May Oxygen-Ozone Therapy Improves Cardiovascular Disorders?

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Abstract: In an aging population vascular disorders well exemplified by the chronic limb ischemia, chronic heart failure, cerebral ischemia and age-related macular degeneration represent a serious medical and socio-economical problem. While there is always a not easily identifiable first pathogenic noxa, all of these diseases are characterized by ischemia, chronic inflammation and tissue degeneration. Orthodox medicine has provided several optimal drugs targeting various pathological situations but, even with their concomitant applications, it is not possible to reduce the chronic oxidative stress. Here it is proposed to associate the approach of ozonated autohemotherapy as a modifier of the biological response capable to block the pathological progress.

Key Words: Chronic limb ischemia, chronic heart failure, ictus, age-related macular degeneration, chronic oxidative stress, ozone therapy.

INTRODUCTION

Cardiovascular diseases including chronic limb ischemia (CLI), chronic heart failure (CHF) and cerebrovascular ischemia (CVI) not only compromise a productive life but markedly increase health-care costs and eventually represent the first cause of death. In affluent Countries, an excessive and incorrect diet, sedentary lifestyle, stressful working conditions and genetic factors are the main causes responsible for enhancing atherosclerosis, obesity and diabetes, premonitory of vascular progressive disorders. The complexity of the metabolic disorders has been aptly summarized by the concept of the "metabolic syndrome" [1,2]. The atrophic form of age-related macular degeneration (A-RMD) with the initial ischemia of the choroidal vessels, the progressive degeneration of the retinal pigment epithelium (RPE) and the retinal photoreceptors death can also be included, because increasing the oxygenation of the retina, which has a very high oxygen consumption, has a decisive relevance [3].

Orthodox medicine has provided a series of antidiabetics, antihypertensive agents, antiplatelet and antithrombotic drugs, statins possibly associated with etizimide or/and fibrates. All of these drugs, by normalizing the glycemia, arterial pressure, hypercoagulability, cholesterol and low-density lipoproteins levels are able to delay ischemic episodes, particularly when these drugs are associated to a moderate diet and physical activity.

Although the life-span of cardiovascular patients has been markedly prolonged, the pathological process remains active because of the ensuing chronic inflammation at vascular level, which provokes a chronic oxidative stress due to an imbalance between either an excess of oxidant molecules or/and the progressive weakening of the natural antioxidant capacity [4]. Among the formers there are the reactive oxygen species (ROS) represented by anion superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), possibly hydroxyl radicals ($OH^{\cdot}$), peroxynitrite ($ONOO^-$), and hypochlorous acid (HClO). Moreover, the peroxidation process, the complement activation, the release of coagulation, metalloproteinases and growth factors, thromboxane A and leukotrienes, cytokines such as tumor necrosis factor α (TNFα), interferon γ and interleukins (IL-1, IL-2, IL-6) perpetuate the inflammatory process.

The progressive release of toxic compounds and of abnormal concentrations of pro-inflammatory cytokines is responsible for cell death or degeneration or a proliferative dysregulation leading to a steady unbalance of the redox state owing to the cell inability to efficiently neutralize the excess of oxidants and toxic compounds. Thus the chronic inflammation worsens with time, possibly becoming irreversible unless we can dampen the process and restore a normal redox state. A vicious imbalance between oxidants and antioxidants is now firmly established in atherosclerosis, diabetes, ischemia, nephropaties, hyperhomocysteinemia, neurodegeneration, infections, autoimmune diseases, asthma, chronic obstructive pulmonary disease and cancer, just to name the most common pathologies. Unless we can interrupt this imbalance a more or less rapid progression is unavoidable. Numerous strategies potentially capable to reduce or contain the oxidative stress in these diseases have been devised as follows:

THE STATE OF THE ART

1). Administration of corticosteroids is one of the most frequently used procedures for reducing inflammation. Corticosteroids have been and are being used in a wide variety of pathologies ranging from dermatitis to brain tumors. Unfortunately the anti-inflammatory effect is temporary and serious adverse effects (diabetes, osteonecrosis, osteoporosis, Cushing's syndrome, glaucoma) can ensue with broad immunosuppression

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1871-529X/09 $55.00+.00

[5]. Moreover glucocorticoid resistance may result from a sustained reduction of histone deacetylase-2.

2). Non-steroidal anti-inflammatory drugs (NSAID) are also widely used mostly to overcome prostaglandin-related pain states. Different types of drugs can block either or both COX-1 and COX-2 isoforms and, they are effective analgesics, but, a prolonged use may cause gastric ulcerations and undesirable cardiovascular events [6,7].

