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Advances in Clinical Toxicology
ISSN: 2577-4328

The Role of Mitochondria in Ozone Therapy

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Mini Review
Volume 7 Issue 3
Received Date: June 25, 2022
Published Date: July 14, 2022
DOI: 10.23880/act-16000247

Abstract

Inhaled ozone can react with the biomolecules present in the epithelial lining fluid, causing the depletion of antioxidants. It can react with the surfactant polyunsaturated fatty acids (PUFA) present at the air-ELF interface to form several reactive oxygen species (ROS).

High ozone exposure resulted in sustained ventricular tachycardia in male and female rats. Ozone can induce oxidative stress, alter some inflammatory factors in tissue, and induce mitochondria-dependent apoptosis. It is not surprising that the mitochondria could be putatively affected by ozone as this organelle is highly sensitive to oxidative stress. Exposure of blood to a few micrograms of ozone was shown to cause a decrease in mitochondrial function and energy metabolism, as it caused a decline in ATP levels and an increase in the NADH/NAD+ ratio. It was also found that cytochrome-c-oxidase was almost wholly inhibited under these conditions. However, in a specific range of concentrations, ozone can induce a beneficial modulation in the anti-inflammatory and antioxidant systems. Some of the biological responses induced by ozone may be potentially suitable to become an active part of the various mechanisms of metabolic regulation, with positive effects on several pathologies. The therapeutic efficacy of ozone therapy may result from controlled and moderate oxidative stress produced by ozone's reactions with various biological components. Although there is not enough data, there are indications that ozone may induce good responses in mitochondria.

Keywords: Reactive Oxygen Species; Polyunsaturated Fatty Acids; Vascular Endothelial Growth Factor; Central Nervous System

Abbreviations: PUFA: Polyunsaturated Fatty Acids; ROS: reactive oxygen species; VEGF: vascular endothelial growth factor; CNS: central nervous system; TNF: tumour necrosis factor; TGF: transforming growth factor; IFN: interferon; IL: interleukins; OOP: oxidative ozone preconditioning; CO: carbon monoxide.

Introduction

Ozone is a molecule of three oxygen atoms soluble in water even at high temperatures. The high solubility of this molecule allows an immediate reaction with other soluble compounds and the biomolecules present in biological fluids [1]. Once ozone is one of the most reactive oxidants known, it has a high capacity to react with polyunsaturated fatty acids, low molecular weight molecules such as uric acid and ascorbic acid, and molecules that contain –SH groups, such as cysteine, methionine and reduced glutathione, leading to their oxidation. Therefore, excessive amounts of ozone can cause oxidative damage to biomolecules such as carbohydrates, enzymes, and nucleic acids [2,3]. Its primary function is to protect human beings from the harmful effects

of ultraviolet radiation. It is present in the stratosphere at 16-20 mg/m3 and on the Earth's surface at around 20 µg/m3, which is compatible with life [4,5].

Due to its oxidizing properties, ozone is a potentially toxic agent that can modulate several biochemical pathways, even at low concentrations, causing damage to vital organs [6]. Ozone inhalation increases lipid peroxidation and causes a decrease in the content of dopaminergic neurons [7], increases vascular endothelial growth factor (VEGF), levels of interleukin 6 (IL-6) and tumour necrosis factor (α-TNF) [8], as well as c-Fos expression in different brain regions [9]. These facts suggest that ozone significantly impacts central nervous system (CNS) physiology, cognitive processes, and emotions.

Humans exposed to atmospheric ozone cause a slight acceleration in breathing with tracheal and laryngeal irritation and chest tightness. It should be noted that notable differences in response between subjects are observed [10]. The threshold for significant changes in respiratory compromise ranges from 0.15 ppm to 0.25 ppm [11]. Higher concentrations of ozone cause hyperresponsiveness, bronchoconstriction of the corresponding airways and damage at the bronchial mucosa and pneumocytes, which may lead to pulmonary edema [5]. Inhalation of ozone causes histological changes such as inhibition of hair cells, proliferation of type 2 cells, and increased membrane permeability, as well as a variable inflammatory response [6].

Ozone in Medicine

Ozone therapy has been used for over 150 years to treat many diseases and infections [12]. It is a moderately invasive procedure based on the tissue regeneration capacity presented by the action of low ozone concentrations and is used as an alternative or adjuvant treatment [13]. Medical ozone therapy implies that a mixture of gaseous ozone and oxygen [14] is administered to the patient, who is indicated in treating inflammatory-mediated diseases such as burns and advanced ischemic diseases [15].

