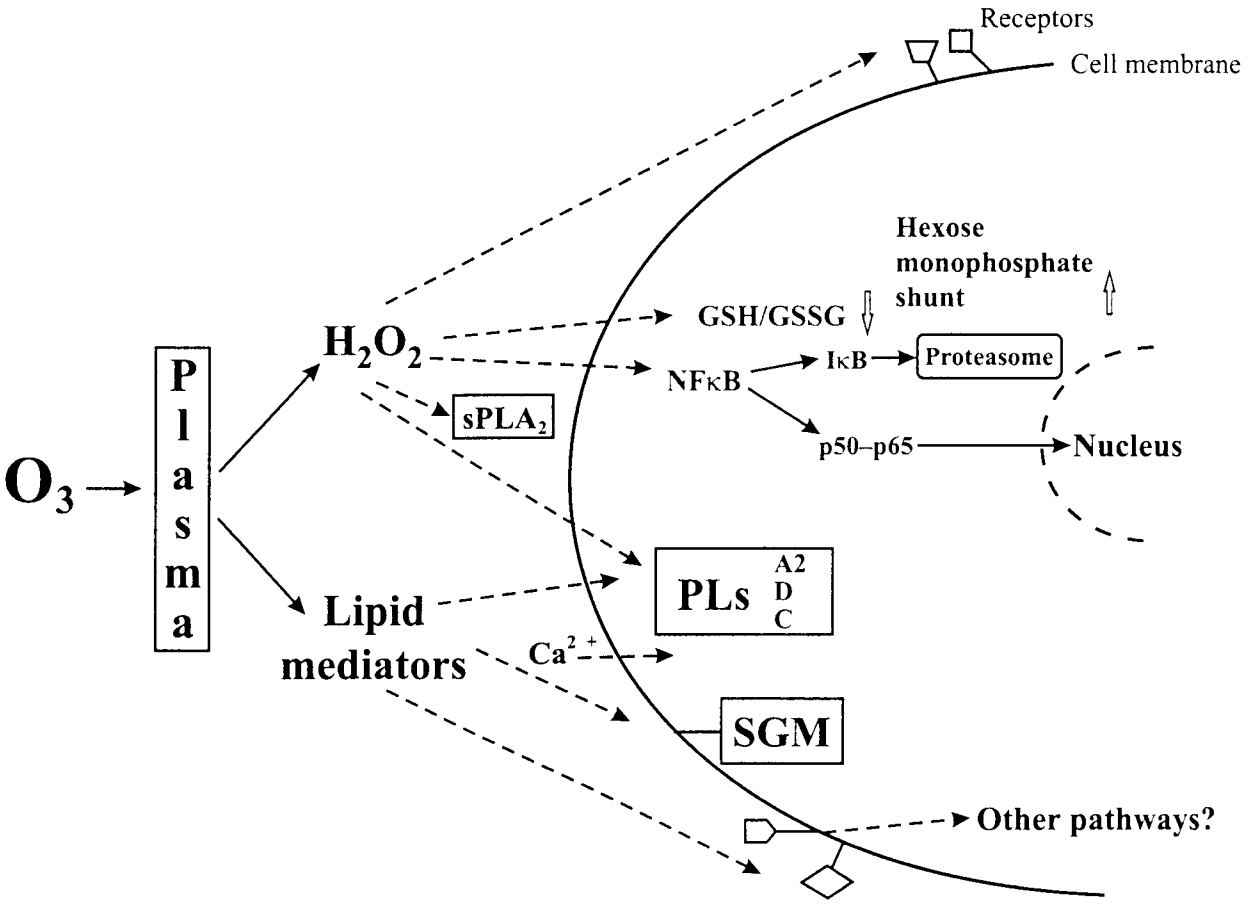

Fig. 1. Hypothetical sites and mechanisms of action of mediators generated from the reaction between O_3 and plasma. The aldehyde 4-hydroxynonenal favours the influx of Ca^{2+}, which in turn enhances intracellular events. GSSG, oxidised glutathione; NFκB: nuclear factor κB; PLs, phospholipases; SGM, sphyngomyelinase; $sPLA_2$, 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 μg/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 μg/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 μg/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 μmol/L or <1 μmol/L), they may be either harmful[89] 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 sphyngomyelinase (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 transmitting[92] peroxidative stress information and possibly inducing up-regulation of antioxidant enzymes, hence the tolerance to O_3. This interesting phenomenon, termed oxidative stress adaptation,[2,42] has an important practical implication in the sense that judicious use of O_3AHT 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 O_3 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 O_3AHT in the treatment of chronic viral infection.

It would appear that O_3AHT simultaneously triggers a range of biological effects that work together in hypoxic tissues, where, for example, vasodilation, enhanced O_2 delivery and release of growth factors accelerate healing of chronic ulcers.[98]

Finally, the critical problem of the O_3 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 O_3 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 μg/mL of O_3 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 (μg/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 μg/mL per gram of blood to the highest level. As treatments are performed on a day-hospital basis, O_3AHT 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 O_3AHT. 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 O_3 and the harmful effects of acute and chronic exposure on the respiratory tract. Moreover, direct IV administration of O_3 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 O_3 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_3AHT;
- 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.

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Ozone Therapy in Critical Patients. Rationale of the Therapy and Proposed Guidelines

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Key words: major ozonated autohemotherapy (O-HAT), Minor O-HAT, albumin, n-acetyl cysteine, ascorbic acid chronic oxidative stress, lung shunt

SUMMARY – In combination with the most suitable orthodox therapy, there are rational bases for justifying the use of ozone therapy in critical patients. This approach is difficult to implement in intensive care units, not for technical reasons, but because ozone therapy is regarded with suspicion and scepticism. However, assuming that appropriate permission and informed consent are obtained, which is the best way to proceed? We should aim to improve oxygen delivery to vital organs and ischemic areas and to support respiratory, cardiac and renal functions. If the patient's metabolic conditions are not excessively deteriorated, within 3-4 days of daily ozonated autohemotherapy treatments the increased synthesis of antioxidant enzymes and the induction of heme-oxygenase-1 may reduce the chronic oxidative stress simultaneously caused by infection-inflammation-tissue necrosis and dysmetabolism. We suggest some guidelines with the proviso of being flexible for each clinical case. The aim is not to achieve a scientific result but to save human lives.

Introduction

Visiting an ordinary intensive care unit, one can observe a heterogeneous group of patients, all at risk of losing their lives owing to traumatic events, severe burns, stroke, gangrene of the limbs, abdominal or pulmonary infections with various degrees of septic shock. We have often wondered if an intensive application of ozone therapy combined with the best conventional therapies may improve the prognosis [1]. However, at Siena hospital the chief doctor has been always concerned about the legal aspects because if the patient dies he will be accused of having used a non-validated therapy. The Ethical Committee also refuses to give permission for a trial because there is not yet any prospect that ozone therapy could represent a valid support.

Recently, Dr. Brito urgently requested a scheme and schedule for treating a critically ill patient with ozone therapy. The patient was a Brazilian colleague who presented a multiple critical dissection of the aorta. Luckily an experienced surgeon was able to correct it by placing an aortic prosthesis, an aortic valve prosthesis and suitable stents in the aortic descendent and thoracic aorta. The patient was under extracorporeal circulation for about six hours and although he was aided by multiple blood transfusions (40 units) he developed a critical situation with lung shunt, pneumonia, fever and serious respiratory difficulties documented by very poor respiratory parameters and a gram negative bacterium on bronchoscopy aspiration. Fortunately, Dr. Brito was a dear friend of the patient, and a disciple of ozone therapy. Having read section 15 in my latest book1 concerned with ozone therapy in emergency conditions, he decided that it was worthwhile to combining the orthodox therapy with ozone therapy. After obtaining prompt permission from the director of the intensive care unit, on the basis of the family's request, an informed consent form signed by the family members and a special authorization from Ministry of Health regulatory agency on medical practice, he performed four major ozonated autohemotherapy (O-HAT) treatments daily for three consecutive days (October 4-6, 2005), using a blood volume of 200 ml each time and an ozone concentration of 40 mcg/ml on the first day and 25 mcg/ml on the 2nd and 3rd days. As the patient conditions started to improve, he reduced the number of treatments to two on the fourth day and to one daily for the following week. The patient had a remarkable improvement, characterized by normalization of body temperature

This paper is dedicated to the memory of Dr. Edison de Cesar Filippi, the most experienced ozone therapist in Brazil.

and improvement of respiratory parameters. He was then moved from the intensive care unit to a regular room, in fairly good health, walking, eating, and starting working on his laptop. Once all intravenous catheters were removed because they were no longer necessary, autohemotherapy was stopped. Moreover, because of the recent extensive surgery, it was decided to stop heparinization to avoid bleeding at surgery sites. This may have been an untimely decision because he had a sudden stroke with high intracranial pressure probably caused by an embolus from the heart or aorta. Any further attempt to save him was unsuccessful due to extensive brain swelling and cerebral death. Unfortunately this outcome is fairly frequent in patients with severe vascular disease.

What could be the role of ozone therapy and was it reasonable to undertake it? Dr G.S. Brito, who closely followed the patient and performed the ozone therapy during the first phase, is convinced that ozone therapy corrected a dangerous post-operative course. Needless to say, the initial surgery and conventional treatments were absolutely indispensable.

If clinical conditions tend to further deteriorate, before multiorgan failure develops, a prompt and appropriate use of ozone therapy may improve the situation even though its intrinsic validity remains a matter of opinion. Nonetheless, in such cases, the scientific rigor is less important than the patient's life. According to Paragraph 32 of Helsinki Declaration the assumption is: In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

Dr G.S. Brito felt that we should propose a guideline taking into account the dysmetabolic and most frequent septic conditions of critically ill patients.

A brief analysis of the problem

Owing to the extreme variability of the aetiology and pathogenesis of the above indicated pathologies, it is reasonable to ask if they share a common denominator which may justify the use of ozone therapy. Is it inflammation, or tissue degeneration, or infection? We feel that the most important and common problem is the presence of chronic oxidative stress (COS) which is a persistent and progressive imbalance between decreasing antioxidants and prevailing oxidants, able to induce a generalized cellular apoptosis and death of the patient. During an infection and/or a chronic inflammation, leukocytes and macrophages generate excessive amounts of reactive oxygen species (ROS, such as anion superoxide, hydrogen peroxide, hypocloric acid) that unselectively destroy pathogens as well as normal cells. Ozone therapy is currently the only procedure able to block and reverse this negative process because it can:

1) Enhance the transport and release of oxygen in ischemic areas. One may think that reperfusion may further damage hypoxic tissues, but if the degeneration has not gone too far, the messengers generated by ozone, namely the lipid oxidation products (LOPs), can, after binding to cell receptors, stimulate the synthesis of antioxidant enzymes such as superoxide dismutase (SOD), catalase (Cat), glutathione peroxidase, reductase and transferase (GSH-Px, Red. and Tr.) [2-6]. Moreover and most importantly, small calculated and transitory acute oxidative stress is one of the best stimuli for inducing the synthesis of some acute oxidative stress proteins, of which the most protective is the inducible heme-oxygenase-1 (HO-1) [1]. This enzyme (OSP-32) allows the degradation of heme from over-abundant hemoproteins and hemoglobin and results in the formation of biliverdin (hence bilirubin that is a valuable lipophilic antioxidant) and carbon monoxide (CO). The concomitant co-induction of ferritin, due to the release of iron, allows the beneficial sequestration of redox-active iron, thus avoiding formation of hydroxyl radical ($OH^{\cdot}$) by the Fenton reaction. Since 1978, the properties and protective effects of HO-1 overexpression have been described in over 3600 publications! It is impossible to enumerate all of the functions of this Herculean enzyme able to prevent or improve different pathological conditions.

2) During infusion of the ozonated blood into the donor, LOPs enter into contact with the vast expanse of the endothelium and stimulate an increased synthesis of nitric oxide (NO) via NO-synthase and arginine [7]. NO (and NO-thiols) and CO are the crucial physiological gases able to activate guanylate cyclase, so that the release of cyclic GMP enhances the vasodilation. The combination of these processes can, in not too advanced diseases, reduce infection, inflammation and cell degeneration.

3) Ozonation of blood ex vivo, by the controlled release of small amounts of the generated

hydrogen peroxide, allows a mild activation of neutrophils and the induction of the production of some cytokines [8-12]. After re-infusion of the ozonated blood, the activated or primed leukocytes migrate all over the body and can slowly improve the response of the adaptive immune system. Obviously, with the crucial help of appropriate antibiotics, even chronic infections can be controlled.

4) Some of our experimental data suggest that stimulation of platelets [13,14] and endothelial cells [7] by LOPs may favour the release of growth factors and autacoids, one of which may be prostacyclin. Surprisingly, LOPs may also inhibit cyclooxygenase II with the beneficial consequences of reducing hyperpermeability, edema and pain [15].

5) Once LOPs and nitroso-thiols reach the bone marrow microenvironment, they may activate metalloproteinase 9, a critical enzyme favouring the release of staminal cells. After their mobilization, these cells may enter the general circulation and home in infarcted areas. Although this idea has not yet been experimentally proved1, it is a likely possibility that must be pursued because it may acquire practical importance.

6) Ozonation of blood performed using sodium citrate (1 ml of citrate 3.8% solution/ 9 ml of blood) does not cause any dyscoagulation during slow blood infusion. Citrate is rapidly metabolized while heparin is less safe.

7) It is a general observation that the majority of patients undergoing ozone therapy report a feeling of well-being and euphoria. Although we have no experimental data, it has been speculated1 that by influencing cerebral, hypothalamic neurons and endocrine cells, LOPs may induce the release of some hormones (ACTH, cortisol, dehydroepiandrosterone, serotonin, endorphins) able to induce a reduction of pain and a feeling of wellness.

8) Provided that ozone therapy is performed correctly, after millions of treatments performed all over the world during the last three decades, there is no record of acute or chronic toxicity1. Against scepticism and the dogma that "ozone is always toxic", we know that the ozone dose (calculated as the product of the ozone concentration per gas volume), representing the acute stressor, must be perfectly calibrated against the potent antioxidant capacity of blood in such a way as to never overwhelm it. Within the established therapeutic window (10-80 mcg/ml ozone per ml of blood), no more than 30% of the antioxidant capacity of blood is oxidized during the ozonation reactions and is rapidly (in about 20 min) reconstituted by a very efficient biochemical recycling of antioxidants [1,16].

9) According to a Cuban study, ozone may inhibit platelet aggregation, and at least theoretically, had ozone therapy been continued, it may have avoided thromboembolism in our case.

In conclusion, each autohemotherapic treatment, equivalent to a precisely calculated chemical shock, appears able to trigger a multitude of biological processes relevant for correcting the complex pathology present in critically-ill patients. The consequent possible correction of the chronic oxidative stress is particularly important.

How and when to perform ozone therapy. Tentative guidelines

We propose to perform the following procedures:

Major O-HAT. Depending on the hemodynamic status of the patient, Major O-HAT can be carried out by collecting from 50 up to 225 ml of venous blood in a sterile glass bottle (250-500 ml) under vacuum. Sodium citrate solution (3.8%) must be added to the bottle before the blood in the proportion of 1:9 ml blood. To avoid any risk of haemorrhage, heparin must be used cautiously by first ascertaining the coagulation parameters. The gas volume must be added in a 1:1 volume ratio using an initial ozone concentration of 10 mcg/ml per ml of blood. Five minutes of slow mixing to avoid foaming is sufficient to complete the ozone reaction before re-infusion of the ozonated blood into the donor. The ozone concentration can be slowly increased to 15- 20-25 mcg/ml during the next few days, but, because the patient is under COS, a higher concentration of ozone should be avoided because more deleterious than advantageous. Frequency of O-AHT can be up to three (about every 8 hours) on the first few days and then, if the patient improves, it can be reduced to two and one.

Minor O-AHT. Very simply, the residual 3-4 ml of blood remaining at the end of the infusion tubing during each O-AHT can be withdrawn in a 10 ml syringe just filled with 5 ml of gas (ozone concentrations at 80-100 mcg/ml for a total dose of 400- 500 mcg). After inserting a G21 needle, the blood is rapidly mixed with the gas by rotating the syringe for 1-2 min and then promptly injected intramuscularly (glutei), with the foam. The very high ozone concentration is purposely used to provoke some hemolysis in order to activate the induction of OSP, particularly heme–oxygenase-I. We suggest the same frequency of administration indicated for major AHT. This i.m. injection is intended to act as a minor acute oxidative shock that, in the hands of one of us (VB), greatly enhances the overall treatment.

An important and frequently overlooked aspect is the possibility that the critically ill patient, under pronounced COS, has a low blood antioxidant capacity. Although we are unable to correct the COS by administering megadoses of antioxidants, we must recommend intravenous administration of selected antioxidants immediately after the auto-hemotherapy treatment for 2-3 hours, hence 5-6 hours before the next O-AHT. For several reasons we suggest the infusion of human albumin (20% concentration), possibly diluted with 100 ml 5% glucose solution with additional 0.5 g of ascorbic acid. Unfortunately, N-acetyl-cysteine (NAC), the best precursor of reduced glutathione (GSH) is not yet available for infusion and therefore can only be administered per os, but this is rarely possible. A compromise is the i.v. infusion of GSH, which will transiently increase the plasma levels but will not increase the critical cellular level because, there is no membrane transport for GSH - at variance with what is commonly believed. Needless to say, depending on the hemoglobin content, we must be ready to perform allotransfusions because it will be useless to administer ozone therapy if the hemoglobin level falls below 11 g/dL.

