

Medical ozone is now ready for a scientific challenge: current status and future perspectives

Lamberto Re^{1,2}, Giuseppe Malcangi¹, Gregorio Martinez-Sanchez¹

¹Medinat srl Clinic, Camerano; ²Molecular and Clinical Pharmacology, University of Ancona, Ancona; Italy.

Abstract

The aim of the present review is to clarify some of the basic mechanisms underlying ozone therapy. Indeed, after its empiric use started at the beginning of the last century, science is now ready to give a chance to the more and more medical doctors working in this field. Unfortunately, the lack of a full recognition by the health authorities and some ostracism against it is, up to date, the major obstacle for its full medical acceptance. Anyway, in the last years and thanks to the contributions of several scientists, most of the mechanisms characterizing the bio-humoral activity of ozone have been scientifically outlined. The built up of randomized clinical studies is going on slowly despite the lack of funds and the difficulties bound mainly to the huge variability of the ozone action.

The thousand and thousand medical doctors involved in the use of ozone as emerging therapy, must be fully educated about the properties of this gas in the aim to counteract scientifically the criticisms of colleagues devoted to other field of medicine and not expert of the ozone pharmacological properties. Is for this reason that we encourage all the professionals to deeply increase the knowledge related to the scientific data produced and published on the international literatures in the field of the ozone therapy.

For the future we suggest the use of ozone not in alternative but as a complement of the most appropriate pharmacological treatments also in the aim to reduce some side effects derived from a chronic drug use. The lack of a well-defined binding site for the ozone molecule could suggest the introduction of virtual receptors for the supposed biological activity of ozone acting mostly throughout second messengers pathways.

Key words:

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Correspondence:

Lamberto Re;
Via Fazioli 22,
60021 Camerano, Italy.
lambertore@univpm.it

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Introduction

When many decades ago we started our scientific work at the Department of Clinical Pharmacology of the Medical Faculty of the Ancona University, we were fascinated in discovering the most intimate mechanisms of the drug action. During these years our prevalent interest was to investigate how cells communicate and how neuronal mediators are released with a highly recognized technique like Patch Clamp [1, 2], The results were encouraging and some pioneering work [3] anticipated important discoveries regarding some new ion channels whose encoding genes were named Slick and Slack [4].

This preface is essential to understand our aim also in different field of medicine like “**ozone therapy**” (OT) that must be, as has been in the past, joined to science following the most rigorous protocol to validate any new proposed therapeutic potential. This was the reason and the condition that we put over any other things when we decided more than 20 years ago to share our science with the OT. Indeed, the jump to this new field of

interest occurred in one period were only a few papers dealing on ozone mechanisms appeared on indexed scientific journals.

The most criticism that we received that time was mainly addressed to the fact that this new field, OT, was apparently out of any reasonable interest either of the pharmaceuticals or of the clinicians. Anyway, we followed because we thought, and we already think, that scientific challenge must be devoted to any field of possible interest, without any exclusion that can be eventually stated only after and not before the demonstration of any scientific data supporting it.

One more reason that stimulates us to follow in the field of OT was the lack of any therapeutic potential against some rare illnesses and the possibility that a deep scientific characterization of the ozone potential could be useful, almost in reducing symptoms, in many conditions so far orphan of adequate pharmacological treatment. More, regarding the autonomic nervous system, looking at the complexity of its organization, with

cholinergic receptors represented both at the preganglionic (nicotinic) and postganglionic (muscarinic or adrenergic) sides, appeared not easy a therapeutic intervention following the conventional schemes. In this context, we can imagine OT not against but as a promising allied of the orthodox medicine treatment.

