

Has oxygen-ozonotherapy a future in medicine?

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Abstract

This review aims to focus attention on the bio-oxidative therapy from the very beginning to date. While ultraviolet blood irradiation is now hardly performed, oxygen-ozone therapy, after an empirical stage, has grown to be precise and safe. Meantime, there is a renewed interest in the infusion of a dilute solution of hydrogen peroxide in infectious diseases antibiotic-resistant.

On a parallel line of research, hyperbaric oxygen therapy has been already accepted by official medicine for the treatment of specific diseases. Meantime it is unfortunate that, owing to quacks' interest in using ozonated physiologic saline and in claiming to "cure" type-2 diabetes in 20 days with the unprecise rectal ozone administration, ozone therapy is losing hope to be acknowledged as a valid therapeutic approach.

Key words:

Antioxidants; Hyperbaric oxygen;
Oxidative stress; Ozone messengers;
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Introduction

The first idea of using ultraviolet blood irradiation (UBI), also known as biophotonic therapy or photophoresis for exploiting its bactericidal and tissue-stimulating effects on the disfiguring lupus vulgaris was born in the genial mind of Niels Ryberg Finsen (Nobel Prize for Medicine in 1903) [1]. Like all Nordish people, he loved the light and sunshine and he treated his own disease bathing in the sun. Most importantly he was able to successfully promote this therapy in tuberculosis.

In the 1920s, UBI was popularized by Emmet Knott [2] by treating severe infections but it fell out of use after the power of antibiotics and because Schwartz *et al* [3] judged the procedure too empirical. UBI was and is still partly considered useful for pathogen reduction, increase tissue oxygenation, and induction of antioxidant enzymes as well as activation of the immune system.

Another type of bio-oxidative therapy was proposed by Dr. I.N. Love working in St. Louis (USA) when, in 1888, published a paper entitled "Hydrogen peroxide as a remedial agent" [4]. After almost a century, in 1993 Dr. C.H. Farr promoted the use of intravenous administration of a dilute solution (0.03-0.15%) of H₂O₂ in several illnesses, but it hardly became known because Werhly and Steinbarth [5] had proposed to collect blood in a special quartz ampoule and to expose it for a short

time to UV light in the presence of pure oxygen followed by re-infusion into the donor. This method was a re-edition of UBI and it had been mostly criticized because the ampoule had to be sterilized each time for avoiding the risk of viral cross-infections. Unfortunately, such a drawback has occurred in several occasions [6, 7]. Moreover, UBI remains an uncertain procedure because one never knows the irradiation efficiency due to progressive decay of the UV source and the variable time of irradiation with a possible damage of blood cells.

Luckily, by the 1970's the physicist Joachim Hansler invented a reliable ozone generator for medical use and Hans Wolff [8] realized the need to expose blood to a mixture of oxygen-ozone in a dispensable, ozone-resistant glass bottle. Wolff deserves the credit for having developed the new type of bio-oxidative therapy in the safe form of ozonated autohemotherapy (AHT).

After three decades, the concept of ozone therapy remains valid because the most recent ozone generators incorporate an UV photometer able to precisely monitor the ozone concentration in real-time. However, as it will be discussed later, the correct procedure is not accepted by all ozonetherapists and several pejorative modifications have been introduced. This is not surprising because in the medical field, drugs and methodologies undergo recurrences. While UBI is

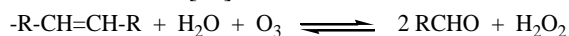
hardly used, infusion of a diluted solution of H₂O₂ has come back to light owing to antibiotic resistance and because H₂O₂ has a milder anti-oxidant potential of 1.78 V compared to ozone, with E° = +2.07 V. H₂O₂ is a physiological molecule considered essential for the cell life and also because during the “oxidative burst” is produced for inactivating pathogens [9].

