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Research Article

Ozone effect on morphology erythrocytes and leukocytes in blood samples: *in vitro* Approach

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ABSTRACT

Clinical studies showed gas ozone use is safe; the blood cells can enzymatically control a certain number of antioxidant molecules; in low concentrations, these mechanisms can be triggered, turned on, and stimulated by other leukocyte mechanisms of defense. To observe the effect of ozone on blood erythrocytes and white blood cells when are exposed to therapeutic doses of ozone at concentrations (20, 40, 60, and 90 µL/mL) in volunteer subjects. Blood samples were exposed to concentrations of ozone (20, 40, 60, and 90 µl/mL), evaluated in a hematometer, and blood stains. An alteration in the morphology of leukocyte and erythrocyte cells was observed in blood samples of all concentrations, with statistical significance for neutrophils and monocytes. Ozone in blood in vitro causes a morphological affectation of erythrocytes, monocytes and neutrophils.

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Introduction

Ozone has been applied in countless patients for its bactericidal and anti-inflammatory action due to its oxidative and chemical capacity to form peroxides, in addition to stimulating immune responses by different mechanisms through major autohemotherapy. In wounds that did not heal due to antibiotic resistance (Zeng et al., 2020; Zeng, Lei, et al., 2020), it has been shown in patients that it promotes a decrease in pain and inflammation, improves motor disability by reducing inflammation in the joints, and has been observed to stimulate the regeneration of articular cartilage (Dhamnaskar et al., 2021; Di Paolo et al., 2005). Since the last century, ozone has been used in several human clinical pathologies with controlled doses and documented the responses to different diseases and with heterogeneous grades of pain. Since the last century, efforts have been made to better understand the effect on the human body (Serra et al., 2023). Although millions of treatments have been performed on patients, their mechanism of action is not fully resolved.

It has been suggested ozone molecule (O_3) acts as a pro-oxidant agent that stimulates the different formation of antioxidant molecules and transforms biomolecules into small molecules under relatively controlled oxidative stress, which can be helpful in situations of immunological dysfunctions and chronic and degenerative diseases (Di Paolo et al., 2005).

Beyond the benefits obtained in clinical use against diseases, unraveling the biochemical and cellular mechanisms has become a scientific challenge. There are different ways to administer ozone, like autohemotherapy in blood, which is carried out by mixing different ozone doses in blood obtained from patients, which is returned ozonized. The Hemotherapy Manual describes concentrations to be used according to the diseases (Re, 2022; Schwartz, 2011; V. Bocci et al., 1998). Bocci, et al. Proposed blood has a powerful antioxidant system, so plasma and its components can regulate O_3 gas in a certain range of concentrations. This author reports that between 40 and 80 / ml per blood gr. (approximately 0.83-1.66 mM) are effective in stimulating some cellular pathways. V. Bocci et al. (1998) describe, after added ozone into the blood, the antioxidant plasma status proteins and the thiol (SH) groups of glutathione change, and Thiobarbituric acid (TBA) increases due to molecular restructuring of plasma fatty acids and causes mild hemolysis (V. Bocci et al., 1998).

An experimental study with O_3 in mice showed red blood cells when were exposed to O_3 in vivo followed by incubation in vitro caused inhibition of membrane deformation, after O_3 exposure at [1 ppm], while control red blood cells apparently after 6 hours of incubation suffered changes in their membrane, an increase in SH groups was quantifying an increase in ATPase function for maintain the deformation of red blood cells to allow them to cross blood vessels, however, the exposed red blood cells do not show the deformation in the membrane of these cells because the