If major O-AHT cannot be performed, as a last option we can resort to rectal insufflation of gas every 8 hours. A volume of 300-400 ml can be insufflated very slowly using an initial concentration of 5 mcg/ml that can be progressively increased to a maximum of 25 mcg/ml. In the case of abdominal or pulmonary lesions, particularly after trauma and infections, it is advisable to use intraperitoneal and intrapleuric insufflation of gas via, as usual, a polypropylene catheter. Ozone can exert both a direct disinfectant activity on these cavities as well as immunomodulatory effects without any discomfort or toxicity.

In the case of severe sepsis and/or septic shock the mortality can be as high as 50% and during the last two decades antibodies against endotoxin and TNF alpha as well as other approaches have yielded negligible results. However, several clinical trials have shown that infusion of recombinant human activated Protein C (Drotrecogin alpha activated) can markedly decrease morbility and mortality and therefore should be kept in mind because this protein reduces inflammation and overt dyscoagulation. Similarly, whenever surgery appears necessary, antibiotics and all the other supportive orthodox drugs must be applied because in our mind ozone therapy can only benefit the patient if used in combination.

Ozone may be able to reverse disseminated intravascular coagulation.

Conclusions

We have outlined a possible scheme and schedule for treating severely ill patients in intensive care units with ozone therapy. In spite of a minimal practical experience, there are good rational bases for suggesting the use of ozone therapy in combination with the best orthodox therapy to reduce the morbility and high mortality of these patients. It would be extremely gratifying if other ozone therapists would like to share their experience with us, so that we may be able to further improve the treatment. Once the patient, if mentally alert, and/or the family desiring to receive a specific treatment according to the Helsinki Declaration have signed an informed consent, the doctor should be allowed to proceed with the treatment. Dr. Brito is currently developing the design of a Phase I study for treatment of sepsis cases in the Intensive Care Unit of Trauma at the Emergency Surgery Department of his Medical School Hospital in Sao Paulo, Brazil.

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REVIEW ARTICLE

Scientific and Medical Aspects of Ozone Therapy. State of the Art

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The aim of this review is to dispel misconceptions and skepticism regarding ozone therapy and to clarify the biochemical and pharmacological mechanisms of action of ozone dissolved in biological fluids. The work performed in the last decade in our laboratory allows drawing a comprehensive framework for understanding and recommending ozone therapy in some diseases. It is hoped that this report will open a dialogue among clinical scientists and will inform physicians about the beneficial effects of ozone therapy. © 2006 IMSS. Published by Elsevier Inc.

Key Words: Ozone, Antioxidants, Oxidative stress, Ozone tolerance, Ozone therapy.

Introduction

It is distressing to note that often ozone therapists are more interested in simply knowing the ozone dosage rather than to understand how ozone acts and why we can avoid toxicity. This behavior reveals a lack of knowledge of the fundamental bases regulating a judicious use of ozone and is the result of a superficial preparation acquired during an occasional ozone therapy's course of a few hours. This is not surprising because during the last three decades, on the basis of Wolff's suggestion (1), ozone therapy has been used by practitioners in Europe in an empirical fashion. Unfortunately, even today, most ozone therapists have either a misconception or know only a few technical tips for performing ozone therapy. This problem, associated with the difficulties and cost of performing extensive clinical studies, has hindered real progress, and ozone therapy remains a scarcely known and objected complementary practice. Worst of all, in some countries, often without any medical qualification, quacks continue to inject either ozone intravenously, a procedure prohibited since 1984 in Germany because of the risk of pulmonary embolism and death, or ozonated saline containing a certain toxic amount of hypochloric acid. Moreover, a distinguished American chemist has affirmed the dogma that ''ozone is toxic any way you deal with it,'' reinforcing the concept that ozone should never be used in medicine. This situation has generated a sort of crusade against ozone therapy in spite of the fact that ozone is considered one of the best disinfectants capable of preventing infection outbreaks. This is becoming a crucial advantage because critically ill patients acquire infections while in hospitals and a number of them die every year as a result.

Table 1 summarizes several good reasons for refusing ozone therapy by orthodox medicine. However, problems 1–5 have now been practically overcome, whereas the remaining 6–9 are stumbling blocks hindering progress. During the last 14 years, we have made a great effort to examine ozone therapy in a scientific fashion both at a basic and clinical level, and we now have some ideas how ozone acts, how and why its toxicity can be controlled and how therapeutic effects can be exerted (2–11). There is no need to invoke philosophical speculations because the mechanisms of action are in the realm of classical biochemistry, physiology and pharmacology.

This review aims to give the reader the essential information and the frame of mind to operate as a real physician. An extensive description is available in three recent books (9–11).

What Is Ozone and How Can We Use It?

Ozone is normally present as a 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:

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

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Table 1. Why oxygen ozone therapy has not yet been accepted by orthodox medicine

1. Excessive empiricism
2. Lack of standardization
3. No precise ozone generator
4. Lack of solid scientific biological and clinical data
5. Ozone toxicity
6. The problem of charlatans
7. Lack of regulation and disinterest of health authorities
8. Lack of financial support
9. Skeptical and uninformed scientists

Consequently, we always collect a gas mixture comprising no less than 95% oxygen and no more than 5% ozone. Air must be excluded because toxic nitrogen dioxide (N_2O_2) will be formed as well as ozone and it is imperative that generators are made of high quality, ozone-resistant materials such as stainless steel, neutral glass and Teflon.

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 ($E^\circ = +2.076$ 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.

Ozone is a controversial gas because, although it is very useful in the stratosphere by absorbing dangerous B and C ultraviolet radiations, it is toxic for the pulmonary tract in the troposphere, particularly mixed with carbon monoxide (CO), N_2O_2 and traces of acids as occurs in photochemical smog.

It must be clear that if we want to use ozone in medicine, we must avoid its toxicity that can be controlled only if we operate cautiously by 1) using a precise ozone generator equipped with a well-standardized photometer, which allows us to determine the ozone concentration in real time, 2) by collecting a precise gas volume with a defined ozone concentration. The total dose is simply calculated by multiplying the ozone concentration with the gas volume. As an example, if we ozonate a blood volume of 225 mL with 225 mL of gas with an ozone concentration of 30 μg/mL, the total dose is equivalent to 6.75 mg of ozone. 3) We must know the optimal dose for achieving a therapeutic effect without any toxicity.

At variance with blood, the eyes and the lungs are very sensitive to ozone because they have minimal antioxidant and neutralizing capabilities and therefore ozone should never contact these organs.

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

$$O_3 + \text{biomolecules} \rightarrow O_2 + O^{\bullet}$$

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:

$$\text{R-CH=CH-R}' + O_3 + H_2O \rightarrow \text{R-CH=O} + \text{R}'\text{-CH=O} + H_2O_2$$

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 $OH^{\bullet}$ (hydroxyl radical).

$$Fe^{++} + H_2O_2 \rightarrow Fe^{+++} + OH^{\bullet} + OH^{-}.$$

Interestingly, we (15) have also determined the formation of nitrogen monoxide ($NO^{\bullet}$) 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 μg/mL of gas per mL of blood in 1:1 ratio) against the antioxidant capacity of blood that can be measured and, if necessary,

strengthened by oral administration of antioxidants before and throughout ozone therapy. d) LOPs production follows peroxidation of PUFA present in the plasma: they are heterogeneous and can be classified as lipoperoxides (LOO•), alkoxyl radicals (LO•), lipohydroperoxides (LOOH), isoprostanes and alkenals, among which are 4-hydroxy-2,3 transnonenal (HNE) and malonyldialdehyde (MDA). Radicals and aldehydes are intrinsically toxic and must be generated in very low concentrations. They are *in vitro* far more stable (6) than ROS but fortunately, upon blood reinfusion, they undergo a marked dilution in body fluids, excretion (via urine and bile), and metabolism by GSH-transferase (GSH-Tr) and aldehyde dehydrogenases. Thus, only submicromolar concentrations can reach all organs, particularly bone marrow, liver, central nervous system (CNS), endocrine glands, etc., where they act as signaling molecules of an ongoing acute oxidative stress (16).

If the stage of the disease is not too far advanced, these molecules can elicit the upregulation of antioxidant enzymes such as superoxide dismutase (SOD), GSH-peroxidases (GSH-Px), GSH-reductase (GSH-Rd) and catalase (CAT). Interestingly, Iles and Liu (17) have just demonstrated that HNE, by inducing the expression of glutamate cysteine ligase, causes an intracellular increase of GSH, which plays a key role in antioxidant defense. Furthermore, LOPs induce oxidative stress proteins, one of which is heme-oxygenase I (HO-1 or HSP-32) which, after breaking down the heme molecule, delivers very useful compounds such as CO and bilirubin (18). Bilirubin is a significant lipophilic antioxidant and a trace of CO cooperates with NO in regulating vasodilation by activating cyclic GMP. Fe^{2+} is promptly chelated by upregulated ferritin. The induction of HO-1 after an oxidative stress has been described in hundreds of papers as one of the most important antioxidant defense and protective enzyme. Moreover, LOPs exert a neuroimmunomodulatory effect highlighted by a feeling of well being reported by patients during ozone therapy.

Although it remains hypothetical, it is possible that LOP, throughout the treatments, acting as acute oxidative stressors in the bone marrow microenvironments activate the release of metalloproteinases, of which MP-9 particularly may favor the detachment of staminal cells (11). These cells, once in the blood circulation, may be attracted and home at sites where a previous injury (a trauma or an ischemic-degenerative event) has taken place. The potential relevance of such an event would have a huge practical importance and will avoid the unnatural, costly and scarcely effective practice of the bone marrow collection with the need of the successive and uncertain reinfusion (19).

It is emphasized that submicromolar LOPs levels can be stimulatory and beneficial, whereas high levels can be toxic. This conclusion, based on many experimental data (16), reinforces the concept that optimal ozone concentrations are critical for achieving a therapeutic result: too low concentrations are practically useless (at best elicit a placebo effect), too high may elicit a negative effect (malaise, fatigue) so that they must be just above the threshold level to yield an acute, absolutely transitory oxidative stress capable of triggering biological effects without toxicity.

In conclusion, it must be clear to the reader that the ozonation process either happening in blood, or intradiscal or in an intramuscular site represents an acute oxidative stress. However, provided that it is precisely calculated according to a judicious ozone dosage, it is not deleterious but is actually capable of eliciting a multitude of useful biological responses and, possibly, can reverse a chronic oxidative stress due to aging, chronic infections, diabetes, atherosclerosis, degenerative processes and cancer. Indeed, the ozonotherapeutic act is interpreted as an atoxic but real ''therapeutic shock'' able to restore homeostasis.

Which Are the Biological Effects Elicited by ROS and LOPs?

The ozonation process is therefore characterized by the formation of ROS and LOPs acting in two phases. This process happens either *ex vivo* (as a typical example in the blood collected in a glass bottle) or *in vivo* (after an intramuscular injection of ozone) but, while ROS are acting immediately and disappear (early and short-acting messengers), LOPs, via the circulation, distribute throughout the tissues and eventually only a few molecules bind to cell receptors. Their pharmacodynamics allow minimizing their potential toxicity and allows them to become late and long-lasting messengers.

Formation of ROS in the plasma is extremely rapid and is accompanied by a transitory and small ozone dose-dependent decrease (ranging from 5 to 25%) of the antioxidant capacity. Importantly, this return to normal within 15–20 min owes to the efficient recycling of oxidized compounds such as dehydroascorbate to ascorbic acid (20). H_2O_2 diffuses easily from the plasma into the cells and its sudden appearance in the cytoplasm represents the triggering stimulus: depending upon the cell type, different biochemical pathways can be concurrently activated in erythrocytes, leukocytes and platelets resulting in numerous biological effects. It must be noted that between the plasma and the cytoplasm compartments there is a gradient and the intracellular H_2O_2 concentration is only about 1/10 of the plasmatic one (21). The rapid reduction to water is operated by the high concentration of GSH, CAT and GSH-Px; nonetheless, H_2O_2 must be above the threshold concentration for activating several biochemical pathways.

Let us now examine how hydrogen peroxide, now universally recognized as one of the main intracellular signalling molecules (13), acts on the different blood cells. The mass of erythrocytes mops up the bulk of hydrogen peroxide: GSH is promptly oxidized to GSSG and the cell, extremely sensitive to the reduction of the GSH/GSSG

ratio, immediately corrects the unbalance by either extruding GSSG or reducing it with GSH-Rd at the expense of ascorbate or of the reduced nicotinamide adenine dinucleotide phosphate (NADPH), which serves as a crucial electron donor. Next, the oxidized NADP is reduced after the activation of the pentose phosphate pathway, of which glucose-6-phosphate dehydrogenase (G-6PD) is the key enzyme. We have determined a small but significant increase of ATP formation (10,11), but whether this is due to the activation of the pentose cycle or to phosphofructokinase or to both remains to be clarified. Moreover, for a brief period the reinfused erythrocytes enhance the delivery of oxygen into ischemic tissues because of a shift to the right of the oxygen–hemoglobin dissociation curve, due either to a slight decrease of intracellular pH (Bohr effect) or/and an increase of 2,3-diphosphoglycerate (2,3-DPG) levels. Obviously, one AHT treatment has a minimal effect and we need to ozonate at least 2.5–4 L of blood within a period of 30–60 days. During this period, LOPs act as repeated stressors on the bone marrow and these frequent stimuli cause the adaptation to the ozone stress during erythrogenesis with upregulation of antioxidant enzymes. As a consequence, a patient with chronic limb ischemia undergoing ozone therapy can have a clinical improvement due to the formation of successive cohorts of erythrocytes progressively more capable of delivering oxygen to his/her ischemic tissues. However, the final improvement is also due to the localized release of NO, CO and growth factors released from platelets and endothelial cells.

Although ozone is one of the most potent disinfectants, it cannot inactivate bacteria, viruses and fungi *in vivo* because, paradoxically, the pathogens are well protected, particularly inside the cells, by the powerful antioxidant system. Thus, as I proposed a long time ago (22,23), ozone acts as a mild enhancer of the immune system by activating neutrophils and stimulating the synthesis of some cytokines (2,5–7). Once again, the crucial messenger is hydrogen peroxide, which after entering into the cytoplasm of blood mononuclear cells (BMC) by oxidizing selected cysteines, activates a tyrosine kinase, which then phosphorylates the transcription factor nuclear factor κB (24), allowing the release of a heterodimer (p50+p65). This complex moves on to the nucleus and switches on some hundred genes eventually responsible for causing the synthesis of several proteins, among which are the acute-phase reactants and numerous interleukins. In the past, we have measured the release of several cytokines from ozonated blood upon *in vitro* incubation (2–7). Once the ozonated leukocytes return to the circulation, they home in lymphoid microenvironments and successively release cytokines acting in a paracrine fashion on neighboring cells with a possible reactivation of a depressed immune system (25). This process, described as the physiological cytokine response, is part of the innate immune system and helps us to survive in a hostile environment.

During ozonation of blood, particularly if it is anticoagulated with heparin, we have noted an ozone dose-dependent increase of activation of platelets (8,26) with a consequent release of typical growth factors, which will enhance the healing of chronic ulcers in ischemic patients. Whenever possible, the use of heparin as an anticoagulant is preferable to sodium citrate because, by not chelating plasmatic Ca^{++}, it reinforces biochemical and electric events.

During reinfusion of the ozonated blood into the donor, the vast expanse of the endothelial cells will be activated by LOPs, resulting in an increased production of NO, plasma S-nitrosothiols and S-nitrosohemoglobin (15,27). Whereas NO has a half-life of less than 1 sec, protein-bound-NO can exert vasodilation also at distant ischemic vascular sites with relevant therapeutic effect.

Moreover, on the basis of the phenomenon of ozone tolerance that says the exposure of an organism to a low level of an agent, harmful at high levels, induces an adaptive and beneficial response (28,29), we have postulated that LOPs, by acting as long-distance messengers, can transmit to all organs the information of an acute oxidative stress (10,11). The bone marrow is particularly relevant because it can upregulate antioxidant enzymes during erythrogenesis and allows the release of staminal cells for possibly regenerating infarcted organs. Moreover, the stimulation of the endocrine and central nervous systems may help to understand why most patients during prolonged ozone therapy report a feeling of euphoria and wellness, probably due to an improved metabolism as well as to an enhanced hormonal or neurotransmitter release.

The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom and there is good experimental evidence (30–34) that this phenomenon is present in the animal and vegetal kingdom. Moreover, it is already supported by our findings of an increased level of antioxidant enzymes and HO-1 during ozone therapy (10,11). It also suggests that a judicious use of ozone, in spite of acting as an oxidant, enhances the antioxidant capacity, which represents the critical factor for overcoming chronic viral infections, ischemia and cell degeneration.

Which Are the Routes of Ozone Administration?

