

## The Bocci Collection

10 studies that are compiled on the Biological effects of Ozone by V Bocci

1. Induction of interferon gamma on human leucocytes.
2. Induction of tumor necrosis factor (TNF-alpha) on human leucocytes.
3. An attempt to define conditions for optimal induction of cytokines
4. Cytokine production and glutathione levels in human erythrocytes.
5. Evaluation of immunological parameters
6. Production of transforming growth factor 1 by human blood after ozone treatment.
7. Generation of (ROS) after exposure of human blood to ozone.
8. Effects on the total antioxidant status and on interleukin-8 production
9. Effects of ozone on human platelets.
10. Release of factors from ozonated human platelets.



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Dr. Adriana Schwartz (MD). Gynecology and Obstetrics, Founder and President of the Spanish Association of Medical Professionals in Ozone Therapy (AEPROMO). Spain

### Other studies

**The ozone paradox: ozone is a strong oxidant as well as a medical drug.**  
Bocci 2009 [Med Res Rev.](#) 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.

**Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients.** Department of Nephrology Transplantology and Internal Medicine 2004

**Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress?** Masaru Sagai<sup>1</sup> and Velio Bocci 2011

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[Haematologica](#). 1990 Nov-Dec;75(6):510-5.

## **Studies on the biological effects of ozone**

### **1. Induction of interferon gamma on human leucocytes.**

[Bocci V, Paulesu L.](#) <http://www.ncbi.nlm.nih.gov/pubmed/2129118>

#### **Source**

Istituto di Fisiologia Generale, Università di Siena, Italy.

#### **Abstract**

In this study we have investigated the effects of ozone on human blood, as well as on resuspended buffy coats and Ficoll-purified mononuclear cells. Samples were exposed at different ozone concentrations (from 2.2 micrograms to 108 micrograms/ml) for 30 sec and then incubated for different times at 37 degrees C in a 95% air-5% CO<sub>2</sub> humidified atmosphere. Supernatants were collected and frozen at -20 degrees C until tested for interferon (IFN) activity. We have determined that the ozone concentration is critical for lymphokine induction. In fact, while low concentrations (2.2 micrograms/ml) are effective in lymphocytes, they do not induce IFN in either whole or diluted (1:1) human blood, or resuspended buffy coats. In such cases levels as high as 42 micrograms/ml are required. On the other hand, a very high ozone concentration (108 micrograms/ml) is not effective and probably toxic. Maximal IFN production occurs 72-96 h after ozone exposure, and the kinetics of IFN release is similar to that after Staphylococcal Enterotoxin B addition. Because ozonization of blood is a medical procedure followed in several countries for treatment of viral diseases, this study can open a new field of investigation that may yield useful results both in biological and practical terms.

[Lymphokine Cytokine Res.](#) 1991 Oct;10(5):409-12.

## **Studies on the biological effects of ozone:**

### **2. Induction of tumor necrosis factor (TNF-alpha) on human leucocytes.**

[Paulesu L, Luzzi E, Bocci V.](#)

#### **Source**

Institute of General Physiology, University of Siena, Italy.

#### **Abstract**

The effect of ozone as a probable inducer of tumor necrosis factor (TNF-alpha) has been investigated on human blood and on Ficoll-purified blood mononuclear cells (PBMC). Samples were exposed at different ozone concentrations ranging from 2.2 to 108 micrograms/ml and incubated at 37 degrees C in an 95% air-5% CO<sub>2</sub> atmosphere. At predetermined times, all cell supernatants were tested for TNF activity and some PBMC cultures were examined for DNA synthesis. We have shown that ozone concentration is critical in terms of TNF production and of cell mitogenesis and that, owing to the presence of erythrocytes, higher ozone concentrations are required to be effective in blood than in PBMC. Because ozonization of blood is a procedure followed in several European countries for the treatment of viral diseases and tumors, the release of factors with antiviral and immunomodulatory activities by leukocytes may explain the mechanism of action of ozone and of autohemotherapy.

Lymphokine Cytokine Res. 1993 Apr;12(2):121-6.