The actual state of art of ozone therapy

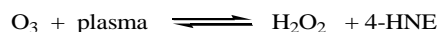
It is unpleasantly surprising that in the field of complementary medicine oxygen–ozonotherapy is not even mentioned because it is a recent development but mostly because it has no sponsors. Although the development of this topic has clearly shown to be well within the classical biochemistry and pharmacology, in contraposition to homeopathy, acupuncture, aromatherapy, Ayurveda and Chinese medicine, it remains unknown. On the other hand there is an ample literature and knowledge of ozone toxicity for the pulmonary system. Indeed as ozone has strong oxidizing properties, it seems paradoxical that this gas may display beneficial effects. Although this appears paradoxical, it has been proven true [10]. On the basis of this synthesis:



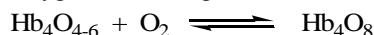
and the fact that the reaction is reversible, it has been shown that ozone readily dissolved in the blood water, releases its energy into hematic components, hence into the body during the reinfusion procedure into the blood donor. Ozone is about ten-fold more soluble in water than oxygen [11, 12] and all the basic chemical reactions have been clarified during the last decade [10, 13-18]. The therapeutic range has been precisely defined to be within ozone concentrations of 20 (0.42 μM) - 80 (1.68 μM) μg of gas (pure O₂: 95% and O₃: 5%) per ml of human blood. Owing to the potent antioxidant capacity of blood due to its hydrophilic, lipophilic and cellular enzymes, a small part of the ozone dose dissolved in the water of plasma is instantly quenched by free antioxidants (mainly uric acid, ascorbic acid and albumin) while the bulk of ozone reacts with polyunsaturated fatty acids (PUFA) mostly present in the three hydrophobic tasks of albumin [19].



Thus the potential energy of ozone is finally transferred into two fundamental messengers such as hydrogen peroxide as a ROS and aldehydic molecules of which 4-hydroxynonenal (4-HNE) is the quantitative relevant lipid oxidation product:



Thanks to the high ozone reactivity these biochemical reactions occur in a few seconds and in fact within the canonical five minutes of mixing an average 200 ml of human blood *ex vivo* in a sterile glass bottle with the 200 ml corresponding volume of the gas mixture (O₂+O₃), ozone is totally exhausted while oxygen is solubilized in plasma and fully oxygenates hemoglobin:



Blood oxygenation is useful, but it has only a little practical relevance because hyperoxygenated-ozonated blood is reinfused via venous route into the donor during the next 20 minutes and it is abundantly diluted with venous blood. Therefore ozone represents the medical drug while the oxygen is only necessary for generating ozone.

Which is the fate of H₂O₂ and 4-HNE? H₂O₂, being unionized, rapidly enters into all blood cells and the chemical gradient between plasma-cells is about 10% of the extracellular concentration [20-22]. In other words, when the highest ozone concentration is mixed with blood, depending upon the modest interindividual variability of antioxidant potency (1.28-1.83 mmol/L plasma) [23] the highest H₂O₂ concentration measured in plasma is 40-50 μM [24] and therefore inside the cells is at most 4-5 μM. This sudden inflow of this small amount of H₂O₂ inside blood cells is the stimulus necessary to activate a series of biochemical reactions as follows:

a) in the erythrocytes: activation of glycolysis with increase of ATP and 2,3-diphosphoglycerate. Functionally, the oxyhemoglobin sigmoid curve shifts to the right and increases the release of oxygen at the tissue level. The erythrocytes mop up most of the H₂O₂ and promptly reduce it to water by catalase, thioredoxin, glutathione peroxidase (GSH-Px) and reduced glutathione (GSH). The sudden formation of oxidized glutathione (glutathione disulphide; GSSG) alters the GSH/GSSG ratio but the cell quickly correct it by either extruding some glutathione disulphide or by reducing it via GSH reductase at the expense of either ascorbic acid or thioredoxin. This important recycling process occurs in about three minutes [25,26], Alternatively, activation of glucose-6-phosphate dehydrogenase (G6PDH) provides reducing power and activates glycolysis.

b) in the leukocytes: neutrophil phagocytic activity is enhanced. Inside monocytes and lymphocytes, H₂O₂ activates a tyrosin-kinase with consequent phosphorylation of IκB, one of the

trimeric components at rest of the nuclear factor kappa-B (NF- κ B) [27, 28]. The phosphorylated I κ B detaches from the trimer and it is broken down in the proteasome. The remaining heterodimer p50-p65 is transferred into the nucleus where it can activate about 100 genes. Of great significance it is the final release of some cytokines (IFN γ , TNF α and IL-8) and of some acute-phase proteins [10];

e) in the platelets: in relation to the ozone concentration, we have measured release of PDGF-AB, TGF β -1 and IL-8 [29]. Growth factors have a specific relevance in enhancing ulcer's healing.