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concentrations of SH groups and ATPase were not modified, like in control samples, the authors demonstrated that O_3 inhibits ATPase after in vivo exposure (Morgan et al., 1988) report that red blood cells exposed to O_3 alter potassium K^+ concentrations, and observed the alteration of membrane lipids and inhibition enzymatic (Van Der Zee et al., 1987). Since red blood cells are the majority of blood cells, O_3 impacts them by affecting their oxidative activity. It appears that a transient exposure (30 seconds) of up to 78 micrograms of O_3 per ml of blood does not limit cytokine production; a slight increase in hemolysis with a decrease in intracellular reduced glutathione appears on contrary, constant exposure (up to 30 seconds) to a flow of O_3 or a high concentration of this gas (108 micrograms/ml) was observed significantly decreases reduced glutathione levels, which depresses cytokine production. (V. Bocci et al., 1993). Other studies have found that ozonized autohemotherapy concentrations of 24, 40, 60, and 100 µl/mL in blood decreases concentration in Vitamin C and E with increasing O_3 concentrations suggest concentrations of vitamins decreases for protect the erythrocyte membrane is not affected, although the amount of fatty acids in plasma increased when decreased glutathione and erythrocyte enzymes, affecting their ability to regulate oxidative stress (Shinriki et al., 1998). O_3 has polar molecular structure, carries out changes in erythrocytes and reacts preferentially with the double bonds of fatty acids, which are in large quantities in cell membranes (Viebahn-Haensler & Fernández, 2023) as a result of this series of reactions, ozone peroxide is formed. It acts as a second messenger, transducing glutathione and activating nuclear factors with an immunomodulatory function such as Nrf2, who is a regulator of the enzymatic antioxidant system together with glutathione (Viebahn-Haensler & Fernández, 2023). Belyck et al. mention that a low dose of ozone reduces the bilipid layer viscosity of the erythrocyte membrane at a [0.16 mg/L] ozone, causing the hemolysis caused in erythrocytes to deform depending on the affectation of their cytoskeleton and the spectrin-actin network, favoring their movement. (Belykh et al., 2007). The theories of what happens in major autohemotherapy depend on their concentration; red blood cells are stimulated by O^3, increasing ATP through glycolysis, causing dissociation changes in hemoglobin, apparently favoring the entry of oxygen into the tissues (Tricarico & Travagli, 2021). Leukocytes benefit from the process of major autohemotherapy because the mechanisms that O3 indirectly stimulates the second messengers involve the activation of NF-Kb, which in turn activates heme oxygenase; this enzyme also involves the Nrf2 factor, and these activated factors protect cells from free radical damage, which favors the stimulation of a depressed immune system. (Togi et al., 2021; Van Der Zee et al., 1987) It has been suggested the molecular union O_3-Nrf2-vitagene also plays a role in regulating cellular REDOX balance and in reducing oxidative stress; it cooperates in the modulation of the apoptosis process and autophagy cellular mechanism (Scassellati et al., 2020). When ozonized blood is introduced, it is considered O_3 is deactivated in relatively seconds or minutes by the enzymatic systems and biochemical components of the blood, since 78% reacts with circulating vitamin C and 20% of O_3 reacts with uric acid, forming allantoin, and the remaining 2% reacts with lipids (Chirumbolo et al., 2023; V. A. Bocci et al., 2011). Recently has been found the molecules resulting from auto-hemotherapy with O_3 products, such as H_2O_2. Other products that result in lipid oxidation, albumin, plasma proteins, and erythrocyte hemoglobin are affected in their structural changes originating alteration of normal functions, caused by incorrect therapies with O_3 reactions with hemoglobin and albumin molecules can lead to the formation of high-weight molecular species toxic in the blood (Mehraban & Seyedarabi, 2023). Other studies have manifested the phagocytic function of O_3 is an improvement at concentrations of [20 and 40 µl/mL] and observed an increase in the phagocytic process between concentrations at [10 and 80 µl/m] at lower or higher concentrations; it did not have the same effect, so O_3 presented a dose-dependent behavior, which may or may not stimulate the phagocytic function (Diaz-Luis et al., 2015). In a study where O_3 administration in rabbits by inhalation, the erythrocytes changed the original form to spherocytes due to a decrease in the oxygen rate causing hemoglobin release and osmotic fragility due to spherocytosis, as well as metabolic changes in the erythrocyte and reporting precipitation and denaturation of globin with the formation of Heinz bodies, so that suggests damage in the erythrocyte's membrane affecting the ability to distribute oxygen (Tricarico & Travagli, 2021). Another study on the ozone effect mentioned the affectation of the erythrocyte membrane in a transitory manner to favor the transport of oxygen although these benefits are limited to the concentrations of O_3 however, reports are mentioning the shape alterations over erythrocyte to spherocytes, were due to the biochemical changes were triggered in plasma and blood, although is true that the biochemical compensatory stimulus occurs favoring the redox systems, the structural changes in the shape of the erythrocyte have been reported, therefore, it is considered important to examine what happens in blood cells by exposure to O_3 in vitro for 24 hours and several days, in the same blood sample, the objective of this study is to examine the morphological alterations that erythrocytes and leukocytes of whole blood could present at autohemotherapy concentrations, the exposure will be at the recommended concentrations with exposure of up to 5 days in the same sample.

Materials and Methodology

Selection of volunteers and samples: Healthy, non-smoking volunteers were selected, including three subjects who did not show comorbidities and signed the informed consent. A blood sample was taken, 5 mL of peripheral blood, one sample for each ozone concentration per volunteer.

Ozone dispensing: Ozone dispensing was performed with Forensics Medical Ozone equipment; ozone gas was dispensed at therapeutic doses, and the gas flow for the concentrations was always controlled. Concentrations used per volunteer [20, 40, 60, and 90 µl/mL]. Blood examination was performed in Zibio Z5 hematometer, staining readings at 40X and 100X with Cloud Smart Panther L digital optical microscope and Wright staining for smears.