The fact that ozone interaction with the biological environment is not ruled by the classical *Lineweaver-Burk* plot was not a reason so strong to convince us to leave the challenge, as some colleagues dealing in the pharmacological field suggested to us too many times. Indeed, new concepts or models must be outlined to fit the conditioning mechanisms described for the ozone activity. The pharmacological approach to patients is rationally conducted with drugs and surgery methodologies underwent, before marketing, to the most rigorous scientific and clinic characterization. The fact is one of the most relevant criteria for drug registration and diffusion from the health authorities all around the world. Ozone was introduced to the medical attention more than two decades ago, mainly used as a support therapy in the aesthetic and rare illnesses fields. Unfortunately, beside the wide use in many countries, its characterization is still lacking of a deepening clinical evaluation throughout well-defined randomized and blind studies. The problems behind a more large acceptability of the technique are mainly due to the difficult in classifying its supposed pharmacological effects and, because of its brief lifetime, to evaluate some pharmacokinetic parameters. Furthermore, if we consider that it is also difficult to grant researches that can't warrant an adequate marketing interest, we can have an idea of a very complicated pattern reflecting negatively against ozone. One of the first international reports of ozone activity was done at the beginning of the past century [5] and described its action as antiseptic at high doses. Later on the concepts of low doses started to be the more appropriate for such agent that, as known, is a strong oxidant and considered very toxic for living organisms and very dangerous when breathed [6]. The hypotheses that low ozone doses, non-toxic and well tolerated by biological tissues which possess an own antioxidant defences, seems to be now supported by some experimental data. In this context some works of the toxicologist Edward Calabrese concerning hormesis could be indicative of such action [7]. Although not completely accepted, the theory is well known to most of the pharmacologists working with *in vitro* assays.

Indeed, it could be easily proven that while higher doses of a drug could activate a receptor, low doses could inhibit it. Looking to the wide basic and clinical studies reported in the next session, it could be not without sense the theory proposed for the ozone mechanism [8, 9].

Current status

By a pure pharmacological point of view ozone can't be considered a drug. However, looking at the multiple second messenger cascades that it activates could be classified as a physio-pharmacological agent. In other words, some physiological mechanisms are involved as an adaptive response from the biological environment but not as a direct consequence of a binding reaction.

This makes more difficult the efforts of scientists in the evaluation of the molecular events underlying its clinical efficacy. The physiological formation of an ozone-like mediator during inflammation is indicative of the striking potential of ozone as a new bio-molecule. The fact imposes hard efforts to clarify the hypothesized mechanism following new strategies with newly constructed randomized-standardized clinical trials. Moreover, the mechanisms of action of ozone on blood bio-molecules with the generation of several messengers responsible of its biological effects have been well clarified since 2002.

Ozone used in appropriate doses is characterized by the formation of reactive oxygen species and lipid peroxides allowing them to become late and long-lasting messengers. The paradoxical concept that ozone eventually induces an antioxidant response capable of reversing a chronic oxidative stress is common in the animal and vegetal kingdom; it is already supported by findings of an increased level of antioxidant enzymes during OT. Those facts can include ozone as a hormetin.

After ozone administration an acute oxidative stress has occurred that leads to a number of phenomena such as up-regulation of antioxidant enzymes. An example of this dose dependent induction was evident in a study in rabbits, even when was imperceptible for the authors the hormetic behavior. A second look to their results shown a significant increase in level of manganese superoxide dismutase (MnSOD) and myeloperoxidase after treatment, only in the central doses (Fig.1)[10].

When ozone treatment was combined with different drugs, used as a model of tissue damage, it was evident that optimal doses of ozone could avoid the drug damage. For example, in a rat model

of nephrotoxicity induced by cisplatin, central doses of ozone reduce the renal damage (serum creatinine decrease), at the same time ameliorating the SOD activities (Fig.2)[11].

Other animal models using combination of drug and ozone treatment showed similar results. For example, ozone reduces methotrexate-induced intestinal injury in rats. The mechanism of protection involves also an overexpression of SOD and glutathione peroxidase in tissue (intestine)[12]. The same mechanism is involved in the protection observed in nephrotoxicity induced by acetaminophen [13].

The integrative effect of OT is also evident in clinical studies. For example, in a multicentre randomized, double-blind controlled trial in patients with acute low back pain due to lumbar disc herniation was found that paravertebral injections of ozone seems to safely and effectively relieve pain, as well as reduce both disability and the intake of analgesic drugs [14]. In addition, two recent meta-analysis studies showed that ozone treatment of herniated discs is an effective and extremely safe procedure. Pain and function outcomes are similar to the outcomes for lumbar discs treated with surgical discectomy, but the complication rate is much lower (<0.1%) and the recovery time is significantly shorter [15, 16]. Ozone treatment also reduces the microorganism resistance and consequently makes more efficient the antibiotic therapy [17, 18]. During treatment of diseases that involve a chronic disruption of redox status (oxidative stress), up-regulation of antioxidant enzymes is far more beneficial than oral antioxidant supplementation. However, the key to reach the optimal effect of ozone depend on the doses.