Table 2 shows that ozone can be administered with great flexibility but it should not be injected intravenously as a gas because of the risk of provoking oxygen embolism, given the fact that the gas mixture contains always no less than 95% oxygen.

So far the most advanced and reliable approach has been the major ozonated AHT because, on the basis of the patient's body weight, a predetermined volume of blood (200–270 mL) can be exposed to an equal volume of gas

Table 2. Routes of ozone administration

Parenteral
Intravenous, intra-arterial,[a] intramuscular, subcutaneous, intraperitoneal, intrapleural, intra-articular, periarticular, myofascial, intradiscal, intraforaminal, intralesional[b]
Topical or locoregional
Nasal,[c] tubal,[c] auricular, oral,[c] vaginal, urethral and intrabladder, rectal, cutaneous, dental

[a]No longer used for limb ischemia. Hepatic metastasis could be embolized via the hepatic artery.
[b]Intratumoral or via a fistula.
[c]To be performed during 30–40 sec apnea.

(O_2–O_3) in a stoichiometric fashion, with the ozone concentration precisely determined. Figure 1 shows a schematic drawing of the components necessary to perform AHT with an ozone-resistant glass bottle (plastic bags must be avoided because they are not ozone resistant and contaminate blood with pthalates and plastic microparticles). Blood, drawn from a cubital vein via a G19 Butterfly needle, is rapidly sucked inside the bottle under vacuum via Segment A. Then a precise volume of gas is delivered via segment B. With gentle mixing to avoid foaming, ozonation of blood is completed in 5–10 min and the ozonated blood is reinfused, via suitable tubing with blood filter, into the donor in about 15 min. This simple, inexpensive (all the necessary disposable material costs about 12 US$) procedure has already yielded therapeutic results in vascular diseases superior to those achieved by conventional medicine. Moreover, the therapeutic modalities, until now restricted to major AHT and to the empirical and imprecise rectal insufflation of gas (11), have been extended: they include the quasi-total body exposure to O_2–O_3 (35) and the extracorporeal blood circulation against O_2–O_3 (36). The latter procedure is rather invasive because blood collected from a vein circulates through an ozone-resistant gas exchanger and, with the help of a peristaltic pump, returns to the circulation via a contralateral vein. On the other hand, the partial cutaneous exposure to oxygen-ozone does not need any venous puncture and, owing to the vast expanse of the skin, allows a generalized and beneficial effect. Clearly, today we can select the most suitable method for different pathologies, their stage and the patient's condition.

A discussion on its own is needed for the minor AHT, which basically consists of withdrawing 5 mL of blood to be immediately and vigorously mixed for 1 min with an equal volume of O_2–O_3 at an ozone concentration ranging between 80 and 100 µg/mL of gas per mL of blood. It has been extensively described in Bocci (11). The strongly oxidized blood, including the foam, is promptly injected into the gluteus muscle without the need of any anesthetic. As an unspecific immunomodulatory approach, I have used this treatment since 1953 and, during the last two decades, several ozone therapists have successfully treated herpetic infections (for review, see Reference 11). I have speculated that blood infiltrated into the muscular tissue will undergo coagulation due to platelet and prothrombin activation.

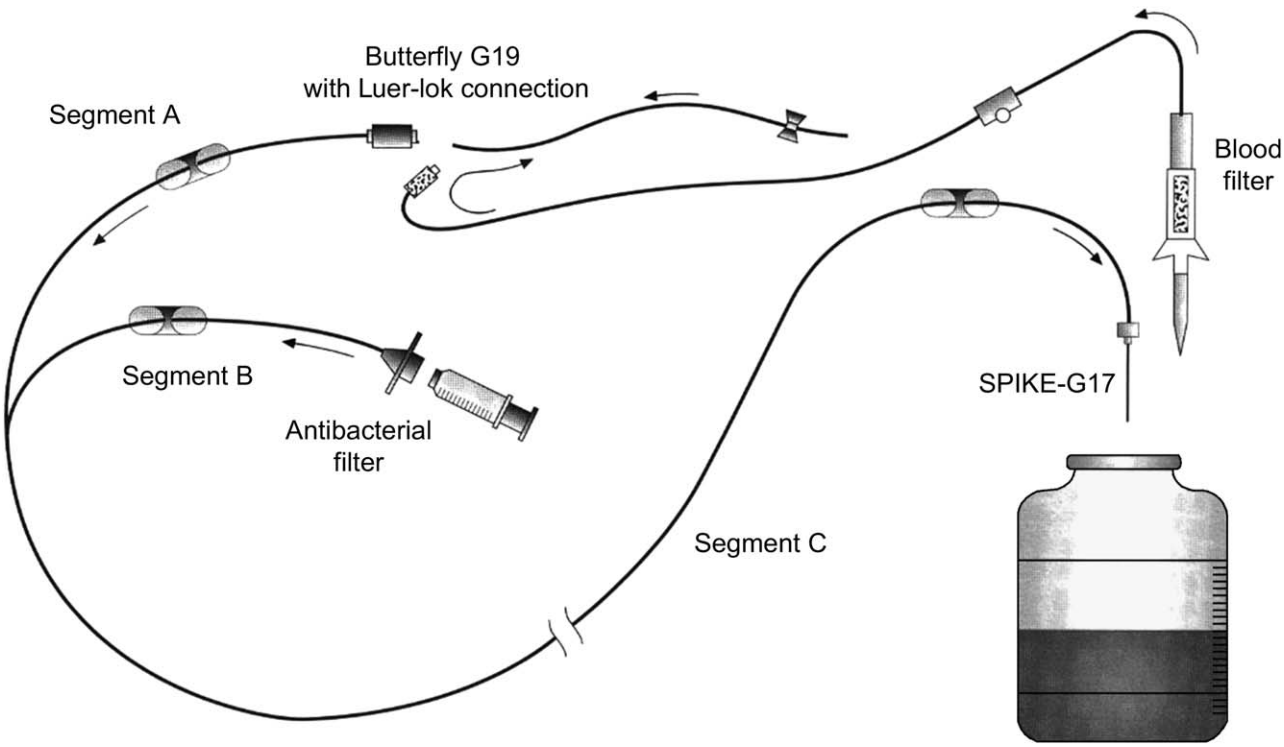

Figure 1. Schematic drawing of the components necessary to perform the ozonated autohemotherapy with an ozone-resistant glass bottle under vacuum.

Although patients rarely report a slight swelling and pain at the injection site, a mild sterile inflammatory reaction may take place with infiltration of monocytes and neutrophils scavenging denatured proteins, lysed erythrocytes and apoptotic cells. If plama contains some free virions (HCV, HBV, HHV, HIV and so on), these will be inactivated by the high ozone concentration and may act as an autovaccine. At the same time a moderate release of cytokines will modulate the physiological response (25), and the abundance of heme will upregulate the synthesis of both antioxidant enzymes and oxidative stress proteins, particularly of heme oxygenase I. It is wonderful that such a simple and autologous treatment can act as a powerful enhancer of several biological responses.

A variant and unnecessarily complicated procedure proposed in the 1990s consists of treating a similarly small volume of citrated blood with ozone, ultraviolet light (obviously generating more ozone and ROS) and heat (42.5°C) for 3 min. To my knowledge, without clarifying the rationale of using three physicochemical stresses, this method appears superfluous because ozone, as an oxidizer, is more than enough and the addition of other stresses makes the interpretation of the response very difficult. A first pilot study by Garber et al. (37) testing this technique in HIV patients was badly conceived and showed neither toxicity nor efficacy, but it has amply discredited the use of ozone. This approach has been subsequently used in patients with either vasculitis (38) or advanced chronic heart failure (39). As might be expected, two biological studies (40,41) have shown the possibility of controlling a chronic oxidative stress (33) and of activating regulatory T cells for downregulating a chronic inflammation. In conclusion, while I am not using this variant, I systematically couple the major and minor AHT as above described in all patients because I have noticed a potentiation of the biological and therapeutic effects. My opinion is that only by using a double-focused approach (it is less expensive than the variant minor AHT), able to simultaneously expand the interaction of ozonated messengers with both blood and muscular tissue, one can achieve a more rapid and intense therapeutic efficacy.

On the basis of experimental data obtained during the last decade (3–11) and on the average antioxidant capacity of human blood, we have determined the so-called 'therapeutic window,' that is the range of ozone concentrations (expressed as μg/mL of gas per mL of blood) within which ozone can exert therapeutic effects without toxicity with regard to major AHT. The range is surprisingly wide: 10–15 μg/mL as a minimum and 80 μg/mL as a maximum. Above 90 μg/mL, an incipient hemolysis (4–5%) warns about toxicity. The threshold level varies between 15 and 20 μg/mL, depending upon the individual antioxidant capacity. The scheme presented in Figure 2 is meant to illustrate the breadth of action expressed by the ozonated blood throughout the whole organism.

It is clear that the ozone oxidative activity is efficiently counteracted by the wealth of plasmatic and intracellular antioxidants so that an ozone concentration of 5–10 μg/mL per mL of blood is practically neutralized: only a trace of ROS and LOPs become detectable and therefore, at this very low level of ozonation, AHT may only have a placebo

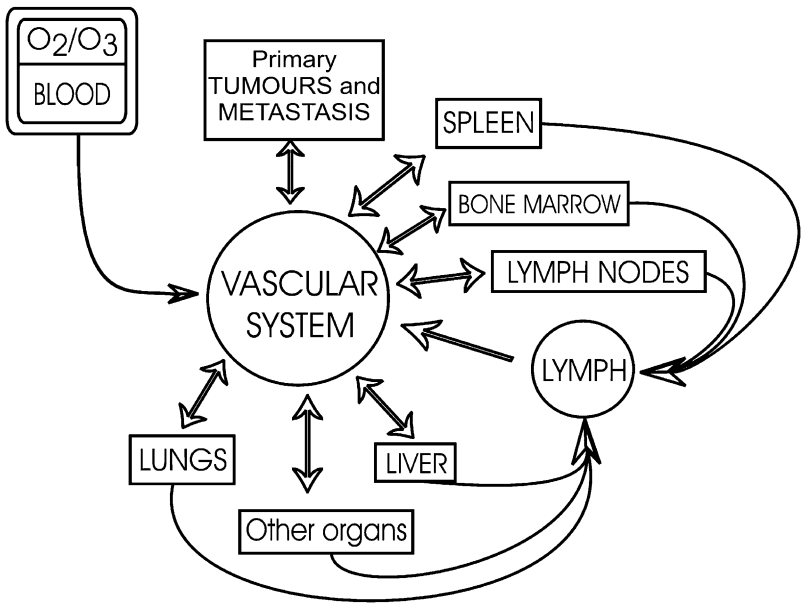

Figure 2. Ozonated blood, after reinfusion into the donor patient, is distributed throughout the whole organism. Erythrocytes continue to circulate in the vascular system delivering more oxygen into ischemic areas while leukocytes, migrated through post-capillary venules into various organs, slowly induce an immune response. Platelets will release their hormonal contents into the blood and will disappear. The reinfused LOPs undergo dilution into about 3 L plasma and 9–11 L interstitial fluid but will deliver the message of an acute oxidative stress to the whole body.

effect. As we are particularly conscious of ozone toxicity, we always apply the strategy ''start low go slow'' and, depending on the stage of the disease and the patient's condition, we usually scale up the concentrations from 15, then 20, 30 and 40 μg/mL, and more when necessary, during the 1st, 2nd, 3rd and 4th weeks, respectively. By using this strategy, after many thousands of autotransfusions, we have never recorded any acute or chronic toxicity. The venous puncture is usually well tolerated because it is performed with a G19 Butterfly needle (quite suitable for withdrawing blood into the glass bottle under vacuum) that remains inserted throughout the 35–40 min treatment. However, a small percentage of women have a very poor venous access: in this case we can select one of the following three options: rectal insufflation of gas, body exposure to gas, or the slow infusion into a visible vein on the hand dorsum, via a G25–27 needle, of an isotonic glucose solution containing a final concentration of 0.03–0.06% (8.8–17.6 mM) hydrogen peroxide (11,14). This last approach cannot be as effective as the classical ozonated AHT, but it is useful. We absolutely discourage the use of ozonated saline because it contains sodium hypochlorite and can cause phlebitis (14).

Normally we perform the treatment bi-weekly but, if necessary, we can do it every day or even three times daily.

When Ozone Therapy Should Be Used?

Whenever orthodox medicine fails to solve the medical problem, the physician has the duty to fully inform the patient of all valid options available before beginning ozone therapy. I dislike antagonizing ozone therapy to orthodox medicine because I believe that there is only good medicine, which is the one that is able to cure the patient.

So far our experience is ample only for chronic limb ischemia (11,42–45), cutaneous chronic ulcers due to ischemia and diabetes (10,11), and in the atrophic form of age-related macular degeneration (ARMD) (11). In chronic limb ischemia, the orthodox treatment is performed by prostanoid infusions, but the benefit is inferior and far more expensive than ozone therapy. Ozone therapy really helps about 70% of the ARMD (dry form) patients (11) because there is no other conventional option. The neovascular, exudative (or wet form) must be first treated with photodynamic therapy (46) or radiation (47) or with other experimental approaches based on blocking the activity of extracellular vascular endothelial growth factor (48).

I will then enumerate other pathologies where ozone therapy can be proficiently combined with orthodox therapies: 1) Acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi (11). Even parasitic infections such as giardiasis and cryptosporidiosis have been treated in children by Cuban physicians after administration of ozonated oil (11). 2) Osteomyelitis, pleural empyema, peritonitis, abscesses with fistulae, bed sores, chronic ulcers, diabetic foot, burns, insect and jellyfish stings, infected wounds, onychomycosis and candidiasis. These infections, often supported by antibiotic-resistant bacteria, like methicillin-resistant *Staphyloccocus aureus* and poor penetration of antibiotics into infected areas, are responsible for too many cases of death occurring in hospitals of even the most advanced countries. In such cases, ozonated AHT associated with the topical application of ozonated olive or sunflower oils allows a rapid disinfection and enhances healing tremendously. Unfortunately, the use of ozonated oils is hardly known and a detailed description of their preparation, application and results is reported in my most recent book (11). It is most interesting that ozone, an unstable gas, can be stably trapped as an ozonide between a double bond of a PUFA: $—(CH_2)_7—O_3—(CH_2)_7CH_3$. When the ozonated oil is layered over the ulcer's exudate at the oil−water interface, the ozone moves slowly into the water and, by reacting with biomolecules, generates a steady flow of H_2O_2. The effects of sterilization and improved oxygenation are responsible for the accelerated cicatrization. In comparison to pharmaceutical creams often containing useless antibiotics and growth factors, once ozonated oil is known and used, it will be extremely beneficial to millions of patients. 3) Herpetic infections (HHVI and II), herpes zoster and papillomavirus infection. The modality of the intramuscular injection of minor ozonated AHT, used as an autovaccine and associated with the topical therapy with ozonated oil, is very effective in preventing relapse of herpetic infections. This approach, particularly when used in combination with the acyclovirs, can cure herpetic infections in the majority of patients (11). It must be mentioned that a new vaccine can significantly reduce the incidence of herpes zoster infection and post-herpetic neuralgia (49). Chronic hepatitis-C and HIV infections, whenever possible, must be basically treated with either PEG-interferon alpha + ribavirin or highly active anti-retroviral therapy, respectively, because these drug combinations usually lower the viral load rapidly. However, ozone therapy could be simultaneously performed as a useful adjuvant treatment (11). 4) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Crohn's disease): results with AHT seem encouraging but are anecdotal. 5) Other chronic ischemic diseases (cerebral and heart ischemia). Ozone therapy exerts beneficial effects because it can a) increase oxygen, glucose and ATP delivery within ischemic tissues, b) enhance neoangiogenesis and possibly facilitate the implantation of bone marrow stem cells, which can provide neovascularization and tissue regeneration, c) induce the preconditioning phenomena by upregulating the expression of antioxidant enzymes and heme oxygenase I and d) trigger a neurohumoral response for improving quality of life. Our preliminary study (11) in end-stage cardiopathic patients, when either transplantation or