3). Administration of allopurinol improves endothelial dysfunction in CHF [8]. A randomized, double-blind crossover study on 11 patients with NYHA class II-III chronic heart failure received 300 mg allopurinol daily versus placebo. Allopurinol appeared to have inhibited xanthine oxidase thus reducing oxidative stress, a finding also supported by the significant reduction of plasma malondialdehyde.

4). Inhibition of NADPH oxidase present in the plasma membrane of phagocytes [9], by limiting the production of superoxide, may reduce oxidative stress, but a direct action remains a difficult pharmacological problem with the further risk of increased bacterial infections.

5). There is good evidence that inhibition of the renin-angiotensin system can reduce cardiovascular events [10]. Angiotensin-converting enzyme (ACE) inhibitors and Ang-II receptor antagonists associated with diuretics are broadly used drugs for effectively reducing blood pressure and partly inhibiting NADPH oxidase. Interestingly, several other antihypertensive drugs do not improve the antioxidant status in patients [11].

6). The inhibition of the 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (or statins), the key enzyme of cholesterol biosynthesis may be beneficial [12]. Surprisingly statins, not only are able to lower serum cholesterol levels and increase the number of hepatic LDL receptors, but they are able to modulate pathophysiologic processes in patients with acute coronary syndromes [13]. By blocking the synthesis of critical isoprenoid intermediates [14], they express other effects such as a limited inhibition of NADPH oxidase, the increased expression of endothelial NO synthase and of tissue-type plasminogen activator, while they inhibit the expression of plasminogen activator inhibitor and endothelin-1. Indeed this "miracle drug" [15] seems to reduce inflammation, inhibits lymphomas progression [16] and the inflammatory components of multiple sclerosis [17]. Although rarely, statins may provoke rabdomyolysis [18].

7). It has been claimed that an excessive production of superoxide could be inhibited by long-term administration of L-arginine, which is the substrate for NO synthesis [19,20].This approach and autohemotherapy may also reduce the many complications observed in sickle-cell anemia patients [21].

8). An excessive production of superoxide could be also quenched by the administration of superoxide-dismutase (SOD) or, better, of enzyme mimetics able to enter into the cells [22,23]. This is an interesting possibility because exogenous enzymes are antigenic and unable to enter into the cell. For some of these mimetics, the pharmacology and toxicity remain to be defined. Nonetheless, in order to investigate the therapeutic potential of these drugs, extensive clinical trials are warranted.

9). The potential of inducing a therapeutic advantage through gene transfer of antioxidant enzymes has been taken into consideration in rabbits [24] but some relevant issues have to be solved prior to clinical application, including the understanding of the most suitable vectors, the feasibility and stability of gene delivery to different tissues or/and organs affected by the oxidant stress. Studies on longevity have shown some correlation with increased resistance to oxidative stress and an intriguing relationship among intracellular oxidants, *p66shc*, forkhead protein and *p53*. This aspect may become important but again we are not yet able to practically manipulate the *p66shc* gene [25,26].

10). Whenever the case, the increase of homocysteine levels in the plasma must be inhibited because the autoxidation of its sulfhydryl group generates superoxide and hydrogen peroxide that are cytotoxic for the endothelium. Hyperhomocysteinemia can be kept under control by the daily administration of folic acid and vitamins B6 and B12 [27] that may allow a normalization of adenosine plasma levels [28].

11). It is well known that an abnormal platelet aggregation has dire consequences and can be inhibited with a variety of antithrombotic agents from the old aspirin to the recent clopidrogrel [29].

12). Moreover the synthesis of pro-inflammatory autacoids and platelet aggregability can be inhibited by the administration (2-3 g daily) of n-3 PUFAs present in fish oil. These particular unsaturated fatty acids enhance the generation of 3-series prostaglandins and 5-series leukotrienes, which are anti-inflammatory [30,31].

13). It is well known that oxidative stress due to formation of $OH^{\cdot}$ is enhanced by free transition metals (Fe^{2+}, Cu^{+}). For some time chelation therapy with EDTA, deferoxamine and defasirox have been popular, but there is now a broad consensus to abandon this approach [32,33].

14). A hybrid of K_{ATP} channel opener and nicotinamide nitrate (Nicorandil) was found to improve cardiac function in acute myocardial infarction by probably reducing ROS formation [34].