It must be said that the H₂O₂ concentration in the cells (2-5 μ M) is the minimal necessary to switch on cellular responses and it probably lasts few seconds as GSH-Px and catalase promptly reduce it to H₂O. An ozone concentration below 10 μ g/ml gas per ml of blood is most likely totally quenched by the hydrosoluble antioxidants and therefore no biological effects can be elicited and, at the best, the procedure has only a placebo effect. In plasma, the H₂O₂ half-life is less than 1 minute and it is absent during blood reinfusion. On the other hand, among a variety of lipid oxidation products (LOPs), 4-HNE remains fairly stable. A small part is broken down at once by enzymes such as GSH-S-transferases and aldehyde dehydrogenase [30] but the bulk forms an adduct with the -SH group of Cys34 present in domain-I of albumin. Furthermore, eleven nucleophilic residues (Lys199 and His146) can also bind up eleven 4-HNE molecules. Thus, owing to the high albumin amount (about 125 g intravascular and 160 g extravascular) the bound alkenals undergo a great dilution in the body fluids implying a most important loss of toxicity.

An interesting aspect is that albumin can transfer 4-HNE to ALL body tissues, from liver to endocrine glands and the CNS. Thus 4-HNE-Cys adducts can be released at many sites and inform a variety of cells of a transient, acute oxidative stress. At submicromolar or picomolar levels, 4-HNE can act as a well-known signaling molecule [31] able to activate the synthesis of γ -glutamate cysteine ligase, γ -glutamyl transferase, γ -glutamyl transpeptidase, HSP-70, heme-oxygenase-I (HO-1), and antioxidant enzymes such as superoxide dismutase (SOD), GSH-peroxidase, catalase and, last but not least important, G6PDH, a critical enzyme electron-donor 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 [32-38]. In other words, the concept that a precisely

controlled oxidative stress can strengthen the antioxidant defenses is well accepted today and represents a conditioning or adaptation effect. 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. This crucial mediator on its own or as a nitrosothiol, with trace of CO released with bilirubin via HO-1 activity allows vasodilation, thus improving tissue oxygenation in ischemic tissues. H₂S is another potentially toxic molecule that, when released in trace amounts, becomes another important physiological mediator like NO and CO [39, 40].

Another very interesting aspect observed in 67-78 years old subjects affected by the dry form of age-related macular degeneration (ARMD) is that a majority of them report a feeling of euphoria and a sense of wellness and physical energy throughout the ozonotherapy cycle of 14-16 treatments lasting about two months [41]. Whether these feelings are simply due to faith in the medical treatment (the power of the mind!), i.e. the power of the placebo effect [42] or is caused by the generated ozone messengers able to modify or improve the hormonal secretion is not yet known. Unfortunately, lack of funds has always prevented to perform a study in normal volunteers where, before and after ozonotherapy, the complete hormonal pattern and cycling in the plasma throughout the 24 hours could have been determined. This study would be very informative and helpful to understand why the patients experiment a feeling of well-being. This may be due to improved oxygenation or/and enhanced secretion of human growth hormone, cortisol, and dehydroepiandrosterone. Patients with arthrosis pain have also reported a marked improvement and less pain that may be due to release of ACTH-cortisol or to a limited stimulation of COX-2 with enhanced release of prostacyclin (PGI₂). It is also possible that LOPs reaching the hypothalamic area improve the release of neurotransmitters such as serotonin, dopamine, and endorphins as it has been observed after an intense physical exercise [43-45].

It is important to note that neither acute nor chronic toxicity has been ever observed during or after ozone therapy. Several studies [17, 19, 46] performed to evaluate possible hematochemical biochemical enzymatic modifications have clearly demonstrated that ozone concentrations of 20-80 (even up 160) μ g/ml of gas per ml of blood do not damage blood cells or other components. Indeed during the ozonation process no more than 35% of

the total antioxidant capacity is oxidized and it is noteworthy that is reconstituted during the next twenty minutes *in vitro* and in a shorter time *in vivo* [47].