### **Studies on the biological effects of ozone:**

#### **3. An attempt to define conditions for optimal induction of cytokines.**

[Bocci V](#), [Luzzi E](#), [Corradeschi F](#), [Paulesu L](#), [Di Stefano A](#).

<http://www.ncbi.nlm.nih.gov/pubmed/8324077>

#### **Source**

Institute of General Physiology, Faculty of Pharmacy, University of Siena, Italy.

#### **Abstract**

Ozonization of blood, normally carried out with citrated blood, may be fine for the autohemotherapy of ischemic diseases but it may be at a loss when employed in viral diseases or in immunodeficiencies. We have shown that heparin, used as an anticoagulant, with the addition of 5 mM CaCl<sub>2</sub> favors production of cytokines by leukocytes with only a modest increase in hemolysis. High plasmatic levels of glucose, glutathione, and ascorbic acid decrease cytokine's yield because these compounds act as antioxidants and quench the inducing activity of ozone. Autohemotherapy with heparinized and Ca(2+)-supplemented blood has not revealed any side effects in volunteers.

[J Biol Regul Homeost Agents](#). 1993 Oct-Dec;7(4):133-8.

### **Studies on the biological effects of ozone:**

#### **4. Cytokine production and glutathione levels in human erythrocytes.**

[Bocci V](#), [Luzzi E](#), [Corradeschi F](#), [Paulesu L](#), [Rossi R](#), [Cardaioli E](#), [Di Simplicio P](#).

#### **Source**

Institute of General Physiology, Faculty of Pharmacy, University of Siena, Italy.

#### **Abstract**

We have investigated the effect of various concentrations of ozone on human blood aiming to correlate the production of cytokines with depletion of reduced glutathione and hemolysis. As erythrocytes constitute the bulk of blood cells and represent the main target of ozone they have been taken as a useful marker of its oxidative activity. It appears that a transient exposure (30 sec) of blood of up to 78 micrograms ozone per ml of blood does not depress the production of cytokines even though there is a slight increase of hemolysis and a small decrease of intracellular reduced glutathione. In contrast either a constant (up to 30 sec) exposure to an ozone flux or a high ozone concentration (108 micrograms/ml) markedly decreases reduced glutathione levels and depresses cytokine production.

[Biotherapy](#). 1993-1994;7(2):83-90.

### **Studies on the biological effects of ozone:**

#### **5. Studies on the biological effects of ozone: Evaluation of immunological parameters and tolerability in normal volunteers receiving ambulatory autohaemotherapy.**

[Bocci V](#), [Luzzi E](#), [Corradeschi F](#), [Paulesu L](#).

#### **Source**

Faculties of Pharmacy, Institute of General Physiology, Siena, Italy.

#### **Abstract**

Autohaemotherapy, after a bland treatment ex vivo of blood with ozone, is a fairly unknown medical procedure claimed to have therapeutic value in viral diseases and neoplasms. Having already shown that ozone acts as a mild inducer of cytokines, we have undertaken an investigation in normal rabbits and in normal volunteers aiming to evaluate eventual changes of some cytokine levels in plasma as well as of immunological parameters such as the Mx protein, neopterin, beta 2-microglobulin and of some acute-phase proteins after single or repeated autohaemotherapy. We have also evaluated the potential development of side-effects. This study is the first one to show that autohaemotherapy can activate an immunological marker in normal subjects without procuring any toxic effects.

[J Biol Regul Homeost Agents](#). 1994 Oct-Dec;8(4):108-12.

### **Studies on the biological effects of ozone:**

#### **6. Production of transforming growth factor 1 by human blood after ozone treatment.**

[Bocci V](#), [Luzzi E](#), [Corradeschi F](#), [Silvestri S](#).

#### **Source**

Institute of General Physiology and Nutritional Sciences, Siena, Italy.

