OZONE THERAPY AND ITS SCIENTIFIC FOUNDATIONS

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За последние годы озонотерапия утвердилась как эффективный развивающийся метод лечения широкого круга заболеваний. В России и на Кубе применение озонотерапии разрешено официально, как и на 76% территории Испании и в четырех регионах Италии. Особенностями озонотерапевтического лечения служат простота в применении, высокая эффективность, хорошая переносимость и практически полное отсутствие побочных эффектов. Данная обзорная статья, базирующаяся на последних тематических научных публикациях и монографиях, объединяет современные представления, обосновывающие медицинское применение озона. На протяжении многих лет применение озона в медицинской практике было существенно затруднено в связи с распространенным мнением о токсических эффектах высоких концентраций соединения, используемых в промышленности. Как и в отношении других лечебных технологий, эффекты озонотерапии зависят от используемой дозы вещества. При этом важно понимать, что концентрации озона, применяемые с лечебной целью, значительно ниже тех, которые вызывают развитие токсических эффектов. В этом случае озон, выступая как фармакологический агент, способен оказывать иммуномодулирующий, противовоспалительный, бактерицидный, противовирусный, фунгицидный, анальгетический и другие саногенетические эффекты. Они подтверждены клиническим опытом и научными исследованиями, в том числе выполненными в формате мета-анализа. В настоящее время продолжается процесс официального утверждения озонотерапии как полноценной медицинской технологии в мировой медицине.

Ключевые слова: озонотерапия, история, эффекты, эффективность, показания, противопоказания

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.

Key words: ozone therapy, history, effects, efficiency, indications, contraindications

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 technicalozonization 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 Current s*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 hasbecome 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 S.P. Peretyagin, G.A. Boyarinov, A.N. Monachov 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 properties6-8. 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 therapy10, 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 "*Guia para el uso médico del ozono: fundamento sterapéuticos 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].

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 health care 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,* 11signed 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].

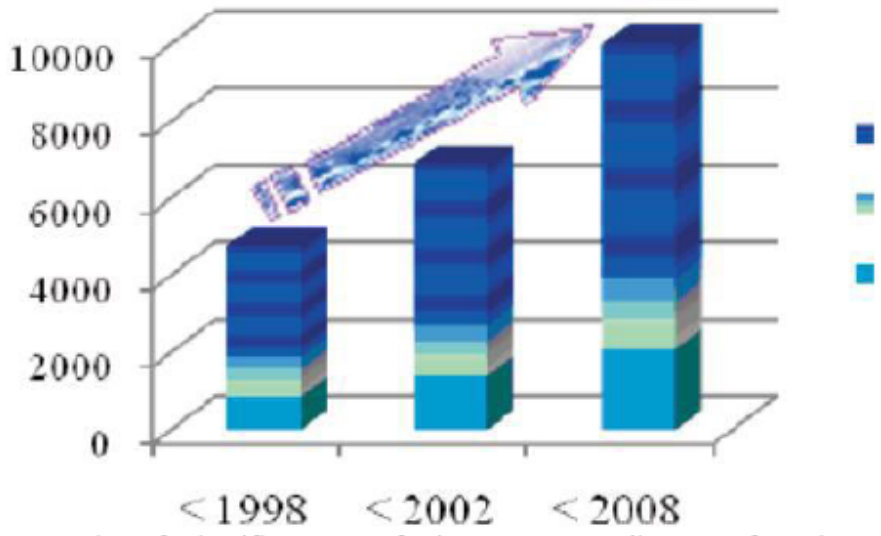

Figure 1. Number of scientific papers referring to ozone studies. Data from the PubMed 2009 database [12].

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].

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 theem piricism 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].

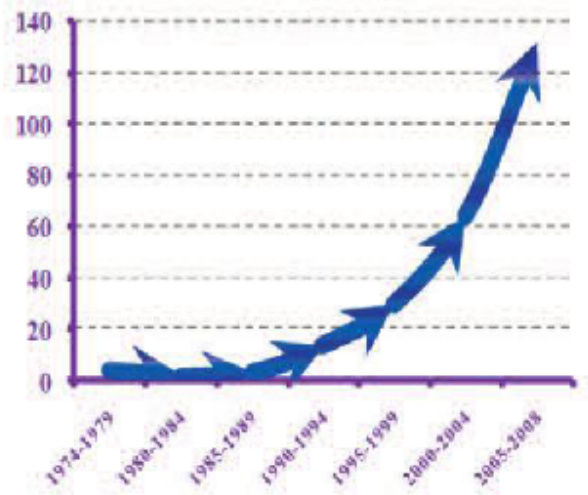

Figure 2.Number of papers on the use of ozone in medicine (Web of Science) [2].

Physico-chemical properties and action mechanism of the ozone

Ozone is the most important gas of the stratosphere, reaching its maximum concentration (above 1000 mcg/m3) 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 mcg/m3-19.63 mcg/m3. 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 105-106mol/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 20ºC [3].

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].

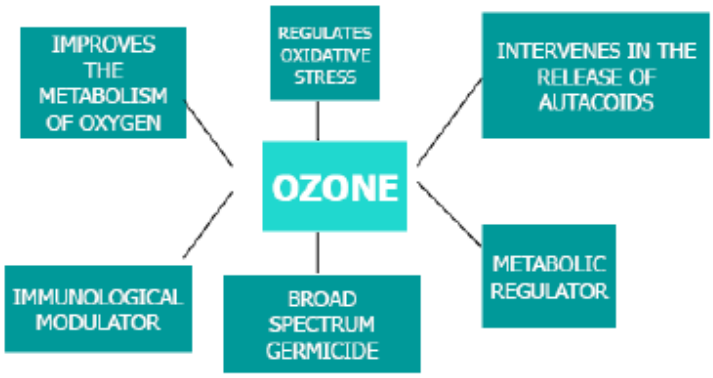

Figure 3.Biological and therapeutic effects of ozone.