Procedure: First phase: A blood sample was taken in a 5 ml tube with EDTA, which was transferred to a three-way syringe where the ozone concentration was added according to doses (20, 40, 60, 90 µl/mL), one dose for the tube, and gently shaken to mix the gas with light shaking for 30 seconds, and was returned original tube, after one-hour cell blood count and blood smear were done, the measurements were performed consecutively for 5 days at fixed time to obtain equivalent and comparative results. The stains corresponding to each sample and concentration were observed under an optical microscope. A control sample was the whole blood of each volunteer; the samples were left at a laboratory temperature of 25°C in racks without movement

The second phase: started adding ozone at the concentrations indicated for each sample to be evaluated. In this phase, ozone was added daily for 5 days, and a smear with Wright stain was performed each day. Hematological measurements were performed on the first and fifth days after sampling. With the results of blood measurements, it created a database for the statistical test, performing the Anova test to compare means and, if there were significant differences, plus any possible graphs. The

smears were reviewed at 40X and 100X on optical microscopy. 150 stains were reviewed.

Results

The samples obtained were evaluated at the concentrations mentioned for O_3, two methods. In none of the samples was hemolysis presented after adding the O_3 gas sample. Observations were made for 5 consecutive days, regardless of the method, of the measurements of blood, it was observed that the hematocrit value increased, morphological and numerical changes occurred in monocytes and neutrophils, and the hemoglobin values did not show any changes. Fluctuations in daily blood measurements of monocytes, neutrophils, and lymphocytes can be seen in Figures 1, 2, and 3.

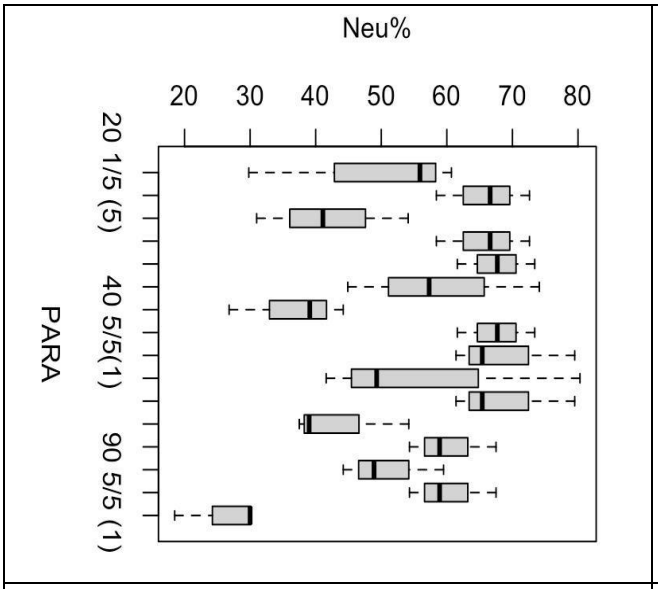


Figure 1. Neu% evaluated with the ANOVA test.

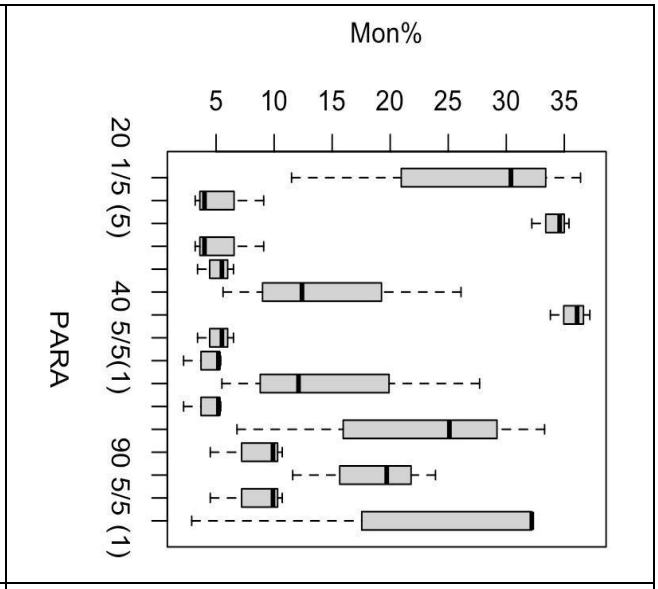


Figure 2. Mon% evaluated with the ANOVA test.