In medicine, it is considered an alternative treatment for different pathologies, such as arthritis, cardiovascular problems, asthma, emphysema and multiple sclerosis [16-18]. Ozone treatment has also been recommended to improve metabolic activities in older people [19-21]. It can prevent liver necrosis by modulating the antioxidant defence system, improving O_2 supply and increasing vascular nitric oxide release [15]. It can also alter the levels of inflammatory cytokines, such as tumour necrosis factor (TNF) [22], transforming growth factor (TGF) [23], interferon (IFN) [24] and interleukins (IL) [25]. The potential benefit of ozone therapy in many pathologies has been associated with the activation of immune cells, production and consequent release of cytokines [26,27]. Moderate oxidative stress and ozone seem to have a direct effect in activating the Nrf2 metabolic pathway that modulates the expression of many genes, including not only de antioxidant enzymes but also the ones above mentioned immune and inflammatory responses [14,28,29]. Ozone autohemotherapy (O_3-AHT) is a form of therapy in which the patient's blood is exposed to a predetermined concentration of ozone and then reinfused into the patient. In plasma, ozone can react with organic molecules, forming lipid ozonation products, reactive oxygen species (mainly H_2O_2) and ozonides. These substances are responsible for the therapeutic response, specifically for the induction of cytokine release by peripheral blood mononuclear cells [30]. On the other hand, these substances are also responsible for several toxic effects in the same cells, including apoptosis [31] and DNA damage [32].

Another benefit of this therapy is resistance to oxidative stress through the induction of antioxidant systems [33,34]. In addition, oxidative ozone preconditioning (OOP), a type of ozone therapy [35], has been reported to protect against damage caused by ischemia and reperfusion in the liver and lungs. On the other hand, chronic exposure to ozone has severe adverse effects.

Ozone Therapy and Mitochondria

Mitochondria are an intracellular structure defining and ubiquitous among eukaryotes as the nucleus. The primary function attributed to mitochondria is the production of energy, in the form of ATP, through the Krebs cycle and oxidative phosphorylation and β-oxidation [36]. Nevertheless, it is also responsible for phospholipid and heme synthesis, calcium homeostasis, including phospholipid synthesis, redox signalling, hormone and vitamin biosynthesis, apoptotic activation, and cell death [37,38].

The central role mitochondria play within cells is evidenced by the broad impact of mitochondrial diseases caused by defects in mitochondrial or nuclear genes encoding mitochondrial proteins in different organ systems. Mitochondrial diseases are the most common inherited metabolic disturbances and are among the most typical forms of inherited neurological disorders [39].

Mitochondrial functions are susceptible to toxic agents that exert their adverse action mainly at the level of the respiratory chain, as it causes changes in the enzymes of the mitochondrial respiratory chain [40]. Dysfunction in the respiratory chain results in a reduction in mitochondrial membrane potential and consequently in a decrease in ATP production and an increase in ROS production [41]. Despite

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the high efficiency of the respiratory chain, a small number of electrons is diverted directly to oxygen leading to the formation of the superoxide anion ($O2•^-$) [42]. Excessive ROS production that is not counterbalanced by increased antioxidant capacity leads to oxidative stress, which causes damage to the mitochondrial structure, including mitochondrial DNA (mtDNA), which will inevitably lead to mitochondrial dysfunction.

A recent study demonstrates that small ozone concentrations can have positive cellular responses in mitochondrial activation [13].

Mitochondria are sensitive to oxidative stress [43], making them a susceptible target to the ozone action. Oxidative stress can induce mitochondrial fission. However, low concentrations of ROS can activate various protective responses, such as the expression of mtHsp70, a protein of the Hsp70 family, which is involved in multiple mitochondrial and extra-mitochondrial functions [44].

The mitochondria and the endoplasmic reticulum simultaneously control intracellular calcium homeostasis. However, if the concentration of calcium stored by the mitochondria exceeds certain limits, it can be toxic. It can induce an increase in ROS production and even open the transient permeability pore with a simultaneous release of apoptotic factors that will lead to cell death [45].

Ozone exposure in non-human primates increased mitochondrial damage in the vascular system, consistent with the susceptibility to developing atherosclerotic lesions known in humans [46]. It has also been shown that ozone reacts selectively with the heme groups of haemoglobin, causing the oxidation of Fe^{2+}, followed by a decomposition of the porphyrin rings. This reaction is similar to the one with carbon monoxide (CO) and haemoglobin [47].

Leist, et al. found that ozone treatment decreases ATP levels and increases the NADH/NAD^+ ratio and the caspase activity. These are clear indicators that mitochondrial function is affected. At the concentrations used (20 and 80 µg/mL), ozone treatment inhibits the activity of complex II and III, but mainly of complex IV, due to the interaction with the Fe-S clusters in the respiratory complexes. The direct binding of ozone to complex IV may have caused the decrease in complex IV activity, similar to the reaction in haemoglobin [47]. The results obtained by these authors agree with previous work, which demonstrated that low concentrations of ozone cause 20% inhibition of complex IV activity in fibroblasts [24].

Despite being a potent oxidizing agent capable of biomolecular oxidation, leading to inhibiting some mitochondrial CTE complexes, ozone in adequate concentrations may induce some adaptive responses. Ozone concentration and effects do not follow a linear relationship: very low concentrations have no effect. However, high concentrations can lead to harmful effects contrary to the benefic effects observed at the medium/low concentration [48]. It is essential to understand the proper relationship between ozone and endogenous antioxidants to define the proper concentration of ozone to be administered. Although the mechanisms by which ozone can model mitochondrial function and redox balance are not yet fully understood, there is numerous evidence for this capability. It is also evident that the impact of ozone depends on the concentration, duration of treatment, exposure route, target organ and animal model used in the study. Therefore, it is essential to carry out further studies using different animal models and different concentrations and routes of ozone administration to understand the molecular mechanism underlying the observed effects on mitochondria.

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