#### **Abstract**

After exposing human whole blood from normal volunteers to ozone concentrations ranging from 22 to 156 micrograms/ml, we have shown that, upon incubation of up to 8 hours, there is a significant release of transforming growth factor beta (TGF-beta 1). In comparison to TGF-beta 1, TGF-beta 2 production is not influenced by ozone concentrations. In line with our previous findings it appears that blood, in the presence of heparin and 5mM Ca<sub>2+</sub> allows a consistent production of tumor necrosis factor alpha (TNF alpha) and the release of low and non-hazardous levels of free hemoglobin. These data support the contention that autohemotherapy performed after treating blood with ozone followed by reinfusion into the donor, may represent a valuable therapeutic approach for achieving immunoregulatory effects

[J Biol Regul Homeost Agents](#). 1998 Jul-Sep;12(3):67-75.

## **Studies on the biological effects of ozone:**

### **7. Generation of reactive oxygen species (ROS) after exposure of human blood to ozone.**

[Bocci V](#), [Valacchi G](#), [Corradeschi F](#), [Aldinucci C](#), [Silvestri S](#), [Paccagnini E](#), [Gerli R](#).  
<http://www.ncbi.nlm.nih.gov/pubmed/9795834>

#### **Source**

Institute of General Physiology, University of Siena, Italy.

#### **Abstract**

The acceptance of any complementary medical approach is conditioned by the results obtained after the same scientific scrutiny applied in orthodox medicine. Otherwise any claim of efficacy remains in the realm of fiction. In the case of ozone therapy, the mechanisms of action have remained nebulous and in a series of publications we are trying to present the biochemical, immunological and morphological evidence in favour or against ozone therapy. We have now shown that ozone (O<sub>3</sub>) dissolved in the water of either plasma or serum or physiological saline generates reactive oxygen species (ROS), of which hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) can be unequivocally demonstrated by using specific methods for its detection. Lipids present in plasma preferentially those present in lipoproteins, undergo peroxidation that is somewhat O<sub>3</sub>-dose dependent and can be observed by the measurement of thiobarbituric acid reactive substances (TBARS). While the generation of H<sub>2</sub>O<sub>2</sub> is crucial in activating both biochemical (hexose monophosphate shunt) and immunological (via the transcription factor NF-κB) mechanisms, the role of lipid oxidation products (LOP) remains to be investigated. We have shown here that there is a small but consistent induction of some cytokines (TNF-α, IFN-γ and IL-2) when human blood is directly exposed to O<sub>3</sub> concentrations up to 100 micrograms/ml per g of blood. On the other hand, isolated blood mononuclear cells (PBMC) in tissue culture medium are far more sensitive to the oxidant action of O<sub>3</sub> as shown by a progressive reduction of the proliferation index with comparatively far lower O<sub>3</sub> concentrations. On the whole, these results support the concept that much of the O<sub>3</sub> toxicity is neutralized by the powerful antioxidant system of blood. The minimal hemolysis supports this idea but as far as platelets are concerned, we must mention that they tend to aggregate in heparinized blood, even when it is exposed to an O<sub>3</sub> concentration of 40 micrograms/ml. In spite of the lack of side-effects after autohemotherapy, this drawback must be kept in mind and avoided in clinical practice.

[Mediators Inflamm](#). 1998;7(5):313-7.

## **Studies on the biological effects of ozone:**

### **8. Effects on the total antioxidant status and on interleukin-8 production.**

[Bocci V](#), [Valacchi G](#), [Corradeschi F](#), [Fanetti G](#).

#### **Source**

Institute of General Physiology, University of Siena, Italy. [figen@unisi.it](mailto:figen@unisi.it)

#### **Abstract**

Ozone (O<sub>3</sub>) is a controversial gas because, owing to its potent oxidant properties, it exerts damaging effects on the respiratory tract and yet it has been used for four decades as a therapy. While the disinfectant activity of O<sub>3</sub> is understandable, it is less clear how other biological effects

can be elicited in human blood with practically no toxicity. On the other hand plasma and cells are endowed with a powerful antioxidant system so that a fairly wide range of O<sub>3</sub> concentrations between 40 and 80 microg/ml per gram of blood (approximately 0.83-1.66 mM) are effective but not deleterious. After blood ozonation total antioxidant status (TAS) and plasma protein thiol groups (PTG) decrease by 20% and 25%, respectively, while thiobarbituric acid reactive substances (TBARS) increases up to five-fold. The increase of haemolysis is negligible suggesting that the erythrocyte membrane is spared at the expense of other sacrificial substrates. While there is a clear relationship between the ozone dose and IL-8 levels, we have noticed that high TAS and PTG values inhibit the cytokine production. This is in line with the current idea that hydrogen peroxide, as a byproduct of O<sub>3</sub> decomposition, acts as a messenger for the cytokine induction.