Is it possible that ozone can oxidize the phospholipids and cholesterol of the cell membrane? The demonstration that the negative electric charge of the erythrocytic membrane is unmodified even after ozonation at 80 µg/ml ozone per ml of blood excludes this possibility. It is well known that blood cell membranes are always shielded by a cloud of albumin molecules able to prevent any ozone attack that it has been suggested by some authors, who claim to have noticed an increased flexibility (or an enhanced filterability) of erythrocytes after ozonation. These sort of claims have been mentioned before during UBI and, if correct, may have been due to excessive irradiation and oxidation or may well have happened during ozonation of saline-washed erythrocytes [48]. Unfortunately too many artificial experiments performed with saline-washed cells totally deprived of the plasmatic antioxidants have led to wrong conclusions [49]. Moreover the validity of an objective biochemical analysis carried out last century on UBI by practitioners remains doubtful.

A brief comment regarding the efficacy and safety of administration procedures

Of the several procedures currently used for ozone administration on the basis of previous data it is suggested to use either the classical major ozonated autohemotherapy [14] or the quasi-total body exposure to oxygen-ozone (QTBE) [50]. This second approach is very appealing because with the ozonetherapist's guidance can be done at home at any time. With the exception of the neck and head (to absolutely prevent ozone inhalation) all the remaining body surface (about 1.4-1.5 m²) becomes in contact with a final concentration of 0.5-0.9 µg/ml within a tightly closed ozone resistant cabinet where the temperature via a thermostat can be regulated between 30-42°C and 100% relative humidity. This ozone concentration is about 30-fold lower than the one used in the topical treatment of skin ulcers [51]. The subject is comfortably seated inside almost nude and with her/his wet skin surface. This is important because ozone immediately reacts with sweat and lipids normally secreted by sebaceous glands. The skin tolerates well ozone at environmentally realistic level, while it is fairly sensitive to higher levels [52-55]. The QTBE has also the advantage to avoid venipuncture that is sometimes difficult to perform in women.

The extracorporeal blood ozonation within an ozone-resistant gas exchange device has limited value because it is invasive (two venipuncture with G16 needles), more complex and expensive. It can be used only in patients at the risk of either amputation or after a stroke or a heart infarction. On the other hand the rectal insufflation of gas is easy to perform, practically risk-free but it is unreliable because the effective ozone dose acting on the luminal content remains uncertain. Moreover in Europe not all patients accept this procedure.

Which are the pathologies more suitable to be treated with ozonotherapy?

Clinical results so far available have been objectively discussed elsewhere [14] showing that ozonotherapy is often more useful than orthodox treatments in a first category of diseases such as:

a) osteomyelitis, pleural empyema, abscesses with fistulae, infected wounds, bed sores, chronic ulcers, diabetic foot and burns.

b) advanced ischaemic diseases (hind-limb ischemia and heart ischemia, possibly also stroke, when the patients arrive too late for thrombolysis).

c) age-related macular degeneration (atrophic form) because orthodox ophthalmology does not provide a meaningful treatment.

d) orthopaedic diseases and localized osteoarthritis.

e) chronic fatigue syndrome and fibromyalgia.

f) dentistry regarding primary root carious lesions, particularly in children.

g) stomatology for chronic or recurrent infections in the oral cavity.

For these pathologies ozone is a real "wonder" drug.

In a second category of diseases including:

a) acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi (hepatitis, HIV-AIDS, herpetic infections and herpes zoster, papillomavirus infections, onychomycosis and candidiasis, giardiasis and cryptosporidiosis) ozonotherapy represent only a useful support because neither ozone, nor H₂O₂ reach the concentration where free pathogens are protected by plasma antioxidants [56]. Intracellular viruses are inaccessible

b) cancer-related fatigue, ozone therapy, associated with orthodox treatments, may accelerate and improve the outcome. However so far ozonotherapy has been unable to "cure" cancer.

There is a third category of serious diseases such as:

a) autoimmune diseases (multiple sclerosis, rheumatoid arthritis, crohn's disease, psoriasis).

b) senile dementias.

c) pulmonary diseases (emphysema, asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and acute respiratory distress syndrome).

d) skin diseases (psoriasis and atopic dermatitis).

e) metastatic cancer.

f) severe sepsis and multiple organ dysfunction, where the combination of orthodox treatments and ozone therapy, at least on theoretical ground, may be helpful but real clinical evidence is lacking. Anecdotal data are encouraging but in most cases achieved by using several types of therapy.