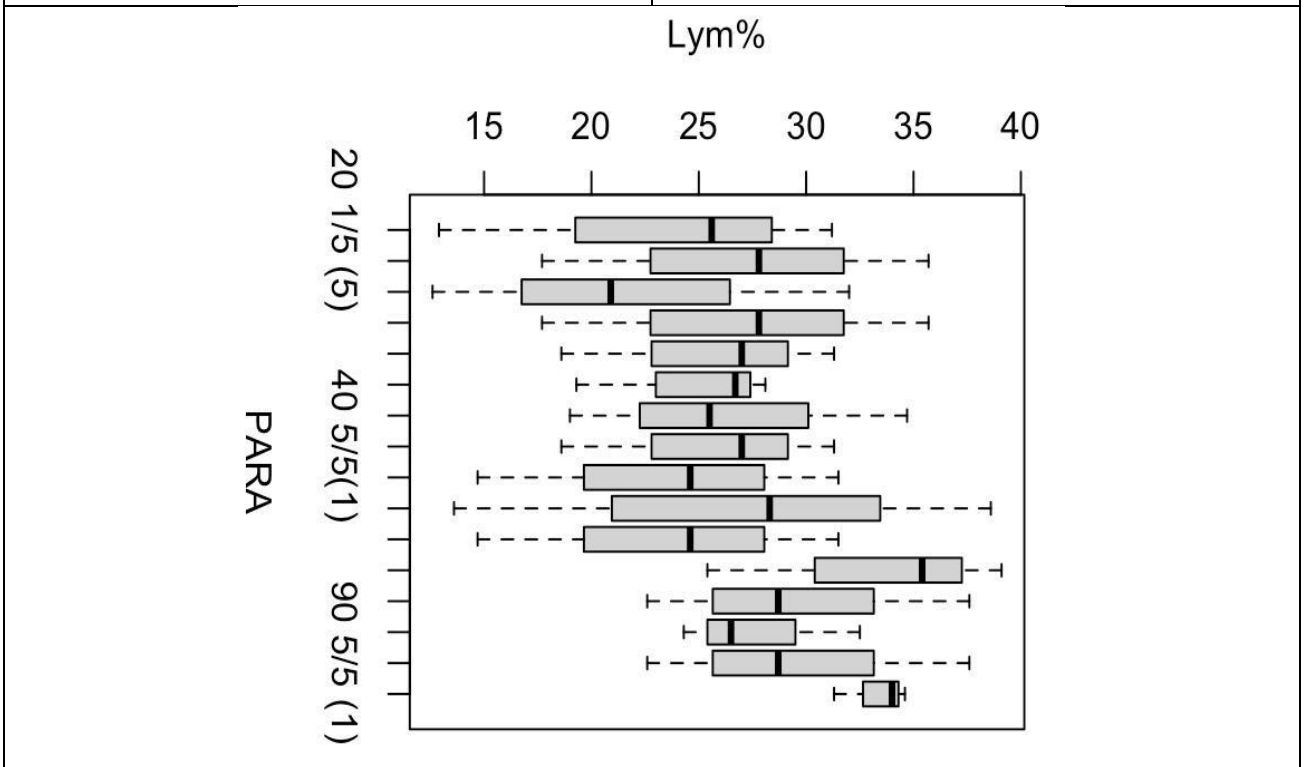
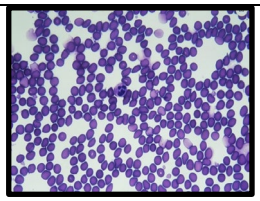
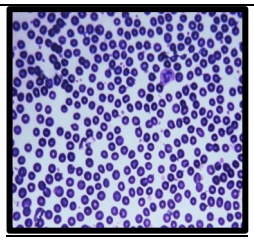
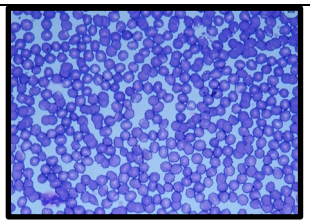
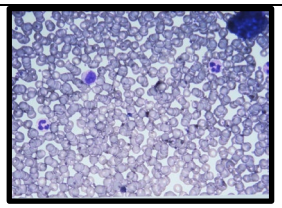
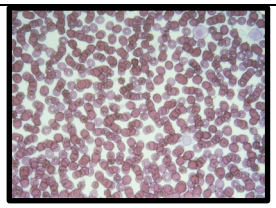


Figure 3. Lym% evaluated with the ANOVA test.