[Platelets](#). 1999;10(2-3):110-6.

### **Studies on the biological effects of ozone:**

#### **9. Effects of ozone on human platelets.**

[Bocci V](#), [Valacchi G](#), [Rossi R](#), [Giustarini D](#), [Paccagnini E](#), [Pucci AM](#), [Di Simplicio P](#).  
<http://www.ncbi.nlm.nih.gov/pubmed/16801079>

#### **Source**

Institute of General Physiology, University of Siena, Siena, Italy. [figen@unisi.it](mailto:figen@unisi.it)

#### **Abstract**

During the course of ozonated autohaemotherapy (O<sub>3</sub>-AHT) using heparin as an anticoagulant, it was occasionally observed that a few clots were retained in the filter during blood reinfusion. This observation prompted an investigation on the effect of ozone (O<sub>3</sub>) on human platelets. We have now shown, both by biochemical and morphological criteria, that heparin in the presence of O<sub>3</sub> can promote platelet aggregation. In contrast, after Ca<sup>2+</sup> chelation with citrate, platelet aggregation is much reduced. The potential role of the transient formation of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) in the presence of Ca<sup>2+</sup> with the possible expression of adhesion molecules is briefly discussed.

[Mediators Inflamm](#). 1999;8(4-5):205-9.

### **Studies on the biological effects of ozone:**

#### **10. Release of factors from ozonated human platelets.**

[Valacchi G](#), [Bocci V](#). <http://www.ncbi.nlm.nih.gov/pubmed/10704074>

#### **Source**

Institute of General Physiology, University of Siena, Italy.

#### **Abstract**

In a previous work we have shown that heparin, in the presence of ozone (O<sub>3</sub>), promotes a dose-dependent platelet aggregation, while after Ca<sup>2+</sup> chelation with citrate, platelet aggregation is almost negligible. These results led us to think that aggregation may enhance the release of platelet components. We have here shown that indeed significantly higher amount of platelet-derived growth factor (PDGF), transforming growth factor beta1 (TGF-beta1) and interleukin-8 (IL-8) are released in a dose-dependent manner after ozonation of heparinised platelet-rich plasma samples.

These findings may explain the enhanced healing of torpid ulcers in patients with chronic limb ischemia treated with O<sub>3</sub> autohaemotherapy (O<sub>3</sub>-AHT).

[Blood Coagul Fibrinolysis](#). 2004 Oct;15(7):619-22.

[Med Res Rev](#). 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.

## **The ozone paradox: ozone is a strong oxidant as well as a medical drug.**

[Bocci V](#), [Borrelli E](#), [Travagli V](#), [Zanardi I](#).

**Source** [Med Res Rev](#). 2009 Jul;29(4):646-82. doi: 10.1002/med.20150.

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### **Abstract**

After five decades characterized by empiricism and several pitfalls, some of the basic mechanisms of action of ozone in pulmonary toxicology and in medicine have been clarified. The present knowledge allows to understand the prolonged inhalation of ozone can be very deleterious first for the lungs and successively for the whole organism. On the other hand, a small ozone dose well calibrated against the potent antioxidant capacity of blood can trigger several useful biochemical mechanisms and reactivate the antioxidant system. In detail, firstly ex vivo and second during the infusion of ozonated blood into the donor, the ozone therapy approach involves blood cells and the endothelium, which by transferring the ozone messengers to billions of cells will generate a therapeutic effect. Thus, in spite of a common prejudice, single ozone doses can be therapeutically used in selected human diseases without any toxicity or side effects. Moreover, the versatility and amplitude of beneficial effect of ozone applications have become evident in orthopedics, cutaneous, and mucosal infections as well as in dentistry.