Whether ozone therapy with the advantages of low cost and no adverse effects, may equal the efficacy of current conventional treatments remains to be explored. However, how and when will we be able to perform these investigations standing the actual situation of total disinterest of health authorities, lack of specific sponsors and the overwhelming power of pharmaceutical industries, which are only interested in pursuing their objectives. Ironically, it is possible that less developed countries with minimal budgets may have an interest in performing pilot trials that can give us precious informations regarding the usefulness of ozone therapy.

A fourth category of diseases such as retinitis pigmentosa, sudden hearing loss and tinnitus where ozone therapy does not yield real therapeutic results must be mentioned. However when ozonotherapy is applied for compassionate reasons, especially in young patients or patients in the acute phase, may yield some benefit that, even temporary, helps anguished patients.

In Table 1 tentative guidelines regarding ozone concentrations within the therapeutic window to be used in different pathologies with the classical ozonated AHT, at least twice weekly are reported. Ozone concentrations are slowly upgraded no more than 5 µg/ml at a time, to achieve the adaptation to oxidative stress in 2-3 weeks.

Table 1. Proposed O₃ concentrations (µg/ml of gas per ml of blood) in different pathologies

Pathology	Initial	Final
<i>Infectious diseases</i>	20-25	70
<i>Vascular diseases</i>	20	40-50
<i>Degenerative diseases</i>	20	30-40
<i>Respiratory diseases</i>	15	30-40
<i>Autoimmune diseases</i>	20-40	80
<i>Metastatic tumours</i>	25	70-90

The examination of the table indicates that:

firstly, the idea "more is better" is not always appropriate for ozone and its concentration must be calibrated in relation to the effector and target cells;

secondly, the frequency of treatment depends on the type and stage of the disease: from daily to two per week, until necessary and

thirdly, the urgent need of further clinical experimentation with appropriate controls to generate definitive clinical data.

Clinical trials are demanding enterprises that require a concerted effort by official medicine and government authorities because the permission of ethical committees is indispensable. A simple but valid randomized study in 100 patients with a control arm (50) using the best orthodox therapy and the experimental arm (50) using well-performed ozone therapy plus the basic therapy even if it is not too expensive, still requires about £ 150.000 for covering insurance, hospital fees, materials and laboratory plus clinical exams. National Health authorities, which are always complaining about the increasing costs of medical assistance, could have an economical advantage if ozonotherapy was widespread and organized in a systematic way in all public hospitals. Although hard data to support this contention are not available, it is likely that the benefit of ozone therapy does outweigh its cost, particularly for the above mentioned first category of diseases. In a public hospital, as an example, ten nurses, under the supervision of an ozonotherapist could easily perform the therapy in about 15 patients per hour. As things are today, it is depressing to realize that ozone therapy will not be applied in public hospitals for years to come, thus depriving many patients of the possibility of restoring their health. On the other hand, some Countries have so many patients and so few resources for treating them that physicians are apparently forced to use procedures such as the infusion of ozonated saline or indiscriminately the rectal insufflation to everyone. This is a very poor compromise because it hinders a real medical progress.

May oxygen ozonotherapy have a future in medicine?

This is an awkward question that needs an objective discussion because all ozonotherapists must understand that ozonotherapy is objected and whenever possible obstructed. Moreover in most countries there are three or more ozonotherapy associations fighting for supremacy. This is simply ridiculous and inadmissible because simply due to

either a different medical speciality or petty commercial interests relative to ozone generators and ancillary materials. This disunity prevents pursuing the fundamental interest of performing valid clinical trials, which are essential for progress. Unless we are able to provide convincing evidence that ozonotherapy is not a panacea but it is useful in some diseases, ozonotherapy will remain in limbo.