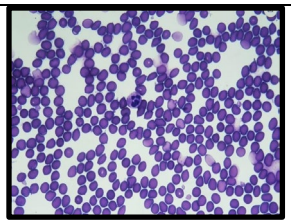
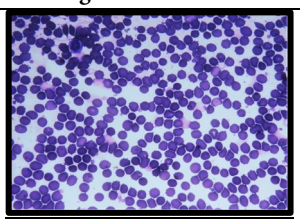
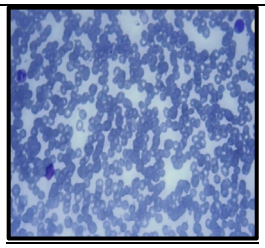
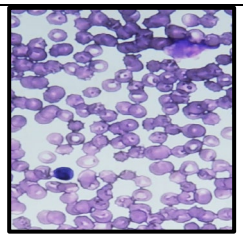
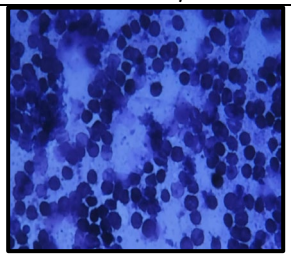
In the smears of the first hours of exposure to ozone, it was observed that the erythrocytes presented morphological changes with projections of their membrane; they were formed of acanthocytes or echinocytes. Rouleaux formation with different patterns in different areas of the smear. As the days passed, an increase of erythrocytes with altered form and white cells showed fewer changes in their normal morphology. On the third day, neutrophils and monocytes began to show changes at the nuclear level, increasing in size, more deformed erythrocytes, and no hemolysis in any sample. The leukocytes showed an increase in size and their nuclei were deformed; most of the erythrocytes were deformed too; and the white cells showed a decrease in neutrophils and an increase in monocytes. No other structures were observed inside the erythrocytes. Table 1 corresponds to the changes observed in one day of exposure, while Table 2 corresponds to the 5 days of exposure.

Table 1. Morphological changes of erythrocytes and leukocytes during 3 days with different ozone concentrations of 20, 40, 60, and 90 µl/mL applied only on the first day.

Ozone concentration	Description	Image
Control	Cells within normal parameters, with normal morphology and without alterations.	 **Image 2.** Control slide.
20	Changes are observed over 3 days by exposing the sample to ozone at a concentration of 20 µl/ml only once: **Day 1:** Presence of poikilo and normal appearance of polymorphonuclear cells (PMN). **Day 2:** Membrane changes are seen in some erythrocytes. **Day 3:** Erythrocytes with an appearance similar to acanthocytes and echinocytes.	 **Image 2.** Concentration of 20 µl/mL of ozone, day 1.
40	Changes are observed over 3 days by exposing the sample to ozone at a concentration of 40 µl/ml only once: **Day 1:** Normal leukocytes and mostly normal erythrocytes; some are seen with slight deformities in the membrane. **Day 2:** Leukocytes with normal appearance; mostly normal erythrocytes, although they begin to deform, taking on a shape similar to that of acanthocytes and echinocytes. **Day 3:** Destruction of PMN and MN and erythrocytes with an appearance similar to acanthocytes and echinocytes, in addition to some beginning to perceive folding of erythrocytes.	 **Image 3.** Concentration of 40 µl/mL of ozone, day 3.
60	Changes are observed over 3 days by exposing the sample to ozone at a concentration of 60 µl/ml only once: **Day 1:** Normal PMN and MN; erythrocytes maintain their shape, although they are enlarged. **Day 2:** Growth of the MN nuclei and the Rouleaux effect can be seen. **Day 3:** Increase in PMN and MN size and persistence of the Rouleaux effect.	 **Image 4.** Concentration of 60 µl/mL of ozone, day 2.
90	Changes are observed over 3 days by exposing the sample to ozone at a concentration of 90 µl/ml only once: **Day 1:** Greater cell separation; in addition, all cells have a more rounded contour. **Day 2:** Structural alterations in leukocytes in addition to erythrocyte destruction. **Day 3:** Rouleaux effect and generalized alterations in cell membranes.	 **Image 5.** Concentration of 20 µl/mL of ozone, day 3.

*Data from this study

Table 2. Morphological changes of erythrocytes and leukocytes at different ozone concentrations of 20, 40, 60, and 90 µl/mL for 5 consecutive days.