15). The famous glycation reaction due to hyperglycemia leads to the formation of advanced glycation end

products (AGEs). The AGEs which are irreversible toxic compounds, deposited in the arterial wall can induce oxidative stress leading to diffused damages and accelerating the progression of diabetes type II, atherosclerosis, renal and retinal damage. Thus hyperglycemia and obesity must be avoided by regulating caloric intake, possibly adopting the norm of caloric restriction [35] with adequate nutrition and by adopting a correct life style without smoking or drinking alcohol and finding time for a daily physical exercise.

16). An excessive paracrine secretion of proinflammatory cytokines such as TNF-α. IL-1, IL-6, IL-8 at the sites of chronic inflammation has been demonstrated in rheumatoid arthritis, Crohn's disease, multiple sclerosis as well as in solid cancer, chronic viral diseases and in chronic pulmonary obstructive disease (COPD) to name a few pathologies. In about 2/3 of rheumatoid arthritis patients, a variety of TNF-α antagonists has significantly improved the clinical outcome by reducing inflammation [36], while antibodies to the other cytokines are far less efficacious. However, TNF-α inhibitors are hardly effective in other diseases with the further caveat that TNF-α antagonists cannot definitively block the disease progression and, with prolonged use, may increase the susceptibility to opportunistic infections [37]. It was postulated that CHF may be due to a continuous release of proinflammatory cytokines, but several clinical trials, aimed to neutralize TNFα in patients with fairly advanced CHF, have resulted in worsening the outcome. It was concluded that neutralization of TNFα alone was inadequate because IL-1β and IL-6 may contribute to the inflammation [38-42]. It was then hypothesized that a new immunomodulation therapy based on treating patients with their own heavily oxidized blood may stop the progression of heart failure [43-46], but a more recent and ample evaluation, further discussed in detail, has yielded disappointing results [47]. At least in theory, immune suppression of the autoimmune reactions may be achieved by the administration of IL-10 and TGF-β1 but to date this remains a possibility to be tested. CD25+ regulatory T cells (T-regs) may be able to control excessive immune responses but, as yet, potentially valid approaches are still in an experimental phase.

17). The discovery of "the cholinergic anti-inflammatory pathway" [48] has clarified that the nervous system can modulate immune responses in real time. The efferent vagus nerve can control the inflammatory response by inhibiting the α7 subunit of the nicotinic acetylcholine receptors expressed on macrophages. Practical therapeutic modalities are being explored [49] for their possible application in the treatment of endotoxemia, myocardial depression and cancer.

18). For a long time, Dr. Richard Spears has pioneered the direct intravascular application of a high oxygen tension in ischemic tissues especially after coronary occlusion. He has proposed to use an extremely high concentration of oxygen dissolved in a saline solution as a more effective alternative to the hyperbaric oxygen chamber [50]. Anoxic myocardium needs oxygen, but the result depends upon a precise time interval as otherwise oxygen may aggravate the damage. Indeed, so far the reperfusion appeared safe, but a modest effect was observed only when reperfusion was performed < 6h from symptom onset. Similar to what may happen during a treatment with the hyperbaric oxygen chamber, an excessive oxygen concentration may well represent a double-edged sword [51].

19). The use of small interfering RNA (siRNA) is promising but, owing to the fact that the chronic oxidative stress is due to a multitude of factors, it will be necessary to first evaluate the most important target(s) and then assess the difficult problem of a suitable delivery system of the selected dsRNAs [52].

20). As the chronic oxidative stress is due to an imbalance between an excess of ROS and LOPs and a deficit of antioxidants, it has become obvious that a supplementary administration of antioxidants may eliminate the stress [4]. This idea has become a fashionable theme, amply discussed by vitaminologists with the risk of intoxicating patients with megadoses of Vitamin A, C and E plus selenium and zinc. A fair conclusion is that the recommended dietary allowance (RDA) of micronutrients, supplemented by a rich dietary intake of fresh fruit and vegetables, while it is important during growth or denutrition or cachexia, it may not have a crucial relevance in oxidative stress-related conditions. Indeed the evidence that it can be a real remedy remains controversial [53-67]. On the other hand, megadoses may be either toxic or counterproductive in the sense that, at the very least, they may inhibit the synthesis of heme-oxygenase-I (HO-I) as described by Peng *et al.* [68]. The following factors can explain why an excessive oral supplementation can be of scarce value:

i. the uncertainty of intestinal absorption. Usually the higher the vitamin dose, the lower is the percentage of absorption.

ii. the difficulty of maintaining a steady plasma level,

iii. the individual variability of metabolism and excretion,

iv. of utmost importance is the variable and limited uptake of antioxidants tightly regulated by either the cell membrane active- or facilitative-transport. The fashionable idea that intravenous injection of megadoses of vitamin C and reduced glutathione do miracles ought to be firstly well documented by pharmacological and clinical data. It is well known that cellular uptake of GSH is inefficient and that it is preferable to administer orally three times daily