In countries with small resources such as Cuba and surprisingly in the Russian Federation, it has been established to use in all patients either the rectal insufflation or the infusion of ozonated saline, respectively. They have presented clinical results such as the “cure in diabetic patients with only twenty daily treatments (10 mg daily for twenty sessions) or excellent results in all diseases with the infusion of few bottles of ozonated saline. This ozonated saline solution, although cheap and quick to deliver, at low ozone concentrations acts as a placebo or it can be toxic. Foksinski *et al*. [57], after the infusion of 500 ml of ozonated saline, detected 8-oxodeoxyguanosine, a typical oxidative DNA marker, in lymphocytes of atherosclerotic patients. If this was not enough there are some physicians still delivering the gas mixture intravenously with the risk of oxygen embolism and patient's death. Other ozonotherapists, for

stinginess, use potentially toxic plastic bags instead of the safe, ozone-resistant glass bottles. Any time this happens ozone therapy loses any possibility to be seriously considered.

Official medicine that is not also free of mishaps owing to the urgency of recovering money spent by big pharmaceuticals, follows the rule of the Helsinki regulations and often achieves valid clinical results.

On this basis, the danger of jeopardizing ozone therapy is high and all ozonotherapists must understand this risk and try to follow only well-established guidelines.

Concluding remarks

Various types of bio-oxidative therapy have been proposed but today the classical ozone therapy or/and infusion of a dilute solution of hydrogen peroxide appear the safest and useful provided that they are correctly performed. All ozonotherapists must now realize that the future of these procedures depends upon the demonstration achieved by valid clinical trials that they are efficacious and without side-effects. Ineffective compromises will hinder for ever the acceptance of bio-oxidative therapy by official medicine.

References

- Møller KI, Kongshoj B, Philipsen PA, Thomsen VO, Wulf HC. How Finsen's light cured lupus vulgaris. *Photodermatol Photoimmunol Photomed* 2005; 21:118-24.
- Knott EK. Development of ultraviolet blood irradiation. *Am J Surg* 1948; 76:165-71.
- Schwartz SO, Kaplan SO, Stengle J, Stevenson FL, Vincenti M. Ultraviolet irradiation of blood in man; studies of sixty-eight patients. *JAMA* 1952; 149:1180-3.
- Love IN. Peroxide of hydrogen as a remedial agent. *JAMA* 1888; 10:262-265.
- Wehrli F, Steinbart H. Erfahrungen mit der Haematogenen Oxydations-Therapie (HOT). *Ars Medici* 1954; 10:44-51.
- Gabriel C, Blauhut B, Greul R, Schneeweis B, Roggendorf M. Transmission of hepatitis C by ozone enrichment of autologous blood. *Lancet* 1996; 347:541.
- Webster GJ, Hallett R, Whalley SA, Meltzer M, Balogun K, Brown D, Farrington CP, Sharma S, Hamilton G, Farrow SC, Ramsay ME, Teo CG, Dusheiko GM. Molecular epidemiology of a large outbreak of hepatitis B linked to autohaemotherapy. *Lancet* 2000; 356:379-84.
- Wolff H. Das medizinische Ozon. Theoretische Grundlagen, therapeutische Anwendungen (Gebundene Ausgabe). Verlag für Medizin, Heidelberg, 1979.
- Forman HJ. Hydrogen peroxide: The good, the bad and the ugly. In: Valacchi G, Davis P (eds) *Oxidants in biology*. Springer, Netherlands, pp 1-17, 2008.
- Bocci V, Borrelli E, Travagli V, Zanardi I. The ozone paradox: ozone is a strong oxidant as well as a medical drug. *Med Res Rev* 2009; 29:646-82.
- Battino R, Rettici TR, Tominaga T. The solubility of oxygen and ozone in liquids. *J Phys Chem Ref Data* 1983; 12:163-78.
- Alborzfar M, Escande K, Allen SJ. Removal of 3,4-dichlorobut-1-ene using ozone oxidation. *Water Res* 2000; 34:2963-70.
- Pryor WA, Squadrito GL, Friedman M. The cascade mechanism to explain ozone toxicity: The role of lipid ozonation products. *Free Radical Biol Med* 1995; 19:935-41.
- Bocci V. *Ozone. A new medical drug*. 2nd edition, Springer, Dordrecht, Netherlands, 2011.
- Bocci V. Scientific and medical aspects of ozone therapy. State of the art. *Arch Med Res* 2006; 37:425-35.
- Bocci V, Aldinucci C. Biochemical modifications induced in human blood by oxygenation/ozonation. *J Biochem Mol Toxicol* 2006; 20:133-8.
- Travagli V, Zanardi I, Silviotti A, Bocci V. A physicochemical investigation on the effects of ozone on blood. *Int J Biol Macromol* 2007; 41:504-11.
- Bocci V, Zanardi I, Michaeli D, Travagli V. Mechanisms of action and chemical-biological interactions between ozone and body compartments: a critical appraisal of the different administration routes. *Curr Drug Ther* 2009; 4:159-73.