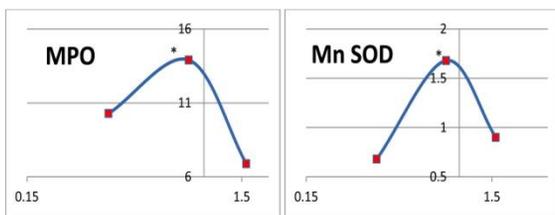


Figure 1. Hormetic shape of antioxidant enzymatic activities after 90 days of ozone treatment in rabbits. Dose log (0.36, 0.85 and 1.57 mg/kg) vs enzymatic activities. Animals were treated 3 times a weeks.

MPO; myeloperoxidase was assayed in lung tissues and expressed in U per g of tissues. **MnSOD;** manganese superoxide dismutase, was assayed in liver homogenates and expressed as U per mg of protein. *significant differences ($p < 0.05$) compared to control group (animals treated with O_2) and low and high O_3 doses. Data were represented using values took from Martinez *et al* [10] Doses were calculated from total dose to mg/kg taking 1.65 kg as median body weight of animals.

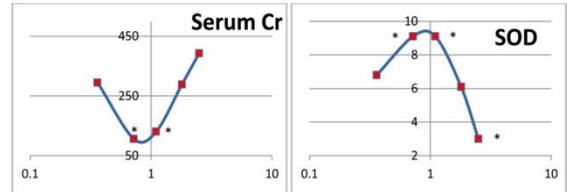


Figure 2. Hormetic shape of serum creatinine (Cr) and superoxide dismutase (SOD) after 15 days of ozone treatment and cisplatin-induced nephrotoxicity in rats. Ozone was administered by rectal way 15 days after cisplatin-induced nephrotoxicity. Dose log (0.36, 0.72, 1.1, 1.8 and 2.5 mg/kg) vs Cr or enzymatic activity.

Cr was expressed in μM . SOD was assayed in kidney homogenates and expressed as U per mg of protein. *significant differences ($p < 0.05$) compared to control group (animals treated with O_2). Data were represented using values taken from Borrego *et al* [11].

Up to now, ozone doses are empirically established based on the clinical experience of the different school of OT. In general doses are ranged in 3 intervals: low, medium and high and it is recommended to begin from low doses to high doses depending by the clinical evolution of the patients. In the future, this therapy will be close connected with a diagnostic of the redox status of the patients. In addition, the mathematical tool based on the hormetic response of ozone to calculate the doses should be developed in order to optimize the therapeutic response. As stated above, we must always take into account that the pharmacological models used to define the mechanism of action of drug are usually based upon the *Michaelis-Menten* kinetics. We know that either the metabotropic or the voltage operated receptors are characterized by an extracellular binding site for the drug interaction and an intracellular transduction mechanism [19].

Usually, *in vitro* assays are very useful to determine with the above model, or with the linearization of the *Lineweaver-Burk* plot, the pharmacodynamic behaviour of drugs and make easier the evaluation of the affinity constant for each ligand-receptor interaction. In the case of ozone or other substances orphan of a stereo chemical ligand site we can postulate the presence of a “**virtual receptor**” characterized by some cellular components like lipids or other membrane substrates. Many scientific data are consistent with a first interaction between ozone molecule and double-binds of membrane fatty acids and the subsequent formations of second messengers like hydroperoxides or lipoperoxides [20]. These products could in turn activate a cascade of molecular events responsible of the modulating

effects induced by the ozone molecule at the intracellular environment and mainly in the mitochondria. By a certain point of view we can state that ozone do not fit any pharmacodynamics or pharmacokinetics model but simply acts as a pharmacomodulator due to an adaptive response following its oxidative reactions. These effects must be clearly stated also in the case of the positive effects of ozone in the disk herniation avoiding referring to terms like lysis or disk disruption that, even occurring, do not represent the main mechanism responsible of the pain relief and could induce some confusion to the patients as well.

Conclusion

In conclusion, the hormesis model could fit well the wide reports describing some biochemical activities induced by ozone. Ozone could not be

used as a regular drug, of which appear to be very far, but as a conditioning agent that could be helpful in modulating some key functions and finally ameliorate the physical status of the patients. In this context, the term *ozohormesis* or *ozormesis* could better explain some positive action of the gas simply as complement or as integrative support to the inalienable orthodox treatment.

Competing interest statement

Authors declare that no support from any organisation for the submitted work was received. Authors have no financial relationships with any organisations that might have an interest in the submitted work in the previous three years and no other relationships or activities that could appear to have influenced the submitted work.

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