600 mg of N-acetylcysteine (NAC) [69]. Moreover the lysine salt of NAC, N-acystelyn (NAL), by forming a neutral pH when in solution, is more effective than NAC [70].

v. the possibility that some antioxidant vitamins may exert oxidant activity.

vi. the inability of vitamins to stimulate the synthesis of cellular antioxidant enzymes which represent the crucial defence. Thus the approach of an antioxidant supplementation must be well weighed and, although seems useful to administer an equilibrated amount, it remains doubtful if it can correct the chronic oxidative stress, unless the patient's organism has become unable to efficiently recycle dehydroascorbate and GSH disulphide to ascorbic acid and GSH. The problem with the chronic oxidative stress is not necessarily the lack of vitamins as much as the constant and elevated intracellular levels of oxidants accompanied by a chronic deficiency of reduced glutathione and thioredoxin due to the inability of producing sufficient reductive power. As beautifully exposed by Mendiratta *et al.* [71], Dickinson and Forman [72], and Wilson [73], low molecular weight thiol compounds and ascorbic acid play an essential role in many reactions "due to the ease with each they are oxidized, and the rapidity with which they can be regenerated." During a chronic inflammation, besides a reduced generation of ATP, the main problem is linked to the defective supply of the major electron donor, NADPH and of antioxidant enzymes such as GSH peroxidases (GSH-px), GSH reductases (GSH-rd) and GSH S-transferases (GST), the latter performing conjugation reactions able to neutralize toxic aldehydes. Obviously a concomitant deficit of SOD and of glucose 6-phosphate dehydrogenase (G6PD) is important while we have rarely observed a deficit of catalase. The crucial advance will come if it will be possible to reverse the chronic oxidative stress by actively inducing the upregulation of intracellular antioxidant enzymes

THE POSSIBILITY OF CORRECTING THE CHRONIC OXIDATIVE STRESS

This problem has been investigated during the last two decades and it has been clearly shown that a small oxidative stress due to hyperoxia as well as ROS [74-78] and, more recently, therapeutic doses of ozone [79-85] can upregulate the synthesis of antioxidant enzymes and of HO-I, which is now one of the most interesting enzymes because it has antioxidant and multiform protective activity [86-89]. It has become clear that any change of the external environment disturbs cell homeostasis, but if the stress (hyperthermia, hyperoxia, radiation, ischemia, xenobiotics and so on) is tolerable, or graduated in intensity, the cell can adapt to it and survive. On the other hand, if the stress exceeds the cell capabilities, the cell programmes its own death. This behaviour is universally present from bacteria to fungi to plants to mammals and the concept of ischemic and oxidative preconditioning has been extensively discussed elsewhere [90]. Goldman [91] and Calabrese [92-2005] have presented examples of stimulatory responses following stimuli below the toxicological threshold and have coined the term "hormesis." As we have used small, precisely calculated and repeated ozone stresses [79,81,83], we have selected to use the term of *induced adaptation to a calibrated oxidative stress*.

There are several modalities but the most precise, where ozone and blood antioxidants can really stoichiometrically react is ozone therapy, under the form of a major autohemotherapy. The procedure consists in 2-3 weekly intravenous reinfusion of 200-250 ml of the patient's blood mixed with an equal volume of a gas mixture composed of medical-grade oxygen (~96%) and ozone (equivalent to progressively increasing ozone doses just five minutes after gently mixing the gas mixture with blood). We have adopted the strategy of slowly increasing the ozone dose from 4-5 mg up to 16-20 mg (200-250 ml of blood, respectively) to gradually improve the adaptation to the ozone stress. Thus, ozone induces a calibrated acute oxidative stress during which a number of well defined messengers (H_2O_2, 4-hydroxynonenal) interact with blood and parenchymal cells and induce the up-regulation of antioxidant enzymes and HO-1.

The practice of autohemotherapy is some 40 years old and it has been performed in many Countries showing to be effective, atoxic and well-tolerated [93,94], especially in A-RMD [3] and chronic limb ischemia [95] which are the most frequently treated disorders.