19. Travagli V, Zanardi I, Bernini P, Nepi S, Tenori L, Bocci V. Effects of ozone blood treatment on the metabolite profile of human blood. *Int J Toxicol* 2010; 29:165-74.
20. Antunes F, Cadenas E. Estimation of H₂O₂ gradients across biomembranes. *FEBS Lett* 2000; 475:121-6.
21. Stone JR, Collins T. The role of hydrogen peroxide in endothelial proliferative responses. *Endothelium* 2002; 9:231-8.
22. Stone JR, Yang S. Hydrogen peroxide: A signaling messenger. *Antioxid Redox Signal* 2006; 8:243-70.
23. Miller NJ, Rice-Evans C, Davies MJ, Gopinathan V, Milner A. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci* 1993; 84:407-12.
24. Valacchi G, Bocci V. Studies on the biological effects of ozone: 11. Release of factors from human endothelial cells. *Mediat Inflamm* 2000; 9:271-6.
25. Mendiratta S, Qu ZC, May JM. Erythrocyte ascorbate recycling: antioxidant effects in blood. *Free Radic Biol Med* 1998; 24:789-97.
26. Mendiratta S, Qu ZC, May JM. Enzyme-dependent ascorbate recycling in human erythrocytes: role of thioredoxin reductase. *Free Radic Biol Med* 1998; 25:221-8.
27. Sen R, Baltimore D. Inducibility of kappa immunoglobulin enhancer-binding protein Nf-kappa B by a posttranslational mechanism. *Cell* 1986; 47:921-8.
28. Baeuerle PA, Henkel T. Function and activation of NF-kappa B in the immune system. *Annu Rev Immunol* 1994; 12:141-79.
29. Valacchi G, Bocci V. Studies on the biological effects of ozone: 10. Release of factors from ozonated human platelets. *Mediat Inflamm* 1999; 8:205-9.
30. Awasthi YC, Ansari GA, Awasthi S. Regulation of 4-hydroxynonenal mediated signaling by glutathione S-transferase. *Methods Enzymol* 2005; 401:379-407.
31. Poli G, Schaur RJ, Siems WG, Leonarduzzi G. 4-hydroxynonenal: a membrane lipid oxidation product of medicinal interest. *Med Res Rev* 2008; 28:569-631.
32. Takahashi Y, Takahashi S, Yoshimi T, Miura T, Mochitate K, Kobayashi T. Increases in the mRNA levels of γ -glutamyltransferase and heme oxygenase-1 in the rat lung after ozone exposure. *Biochem Pharmacol* 1997; 53:1061-4.
33. Dianzani MU. 4-Hydroxynonenal and cell signalling. *Free Radic Res* 1998; 28:553-60.
34. Cheng JZ, Sharma R, Yang Y, Singhal SS, Sharma A, Saini MK, Singh SV, Zimniak P, Awasthi S, Awasthi YC. Accelerated metabolism and exclusion of 4-hydroxynonenal through induction of RLIP76 and hGST5.8 is an early adaptive response of cells to heat and oxidative stress. *J Biol Chem* 2001; 276:41213-23.
35. Yang YS, Sharma R, Sharma A, Awasthi S, Awasthi YC. Lipid peroxidation and cell cycle signaling: 4-hydroxynonenal, a key molecule in stress mediated signaling. *Acta Biochim Pol* 2003; 50:319-36.
36. Petersen DR, Doorn JA. Reactions of 4-hydroxynonenal with proteins and cellular targets. *Free Radic Biol Med* 2004; 37:937-45.
37. Poole LB, Karpus PA, Claiborne A. Protein sulfenic acids in redox signaling. *Annu Rev Pharmacol Toxicol* 2004; 44:325-47.
38. Markovitch D, Tyrrell RM, Thompson D. Anticipation of subsequent demanding exercise increases the expression of haem oxygenase-1 mRNA in human lymphocytes. *Stress* 2008; 11:79-82.
39. Nakao A, Sugimoto R, Billiar TR, McCurry KR. Therapeutic antioxidant medical gas. *J Clin Biochem Nutr* 2009; 44:1-13.
