On the Action Mechanism of Ozone Therapy
オゾン療法の作用メカニズムについて

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The low-dose ozone acts as mild oxidative stress and induces both inflammatory and anti-inflammatory responses which are mainly regulated by two transcriptional factors, NFκB and Nrf2. NF-κB is involved in the expressions of cyclooxygenase-2 and various inflammatory cytokines whereas Nrf2 is involved in the up-regulations of numerous antioxidant enzymes and cytoprotective proteins. In major autohemotherapy, the most used application form in ozone therapy, ozone in contact with blood instantaneously reacts with unsaturated fatty acids generating mainly H_2O_2 and 4-hydroxynonenal which are responsible for activations of NFκB and Nrf2, respectively.

1. Ozone Therapy

Owing to its potential oxidant properties, ozone exerts damaging effects on the respiratory tract [1]. However, the low-dose ozone has been used for therapy of various diseases in European countries during the last six decades. In ozone therapy, the ozone-oxygen mixture is administered by various routes: topical application on skin and mucosa, parenteal injections. Of these various routes, major autohemotherapy (MAH) is the most used route for the administration of ozone and it involves the withdrawal of blood (100-200 mL) from subjects and then reinfusion of the blood back into a vein of subjects after mixing with the same volume of ozone -oxygen gas (10-80 μgO_3/ mL). Now MAH is a common therapy in Europe, South America and Cuba and is performed to treat inflammation, circulatory disturbances, infections and pain. Clinical studies have demonstrated that MAH induces the following biological effects [2]:

(1) Improvement in blood circulation and oxygen delivery to ischemic tissues.
(2) Enhancement in general metabolism through improved oxygen delivery.
(3) Upregulation of cellular antioxidant enzymes and cytoprotective proteins.
(4) Induction of mild activation of the immune system and enhanced release of growth factors.

Action mechanism of ozone responsible for some of these biological effects can be explained form the recent studies [3, 4].

2. Action of Ozone on Blood

Because human blood plasma contains various antioxidants such as ascorbic acid, tocopherols, uric acid and thiol (SH) containing substances (albumin, glutathione, cysteine, etc.) with high reactivity to ozone, most of ozone is consumed by these antioxidants upon contact with blood. However, a small portion of ozone escaped from plasma antioxidant defenses can react with unsaturated fatty acids to give reactive oxygen species and lipid oxidation products [5]. Bocci, et al. revealed the formation of hydrogen peroxide (H_2O_2) and 4-hydroxy-2 nonenal (4-HNE) in the reactions of the low-dose of ozone with human blood and serum [3, 4]. Since H_2O_2 and 4-HNE are unionized and their life spans are longer than ozone, they can freely diffuse into cells and trigger several biochemical reactions. Therefore, H_2O_2 and 4-HNE act as short-lived and long-lived second messengers, respectively, in MAH.

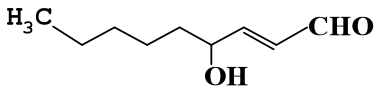

Fig.1. Structure of 4-hydroxy-2-nonenal

3. Roles of Second Messengers in MAH

It is now well-documented that both H_2O_2 and 4-HNE are “double edged” molecules. They can have either protective (adaptive response) or noxious effect on the cells and their effects depend on the dosage [6]. In MAH, the exposure of human blood to the low-dose ozone induces adaptive and beneficial responses through the formation of small amounts of H_2O_2 and 4-HNE.

Recent studies demonstrated that two nuclear transcriptional factors, nuclear transcriptional

factor κB (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2) are involved in such adaptive responses induced by H_2O_2 and 4-HNE.

NF-κB is involved in inflammatory or immune responses by regulating expression of inflammatory mediators such as cyclooxygenase-2 (COX-2) and various inflammatory cytokines. Whereas Nrf2 is involved in anti-inflammatory responses by up-regulations of numerous antioxidant enzymes and cytoprotective proteins.

In fact, ozonation of human blood produces various cytokines such as α-, β- and γ-interferons, tumor necrosis factor-α, interleukin-2,-4, -6-, -8, -10 and nitric oxide (NO). Blood also produces some growth factors (GM-CSF, PDGF, TGF-β_1) by ozonation (10-80 μgO_3/ mL). Up-regulations of these cytokines can be explained by activation of NFκB of leucocytes and platelets by H_2O_2. H_2O_2 also accelerates the glycolysis in erythrocyes by enhancing the recycle of the reduced glutathione, which increases ATP and 2,3-diphosphoglycerate (2,3-DPG). Mild induction of immune responses may be usful in immune-depressed patients after chemotherapy, or in chronic infectious diseases and the increase of growth factors in circulation may be beneficial for enhancing the wound healing. In addition, NO and 2,3-DPG are effective for the improvement in blood circulation and oxygen delivery to ischemic tissues.

On the other hand, 4-HNE induces the up-regulation of antioxidant enzymes through activation of Nrf2. The mechanism of activation of Nrf2 is well known. Briefly, Nrf2 is present in cytoplasm as the complex with its inhibitory protein, Keap1, in the absence of a specific stimulus. Many sources of stimulus such as oxidants and electrophiles activate Nrf2 by reacting with cysteine residues of Keap1 and release Nrf2 from the Complex. Once released, Nrf2 migrates into nucleus and bonds to the DNA at the location of the ARE which is the master regulator of the entire antioxidant system located in all human cells [7]. Since 4-HNE is powful electrophile, it activates Nrf2 by alkylating thiol groups of cysteine residue in Keap1 and consequently induces antioxidant enzymes and cytoprotective proteins.

Recently, Pecorelli, et al. have revealed that ozonated human serum containing 4-HNE activates Nrf2 of human endothelial cells in a dose dependent manner with the subsequent induction of heme oxygenase-1 (HO-1) and NADPH quinine oxido-reductase (NQO-1) [4]. The results suggest that 4-HNE can distribute in all body tissues and activate Nrf2 after refusion of ozonated blood in MAH. More recently, Re et al. demonstrated that level of Nrf2 in peripheral blood mononuclear cells increased immediately after MAH. Thus, MAH is beneficial for the treatment of the diseases that involved a chronic disruption of redox status (oxidative stress) by up-regulation of antioxidant enzymes.

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