On the basis of the mechanism of action, ozone therapy can induce the following biological responses: a) it improves blood circulation and oxygen delivery to ischemic tissue owing to the concerted effect of NO and CO and an increase of intraerythrocytic 2,3-DPG level; b) by improving oxygen delivery, it enhances the general metabolism; c) it upregulates the cellular antioxidant enzymes and induces HO-1 and HSP-70; d) it induces a mild activation of the immune system and enhances the release of growth factors; e) it does not procure acute or late side effects; and, finally, it procures a surprising wellness in most of the patients, probably by stimulating the neuro-endocrine system. It does seem that ozone therapy acts as a biological response modifier on many targets gone astray because of a chronic inflammation. All of these biological modifications have been extensively discussed elsewhere [82,90,95] but it remains still uncertain whether some messengers present in the ozonated blood are able to stimulate the release of staminal cells in the patient's bone marrow. The mobilization of these cells would represent a crucial advantage in both CLI and CHF.

Nonetheless, this simple and inexpensive procedure has already yielded therapeutic results in CLI (grade 2-4 of Fontaine-Leriche's classification) superior to those achieved by orthodox medicine using the gold standard infusion of various prostanoids [96-106]. It appears likely that the exceptional versatility of the procedure makes it amenable to be

evaluated in CHF and ictus. In such a case, by simultaneously using a fibrinolytic agent (tissue plasminogen activator) with mild ozone therapy appears important to rapidly reduce hypoxia of the penumbral zone, thus minimizing further complications [107].

Why our very mild ozonetherapeutic modality should be effective when the Celacade™ system, thought to be an immune-modulation procedure based on the intragluteal injection of 10 ml of the CHF's patient blood (grade II and III of the New York Heart Association, functional classification) after its treatment with as much as 75 mg of ozone, plus UVA irradiation and heat stress at 42.5 °C has failed to improve the prognosis? This extremely harsh procedure proposed in 1997 [43] and supported by commercial interest was wrongly believed to reverse *in vivo* the prevalence of the T-helper type 1 (Th1)/T-helper type 2 (Th2) equilibrium and inhibit the vascular flogosis. Already in 1996 the "SIMPADICO" trial programmed for improving the chronic limb ischemia was aborted [108] owing to the lack of a clinical result. However, it has been the final "ACCLAIM" trial published in 2008 [47] that has shown no real clinical improvement in 1213 CHF's patients treated for about 6 months with some 25 i.m. injections of their heavily oxidized blood. Although four critical comments [109-112] on the rationale of this approach have explained this failure, the improper use of an ozone dose at least 92-fold higher than the real therapeutic dose (from 20 μg/ml or 0.42 μmol/ml up to 80 μg/ml or 1.68 μmol/ml gas per ml of blood) as we have determined over the years [82-84] has compromised the future of ozone therapy. The misinterpretation of the real mechanisms of action such as vasodilatation, increased delivery of oxygen in ischemic tissue and upregulation of antioxidant enzymes and HO-1 has completely subverted the proposal of the physiological use of ozone as a valid medical drug.

In spite of this serious pitfall, it is believed that the ozonetherapeutic approach simultaneously applied with the broad range of orthodox drugs, may represent a promising approach for correcting the chronic oxidative stress and improving the uncertain prognosis of many patients. Clinical protocols had been prepared for the four mentioned vascular disorders and will be evaluated during the next two years. Any interested clinicians in this endeavour would receive our best assistance.

CONCLUDING REMARKS AND A PERSPECTIVE

There is a general consensus that vascular disorders such as CLI, CHF, ictus, and A-RMD are progressive diseases where the common denominators are the ischemia and an irreversible chronic inflammation. Owing to a number of concomitant negative factors, it is unlikely that the separate use of the available therapeutic strategies for reducing the chronic oxidative stress may succeed in improving the prognosis. The problem is complex because among obesity, diabetes, hypertension, and the likes there are substantial pathologic differences so that the clinician has the difficult task to select the best medicinal combination for achieving a benefit. Moreover, several of current drugs are too narrowly focused and not all patients are able to be compliant. The basic aim is to decrease oxidative stress and it is felt that a judicious and simultaneous application of ozone therapy able to radically enliven the biological response is an approach that must be explored for the sake of many patients.

Just to mention a few modern approaches such as gene therapy, autologous transplantation of bone-marrow cells and administration of angiogenic factors, how ozonetherapy compares today with them? Ozone acts as a pro-drug: it dissolves almost instantly in the plasma, switches on a number of biochemical reactions and disappears after generating a number of messengers that, by reacting with a great variety of cells, allow the revival of important biological activities went astray during the preceding chronic oxidative stress. Thus, ozone acts as a natural drug which has a minimal cost, no side effects and procures useful clinical benefits that can be maintained with a bimonthly administration. Indeed this review has intended to inform clinicians of the existence of this neglected procedure.

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Received: 13 February, 2008 Revised: 11 March, 2009 Accepted: 12 March, 2009