40. Calabrese EJ. Getting the dose-response wrong: why hormesis became marginalized and the threshold model accepted. *Arch Toxicol* 2009; 83:227-47.
41. Bocci V. Retinal degenerative disorders. In: *Ozone. A new medical drug*. 2nd edition, Springer, Dordrecht, Netherlands, pp 133-145, 2011.
42. Benson H, Friedman R. Harnessing the power of the placebo effect and renaming it "remembered wellness". *Annu Rev Med* 1996; 47:193-9.
43. Viru A, Tenzegolskis Z. Plasma endorphin species during dynamic exercise in humans. *Clin Physiol* 1995; 15:73-9.
44. Hilbert JE, Sforzo GA, Swensen T. The effects of massage on delayed onset muscle soreness. *Br J Sports Med* 2003; 37:72-5.
45. Michaud K, Matheson K, Kelly O, Anisman H. Impact of stressors in a natural context on release of cortisol in healthy adult humans: a meta-analysis. *Stress* 2008; 11:177-97.
46. Travagli V, Zanardi I, Bocci V. A realistic evaluation of the action of ozone on whole human blood. *Int J Biol Macromol* 2006; 39:317-20.
47. Bocci V, Valacchi G, Corradeschi F, Aldinucci C, Silvestri S, Paccagnini E, Gerli R. Studies on the biological effects of ozone: 7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone. *J Biol Regul Homeost Agents* 1998; 12:67-75.
48. Goldstein BD, Balchum OJ. Effect of ozone on lipid peroxidation in the red blood cell. *Exp Biol Med* 1967; 126:356-358.
49. Cataldo F, Gentilini L. Chemical kinetics measurements on the reaction between blood and ozone. *Int J Biol Macromol* 2005; 36:61-5.
50. Bocci V, Borrelli E, Valacchi G, Luzzi E. Quasi-total-body exposure to an oxygen-ozone mixture in a sauna cabin. *Eur J Appl Physiol Occup Physiol* 1999; 80:549-54.
51. Werkmeister H. Dekubitalgeschwüre und die Behandlung mit der Ozon-Unterdruckbegasung In: *Viebahn-Hänsler R, Knoch HG (eds) Ozon-Handbuch. Grundlagen. Prävention. Therapie. Ecomed, Landsberg*, pp V-7.11-V-7.122, 2001.
52. Thiele JJ, Traber MG, Tsang K, Cross CE, Packer L. In vivo exposure to ozone depletes vitamins C and E and induces lipid peroxidation in epidermal layers of murine skin. *Free Radic Biol Med* 1997; 23:385-91.
53. Thiele JJ, Traber MG, Polefka TG, Cross CE, Packer L. Ozone-exposure depletes vitamin E and induces lipid peroxidation in murine stratum corneum. *J Invest Dermatol* 1997; 108:753-7.
54. Thiele JJ, Hsieh SN, Briviba K, Sies H. Protein oxidation in human stratum corneum: susceptibility of keratins to oxidation in vitro and presence of a keratin oxidation gradient in vivo. *J Invest Dermatol* 1999; 113:335-9.
55. Valacchi G, van der Vliet A, Schock BC, Okamoto T, Obermuller-Jevic U, Cross CE, Packer L. Ozone exposure activates oxidative stress responses in murine skin. *Toxicology* 2002; 179:163-70.
56. Burgassi S, Zanardi I, Travagli V, Montomoli E, Bocci V. How much ozone bactericidal activity is compromised by plasma components? *J Appl Microbiol* 2009; 106:1715-21.
57. Foksinski M, Bialkowski K, Skiba M, Ponikowska I, Szmurlo W, Olinski R. Evaluation of 8-oxodeoxyguanosine, typical oxidative DNA damage, in lymphocytes of ozone-treated arteriosclerotic patients. *Mutat Res* 1999; 438:23-7.