Insights into the use of oxygen-ozone therapy in ischemic cardiopathy and cardiovascular disease: a role for mitochondria?

Oxygen-ozone as an adjunct therapy in cardiovascular medicine is increasing its impact in medicine, as it has recently been considered for use in peripheral arterial disease[1] and other vascular disorders.[2] This evidence expands the debate if ozone can help physicians in addressing major and concerning cardiovascular functional impairments.[3]

A possible explanation on how ozone works on cardiovascular physiology may come from considering the role of mitochondria in cardiovascular pathology.[4] Mitochondria are particularly susceptible to ozone, as low doses of ozone modulate mitochondria autophagy (mitophagy)[5] and induce mitohormesis.[6,7] Actually, the quality of mitochondria viability and function greatly affects ischemic events in the cardiac function[8] and mitochondria may be pharmacological targets to be considered in the treatment of ischemic cardiopathy.[9]

If mitochondria are possible targets for treating cardiovascular diseases, then ozone therapy has the potential to find its functional elucidation.

Since nineties, the activity of ozone in ischemic cardiopathy and vascular pathology has been thoroughly reported in the literature. The vascular district and microcirculation are possible target microenvironments to assess the activity of ozone at low doses as a cardioprotective factor.

Orakdogen and colleagues[10] in a model of femoral artery vasospasm in Sprague-Dawley rats, reported that 4 mL of 20 μg/mL ozone daily for 1 week, reduced the morphometric change of artery due to an irregular elastic lamina, inhibited the damage to endothelial cells undergoing vacuolization and reducing vasospasm. The potential of ozone in addressing ischemic concerns in the cardiovascular function starts from previous evidence regarding the effect of ozone in brain reperfusion.[11-13] Even these data suggest that one of the major activities of ozone may be its ability to modulate endothelial physiology.

Actually, oxygen-ozone therapy in patients suffering from ischemic heart disease or myocardial infarction has been tested in Italy as early as 1991, when Prof. Biagio Lettieri, Full Professor of Anesthesia and Intensive Care at the Federico II of Naples, used major oxygen-ozone auto-hemotherapy (O_2-O_3-MAHT) in the treatment of patients with acute-phase myocardial infarction, with good results in terms of pain and prognosis.[14-16]

In 1996, oxygen-ozone therapy was used in the prevention of recurrence of myocardial infarction showing a significant protection from recurrence of infarction.[16] Even recent literature in the field indicates that ozone exerts an important action in modulating the inflammatory response and restoring the proper rheological characteristics of the microcirculation,[17-20] by modulating the endogenous protective enzymatic functions of cells against radical forms, increasing transcription at the DNA level of antioxidant enzymes and reducing the impact of the coagulative system on the inflammatory response.[21,22] Ozone activates the redoxin system, reduces the expression of pro-inflammatory cytokines such as interleukin 1β and tumor necrosis factor α, modulates the nuclear factor-κB system, reduces platelet aggregation and stimulates the release of various growth factors.[17-20,23,24] Thanks to these characteristics, oxygen-ozone therapy is useful in the prevention and treatment of ischemic heart disease and in post-infarction rehabilitation.[14-16,25-27]

The effects of acute administration of an oxygen/ozone mixture on myocardial tissue damage following an experimental myocardial ischemia and reperfusion event were evaluated and it was found that the infarct lesion can be counteracted by a pretreatment with the systemic administration of the gaseous mixture of oxygen-ozone.[25]

Insights into ozone in the cardiovascular physiology: The tale reported so far suggests that low doses of ozone, acting in the hormetic range, may have an impressive impact on microcirculation, cardiovascular function and endothelia. Many recent data about the effect of medical ozone in cardiovascular physiology come from research on experimental animals.

Di Filippo et al.[28] reported an anti-arrhythmic effect of doses from 100 to 300 μg/kg ozone in Sprague-Dawley rats (a dose range corresponding approximately to 11–32 μg/mL), where arrythmias were elicited by a) either ischemia, b) ischemia/reperfusion, c) aconitine 15 μg/kg, intravenous administration and d) 1.5% KCl, intravenous administration. Ozone did not affect the arrhythmias caused by KCl, whereas other forms of induced ischemia were significantly reduced when animals were pre-treated with 150 or 300 μg/kg ozone.[28] Ozone reduces the impact of damages caused by the ischemia/reperfusion injury.[29,30] A possible mechanism involves the Janus kinase 2/signal transducer and activator of transcription 3 signaling and the heat shock protein 70 regulation, as ozone activates the Janus kinase 2/signal transducer and activator of transcription 3 signaling leading to the upregulation of the expression of heat shock protein 70 and reducing the rate of cardiomyocyte apoptosis, which would be induced by ischemia/reperfusion injury.[30] This inhibition on apoptosis may be ruled by an effect on mitochondria.[31]

A way by which ozone can reduce ischemic-induced damage involves the upregulation of the hypoxia factor hypoxia-inducible factor 1α. Actually, rabbit heart is protected by ozone pre-conditioning, which upregulates hypoxia-inducible factor 1α, increasing heart rate, left ventricular filling pressure and the expression of anti-inflammatory cytokines such as interleukin 10, whereas troponin T, troponin I, creatine kinase-MB, interleukin 6, infarcted myocardial area, ventricular tachycardia and ventricular fibrillation were all reduced.[32]

It is paramount to state that ozone in the bloodstream lasts few minutes at 37°C, yet it generates ozonides, lipoperoxides, malonyldialdehyde and 4-hydroxynonenal from polyunsaturated fatty acids, which have bioactive regulatory actions. In particular, 4-hydroxynonenal is able to reduce the activation of the inflammasome NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3), by acting on mitochondria mitophagy and inhibiting pyroptosis.[33] Interestingly, a relationship between ischemic cardiopathy and mitophagy/pyroptosis, has been recently reported.[34]

The effect of ozone therapy in the cardiac activity has been reported also in the context of the heart failure reduced ejection fraction.[15] Buyuklu et al.[15] treated 40 patients with 35% left ventricular ejection fraction with 20–50 μg/mL ozone via O_2-O_3-MAHT for at least 5 weeks. Patients were matched with a paired number of ozone-untreated healthy controls. Following ozone treatment, both left ventricular end-systolic and end-diastolic volumes were reduced, the level of plasma anti-oxidants increased, serum nitric oxide (NO) and malonyldialdehyde reduced.[15] However, the role of NO may be much more intriguing than expected.