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.

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.

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:

$$HbO_2 + 2.3\ DPG \rightarrow Hb + O_2$$

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 improve 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.

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].

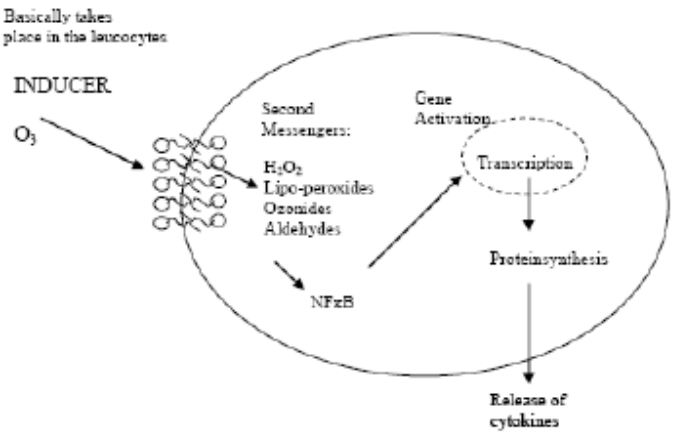

Figure 4. Action mechanism of the ozone as regulator of the cytokine synthesis

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.

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.

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 *Pseudomonas aeruginosa* and *Escherichia 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).

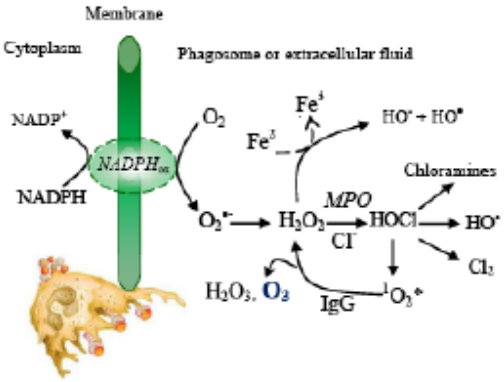

Figure 5. Schematic representation of the processes that lead in vivo to the formation of O_3 by activated neutrophils. O_2*-, superoxide anion radical; $^1O_2$*, singlet oxygen; MPO, myeloperoxidase; HOCL, hypochlorous acid; HO*, hydroxyl radical; NADPHox, NADPH oxidase; IgG, immunoglobulin G; H_2O_2, hydrogen peroxide; H_2O_3, dihydrogen trioxide [2].

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.

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.

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.

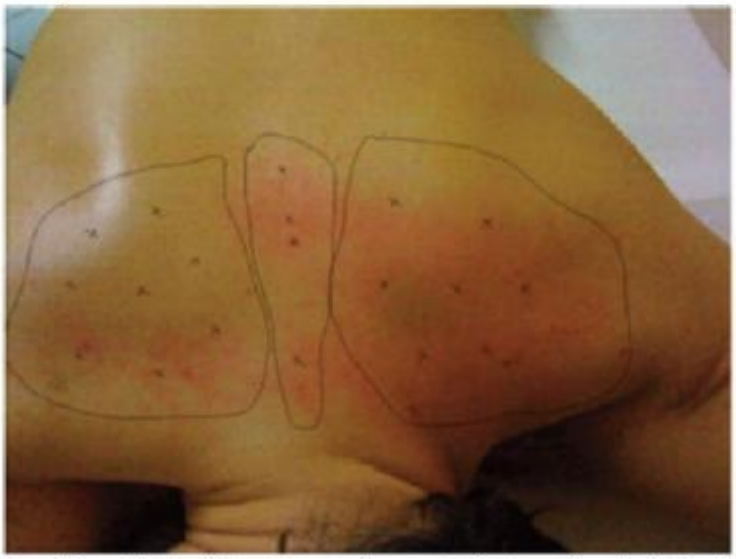

Figure 6. Photo of a patient with sub-scapular pain 1 min. after the injection (injection points marked with an X) with O_2/O_3 8 μg/mL, 2-3 mL. Note that in the area in which pain is reported (central and right of the photo) there is a rosy coloring (erythema) while in the area where there is no pain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

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].

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).

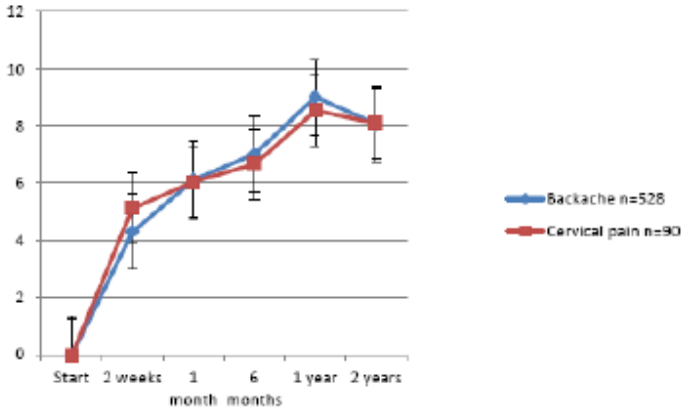

Figure 7.Evolution of patients with back pain and cervical pain treated with ozone with the intramuscular paravertebral technique in time. The scale of 1-10 indicates the perception of pain by the patient (0 means intense pain, 8-10 minimum pain) [12].

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-C32.

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.

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.

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.

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), significant hypo-coagulation syndrome, blood diseases, haemophylia, thrombocytopenia, hemorrhagic vasculitis,

acute myocardium infarctus, hemorrhagic insult, 6) pancreatitis, 7) thyreotoxicosis, 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.

Conclusions

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.

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