surgical revascularization was no longer feasible, has already shown that ozone therapy combined with the conventional best medical therapy can improve a gloomy prognosis. 6) Degenerative disorders: AHT helps patients in the early phase of senile dementia. On the other hand, it is rarely and minimally useful in diabetic retinopathy, retinitis pigmentosa, sudden hearing loss and chronic tinnitus. 7) Pulmonary diseases: emphysema, asthma, chronic obstructive pulmonary disease (COPD) and acute respiratory distress syndrome. COPD is becoming the fourth cause of death in spite of orthodox therapy based on the inhaled combination of corticosteroids plus long-acting β2-agonists and antibiotics, when necessary (50). Unfortunately these drugs, if prolonging the patient's life, do not arrest the progression of the disease. The rationale for using ozone therapy is briefly based upon a) blood reinfusion, LOPs, present in low concentrations act on the vast endothelial surface and enhance the release of prostacyclin and NO while release of endothelin-1 is depressed (8,15). It is known that the release of NO and S-nitrosothiols represents the physiological mechanism for vasodilation (51,52) and contrasts the release of the anion superoxide, which causes vasoconstriction and deploys negative influences on platelets and endothelial cells. Secondly, the delivery of oxygen in ischemic tissues is enhanced and the progressive increase of antioxidant enzymes and heme oxygenase-1 counteracts the chronic oxidative stress, typical of pulmonary diseases. Moreover, the mild stimulation of the immune system helps to contain recurrent and chronic pulmonary infections. Recently, I have been able to treat advanced COPD patients with very encouraging results demonstrated by a marked improvement of the respiratory parameters and the walking test (11). 8) Terminal nephropathies are progressively worsened by a chronic oxidative stress not yet controllable by orthodox medicine and therefore ozone therapy could stabilize this serious dysfunction and improve the quality of life of these patients (11). 9) In a similar manner, ozonated AHT combined with topical application of ozonated oil is proving to be very useful in the metabolic syndrome well exemplified in patients with type 2 diabetes suffering from chronic ulcers with no tendency to heal (11). There is no doubt that patients prefer ozone therapy to hyperbaric oxygen and local larval (maggot) therapy (53). Needless to say, we must continue to strictly control the glycemic level. 9) Skin diseases (psoriasis, atopic dermatitis): available data seem positive but there are no randomized studies. 10) Chemoresistant metastatic cancer; therapy of cancer-related fatigue: we have reported (11,54) that a 6-month, biweekly, ozone therapy session in preterminal patients previously heavily treated with chemo- or/and radiotherapy does improve their quality of life but is unable to block cancer progression. On the other hand, ozone therapy may be far more useful immediately after surgery, possibly combined with chemo- or/and radiotherapy. Not only could it potentiate the effect of the cytotoxic drugs but by inducing the antioxidant response, it could reduce chemotoxicity (55). It is deplorable that oncologists do not want to cooperate and want to apply only their protocols. Meantime, even if survival is moderately prolonged at the cost of a poor quality of life, the mortality remains very high. Peter Boyle, Director of the International Agency for Cancer Research in Lyon, France has communicated that in Europe, in 2004, new cancer cases amounted to 2.9 million with over 1.7 million deaths. These impressive numbers indicate that the war on cancer remains wide open and that a skeptical attitude against the use of ozone therapy is unjustified. 11) Orthopedic diseases (the problem of backache): the direct intradiscal injection of oxygen-ozone is a great success in about 75% of patients (11,56) and is one of the few modern techniques able to solve the problem of a hernial disc with a mini-invasive approach. The indirect procedure that I defined as a ''chemical acupuncture'' consists of injecting 10-20 mL of gas into the paravertebral muscle corresponding to the metameres of the disc; it is also effective in about two thirds of patients but, in this case, the mechanism of action is linked to the activation of the antinociceptor system. The gas injection appears also effective in alleviating osteoarthrosis and several other joint–tendinitis affections. 12) Chronic fatigue syndrome and fibromyalgia: AHT has been found beneficial in the majority of patients (11). 13) Dentistry and stomatology: ozone has been found very useful for treating primary root carious lesions (57). Moreover, local application of ozonated oil in aphthous ulcers (cold sores) occurring on the tongue, lips and cheeks of many people allows an extremely rapid healing and disappearance of pain (11). 14) Emergency situations such as those occurring after extensive trauma, burns, acute peritonitis and toxic sepsis often lead to multiple organ failure and death. The combination of the best orthodox therapy with three to four daily mild ozonated major AHT can prevent or reduce the worsening of the metabolic impairments and reduce mortality. Moreover, patients waiting for organ (particularly heart) transplantation may improve resistance to infections and immunesuppression (due to anesthesia and surgery) if they could undergo six to eight major and minor AHTs presumably during 6–15 days before surgery. During heart transplantation, organs such as the brain and kidneys may be damaged by the ischemia reperfusion syndrome that can be attenuated by previous adaptation to oxidative stress. A similar concept could be adopted for scheduled complex operation or application of joint implants. This sort of prophylactic ozone therapy, with little effort and expense, may reduce the risk of infections, shorten the hospitalization and save money. However, the implementation of the prophylactic ozone therapy remains a dream in so far as World Health Authorities remain aloof and entangled in economic and political problems.

Hyperbaric Oxygen Therapy (HOT) and Ozone Therapy

It appears relevant to briefly clarify the validity and scopes of these two different approaches. In the hyperbaric chamber, the breathing of pure oxygen at 2.6 atmospheres greatly increases the solubilization of oxygen in the plasma (about 5 mL/dL) so that the dissolved oxygen is sufficient to satisfy the cellular requirements even in ischemic tissues. That is the reason why patients with chronic limb ischemia, or with diabetic foot, or ARMD often undergo HOT. Unfortunately, this is only a palliative treatment because, after 2 h, as soon as the patient comes out of the chamber, hypoxia resumes in the ischemic areas and the therapeutic effect is minimal and temporary. On the other hand, during ozone therapy, while the hyperoxygenation of the reinfused blood has a negligible relevance, the ozone triggers a series of biological mechanisms that lead to normalizing the delivery of oxygen for several days with consequent therapeutic effects. Two excellent reviews (58–59) clarify the exclusive role of HOT in air embolism, decompression sickness, CO-poisoning and clostridial myonecrosis but, regrettably, do not examine the relevance of ozone therapy. Indeed, they objectively report that HOT may be useful in chronic limb, heart and cerebral ischemia, autoimmune colitis, sickle cell anemia, chronic osteomyelitis, ARMD, diabetic foot, thermal burns, extensive chronic ulcers and bed sores, but the actual evidence is flawed and anecdotal. All of these latter conditions can instead greatly benefit by the use of parenteral (and when necessary topical) ozone therapy because the multiple mechanisms of action of ozone can correct pathologies linked to ischemia, infections, delayed healing and chronic oxidative stress (reviewed in Reference 11). In conclusion, both HOT and ozone therapy are important, but it is necessary to understand that their respective field of application is different and each approach must be used profitably only in selected pathologies.

Conclusions and Perspectives

I often ask myself if ozone therapy is obsolete or worthwhile being pursued. Our many treated patients answer for me and they loudly say that it is very beneficial. The compliance is excellent and the patients, as soon as the therapeutic effect declines, ask for a new cycle. This is an excellent proof that provided we are using judicious ozone concentrations, there is neither acute nor chronic toxicity. It has been unfortunate that, in the past, the direct intravenous injection of the gas, now prohibited, and misuse of ozone by incompetent quacks has generated the dogma that ozone is toxic and should not be used in medicine. This concept is wrong and has also been based first on non-physiological studies (60) performed in washed erythrocytes, hence unprotected by the plasma antioxidants and second, in not recognizing the profound difference between the endogenous chronic oxidative stress, occurring every day during a lifetime or during a chronic disease, and the calculated, extremely brief and exogenous oxidative stress that we induce on blood by using a precise and small ozone dose. We know that any drug, depending upon its dosage, can be either therapeutic or toxic. The following elementary observation is even more compelling: the normal glucose concentration in the plasma ranges between 0.7 and 1 mg/mL and is essential for survival. However, when this concentration falls below 0.4 mg/mL, the consequent hypoglycemic coma can be deadly. On the other hand, if the glucose concentration remains constantly above 1.3 mg/mL, it induces the metabolic syndrome, as is well exemplified by the current diabetic epidemic. Thus, the dogma about ozone toxicity is futile because, after millions of treatments, we have never observed any acute or chronic toxicity. Moreover, most of the patients report a feeling of wellness.

Needless to say, ozone therapy does not ‘‘cure’’ ARMD or other chronic pathologies but, by performing the maintenance therapy, it does improve the condition and maintain a good quality of life. On the other hand, even orthodox medicine, with the exception of several infectious diseases thanks to antibiotics, antivirals, antibodies and vaccines and far less frequently of cancer thanks to surgery/chemotherapy, is unable to "cure" most human diseases such as atherosclerosis, advanced cancer, diabetes, degenerative, metabolic and autoimmune diseases.

We are certainly not blinded by ozone therapy but the great strides of molecular biology and gene therapy during the last decade have not yet been paralleled by comparable advances in therapeutic innovations and many unforeseen difficulties still have to be overcome (61). I do not want to diminish scientific achievements but simply to point out that we are often unable to predict the pitfalls when new treatments are applied from mice to patients. This is probably one reason for the worldwide boom of complementary medicine, not only in underdeveloped countries but also in the U.S. Patients, as human beings, are often disappointed by the high-tech therapist. Moreover, conventional therapy often has side effects, and about 55,000 Americans may have died as a result of taking the now infamous Vioxx (62,63).

Ozone therapy is capturing increasing attention all over the world, since our studies reported in two books (10,11) have clarified the main biochemical mechanisms of action and the real possibility of taming ozone toxicity. We now have the first comprehensive framework for understanding and recommending ozone therapy in a few diseases as a first choice and in combination with orthodox therapy in many others. Indeed, one important characteristic of ozone therapy is that it can be experimentally verified both at the biochemical and clinical levels.

So far, the most advanced and reliable approach has been the major ozonated AHT but today we also have other technical possibilities and we can select the optimal method for different pathologies. As far as chronic diseases are

concerned, the problem is that official medicine tends to treat symptoms rather than the cause(s) of the disease. Besides the fact that the etiology is too complex or remains obscure, the treatment is often limited and remains unsatisfactory. On the other hand, a simple gaseous molecule like ozone, that probably is even produced *in vivo* (64), by acting on many targets, at least in part can recover functional activities that have gone astray. We have good reasons to believe that the therapeutic power of ozone therapy consists of simultaneously improving circulation and oxygen delivery, in enhancing the release of autacoids, growth factors and cytokines and in reducing the endogenous, chronic oxidative stress. In other words, ozone therapy seems to act as a biological response modifier.

Finally, I cannot omit mentioning some drawbacks. Although the cost of ozone is very low, it represents an impractical drug because it is unstable and cannot be stored in any form. However, by using a portable ozone generator we can perform domiciliary AHT treatments, useful for the elderly and for those patients with chronic diseases. Moreover, rectal insufflation of gas can be easily done by the patient at home, under the ozone therapist's supervision. Topical therapy of chronic ulcers and infectious wounds with ozonated oil is very practical and easy because we have standard and stable preparations. The last, but certainly not the least, problem is the lack of financial support for performing controlled and randomized clinical trials, whose results are critical and urgently needed to prove the validity and atoxicity of ozone therapy in various diseases. Objective results from clinical studies represent the unique possibility of convincing the biased opponents of this approach. The private ozone therapist, or even the small existing national associations, in comparison to the pharmaceutical industries that can register an annual profit of 340 billion dollars, have no financial power and how can an ant compete with an elephant? Really, we do not want to compete with official medicine, but only help patients to regain health.

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Basic and clinical results regarding ozonetherapy during the last three years

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Abstract

During the last three years, our work aimed to further clarify either **(A)** the fundamental aspect of the ozone tolerance and to improve **(8)** the therapeutic results achieved with ozone therapy in some human diseases.

(A) As far as the problem of ozone tolerance is concerned, it has been demonstrated that a differential enhancement of antioxidant enzymes is ozone dose-dependent in Jurkat T cells incubated for 24, 48 and 72 h after an ozonation period of 10 min with a cell/gas volumes ratio of 1. Precise ozone concentrations ranging from 1.5 up to 72 IJg/ml (31.5-1512 IJM) were tested. The proliferation index declined progressively and was ozone dose-dependent. The response of enzymatic activities varied depending upon the enzyme and ozone concentrations: glucose-6-phosphate dehydrogenase (G6PD) began to increase at an ozone dose of 6 IJg/ml (126 IJM), reached a peak at 12 IJg/ml (252 IJM) and rapidly declined thereafter: on the other hand activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-R) increased progressively from the ozone concentration of 12 IJg/ml, clearly showing a protective role (Larini et al.,2003).

The process of ozonation includes the release *ex vivo* of reactive oxygen species (ROS) and *in vivo* of lipid oxidation products (LOPs), which act as early and late messengers, respectively. A successive study has revealed that a typical LOP, 4-hydroxynonenal (HNE), which is one of the major end products of lipid peroxidation, in relation to its dose, can behave either as a physiological or a toxic signals in vitro. We have studied some biochemical effects of HNE, at various concentrations (0.1-100 J..IM), on Jurkat T cells incubated thereafter for 24, 48 and 72 h. HNE at very low concentrations significantly enhanced the proliferation index, whereas at higher concentrations progressively blocked cell proliferation. SOD, GSH-Px and GSH-R increased progressively with HNE concentrations, particularly GSH-Px. G6PD showed a different pattern, increasing at low HNE (1-5J..1M) concentrations and rapidly declined thereafter. These results have shown that HNE induces growth inhibition of Jurkat T cells and regulates the activity of typical antioxidant enzymes. Furthermore the useful protective effect of doubling the volume of fetal calf serum points out the serious and widely overlooked risk that cultured cells undergo oxidative stress during incubation (Larini et aI., 2004).

Furthermore we have investigated the release of cytokines from isolated peripheral human blood mononuclear cells (PBMC) exposed to various ozone concentrations for 10 min and the release of both proinfiammatory and immunosuppressive cytokine after 24, 48 and 76 hours incubation (Larini and Bocci, 2004a). Ozonation was performed by exposing for 10 min equal cell numbers and volumes of cell suspension to equal volumes of a gas mixture (1:1 ratio) composed of oxygen- ozone with precise ozone concentrations ranging from 1.0 up to 80 IJg/ml (0.02 up to 1.68 mM). Markers of oxidative stress showed a significant relationship between ozone dose and both lipid peroxidation and protein thiol group contents. With the exception of the lowest ozone concentration, the cytokine production of PBMC was depressed particularly at concentrations from 40 J..Ig/ml upwards. There was no significant effect on IL-6 production between exposed or unexposed cells, up to 72 h of incubation. IL-4 production was markedly affected by ozone exposure, showing a marked decrease even at the lowest ozone concentration (2.5 IJg/ml) already after 24 hours incubation. On the other hand, production of IFN-y and TNF-a was slightly stimulated by the lowest ozone dose either at all times or only after 72 h incubation, respectively. Analysis of the proliferation index (PI) is consistent with these results showing that, while the lowest concentration stimulates it, progressively increasing O_3 concentrations inhibit the PI. These data show that there is a significant relationship between cytokine production and ozone concentrations and that PBMC are very sensitive to oxidation particularly in presence of serum with low antioxidant capacity and lack of erythrocytes, which can quickly recycle dehydroascorbic acid back to ascorbic acid (May et aI., 1996).

These results have shown the critical protective effect of soluble antioxidants, which are deficient in tissue culture fluids. Both uric and ascorbic acids and particularly albumin are the most effective

and readily available antioxidants during human blood ozonation (Larini and Bocci, 2004b): even more interesting is the fact that dehydroascorbic acid is reduced within three min by GSH and thioredoxin reductase by an extremely efficient recycling system operated by erythrocytes (Mendiratta et aI., 1998). That is one of the reasons why the antioxidant capacity of plasma during ozonation of blood decreases transitorily of only 20-25 and does not procure any cell damage (Bocci, 2002; 2004). On the other hand experiments *in vitro* suffer from a severe limitation of antioxidants and have erroneously led many cell biologists to conclude that ozone is always toxic. Moreover our studies *in vitro* have fully confirmed that a cycle of ozonated autohaemotherapy (AHT) in patients induces an adaptation to the repeated and precisely calculated **acute** oxidative stresses. This phenomenon is one of the most important results obtainable in patients with chronic diseases under **chronic** oxidative stress. This apparently paradoxical (an oxidant like ozone enhances the antioxidant system!) result, shown in both experimental animals (Leon et aI., 1998) and patients (Bocci, 2002) demonstrates that ozone is a drug with the unique property of re-equilibrating the impaired redox balance in several dysmetabolic diseases.

(B) As far as clinical results are concerned, we have had the opportunity of applying ozone therapy to the following diseases:

1) Age-related macular degeneration (ARM D);

2) Chronic limb ischemia in atherosclerotic and diabetic patients;

3) Bed sores and one case of necrotizing fasciitis;

4) Chronic fatigue syndrome and fibromyalgia Borrelli and Bocci, 2002)

5) Chemotherapy -resistant cancer in preterminal patients (Bocci, 2004).

The therapeutic window that we have determined on an experimental basis ranges between 10 and 80 fJg/ml gas per ml of blood for patients undergoing ozonated AHT remains valid and, within this range, there is neither haemolysis, nor methemoglobin formation, nor variations of enzymatic levels (U/g Hb of SOD, GSH-Px, GSH-R and G6PD) assessed in erythrocytes after blood ozonation (Bocci, 2002; 2004). Obviously for each type of pathology we have selected the most suitable method (major and minor HAT, extracorporeal circulation of blood against 0203, infusion of the "gluco-peroxide" solution (Bocci et aI., 2004), rarely rectal insufflation of gas and topical therapy with ozonated water and oil) and what we believe to be the optimal range of ozone concentrations. We use the strategy "start low, go slow" or, in other words, in order to achieve the adaptation to the oxidative therapy, we start with a low dose that is escalated every week to the optimal level. Neither acute, nor chronic side-effects have ever been noted.