## **Platelet function unaffected by ozonated autohaemotherapy in chronically haemodialysed patients.**

[Tylicki L](#), [Lizakowski S](#), [Biedunkiewicz B](#), [Skibowska A](#), [Nieweglowski T](#), [Chamienia A](#), [Debska-Slizien A](#), [Rutkowski B](#). <http://www.ncbi.nlm.nih.gov/pubmed/15389131>

### **Source**

Department of Nephrology Transplantology and Internal Medicine, Medical University of Gdansk, Poland. leszek.tylicki@amg.gda.pl

### **Abstract**

#### **BACKGROUND:**

The therapeutic use of ozone is still a controversial medical strategy due to the potential toxicity of ozone, which is recognized as a highly reactive oxidant. The reactive oxygen species are known to induce platelet aggregation, the process involved in the development of atherosclerosis and cardiovascular events. In the present study, the influence of ozonated autohaemotherapy (O<sub>3</sub>-AHT) on the platelet function was evaluated in chronically haemodialysed patients with peripheral arterial disease.

**METHODS:**

This was an oxygen-controlled, cross-over study, in which nine sessions of autohaemotherapy with oxygen administration as a control were followed by nine sessions of O3-AHT. The platelet function was assessed by the extent of spontaneous aggregation (SPA) and agonist-induced aggregation (AIPA), where different concentrations of adenosine were used as an agonist.

**RESULTS:**

There were no differences between SPA and AIPA assessed after nine sessions of O3-AHT and after nine sessions of autohaemotherapy with oxygen administration. SPA and AIPA did not change after the first session of O3-AHT as compared with the levels before this procedure.

**CONCLUSION:**

O3-AHT with ozone concentration of 50 microg/ml and citrate as an anticoagulant does not induce platelet aggregation.

*Medical Gas Research* 2011, 1:29 doi:10.1186/2045-9912-1-29

## Mechanisms of Action Involved in Ozone Therapy: Is healing induced via a mild oxidative stress?

Masaru Sagai<sup>1</sup> and Velio Bocci<sup>2\*</sup> <http://www.medicalgasresearch.com/content/1/1/29>

### Abstract

The potential mechanisms of action of ozone therapy are reviewed in this paper. The therapeutic efficacy of ozone therapy may be partly due the controlled and moderate oxidative stress produced by the reactions of ozone with several biological components. The line between effectiveness and toxicity of ozone may be dependent on the strength of the oxidative stress. As with exercise, it is well known that moderate exercise is good for health, whereas excessive exercise is not.

Severe oxidative stress activates nuclear transcriptional factor kappa B (NFκB), resulting in an inflammatory response and tissue injury via the production of COX2, PGE2, and cytokines. However, moderate oxidative stress activates another nuclear transcriptional factor, nuclear factor-erythroid 2-related factor 2 (Nrf2). Nrf2 then induces the transcription of antioxidant response elements (ARE). Transcription of ARE results in the production of numerous antioxidant enzymes, such as SOD, GPx, glutathione-s-transferase(GSTr), catalase (CAT), heme-oxygenase-1 (HO-1), NADPH-quinone-oxidoreductase (NQO-1), phase II enzymes of drug metabolism and heat shock proteins (HSP). Both free antioxidants and anti-oxidative enzymes not only protect cells from oxidation and inflammation but they may be able to reverse the chronic oxidative stress. Based on these observations, ozone therapy may also activate Nrf2 via moderate oxidative stress, and suppress NFκB and inflammatory responses. Furthermore, activation of Nrf2 results in protection against neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. Mild immune responses are induced via other nuclear transcriptional factors, such as nuclear factor of activated T-cells (NFAT) and activated protein-1 (AP-1).

Additionally, the effectiveness of ozone therapy in vascular diseases may also be explained by the activation of another nuclear transcriptional factor, hypoxia inducible factor-1α (HIF-1α), which is also induced via moderate oxidative stress. Recently these concepts have become widely accepted. The versatility of ozone in treating vascular and degenerative diseases as well as skin lesions, hernial disc and primary root carious lesions in children is emphasized. Further researches

able to elucidate whether the mechanisms of action of ozone therapy involve nuclear transcription factors, such as Nrf2, NFAT, AP-1, and HIF-1 $\alpha$  are warranted.