Ozone concentration	Description	Image
Control	Cells within normal parameters, with normal morphology and without alterations.	 **Image 1.** Control slide.
20	Poikilo cytosis is observed, and as the days go by, structural changes are observed in the leukocytes. Over 5 days, the following could be seen: ● Day 1: Presence of poi kilo and normal appearance of polymorph nuclear cells (PMN). ● Day 2: A normal appearance of PMN remains. ● Day 3: Involvement of the nucleus of PMN and mononuclear cells (MN). ● Day 4: Enlargement of MN cells is seen. ● Day 5: Destruction of leukocyte nuclei is observed.	 **Image 2.** Concentration of 20 µl/mL of ozone, Day 4.
40	The Rouleaux effect is observed, and, as the days go by, structural changes are observed in the leukocytes. Over 5 days, cells were observed: ● Day 1: No deformities are observed in any cell. ● Day 2: An increase in size is seen in the MN. ● Day 3: The Rouleaux effect and an increase in size in the MN are observed. ● Day 4: An enlarged nucleus is distinguished in PMN and MN. ● Day 5: A clear enlargement of the cells is distinguished.	 **Image 3.** Concentration of 40 µl/mL of ozone, Day 3.
60	Poikilocytosis, acanthocytes, and the Rouleaux effect were observed. Over 5 days, the cells were observed: ● Day 1: Damage is observed in the MN. ● Day 2: Changes in PMN are observed. ● Day 3: The destruction of cell nuclei is observed. ● Day 4: A cellular deformation is observed. ● Day 5: There are no significant changes compared to the previous day.	 **Image 4.** Concentration of 60 µl/mL of ozone, Day 3.
90	Poikilocytosis, acanthocytes, and the Rouleaux effect were observed. Over 5 days, cells were observed: ● Day 1: Rouleaux, poikilocytes, and acanthocytes are observed. ● Day 2: Poikilocyte and acanthocyte are observed, but we see that PMN and MN are affected. ● Day 3: The Rouleaux effect is still visible, and there is an increase in the size of the PMN and MN. ● Day 4: The destruction of the nuclei of PMN and MN cells is visualized. ● Day 5: There are no significant changes compared to the previous day.	 **Image 5.** Concentration of 90 µl/mL of ozone, Day 4.

*Data from this study

The ANOVA shows significant changes in neutrophils and monocytes with a P <0.001 as shown in Table 2, which agrees with the observations seen in the morphological changes of white cells. Table 3.

Table 3. ANOVA statistical test for O3 doses at concentrations of 20, 40, 60, and 90 µl/mL.

ANOVA - Neu%					
Cases	**Sum of squares**	**df**	**Square root of the mean**	**F**	**p**
Parameters	7545.683	15	503.046	4.577	< .001
Residual	3517.153	32	109.911		
ANOVA - Mon%					
Cases	**Sum of squares**	**df**	**Square root of the mean**	**F**	**p**
Parameters	5165.536	15	344.369	5.636	< .001
Residual	1955.253	32	61.102		
ANOVA - PLT					
Cases	**Sum of squares**	**df**	**Square root of the mean**	**F**	**p**
Parameters	28536.646	15	1902.443	0.350	0.983
Residual	174038.667	32	5438.708		

Concerning neutrophils, those exposed to 5 days with the addition of ozone for 5 days were more damaged since they were reduced to almost half of the initial measurement. (Fig. 1) In (Fig. 2) the monocytes of the 5-day doses of O_3 increased significantly; referring to the lymphocytes, their number decreased (Fig. 3); nucleus damage was observed as the most frequent observation. Although there were changes with a decrease in size, the lymphocytes presented a p = 0.901, while the platelets did not show changes or morphological affectation, showing a p = 0.983, while the hematocrit increased with the days statistically showed a p = 0.796. It is not significant for these tests.

Discussion

The most noticeable effect of ozone was the change in the structure of the erythrocyte with the appearance of acanthocytes *vs* echinocytes. According to biochemical hypotheses, ozone affects changes in the membrane due to the reactions ozone originates The changes reported in erythrocytes in other investigations related to a temporary deformation of the membrane in erythrocytes attributed to ozone so that the biochemical changes in it favor oxidation-reduction processes and immunological stimulation, the ozone-treated blood is returned to the whole blood so that in reality it is not known if they remain deformed or die once all the cellular biochemical reactions have been carried out. (Belykh et al., 2007; Morgan et al., 1988; V. Bocci et al., 1993) This work contributes to presenting the forms observed through exposure to ozone; in addition, other works report the formation of spherocytes due to the presence of ozone (Tricarico & Travagli, 2021). Rouleaux's formation was observed in erythrocyte staining two and three days after O3 treatment and causes enzymatic and molecular changes in the protein content of plasma and cells, as shown in this study (Maya, 2008) O_3 alternatively activates messenger molecules of the immune system and simultaneously, ozone participates in highly reactive reactions, transforming many plasma molecules into other products (Diaz-Luis et al., 2015; Togi et al., 2021; V. Bocci et al., 1993). However, exposure to ozone may permanently affect the erythrocyte membrane. In ozone treatments, recovery may occur since in autohemotherapy, the ozonized blood returns to the body diluted with the rest of the blood fluid, and the cells recover the necessary elements to return to their original form. (Belykh et al., 2007; Morgan et al., 1988; V. Bocci et al., 1993), the biochemical and immunological reactions in erythrocytes are explained in the experiments that report the benefit of cellular change. (Belykh et al., 2007; Mehraban & Seyedarabi, 2023; Morgan et al., 1988; Ross et al., 1979; Van Der Zee et al., 1987; V. Bocci et al., 1998), in this study, leukocyte cells presented their transformations until the third day, with an increase in size and shape of their nuclei and deformation may be due to their exposure in closed systems and long exposition products or redox reactions are in plasma. Although changes were observed in the results of blood counts in other parameters, they were not statistically significant such as lymphocytes, platelets, hematocrit and the mean corpuscular volume of erythrocytes. The same morphological changes were observed in the cells of the volunteer samples, confirming the biochemical and structural changes presented in each of the samples are due to the effect of ozone, the cells affected in a closed system in vitro were observed in neutrophils and erythrocytes at the therapeutic concentrations used in this work; we describe acanthocytes *vs* echinocytes, although other authors describe spherocytes (Tricarico & Travagli, 2021). There are reports of hemolysis observed (Belykh et al., 2007; Inguscio et al., 2023; V. Bocci et al., 1998); in this study it was not observed.