Since long time it is well known the role of NO in myocardial ischemic injury.[35] NO is cardioprotective and ozone is able to induce the expression of NO via endothelial NO synthase.[26] The endothelial NO synthase activity and the release of NO trigger

the recruitment of endothelial progenitor cells.[26] A dose range of 100–300 µg/mL ozone in Sprague-Dawley rats induces these mechanisms.[26]

Noteworthy, ozone was reported to have effects on stem cells biology, for example, in neural stem cells from mouse embryo, where 15 µg/mL and 25 µg/mL ozone were able to modulate cytokine production (increasing IL-33, for example), so affecting the development of these neuroectodermal cells.[36] This evidence should suggest if ozone might have a role on cardiac stem cells and progenitor cells.[37]

Actually, despite no evidence so far can be retrieved about a role of ozone in cardiac stem cells, a role of ozone in endothelial progenitor cells can be suggested.[26] As a matter of fact, endothelial progenitor cells are formidable sensors of environmental air pollution, in order to save a healthy functionality of the whole cardiovascular system.[38] This would mean that the role of aryl hydrocarbon receptors in the vascular tissue, as particularly engaged in sensing pollutants and chemical xenobiotics, is crucial and regulate also the fate of stem and progenitor cells in endothelia.[39] Noteworthy, the close relationship between ozone and aryl hydrocarbon receptors has been recently highlighted, and in this sense aryl hydrocarbon receptors would be pertained receptors allowing ozone to modulate endothelial progenitor cells and vascular endothelia activity.[26,40,41] This consideration is particularly crucial, as endothelial cells have a fundamental role in myocardial ischemia/reperfusion injury.[42]

As a matter of fact, ozone protects heart from ischemia/reperfusion injury.[25,26,28]

A look into the molecular mechanisms underlying the cardioprotective role of ozone: The leading property of medical ozone, used in a restricted and low range of doses, is its impact on the oxidative stress response. Cells respond to oxidative stress by finely regulating the genetic expression of the many antioxidant enzymes and scavenging proteins, via the nuclear factor erythroid 2-related factor 2/Kelch-like ECH-associated protein 1/antioxidant response element (Nrf2/Keap/ARE) pathway. This process is controlled by redoxins, which are highly reactive cysteine residues, with rate constants, close to 0.6/s, engaging in nucleophilic substitution a thiol-disulphide with another -SH group in an inter-sulphide transfer reaction. This process is ruled by hydrogen peroxide and has the purpose to tune the correct oxidative transcriptional response via the relationship between these different thiols.[43] Recent data have reported that stimulating redoxins function by low doses of ozone, neutralizes the oxidative damage associated with in-stent restenosis and a poor re-endothelization in cases of coronary angioplasty.[27]

Actually, a proper regulation of the anti-oxidant response is crucial to prevent cardiovascular diseases.[44] The heart-specific knock out of mitochondrial thioredoxin reductase induces cardiac functional impairments.[45]

Torre Amione et al.[46] reported a double-blind, placebo controlled clinical study in patients with heart failure and used ozone at 15–35 g/m^3 (= 15–35 µg/mL) to ozonize autologous blood and then injecting it by intragluteal injection. This procedure was approached by the authors to create a moderate amount of oxidative stress, and they called it immunomodulation therapy.[46] The results they obtained showed a reduction in mortality, an improvement in the quality of life, length of hospital stay and serum inflammatory markers, although these differences were slight.[46]

It is of utmost importance to state that mitochondria play a paramount role in the way by which ozone acts in the cardiovascular system.

First, endothelial mitochondria are fundamental sensing stations, suited to regulate microcirculation and the vascular microenvironment and ensure a correct cardiovascular functionality, particularly in those districts very critical for the onset of ischemic phenomena, such as atherosclerosis.[47-51]

Second, mitochondria, via mitophagy, mitochondria turnover and biogenesis, adjust and modulate the inflammatory and angiogenetic signaling, so that 4-hydroxynonenal, a product of ozone interaction with blood lipids, possesses angiogenetic potential.[52,53]

It is possible that ozone, by interacting with aryl hydrocarbon receptors, modulates the bioactivity of endothelia and their ability in modulating the cardiovascular function.

At the same time, its activity on redoxins balance, the inhibition of the NLRP3 and pyroptosis by 4-hydroxynonenal, the promotion of mitophagy and the control of mitochondria turnover,[54] may be major factors to comprehend the bioactivity of low doses of ozone in cardiovascular disease.

Conclusions: Focusing on the role of mitochondria in the oxygen-ozone therapy may be an intriguing point of view to elucidate the role of ozone in the cardiovascular physiology, in order to deepen the mechanisms by which oxygen-ozone therapy leads to cardiovascular health recovery in ischemic disorders and in endothelial dysfunction involving the microcirculation. Further research will provide insightful clues about the crucial role of the mitochondria-antioxidant-immune axis, where ozone seems to play a paramount role.

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