In diseases 1-4, we have observed good or excellent improvements in the majority of patients and these results confirm the validity of ozone therapy in chronic infectious diseases and in ischaemic-degenerative diseases. However in preterminal, neoplastic patients, we could improve the quality of life for some 4 to 6 months but the already extensive metastatization could not be stabilized (Bocci, 2004). This last result, although discouraging, was not unexpected because we have treated patients at too later stage due to the chemotherapist's obstinacy to continue cytotoxic therapy in already chemoresistant-neoplasms. The rational concept to start ozone therapy immediately after surgery, or during the initial chemotherapy, is unfortunately opposed by the oncologists and it is difficult for the patient to refuse the radio-chemotherapy treatments. However we must persevere in our endeavor because so far gene and vaccine therapy have been disappointing with objective response rate of only 2.6 (Rosenberg et aI., 2004). It is hoped to be able to discuss these clinical data with other ozonetherapists because we feel that there is room for further improvement.

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Original article

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TREATMENT OF HERNIATED LUMBAR DISC BY INTRADISCAL AND INTRAFORAMINAL OXYGEN-OZONE (O_2-O_3) INJECTION

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SUMMARY

Material: We report our experience between May 1996 and May 2003 with 2200 patients affected by low back pain or sciatica due to herniated disk treated by intradiscal and intraforaminal oxygen-ozone injection.

The patients received medical and physical therapy before treatment for at least 2 months; the patients with conus-cauda syndrome and hyperalgesic sciatica were excluded. We never performed discography before the treatment that was performed under CT guidance or fluoroscopy. CT provided monitoring of gas distribution in the disk and epidural space.

Results: No side effects were recorded at short and long-term follow-up. Clinical results were evaluated with the modified McNab method showing an 80% success rate and 20% failure rate in 1750 patients followed up to 6 months while the success rate dropped down at 75% and failure increased at 25% in 1400 followed up to 18 months. CT showed reduction in the size of the herniated disk in only 63% of the followed patients (420 patients).

The failure has been mostly related to: calcified herniated disk; spinal canal stenosis; recurrent herniated disk with epidural fibrosis; small descending herniated disk at the level of the lateral recess.

Key words: oxygen-ozone therapy, CT, Low back pain, sciatica.

RÉSUMÉ

Le traitement des hernies discales lombaires par injection intradiscale et intraforaminale d'O_2-O_3

Matériel : 2 200 patients souffrant de lombalgie ou sciatique en rapport avec une hernie discale ont été traités entre mai 1996 et mai 2003 par injection intradiscale et intraforaminale d'O_2-O_3. Tous les patients avaient bénéficié d'un traitement médical pendant au moins 2 mois. Les patients souffrant de syndrome de la queue de cheval ou de sciatique hyperalgique ont été exclus. L'injection d'O_2-O_3 était faite sous contrôle scanographique ou scopique. La scanographie a permis de contrôler la distribution des gaz dans les espaces discal et extradural.

Résultats : Aucun effet secondaire n'a été observé. Les résultats cliniques ont été évalués selon la méthode de Mac Nab modifiée à 6 mois (1 750 patients suivis) et à 18 mois (1 400 patients suivis) les taux de succès ont été respectivement de 80 % et 75 %. L'examen scanographique a montré une réduction de la hauteur de disque de 63 % de patients suivis (n = 420). Les causes d'échec sont principalement les disques calcifiés, les canaux étroits, les récidives de hernie avec filomes épiduraux, les petites hernies migrées dans le récessus latéral.

Mots-clés : *ozone thérapie, scanographie, lombalgie, sciatique.*

INTRODUCTION

Nerve root pain and low back pain are one of the commonest conditions affecting the lumbar spine. Around 80% of the population in western countries will experience at least one episode of low back pain in their lifetime and 55% suffer from low back pain associated with radicular syndromes [14]. Back pain is often caused by disc disease even though other factors are responsible for nerve root syndromes and should be entertained when clinical symptoms fail to correlate with CT and/or MR findings [17].

In this paper, we report our experience between May 1996 and May 2003 with 2200 patients affected by low back pain or sciatica due to herniated disk treated by intradiscal and intraforaminal oxygen-ozone injection.

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MATERIAL AND METHODS

Material

In the five-year period from Juanary 1997 to May 2003, 2200 patients aged between 13 and 89 years underwent percutaneous chemonucleolysis with periradicular and pariganglionic injection of oxygen-ozone mixture.

The following selection criteria were adopted for inclusion:

1) clinical: low back and/or nerve root pain resistant to previous medical treatment, physiotherapy and other therapies (manipulation, acupuncture, etc.) for a period of not less than two months;

2) psychological: a firm resolve on the part of the patient to recover with a commitment to cooperate and undergo subsequent physiotherapy with postural and motor rehabilitation;

3) neurological: paresthesia or hypoesthesia over the dermatome involved, mild muscle weakness and signs of root-ganglion irritation;

4) neuroradiological (CT and/or MR): a) evidence of small and medium-sized herniated discs correlating with the patient's symptoms with or without degenerative disc-vertebra disease complicated by intervertebral disc changes (protrusion, herniation); b) residue of surgical (micro)-discectomy with herniation recurrence and/or hypertrophic fibrous scarring.

The exclusion criteria were:

1) CT/MR evidence of a herniated disc fragment with symptoms of motor and/or sphincter disturbance;

2) CT/MR evidence of disc herniation corresponding to clinically severe motor deficit and/or sphincter disturbance.

The indications for O_2-O_3 treatment were extended to FBSS patients when it was understood that the ozone mechanisms of action could be exploited to treat the chronic inflammation and venous stasis present in FBSS. Technical approach to the disc is the same as that used for both discography and other percutaneous intervertebral disc procedures. We used an 18-20G Chiba needle inserted from a posterior paravertebral oblique approach under CT or fluoroscopic guidance. The L5-S1 space is not always an easy target to reach and may require additional 30° craniocaudal inclination of the needle. Once the needle has been positioned in the centre of the disc, the gas mixture is injected into the disc (3-4ml) and into the epidural and intraforaminal spaces (10ml) at the concentrations of 30 micrograms/ml *(figures 1 and 2)*.

We no longer perform discography before percutaneous treatment as the procedure adds no further diagnostic information needed for treatment. A CT scan is done before therapy to rule out the presence of a retro-psoas bowel loop.

CT guidance was adopted instead of the well-tested radiological monitoring by isocentric angio suite with double arm due to the need for meticulous positioning of the needle within the nucleus pulposus. In addition, CT avoids the use of intradiscal contrast administration which even in low doses

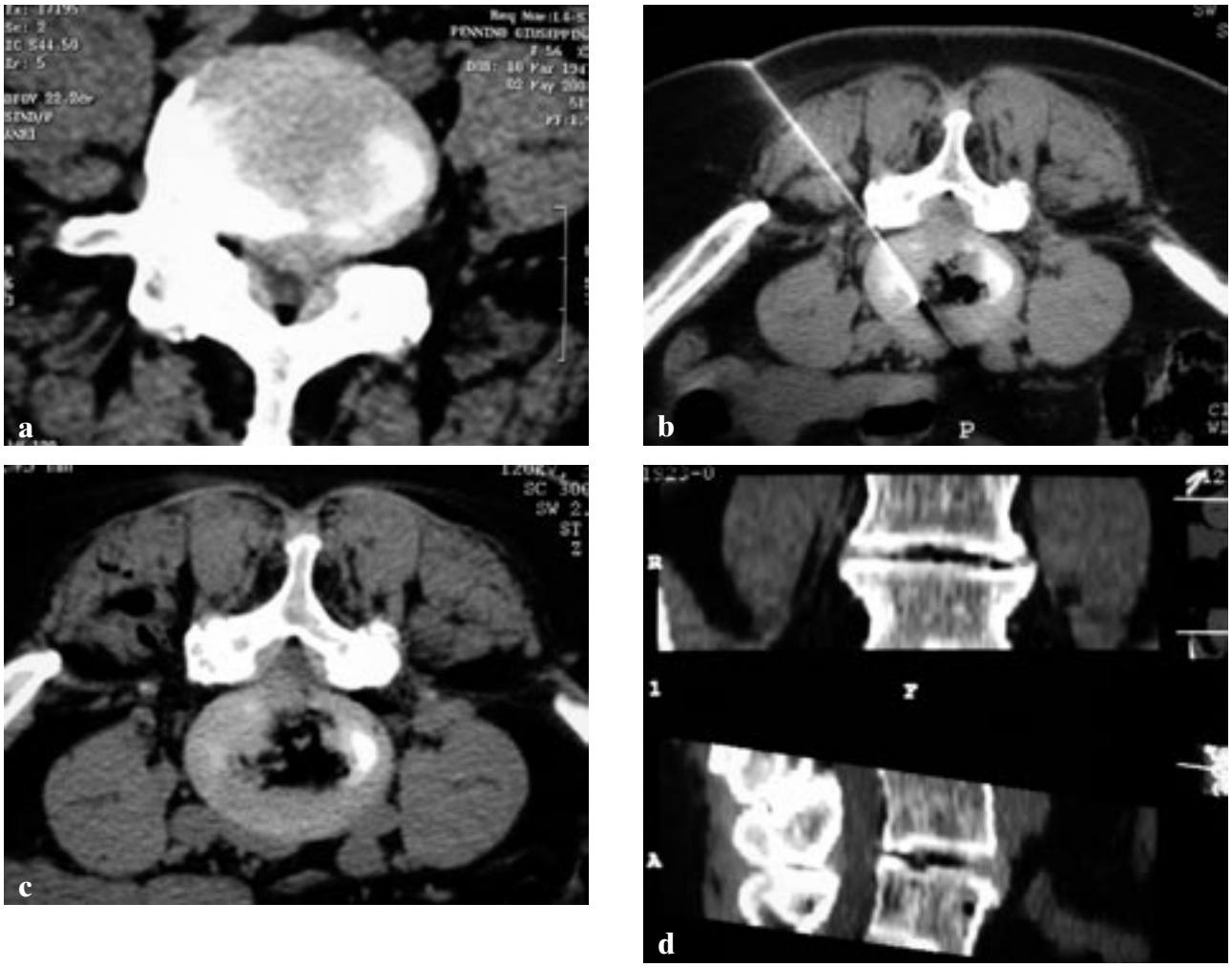

FIG. 1. – Patient with median and left paramedian L4-L5 herniated disk (a). Intradiscal positioning of the 20G Chiba needle under CT guidance (b). CT control after intradiscal injection of 4ml of oxygen ozone mixture (c) with evidence of intramuscular gas diffusion. Coronal and sagittal (d) MPR CT images with evidence of gas within the disk.

FIG. 1. – *Hernie discale postérolatérale L4-L5 (a). Positionnement d'une aiguille de Chiba (20 G) sous scanographie (b). Contrôle scanographique après injection du mélange O_2-O_3, noter la diffusion moléculaire du gaz (c). Reformatage dans les plans frontal et sagittal montrant la présence de gaz dans le disque (d).*

reduces the discal absorption of ozone and the space available and hinders the search for the site of intraforaminal injection of the O_2-O_3 mixture.

A CT scan was always performed to confirm the intradiscal injection, and the epidural and intraforaminal diffusion of the O_2-O_3 mixture.

Methods

There are many and different protocols to analyze in an objective way the clinical results in patients with low back pain and HNP (16-18). We evaluated our results according to a modified Mac Nab method *(table I)* in the following situations: degenerative disease complicated by herniated disk , L4-L5 or L5-S1 herniated discs, multiple disc herniations, FBSS, calcified disc herniations and disc herniations associated with spinal stenosis.

The first three situations represented 78% of all treated patients. Evaluation at 6 months was performed clinically in all 2200 patients with CT or MR Follow-up obtained in 420 patients while clinical follow-up evaluation at 18 months was available for 1400 patients *(table I)*.

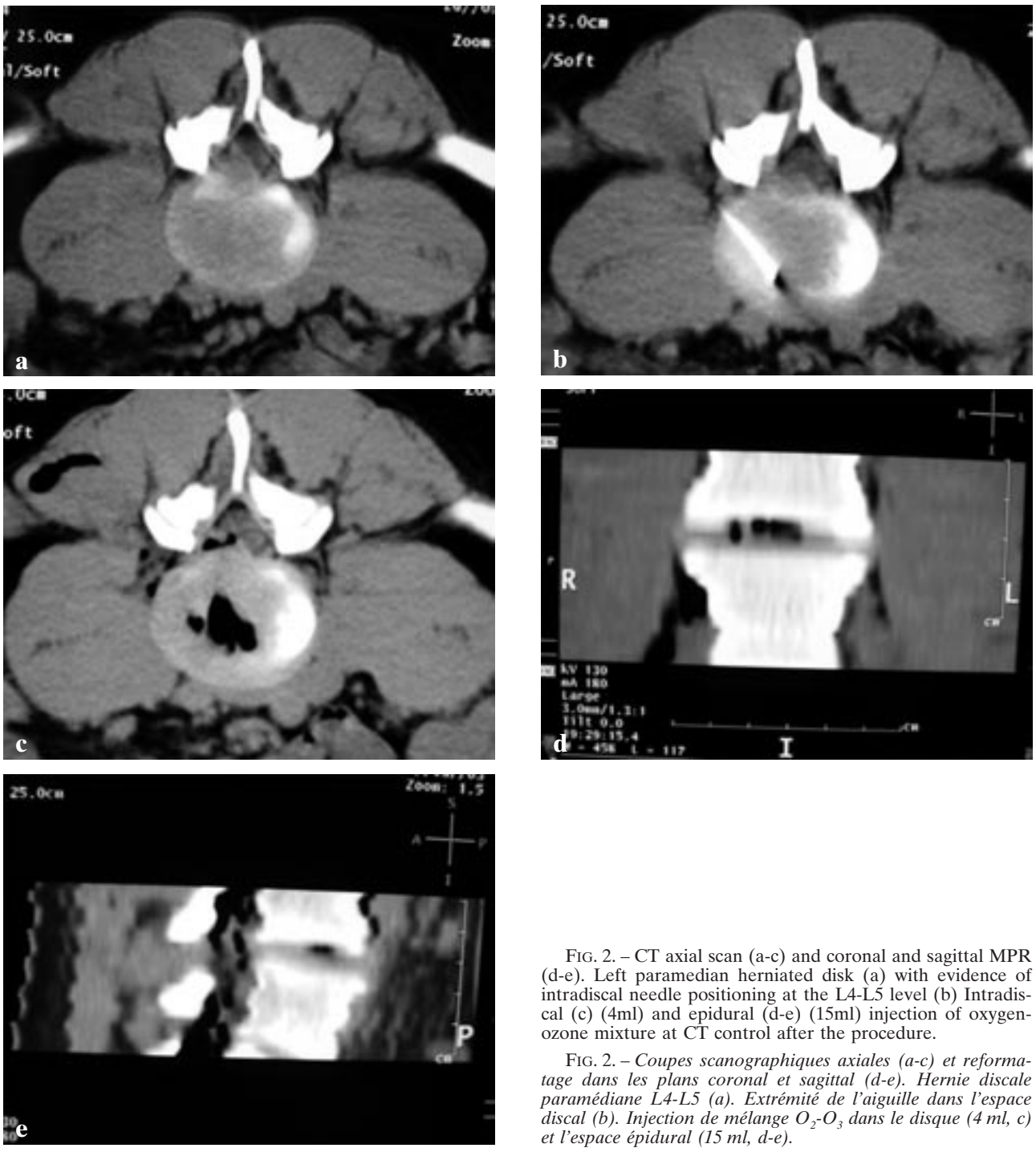

FIG. 2. – CT axial scan (a-c) and coronal and sagittal MPR (d-e). Left paramedian herniated disk (a) with evidence of intradiscal needle positioning at the L4-L5 level (b) Intradiscal (c) (4ml) and epidural (d-e) (15ml) injection of oxygen-ozone mixture at CT control after the procedure.

FIG. 2. – *Coupes scanographiques axiales (a-c) et reformatage dans les plans coronal et sagittal (d-e). Hernie discale paramédiane L4-L5 (a). Extrémité de l'aiguille dans l'espace discal (b). Injection de mélange O_2-O_3 dans le disque (4 ml, c) et l'espace épidural (15 ml, d-e).*

TABLE I. – Modified Mac Nab method.
TABLEAU I. – *Méthode de Mac Lab modifiée.*

Success	Failure
Excellent — disappearance of symptoms — complete recovery in working and sport activities	**Mediocre** — insufficient improvement of symptoms — periodic administration of drugs — limitations of physical activity
Good — occasional episodes of low back pain or sciatica	**No results** — no improvement — surgery required
Fair — improvement of symptoms — limitation of heavy physical activity	**Bad** — worsening of clinical situation — surgery required

RESULTS

The results at 6 and 18 months are summarized in *tables II and III*.

Clinical follow-up for up to 18 months in 1400 patients confirmed persistent good outcome in 75% of cases. CT or MR follow-up was done in 420 patients, documenting a reduction in herniated disc size only in 63% of cases *(figure 3)*. We analyzed the failures reported herein, focusing on possible technical errors to establish whether indications for treatment had been too broad or whether correlations exist between certain types of herniated disc, site of herniation, type of intervention and treatment failure.

Retrospective analysis of our failures disclosed that a successful outcome was much more unlikely in the presence of calcified herniated discs, herniations associated with stenosis of the spinal canal and large extruded herniations.