Conclusion

In this study it is concluded that the effect of ozone acts on the membranes of erythrocytes and leukocytes, specifically neutrophils and monocytes, with statistically significant changes and in the microscopic observation, the explanation is probably due to the prolonged exposure to O_3 in the samples for several days in *in vitro* systems, as was the case. However, many investigations have demonstrated the beneficial effects of these therapies, as well as their clinical application in patients with health benefits, since the effect of ozone in a larger blood volume does not cause these changes in the cells permanently.

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Conflict of interest this study did not have any financial support of any kind, and the authors declare there is no economic or personal conflict of interest for the publication of this manuscript

The authors of this work declare that they will make the data generated by this research available electronically.

References

Belykh, I. A., Volovelskaya, I. L., & Zinchenko, V. D. (2007). Effect of Low Ozone Doses on Erythrocyte Hypertonic Lysis. *Problems Of Cryobiology And Cryomedicine*, *17*(3), 237-242. http://cryo.org.ua/journal/index.php/probl-cryobiol-cryomed/article/view/494

Bocci, V., Luzzi, E., Corradeschi, F., Paulesu, L., Rossi, R., Cardaioli, E., & Di Simplicio, P. (1993). Studies on the biological effects of ozone: 4. Cytokine production and glutathione levels in human erythrocytes. *PubMed*, *7*(4), 133-138. https://pubmed.ncbi.nlm.nih.gov/8023701

Bocci, V., Valacchi, G., Corradeschi, F., & Fanetti, G. (1998). Studies on the biological effects of ozone: 8. Effects on the total antioxidant status and on interleukin-8 production. *Mediators Of Inflammation*, *7*(5), 313-317. https://doi.org/10.1080/09629359890820

Bocci, V. A., Zanardi, I., & Travagli, V. (2011). Ozone acting on human blood yields a hormetic dose-response relationship. *Journal Of Translational Medicine*, *9*(1). https://doi.org/10.1186/1479-5876-9-66

Chirumbolo, S., Valdenassi, L., Tirelli, U., Ricevuti, G., Pandolfi, S., Vaiano, F., Galoforo, A., Loprete, F., Simonetti, V., Chierchia, M., Bellardi, D., Richelmi, T., & Franzini, M. (2023). The Oxygen–Ozone Adjunct Medical Treatment According to the Protocols from the Italian Scientific Society of Oxygen–Ozone Therapy: How Ozone Applications in the Blood Can Influence Clinical Therapy Success via the Modulation of Cell Biology and Immunity. *Biology*, *12*(12), 1512. https://doi.org/10.3390/biology12121512

Diaz-Luis, J., Menendez-Cepero, S., Diaz-Luis, A., & Ascanio-Garcia, Y. (2015). In vitro effect of ozone in phagocytic function of leucocytes in peripheral blood. *Journal Of Ozone Therapy*, *1*(1). https://doi.org/10.7203/jo3t.1.1.2015.10627

Dhamnaskar, S., Gobbur, N., Koranne, M., & Vasa, D. (2021). Prospective Comparative Observational Study of Safety and Efficacy of Topical Ozone Gas Therapy in Healing of Diabetic Foot Ulcers versus Only Conventional Wound Management. *The Surgery Journal*, *07*(03), e226-e236. https://doi.org/10.1055/s-0041-1731447

Di Paolo, N., Gaggiotti, E., & Galli, F. (2005). Extracorporeal blood oxygenation and ozonation: clinical and biological implications of ozone therapy. *Redox Report*, *10*(3), 121-130. https://doi.org/10.1179/135100005x38888

Inguscio, C. R., Cisterna, B., Carton, F., Barberis, E., Manfredi, M., & Malatesta, M. (2023). Modifications of Blood Molecular Components after Treatment with Low Ozone Concentrations. *International Journal of Molecular Sciences*, *24*(24), 17175. https://doi.org/10.3390/ijms242417175

Maya, G. C. (2008). Utilidad clínica del extendido de sangre periférica: los eritrocitos. *Medicina & Laboratorio*, *14*, 311-357. https://www.medigraphic.com/pdfs/medlab/myl-2008/myl087-8b.pdf

Mehraban, F., & Seyedarabi, A. (2023). Molecular effects of ozone on amino acids and proteins, especially human hemoglobin and albumin, and the need to personalize ozone concentration in major ozone autohemotherapy. *Critical Reviews In Clinical Laboratory Sciences*, *60*(5), 382-397. https://doi.org/10.1080/10408363.2023.2185765

Morgan, D. L., Furlow, T. L., & Menzel, D. B. (1988). Ozone-initiated changes in erythrocyte membrane and loss of deformability. *Environmental Research*, *45*(1), 108-117. https://doi.org/10.1016/s0013-9351(88)80013-6

Re, L. (2022). Ozone in Medicine: A Few Points of Reflections. *Frontiers In Physiology*, *13*. https://doi.org/10.3389/fphys.2022.842229

Ross, B. K., Hlastala, M. P., & Frank, R. (1979). Lack of Ozone Effects on Oxygen Hemoglobin Affinity. *Archives of Environmental Health An International Journal*, *34*(3), 161-163. https://doi.org/10.1080/00039896.1979.10667389

Scassellati, C., Galoforo, A. C., Bonvicini, C., Esposito, C., & Ricevuti, G. (2020). Ozone: a natural bioactive molecule with antioxidant property as potential new strategy in aging and in neurodegenerative disorders. *Ageing Research Reviews*, *63*, 101138. https://doi.org/10.1016/j.arr.2020.101138

Schwartz, A. (2011). *Guía para el uso médico del ozono: fundamentos terapéuticos e indicaciones*.

Serra, M. E. G., Baeza-Noci, J., Abdala, C. V. M., Luvisotto, M. M., Bertol, C. D., & Anzolin, A. P. (2023). The role of ozone treatment as integrative medicine. An evidence and gap map. *Frontiers In Public Health*, *10*. https://doi.org/10.3389/fpubh.2022.1112296

Shinriki, N., Suzuki, T., Takama, K., Fukunaga, K., Ohgiya, S., Kubota, K., & Miura, T. (1998). Susceptibilities of plasma antioxidants and erythrocyte constituents to low levels of ozone. *PubMed*, *29*(3), 229-239. https://pubmed.ncbi.nlm.nih.gov/10069448

Togi, S., Togi, M., Nagashima, S., Kitai, Y., Muromoto, R., Kashiwakura, J., Miura, T., & Matsuda, T. (2021b). Implication of NF-κB Activation on Ozone-Induced HO-1 Activation. *BPB Reports*, *4*(2), 59-63. https://doi.org/10.1248/bpbreports.4.2_59

Tricarico, G., & Travagli, V. (2021). The Relationship between Ozone and Human Blood in the Course of a Well-Controlled, Mild, and Transitory Oxidative Eustress. *Antioxidants*, *10*(12), 1946. https://doi.org/10.3390/antiox10121946

Van Der Zee, J., Tijssen-Christianse, K., Dubbelman, T. M., & Van Steveninck, J. (1987). The influence of ozone on human red blood cells. Comparison with other mechanisms of oxidative stress. *Biochimica Et Biophysica Acta (BBA) - General Subjects*, *924*(1), 111-118. https://doi.org/10.1016/0304-4165(87)90077-8

Viebahn-Haensler, R., & Fernández, O. S. L. (2023). Ozone as Redox Bioregulator in Preventive Medicine: The Molecular and Pharmacological Basis of the Low-Dose Ozone Concept—A Review. *International Journal of Molecular Sciences*, *24*(21), 15747. https://doi.org/10.3390/ijms242115747

Zeng, J., Dou, J., Gao, L., Xiang, Y., Huang, J., Ding, S., Chen, J., Zeng, Q., Luo, Z., Tan, W., & Lu, J. (2018). Topical ozone therapy restores microbiome diversity in atopic dermatitis. *International Immunopharmacology*, *80*. https://doi.org/10.1016/j.intimp.2020.106191

Zeng, J., Lei, L., Zeng, Q., Yao, Y., Wu, Y., Li, Q., Gao, L., Du, H., Xie, Y., Huang, J., Tan, W., & Lu, J. (2020). Ozone Therapy Attenuates NF-κB-Mediated Local Inflammatory Response and Activation of Th17 Cells in Treatment for Psoriasis. *International Journal of Biological Sciences*, *16*(11), 1833-1845. https://doi.org/10.7150/ijbs.41940