DISCUSSION

We know from the natural history of herniated disc that clinical symptoms tend to disappear in up to 50% of patients and the disc herniation can shrink at CT or MR scans within eight to nine months after the onset of back pain, but not all patients can wait so long before improve symptoms [5-14]. The short-term success rate after surgery for lumbosacral disc herniation is around 95-98% with a 2-6% incidence of true recurrence of herniation. This percentage decreases to around 80% in the long-term due to the onset of symptoms linked to Failed Back Surgery Syndrome (FBSS) characterised by recurrence and/

TABLE II. – Results at 6 month follow-up.
TABLEAU II. – *Résultats à six mois.*

In patients with degenerative disease complicated by herniation: — excellent in 40% — good or fair in 40% — mediocre or bad in 20%
In patients with L4 L5 or L5-S1 herniated discs: — excellent in 64% — good or fair in 13% — mediocre or bad in 23%
In patients with multiple disc herniations: — excellent in 58% — good or fair in 11% — mediocre or bad in 31%
In FBSS patients: — excellent in 45% — good or fair in 20% — mediocre or bad in 35%.
In patients with calcified disc herniations: — excellent in 35% — good or fair in 20% — mediocre or bad in 45%
In patients with herniated disc associated with stenosis: — excellent in 25% — good or fair in 25% — mediocre or bad in 50%

TABLE III. – Results at 18 month follow-up.
TABLEAU III. – *Résultats à 18 mois.*

In patients with degenerative disease complicated by herniation: — excellent in 40% — good or fair in 38% — mediocre or bad in 22%
In patients with L4 L5 or L5-S1 herniated discs: — excellent in 62% — good or fair in 14% — mediocre or bad in 24%
In patients with multiple disc herniations: — excellent in 56% — good or fair in 12% — mediocre or bad in 32%
In FBSS patients: — excellent in 43% — good or fair in 19% — mediocre or bad in 39
In patients with calcified disc herniations: — excellent in 33% — good or fair in 18% — mediocre or bad in 49%
In patients with herniated disc associated with stenosis: — excellent in25% — good or fair in 25% — mediocre or bad in 50%

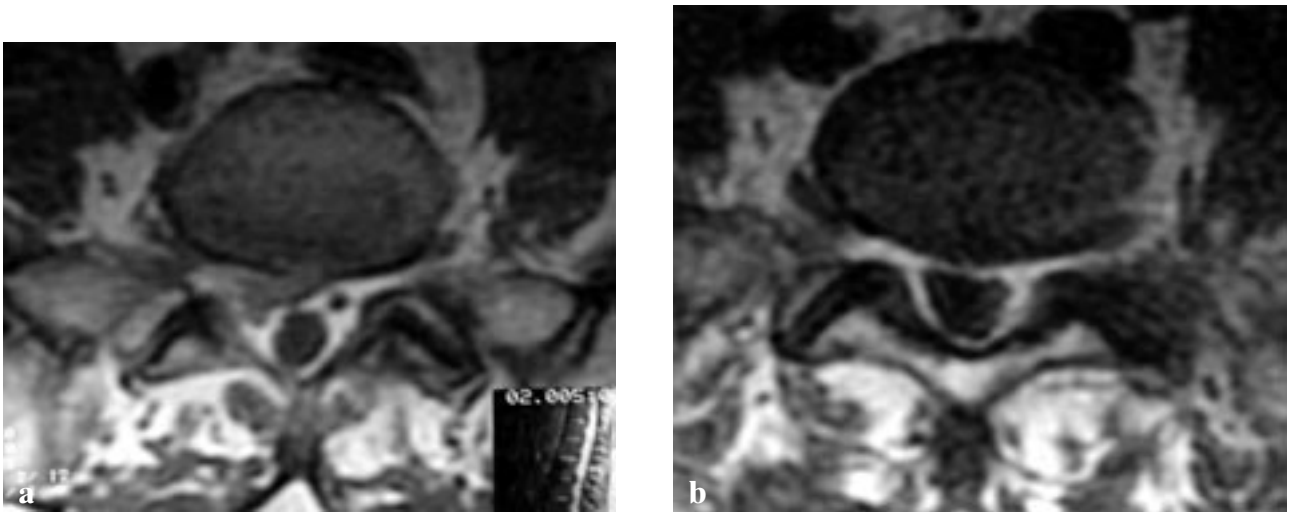

FIG. 3. – Follow-up MR of a patient treated at the L5-S1 level prior to (a) and 9 months after treatment (b).

FIG. 3. – *Hernie discale L5-S1 (a), examen effectué 9 mois après traitement (b).*

or hypertrophic scarring with severe symptoms in 20% of patients and FBSS proper in 15% [9, 10]. These figures have stimulated research into newer minimally-invasive techniques to improve clinical results. At the same time, advances in percutaneous techniques by interventional procedures (chemonucleolysis with chemopapain, nucleo-disckectomy introduced by Onik, IDET, discectomy LASER, and recently nucleoplasty) have minimized the invasive nature of surgical techniques and avoid or decreased complications such as postsurgical infection.

Reducing intervertebral disc size by mechanical aspiration of disc fragments or partially dissolving the herniation by drying reduces the conic pressure on the torn annulus and creates the space necessary for retropulsion whenever the circular fibres of the annulus regain a minimum capacity to contain the disc under tension.

All percutaneous procedures are mildly invasive entailing only a short hospital stay. By avoiding the spinal canal, these techniques also eliminate the risks of post-operative scarring linked to surgery which is often responsible for recurrence of pain. Percutaneous techniques can also be repeated in the same patient without precluding recourse to traditional surgery if they should fail. The success rates reported in different studies vary from 65 to 80% of excellent or good results with chemonucleolysis and aspiration [10]. Epidural steroid injections under CT or fluoroscopic guidance are also used to minimize radicular pain and to try to obtain complete pain relief [2, 4, 8, 15, 20, 21]. Discography is often used to decide regarding the possibility to perform percutaneous discectomy and chemonucleolysis but is useless in case of O_2-O_3 therapy.

Chemonucleolysis with "nucleoptesis" combined with drying the nucleus with an oxygen-ozone mixture (O_2-O_3) uses a colorless irritant gas with a pungent odour which is unstable and has a strong oxidizing power with good antiseptic, disinfectant and antiviral properties. Ozone is prepared and administered as required by transforming a small percentage of oxygen into ozone using special generators. The O_2-O_3 gas mixture produced can be injected into the intervertebral disc and root foramina, 3-4 ml into the disc and 15-20 ml into the neural foramen and root canal. The concentration of the mixture is adjusted by the equipment. The dose mainly administered to treat disc disease is 30 micrograms/ml, a concentration calculated from experimental studies as the amount best suited to dry out the nucleus and minimize inflammation. A number of studies have been published in the literature on the O_2-O_3 treatment of disc herniation with satisfactory results in selected cases [1, 3, 6, 7, 13, 18]. The causes of backache are the topic of scientific investigation. Mechanical and/or inflammatory irritation of the nerve endings is responsible for low back pain [11, 19, 20, 23, 24]. The natural response of any structure to injury is to trigger a repair process by means of an inflammatory response. The number of direct or immune-mediated inflammatory events accounts for decompressive FBSS in some patients.

Mechanism of action of the O_2-O_3 mixture

The nucleus pulposus can set off an immune-mediated inflammatory process as the proteoglycan component of its nucleus is segregated from the immune system after birth. Herniation of the nucleus pulposus would therefore trigger an autoimmune reaction, generating an inflammatory process whose cell component is mainly supported by macrophages. On the other hand, the nucleus pulposus can also give rise to an inflammatory process through a non-immune-mediated mechanism supported by the many inflammatory agents identified in its nucleus.

The reactive tissue surrounding the disc contains histiocytes, fibroblasts in the herniations, and chondrocytes in the disc protrusions able to produce cytochines (Interleukin-1 alpha, Interleukin 6 and TNF-alpha) with an increase in phospholipase A2 leading to the release of prostaglandin E2, leukotrienes and thromboxanes found in larger quantities in non-contained discs hernia-

tions and patients presenting more severe symptoms.

Prostaglandins cause pain. In small amounts, they enhance sensitivity of the nerve roots and other pain-producing substances like bradykinin. Experimental studies have shown that an oxygen-ozone gas mixture at the concentrations used for intradiscal treatment have the same effect as steroids on inhibiting cytokine production and hence the pain induced by the same [12].

The oxygen-ozone mechanisms of action are currently being investigated and include:

1) enhanced oxygenation and reduced inflammation in the disease site due to the oxidizing effect on pain-producing mediators;

2) direct effect of ozone on the mucopolysaccharides making up the nucleus pulposus of the intervertebral disc with rupture of water molecules and shrinkage of the disc exerting compression on the nerve roots;

3) improved microcirculation due to resolution of venous stasis and lack of oxygenated blood supply following mechanical compression of the herniated disc and disc protrusion on the vessel components.

Tissue structure alterations

In vivo experimental studies on swine intervertebral discs and in vitro tests on human discs with intradiscal administration of an O_2-O_3 mixture at a concentration of 27mcg/ml demonstrated dehydration of the fibrillary matrix of the nucleus pulposus disclosing the collagen mesh/network and regressive events (fragmentation and vacuole formation). Neoangiogenesis was sometimes present with mild hyperplasia of the chondrocytes in the matrix periphery. Such changes are thought to be due to the decomposition of ozone with release of free radicals which act directly on the disc matrix or indirectly via proteolytic enzymes.

Complications and risks

No early or late neurological or infectious complications have been reported following O_2-O_3 injection. The results are virtually the same as those of other percutaneous techniques (75-80% success rate), injections can be repeated if necessary, and there are no side effects. However, the low costs of this O_2-O_3 therapy make this the method of choice in the percutaneous treatment of herniated lumbar disc.

CONCLUSION

In our experience, intradiscal O_2-O_3 treatment of herniated lumbar disc has revolutionized the percutaneous approach to nerve root disease making it safer, cheaper and easier to repeat than treatments currently in use. In addition, O_2-O_3 therapy does not preclude later recourse to surgery should patients fail to benefit. The technique is also reliable and compatible with other percutaneous procedures.

O_2-O_3 treatment also has the advantage of being feasible in virtually all patients with root syndromes without the contraindications of chemonucleolysis or nucleoaspiration which must be ruled out by discography.

In our experience the failure has been mostly related to patients with spinal canal stenosis, recurrent herniated disk, calcified herniated disk and small descending herniated disk of the lateral recess with significant compression of the nerve sheath.

On the basis of our results and the assessment of our failures, we recommend careful selection patients to avoid broadening the indications for treatment, thereby ensuring a high success rate.

Accurate diagnosis of the lesion and the spinal level to be treated, accurate technique under CT or fluoroscopic guidance in expert hands and patient follow-up by the neuroradiologist after treatment are key factors in ensuring the successful outcome of percutaneous treatment for this common condition.

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Analyse de livre

"Diffusion-Weighted MR Imaging of the Brain", T. MORITANI, S. EKHOLM, P.-L. WESTESSON, 2004, ISBN 3-540-03439-0

L'imagerie de diffusion est aujourd'hui une des techniques-clé de l'exploration du système nerveux central. Largement utilisée pour le diagnostic des accidents ischémiques à la phase aiguë, son application s'étend à beaucoup d'autres affections. L'ouvrage très pratique de T. Moritani, S. Ekholm, P.L. Westesson, souligne l'intérêt de cette séquence d'imagerie rapide. Les premiers chapitres sont consacrés aux bases techniques de l'imagerie de diffusion, bénéficiant d'illustrations en couleur très utile à la compréhension de ces données, aux résultats normaux, ainsi qu'aux artéfacts observés. Les chapitres suivants sont consacrés à la pathologie cérébrovasculaire, aux affections démyélinisantes, dégénératives, toxiques et métaboliques, aux infections crâniocérébrales et à la pathologie tumorale. Deux thèmes spécifiques sont abordés dans des chapitres distincts : l'épilepsie et la neuro-imagerie de l'enfant. L'ouvrage se conclut par un chapitre particulièrement utile à l'imagerie de diffusion ; celui-ci est fait de tableaux illustrés, où sont classées les affections en fonction du signal observé en diffusion, en T2 et de la valeur de l'ADC. Les auteurs proposent un ouvrage de très grande qualité, parfaitement illustré, pratique, indispensable à tout spécialiste amené dans sa pratique à utiliser l'IRM de diffusion.

Artificial Organs
31(9):743–748, Blackwell Publishing, Inc.

Thoughts and Progress

Oxygenation–Ozonation of Blood During Extracorporeal Circulation: In Vitro Efficiency of a New Gas Exchange Device

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Abstract: We have investigated the performance of a new gas exchange device (GED), named L001, specifically devised for the ozonation of human blood during extracorporeal circulation. This procedure, defined with the acronym "EBOO," means "extracorporeal blood oxygenation–ozonation." The innovative GED is made of microporous, ozone-resistant, polipropylene hollow fibers with an external diameter of 200 μm, a thickness of 50 μm, and a membrane surface area of 0.22 m^2. The material is coated with phosphorylcholine on the external side in contact with the circulating blood, while a gas mixture, necessarily composed of medical oxygen and ozone (about 99 and 1%, respectively), flows inside the fibers in opposite direction. The new GED has been tested by using a buffered saline solution containing KI and by varying several parameters, and it has shown to be very versatile and efficient. Its main characteristics are minimal foreign surface contact, high gas transfer, and negligible priming volume. This device appears to be a practical, nontoxic, and rather inexpensive tool for performing ozonation of blood for already defined human diseases. **Key Words:** Ozone—Extracorporeal circulation—Polipropylene hollow fibers—Phosphorylcholine coating—Gas exchange device.

The idea to realize a dialysis-like system for a mild blood ozonation of blood ex vivo was fairly common in the 90s (1), and unfortunately, even today, dialysis filters are used in private clinics for cancer and human immunodeficiency virus-infected patients hoping for a cure. Dialysis filters are made with hydrophilic hollow fibers to allow passage of water and solutes into the dialysate but are inefficient and not idoneous for gas exchange. Moreover, being made of ozone-sensitive materials, they can release toxic compounds in the circulating blood. Thus, only gas exchange devices (GEDs) made with hydrophobic, water-impermeable, ozone-resistant, polypropylene hollow fibers enclosed in an ozone-resistant housing and connected to the peristaltic pump and ozone generator by silicone tubing must be used. During the last 8 years, we have performed a number of investigations aiming to establish a new method for the oxygenation–ozonation of human heparinized blood during extracorporeal circulation using GEDs, namely, oxygenators, typically used in open heart surgery. We are indeed interested in not simply oxygenating blood because, as explained in the Discussion, our aim was to deliver minimal doses of ozone, which, at appropriate concentrations, can activate a number of biological functions in blood cells without any deleterious effect. In the first article, we have evaluated the methodology and performed a preclinical evaluation in sheep (2), while initial clinical studies have examined the efficacy of the system in a variety of vascular diseases (3) and in one patient with necrotizing fasciitis (4). In contrast to dialysis filters, the GED allows blood to flow outside the hollow fibers while the gas mixture (~99% medical oxygen and ~1% ozone) flows in a counter-current fashion inside the hollow fibers, but we noted some problems related to the scarce bioavailability of the devices. We have also tested GEDs with fibers externally coated with either heparin or human albumin (5,6) but, although albumin reduces platelet adhesion better than heparin, GEDs are not satisfactory. This is so because the strong ozone reactivity stimulates the aggregation of platelets that, in spite of the presence of heparin, adhere onto the external surface of the fibers and cause a progressive reduction of the gas transfer and the GED's efficiency. We have already extensively discussed the problem why, in the presence of ozone, neither heparin nor albumin represents the ideal biocompatible coating for a foreign surface (6). However, the last clinical study (7) has been performed with GEDs coated with phosphorylcholine, which represents a great advance in terms of biocompatibility (8). The present article aims to describe a new GED, specifically engineered for the EBOO. This device tries to optimize the process of oxygenation–ozonation of blood.

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MATERIALS AND METHODS

In this study, we examined in detail a new GED, model L001: the membrane, 50 μm thick, was made of microporous polypropylene, with a gas exchange surface area of 0.22 m^2 coated with phosphorylcoline, lined on the surface in contact with blood to prevent adhesion of platelets and procoagulant proteins. The hollow fibers had an external diameter of 200 μm and an internal one of 150 μm. The device weighed 236.0 g; the maximal blood flow was of 0.7 L/min, and the priming volume was as little as 20 mL. It was produced by SORIN Group Italia, Mirandola (Modena, Italy), patent pending. Figure 1a,b shows the external form and a section, respectively. Figure 1c describes in detail the various parts. We made a partial comparison with the older model L2, which had a surface of 0.65 m^2 (patent no. 0582959) used in the clinical trial published in 2005 (7).

The GED and lines were routinely rinsed with 2-L saline before starting the perfusion. Ozone was produced by three generators by using only pure, medical oxygen constantly delivered at no more than 1.5 bar:

1. Ozonline ECO_3 (Torino, Italy). It delivers ozone concentrations between 0.1 and 3.0 μg/mL of gas, with a gas flow ranging from 250 mL/min (15 L/h) up to 1 L/min (60 L/h).
2. Ozonline. It delivers ozone concentrations between 1 and 15 μg/mL of gas with a gas flow ranging from 1.60 and 6.0 L/min.
3. Ozonosan PM-80 (Hansler GmbH, Iffezheim, Germany), which could deliver ozone concentrations from 2 up to 80 μg/mL with a gas flow ranging between 1 and 8 L/min.

We felt compelled to use these three ozone generators because we needed to test a wide range of ozone concentrations and, to date, a generator able to deliver these concentrations is not yet available.

Ozone was generated from oxygen using electrical corona arc discharge. Silicone and, when necessary, Tygon polymer tubings (Saint-Gobain Performance Plastics, Lyon, France) were used throughout the reaction procedure to ensure containment of ozone and consistency in concentrations. The unused ozone was immediately converted to oxygen by passing through a destructor (Fig. 1c).

Within the ranges of ozone concentrations indicated earlier, medical oxygen represented between 95.0 and 99.8% of the gas mixture, while ozone was the residual percentage. In all cases, the ozone concentration was monitored continuously by photometry at 600 nm (Chappuis band), periodically checked by iodometric titration. GEDs and ancillary materials used in this investigation were sterile and used only once. The precise entity of the gas flow was periodically checked with a Rota Yokogawa, Typ: RAGL 53 (Wehr, Germany).

Most of the chemical determinations were carried out in the Physiology and Pharmaceutical Chemistry Laboratories of the University of Siena, while those performed with the ozone generator B were carried out in the Dialysis Unit of the University Polyclinic, Siena, Italy. The protocol (no. 562/05, June 30, 2005) was approved (February 28, 2006) by the Ethical Committee of the University of Siena.

The solution used throughout the experiments simulated deproteinized serum and consisted of a freshly prepared saline (0.145 M), phosphate buffered (0.05 M) solution adjusted at pH 7.3. KI (0.12 M) was added just before the experiments, and the final solution was clear and colorless.

The efficiency of the GEDs to transfer ozone flowing inside the hollow fibers to this solution, flowing in a counter-current fashion outside the hollow fibers via a precise peristaltic pump (Multimat B, Bellco, Modena, Italy), was determined by the iodometric method according to the revised procedure described by Masschelein (9). All samples were collected in 50-mL sterile polypropylene test tubes immediately closed with Teflon caps (VWR International S.r.l., Milan, Italy). No recirculation of the solution was allowed because it was discarded after sampling. The iodometric reaction was carried out immediately at the end of the experiment. When ozone reacts with the KI solution, iodine is generated and the solution immediately acquires an amber color which, upon reduction with a titrated solution of $Na_2S_2O_3$ and a starch indicator allows the determination of the ozone concentration:

$$O_3 + 2I^- + H_2O \rightarrow I_2 + O_2 + 2OH^-$$

$$I_2 + 2S_2O_3^{2-} \rightarrow 2I^- + S_4O_6^{2-}$$

The concentration of ozone in gram per liter equals 24 × volume of thiosulphate in L × normality of thiosulphate divided by the inlet volume of gas flow in liters. Both the entities of the gas and of the solution volumes per min were precisely determined at 21°C and normal atmospheric pressure.

The detection limit of the analytical procedure was 0.1 mg/L, and the reproducibility was ±2% of the measured ozone concentration. All determinations were repeated at least three times and proved to be very reproducible (coefficient of variation <2%). Figures 2–5 present the mean value.

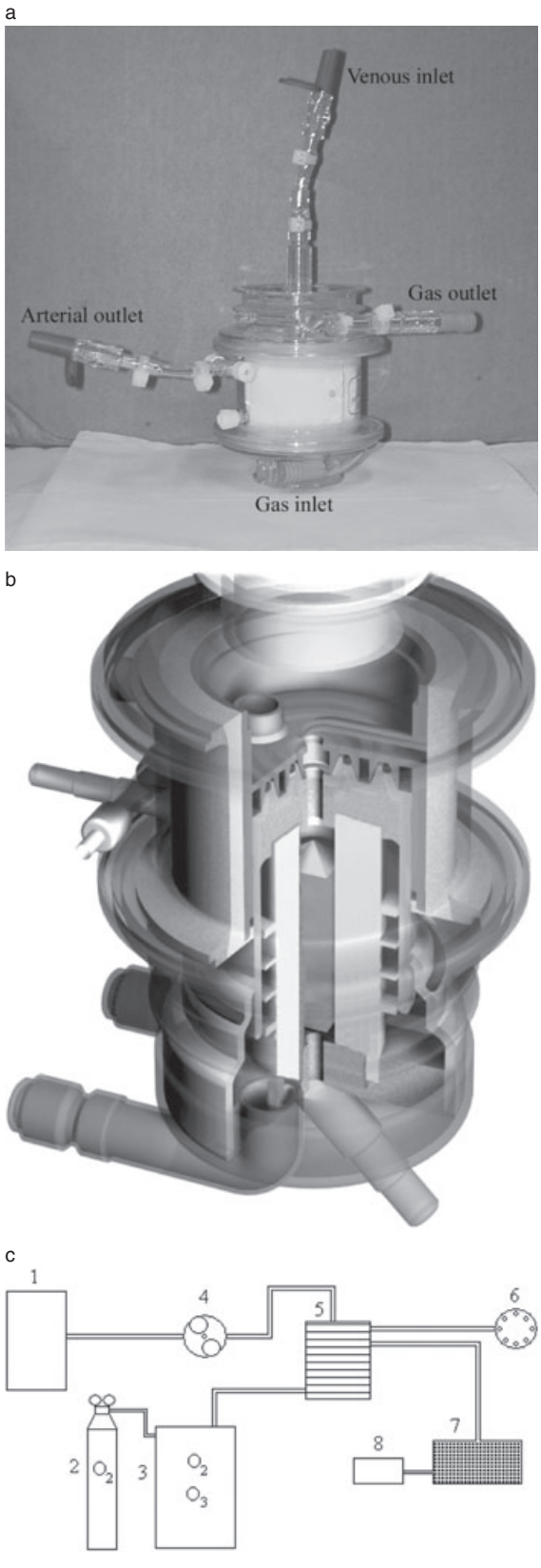

FIG. 1. GED L001 (a) the external aspect, (b) the cross section, and (c) a schematic diagram of the circuit (1, saline solution; 2, oxygen supply; 3, ozone generator with photometer; 4, peristaltic pump; 5, GED; 6, sample collector; 7, silica gel trap; 8, ozone destructor).

RESULTS

Owing to the fact that ozone is a strong oxidant, it must be used with great caution and we must gain a precise knowledge of the efficiency of the GED. For these reasons, we ran the first series of experiments (by using ozone generator A) to evaluate the amount of iodine formation with a constant flow of the saline–KI solution of 80 mL/min, by using very low ozone concentrations (from 0 to 2.4 μg/mL of gas) delivered at a constant gas flow of 250 mL/min. Consequently, the ozone doses range between 0 and 0.6 mg/min. Figure 2a shows the kinetic of the trans-

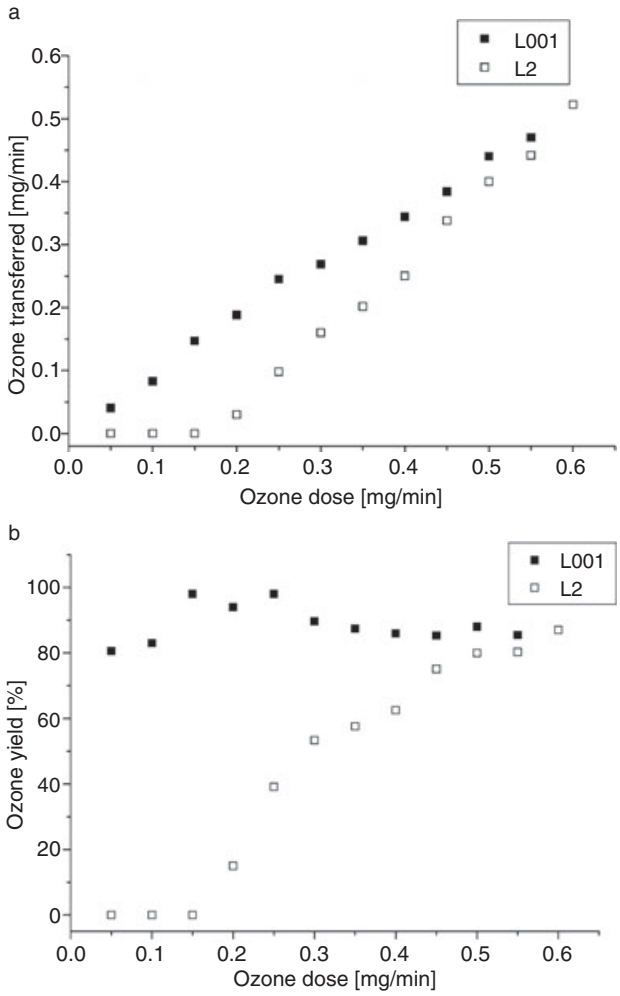

FIG. 2. (a) Transfer and (b) total yield of ozone obtained by using ozone generator A and two different GED sizes.

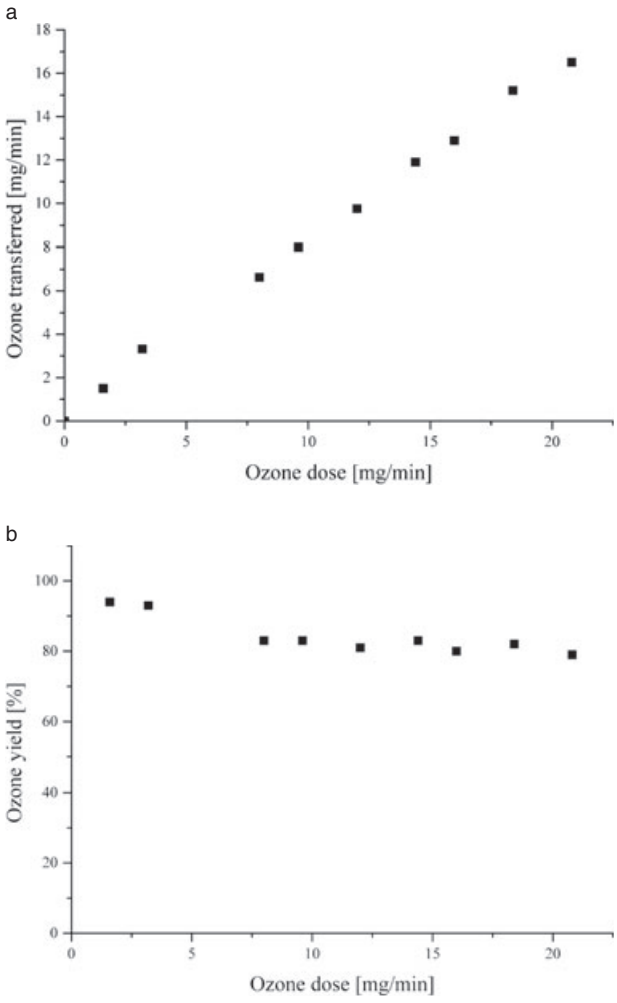

FIG. 3. (a) Transfer and (b) total yield of ozone by using GED L001 and the ozone generator B.

fer of ozone; hence, the iodine formation increased in a linear fashion for the GED L001, while the L2 was defective at the initial ozone doses. Figure 2b demonstrates that the net yield of ozone, expressed as a percentage, is almost quantitative (80–98%) for the model L001.

Next, with the more powerful generator B, we thought that it was worthwhile to investigate the effect of higher ozone concentrations, from 1 to 13 μg/mL of gas delivered at a constant gas flow of 1.60 L/min, and therefore ozone doses ranging between 0 and 20.8 mg/min. The saline–KI solution was pumped constantly at the usual flow of 80 mL/min. Figure 3a shows the linearity of the ozone transferred from the gas phase flowing inside the hollow fibers and the external iodide solution. Figure 3b shows that the ozone yield ranges between 80 and 94%.

During open heart surgery, blood needs to be oxygenated with far higher volumes of the gas mixture (from 1 to 4.5 L/min) and therefore, by using the ozone generator C, we examined the L001 behavior undergoing these gas volumes, with ozone concentrations ranging from 4 to 69 μg/mL and therefore with ozone doses ranging between 15 and 124 mg/min. Figure 4a shows that, in spite of a wide change of gas flow, the amount of ozone interacting with the transferred KI remains fairly constant. However, Fig. 4b shows that the ozone yield is higher for the gas flow of 1 L/min (between 93 and 88%) and decreases from 76 to 65% for a gas flow of 2.5 L/min and from 83 to 60% for the highest flow (4 L/min). These results were somewhat expected because, in a preliminary experiment, we noted that the progressive increase of the ozone concentration and gas flow allowed only a partial transfer of ozone from the gas to the liquid compartment: in fact, an increased amount of ozone could be recovered in a suitable iodide trap posed after the GED. Nonetheless, these data might be useful later on, if we need to perform oxygenation–ozonation of blood during heart surgery.

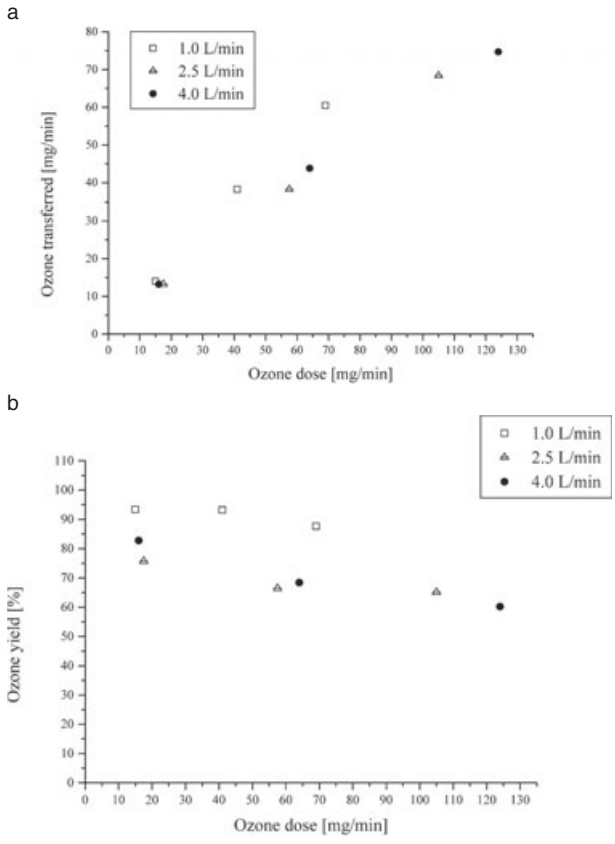

FIG. 4. (a) Transfer and (b) total yield of ozone by using GED L001 and the ozone generator C.

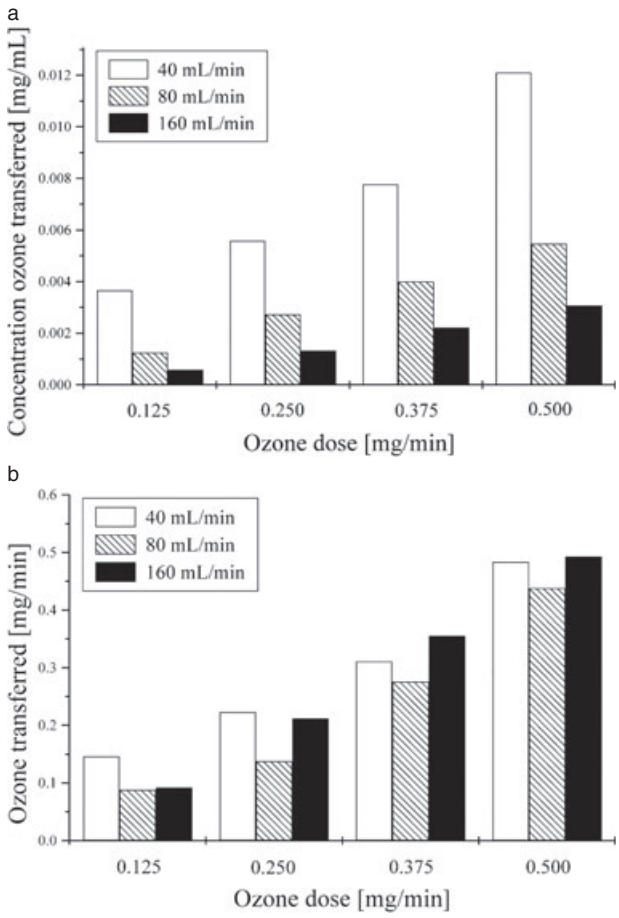

FIG. 5. (a) Transfer and (b) total yield of ozone by using GED L001, the ozone generator A, and three different flows of the buffered saline–KI.

Another critical factor might be represented by the individual caliber's variation of the venous access, which might limit or enhance the volume of blood ozonated per minute. Thus, by using the ozone generator A, we examined four ozone doses (0.125, 0.250, 0.375, and 0.500 mg/min) reacting with the saline–KI solution by testing the model L001 with three different saline–KI flows, namely 40, 80 (as the usual volume), and 160 mL/min. With this system, even by using 16G needles, the patient could not tolerate the withdrawal and return of more than 160 mL/min blood. In all cases, the gas volume delivered per min was of 250 mL (15 L/h). As could be expected, Fig. 5a shows that the amount of ozone reacting with the iodide is inversely proportional to the volume of the saline–KI solution pumped per minute in the GED L001. Moreover, Fig. 5b shows that the total amount of ozone reacted is about constant in relation to the three different saline–KI volumes delivered per min, but, obviously, it increases in relation to the four ozone doses. It appears superfluous to report the diagram showing that in all cases, the percentage of reacted ozone varied between 80 and 98%. On the basis of these results, Table 1 exemplifies how, after having selected the most suitable ozone concentration and dose for the patient, by using the L001 GED, we can define the perfusion time of about an hour.

DISCUSSION

The objective of this study was to evaluate the efficiency of a new GED specifically designed for the ozonation of blood during extracorporeal circulation. Although the total surface of gas exchange has been reduced to only 0.22 m^2, the results obtained, varying several parameters such as ozone concentration, gas volume, and liquid volume, have emphasized the versatility and efficiency of the L001 model. The unique scope of the GED is to allow a mild and well-checked ozonation of human blood during a therapeutic session of about 60 min. Although the gas mixture is usually composed of almost 99% medical oxygen, the residual 1% ozone is quite sufficient for achieving the

TABLE 1. *Definition of perfusion time by using different ozone concentrations and gas volumes*

Ozone (mg/L) Gas flow (15 L/h)	Ozone dose (mg/min)	Ozone transferred (mg/min)	Time of treatment for a 5-mg dose (min)	Time of treatment for a 10-mg dose (min)	Time of treatment for a 15-mg dose (min)	Time of treatment for a 20-mg dose (min)
0.2	0.05	0.0403	124	248	372	496
0.4	0.10	0.0830	60	143	214	285
0.6	0.15	0.1469	34	68	102	136
0.8	0.20	0.1882	27	53	80	106
1.0	0.25	0.2448	20	41	61	82
1.2	0.30	0.2688	19	37	56	74
1.4	0.35	0.3062	16	33	49	65
1.6	0.40	0.3437	15	29	44	58
1.8	0.45	0.3840	13	26	39	52
2.0	0.50	0.4397	11	23	34	45
2.2	0.55	0.4704	11	21	32	43

desired blood activation (10). This is accomplished because ozone dissolves readily in the water of plasma and immediately reacts with hydrophilic reductans and polyunsaturated fatty acids mostly bound to albumin. The reaction generates a cascade of compounds such as hydrogen peroxide and lipoperoxides that (i) improve blood circulation and oxygen delivery to ischemic tissues; (ii) improve the general metabolism; (iii) induce a mild activation of the immune system; (iv) correct a chronic oxidative stress by upregulating the antioxidant system; (v) procure a state of well-being in the majority of patients by activating the neuroendocrine system, and (vi) do not cause any side effects. All of these aspects have been extensively discussed in previous publications (10,11). It goes without saying that blood emerging from the GED is hyperoxygenated (the pO_2 has a tension of about 400 mm Hg), but this aspect is practically irrelevant because the normally used blood flow of 80 mL/min mixes with about 5 L of venous blood before reaching the lungs. However, the new L001 GED can allow, if needed, a blood flow of 700 mL/min, but, obviously, in such a case, we should need to use the ozone generator A, able to deliver ozone at a concentration as low as 0.1 μg/mL.

The small size of the new GED is particularly valid because of the negligible priming volume and the absence of a thermostatically controlled heating system, truly unnecessary. Thus, it is very simple and practical to operate; it is less expensive than previous models; it avoids blood loss, and, being ozone resistant, it does not release foreign compounds in the circulating blood. Having previously used the L2 GED (7), we have already clarified that, in comparison to heparin and albumin, the phosphorylcholine coating on the external surface of the GED in contact with blood is exceptionally biocompatible (8) and avoids the generation of complement complex and the activation of procoagulant factors. Neutrophils, monocytes, and particularly platelets are exquisitively sensitive to ozone (12), but the phosphorylcoline coating and the low ozone concentration avoid the adhesion and the activation of these cells.

Previous clinical experience has already clarified the breadth of therapeutic efficacy of this system in infectious, metabolic, and vascular diseases (3,4,7,11).

Work in progress aims to further clarify a number of biochemical modifications, namely, variation of the blood antioxidant capacity, lipoperoxide concentration, thiol oxidation, hydroperoxide levels, free haemoglobin, and lactic dehydrogenase. The results will be reported in the second part of this series.

Acknowledgments: The authors are grateful to Eng. I. Panzani of SORIN Group Italia and Dr. L. Bigi for providing the GED and useful comments. The linguistic revision by Mrs. H. Carter is gratefully acknowledged.

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6 studies that are compiled on the Biological effects of Ozone by V Bocci

1. Induction of interferon gamma on human leucocytes.

3. An attempt to define conditions for optimal induction of cytokines

7. Generation of (ROS) after exposure of human blood to ozone.

8. Effects on the total antioxidant status and on interleukin-8 production

9. Effects of ozone on human platelets.

10. Release of factors from ozonated human platelets.

Other studies

The ozone paradox: ozone is a strong oxidant as well as a medical drug. Bocci 2009 Med Res Rev. 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.

Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients. Department of Nephrology Transplantology and Internal Medicine 2004

Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress? Masaru Sagai[1] **and** Velio Bocci 2011

Haematologica. 1990 Nov-Dec;75(6):510-5.

Studies on the biological effects of ozone

1. Induction of interferon gamma on human leucocytes.

Bocci V, Paulesu L. http://www.ncbi.nlm.nih.gov/pubmed/2129118

Source

Istituto di Fisiologia Generale, Università di Siena, Italy.

Abstract

In this study we have investigated the effects of ozone on human blood, as well as on resuspended buffy coats and Ficoll-purified mononuclear cells. Samples were exposed at different ozone concentrations (from 2.2 micrograms to 108 micrograms/ml) for 30 sec and then incubated for different times at 37 degrees C in a 95% air-5% CO2 humidified atmosphere. Supernatants were collected and frozen at-20 degrees C until tested for interferon (IFN) activity. We have determined that the ozone concentration is critical for lymphokine induction. In fact, while low concentrations (2.2 micrograms/ml) are effective in lymphocytes, they do not induce IFN in either whole or diluted (1:1) human blood, or resuspended buffy coats. In such cases levels as high as 42 micrograms/ml are required. On the other hand, a very high ozone concentration (108 micrograms/ml) is not

effective and probably toxic. Maximal IFN production occurs 72-96 h after ozone exposure, and the kinetics of IFN release is similar to that after Staphylococcal Enterotoxin B addition. Because ozonization of blood is a medical procedure followed in several countries for treatment of viral diseases, this study can open a new field of investigation that may yield useful results both in biological and practical terms.

Lymphokine Cytokine Res. 1993 Apr;12(2):121-6.

Studies on the biological effects of ozone:

3. An attempt to define conditions for optimal induction of cytokines.

Bocci V, Luzzi E, Corradeschi F, Paulesu L, Di Stefano A.
http://www.ncbi.nlm.nih.gov/pubmed/8324077

Source

Institute of General Physiology, Faculty of Pharmacy, University of Siena, Italy.

Abstract

Ozonization of blood, normally carried out with citrated blood, may be fine for the autohemotherapy of ischemic diseases but it may be at a loss when employed in viral diseases or in immunodeficiencies. We have shown that heparin, used as an anticoagulant, with the addition of 5 mM CaCl2 favors production of cytokines by leukocytes with only a modest increase in hemolysis. High plasmatic levels of glucose, glutathione, and ascorbic acid decrease cytokine's yield because these compounds act as antioxidants and quench the inducing activity of ozone. Autohemotherapy with heparinized and Ca(2+)-supplemented blood has not revealed any side effects in volunteers.

J Biol Regul Homeost Agents. 1998 Jul-Sep;12(3):67-75.

Studies on the biological effects of ozone:

7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone.

Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, Gerli R.
http://www.ncbi.nlm.nih.gov/pubmed/9795834

Source

Institute of General Physiology, University of Siena, Italy.

Abstract

The acceptance of any complementary medical approach is conditioned by the results obtained after the same scientific scrutiny applied in orthodox medicine. Otherwise any claim of efficacy remains in the realm of fiction. In the case of ozone therapy, the mechanisms of action have remained nebulous and in a series of publications we are trying to present the biochemical,

immunological and morphological evidence in favour or against ozone therapy. We have now shown that ozone (O3) dissolved in the water of either plasma or serum or physiological saline generates reactive oxygen species (ROS), of which hydrogen peroxide (H2O2) can be unequivocally demonstrated by using specific methods for its detection. Lipids present in plasma preferentially those present in lipoproteins, undergo peroxidation that is somewhat O3-dose dependent and can be observed by the measurement of thiobarbituric acid reactive substances (TBARS). While the generation of H2O2 is crucial in activating both biochemical (hexose monophosphate shunt) and immunological (via the transcription factor NF-kB) mechanisms, the role of lipid oxidation products (LOP) remains to be investigated. We have shown here that there is a small but consistent induction of some cytokines (TNF-alpha, IFN-gamma and IL-2) when human blood is directly exposed to O3 concentrations up to 100 micrograms/ml per g of blood. On the other hand, isolated blood mononuclear cells (PBMC) in tissue culture medium are far more sensitive to the oxidant action of O3 as shown by a progressive reduction of the proliferation index with comparatively far lower O3, concentrations. On the whole, these results support the concept that much of the O3 toxicity is neutralized by the powerful antioxidant system of blood. The minimal hemolysis supports this idea but as far as platelets are concerned, we must mention that they tend to aggregate in heparinized blood, even when it is exposed to an O3 concentration of 40 micrograms/ml. In spite of the lack of side-effects after autohemotherapy, this drawback must be kept in mind and avoided in clinical practice.

Mediators of Inflammation, **7**, 313–317 (1998)

Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production

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OZONE (O3) is a controversial gas because, owing to its potent oxidant properties, it exerts damaging effects on the respiratory tract and yet it has be in use for four decades as a therapy. While the disinfectant activity of O3 is understandable, it is less clear how other biological effects can be elicited in human blood with practically no toxicity. On the other hand plasma and cells are endowed with a powerful antioxidant system so that a fairly wide range of O3 concentrations between 40 and 80 m g/ml per gram of blood (~0.83–1.66 mM) are effective but not deleterious.

After blood ozonation total antioxidant status (TAS) and plasma protein thiol groups (PTG) decrease by 20% and 25%, respectively, while thiobarbituric acid reactive substances (TBARS) increases up to five - fold. The increase of haemolysis is negligible suggesting that the erythrocyte membrane is spared at the expense of other sacrificial substrates . While there is a clear relationship between the ozone dose and IL-8 levels, we have noticed that high TAS and PTG values

inhibit the cytokine production. This is in line with the current idea that hydrogen peroxide, as a byproduct of O3 de composition, acts as a messenger for the cytokine induction.

Platelets. 1999;10(2-3):110-6.

Studies on the biological effects of ozone:
9. Effects of ozone on human platelets.

Bocci V, Valacchi G, Rossi R, Giustarini D, Paccagnini E, Pucci AM, Di Simplicio P.
http://www.ncbi.nlm.nih.gov/pubmed/16801079

Source

Institute of General Physiology, University of Siena, Siena, Italy. fisgen@unisi.it

Abstract

During the course of ozonated autohaemotherapy (O3-AHT) using heparin as an anticoagulant, it was occasionally observed that a few clots were retained in the filter during blood reinfusion. This observation prompted an investigation on the effect of ozone (O3) on human platelets. We have now shown, both by biochemical and morphological criteria, that heparin in the presence of O3 can promote platelet aggregation. In contrast, after Ca(2+) chelation with citrate, platelet aggregation is much reduced. The potential role of the transient formation of hydrogen peroxide (H2O2) in the presence of Ca2+ with the possible expression of adhesion molecules is briefly discussed.

Mediators Inflamm. 1999;8(4-5):205-9.

Studies on the biological effects of ozone:
10. Release of factors from ozonated human platelets.

Valacchi G, Bocci V. http://www.ncbi.nlm.nih.gov/pubmed/10704074

Source

Institute of General Physiology, University of Siena, Italy.

Abstract

In a previous work we have shown that heparin, in the presence of ozone (O3), promotes a dose-dependent platelet aggregation, while after Ca2+ chelation with citrate, platelet aggregation is almost negligible. These results led us to think that aggregation may enhance the release of platelet components. We have here shown that indeed significantly higher amount of platelet-derived growth factor (PDGF), transforming growth factor beta1 (TGF-beta1) and interleukin-8 (IL-8) are released in a dose-dependent manner after ozonation of heparinised platelet-rich plasma samples. These findings may explain the enhanced healing of torpid ulcers in patients with chronic limb ischemia treated with O3 autohaemoteraphy (O3-AHT).

Blood Coagul Fibrinolysis. 2004 Oct;15(7):619-22.

Med Res Rev. 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.

The ozone paradox: ozone is a strong oxidant as well as a medical drug.

Bocci V, Borrelli E, Travagli V, Zanardi I.

Source **Med Res Rev. 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.**

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Abstract

After five decades characterized by empiricism and several pitfalls, some of the basic mechanisms of action of ozone in pulmonary toxicology and in medicine have been clarified. The present knowledge allows to understand the prolonged inhalation of ozone can be very deleterious first for the lungs and successively for the whole organism. On the other hand, a small ozone dose well calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms and reactivate the antioxidant system. In detail, firstly ex vivo and second during the infusion of ozonated blood into the donor, the ozone therapy approach involves blood cells and the endothelium, which by transferring the ozone messengers to billions of cells will generate a therapeutic effect. Thus, in spite of a common prejudice, single ozone doses can be therapeutically used in selected human diseases without any toxicity or side effects. Moreover, the versatility and amplitude of beneficial effect of ozone applications have become evident in orthopedics, cutaneous, and mucosal infections as well as in dentistry.

Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients.

Tylicki L, Lizakowski S, Biedunkiewicz B, Skibowska A, Nieweglowski T, Chamienia A, Debska-Slizien A,Rutkowski B. http://www.ncbi.nlm.nih.gov/pubmed/15389131

Source

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Abstract

BACKGROUND:

The therapeutic use of ozone is still a controversial medical strategy due to the potential toxicity of ozone, which is recognized as a highly reactive oxidant. The reactive oxygen species are known to induce platelet aggregation, the process involved in the development of atherosclerosis and cardiovascular events. In the present study, the influence of ozonated autohaemotherapy (O3-AHT) on the platelet function was evaluated in chronically haemodialysed patients with peripheral arterial disease.

METHODS:

This was an oxygen-controlled, cross-over study, in which nine sessions of autohaemotherapy with oxygen administration as a control were followed by nine sessions of O3-AHT. The platelet function

was assessed by the extent of spontaneous aggregation (SPA) and agonist-induced aggregation (AIPA), where different concentrations of adenosine were used as an agonist.

RESULTS:
There were no differences between SPA and AIPA assessed after nine sessions of O3-AHT and after nine sessions of autohaemotherapy with oxygen administration. SPA and AIPA did not change after the first session of O3-AHT as compared with the levels before this procedure.

CONCLUSION:
O3-AHT with ozone concentration of 50 microg/ml and citrate as an anticoagulant does not induce platelet aggregation.

Medical Gas Research 2011, 1:29 doi:10.1186/2045-9912-1-29

Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress?

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Abstract

The potential mechanisms of action of ozone therapy are reviewed in this paper. The therapeutic efficacy of ozone therapy may be partly due the controlled and moderate oxidative stress produced by the reactions of ozone with several biological components. The line between effectiveness and toxicity of ozone may be dependent on the strength of the oxidative stress. As with exercise, it is well known that moderate exercise is good for health, whereas excessive exercise is not.

Severe oxidative stress activates nuclear transcriptional factor kappa B (NFκB), resulting in an inflammatory response and tissue injury via the production of COX2, PGE2, and cytokines. However, moderate oxidative stress activates another nuclear transcriptional factor, nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2 then induces the transcription of antioxidant response elements (ARE). Transcription of ARE results in the production of numerous antioxidant enzymes, such as SOD, GPx, glutathione-s-transferase(GSTr), catalase (CAT), heme-oxygenase-1 (HO-1), NADPH-quinone-oxidoreductase (NQO-1), phase II enzymes of drug metabolism and heat shock proteins (HSP). Both free antioxidants and anti-oxidative enzymes not only protect cells from oxidation and inflammation but they may be able to reverse the chronic oxidative stress. Based on these observations, ozone therapy may also activate Nrf2 via moderate oxidative stress, and suppress NFκB and inflammatory responses. Furthermore, activation of Nrf2 results in protection against neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Mild immune responses are induced via other nuclear transcriptional factors, such as nuclear factor of activated T-cells (NFAT) and activated protein-1 (AP-1).

Additionally, the effectiveness of ozone therapy in vascular diseases may also be explained by the activation of another nuclear transcriptional factor, hypoxia inducible factor-1α (HIF-1a), which is also induced via moderate oxidative stress. Recently these concepts have become widely accepted. The versatility of ozone in treating vascular and degenerative diseases as well as skin lesions, hernial disc and primary root carious lesions in children is emphasized. Further researches able to elucidate whether the mechanisms of action of ozone therapy involve nuclear transcription factors, such as Nrf2, NFAT, AP-1, and HIF-1α are warranted.