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# How Ozone Treatment Affects Erythrocytes

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## 1. Introduction

Blood, a non-Newtonian fluid, is a suspension of formed elements in an aqueous solution of organic molecules, proteins, and salts. The rheological properties of blood are generally well-understood under steady-flow conditions. However, the *in vivo* flow is unsteady, because of the heart pumping the blood, and blood has to travel through a complex network of vessels of varying geometric qualities (e.g., lumen shape, diameter, single vessels, bifurcations, valves). What's more, the apparent viscosity of blood, which depends on the existing shear forces, is determined by hematocrit, plasma viscosity, and the properties of red blood cells (RBCs) (Baskurt & Meiselman, 2003; Cokelet, 1980; Lipowsky, 2005). In addition to the concentration of cellular elements in blood, the disturbance of flow streamlines depends not only on the concentration of blood cells but also on the behavior of these cells under shear forces (i.e. their rheological properties). RBCs comprise about 45% of the volume of normal blood and are responsible for its complex, scale-dependent rheology. Due to their fluid-filled bag-like structure, RBCs undergo large deformation as they traverse the microcirculation (Baskurt & Meiselman, 2003; Lee & Smith, 2008).

Blood rheology is altered in various conditions: Changes at the level of hematocrit notably lead to variations in hemorheological parameters. Also modifications of the membrane skeletal proteins, the ratio of RBC membrane surface area to cell volume, cell morphology, and cytoplasmic viscosity influence deformability of RBCs. What's more, RBC aggregation is mainly determined by plasma protein composition and surface properties of RBCs (Baskurt & Meiselman, 2003; Lipowsky, 2005). So tissue perfusion may be notably affected by impaired blood fluidity and this may cause to functional deteriorations, especially if the changes had resulted in disturbance of the vascular properties.

The high deformability property of RBCs significantly contributes to aiding blood flow both under bulk flow conditions and in the microcirculation. With diminishing vessel diameter, the particulate nature of blood dominates the resistance to flow. It is now well understood that in addition to blood cell concentration, red cell deformability and aggregation and white blood cell deformability and adhesion to the endothelium are the principal intrinsic factors that affect resistance to flow. Blood cell deformability affects the entrance of blood cells into capillaries. RBCs with reduced deformability in pathological disorders (e.g., sickle cell disease) may be sequestered at the capillary entrance (Lipowsky, 2005).

The aggregation of RBCs is a natural phenomenon affected by high molecular weight molecules, the most important of which is fibrinogen. Erythrocyte aggregation as a reversible

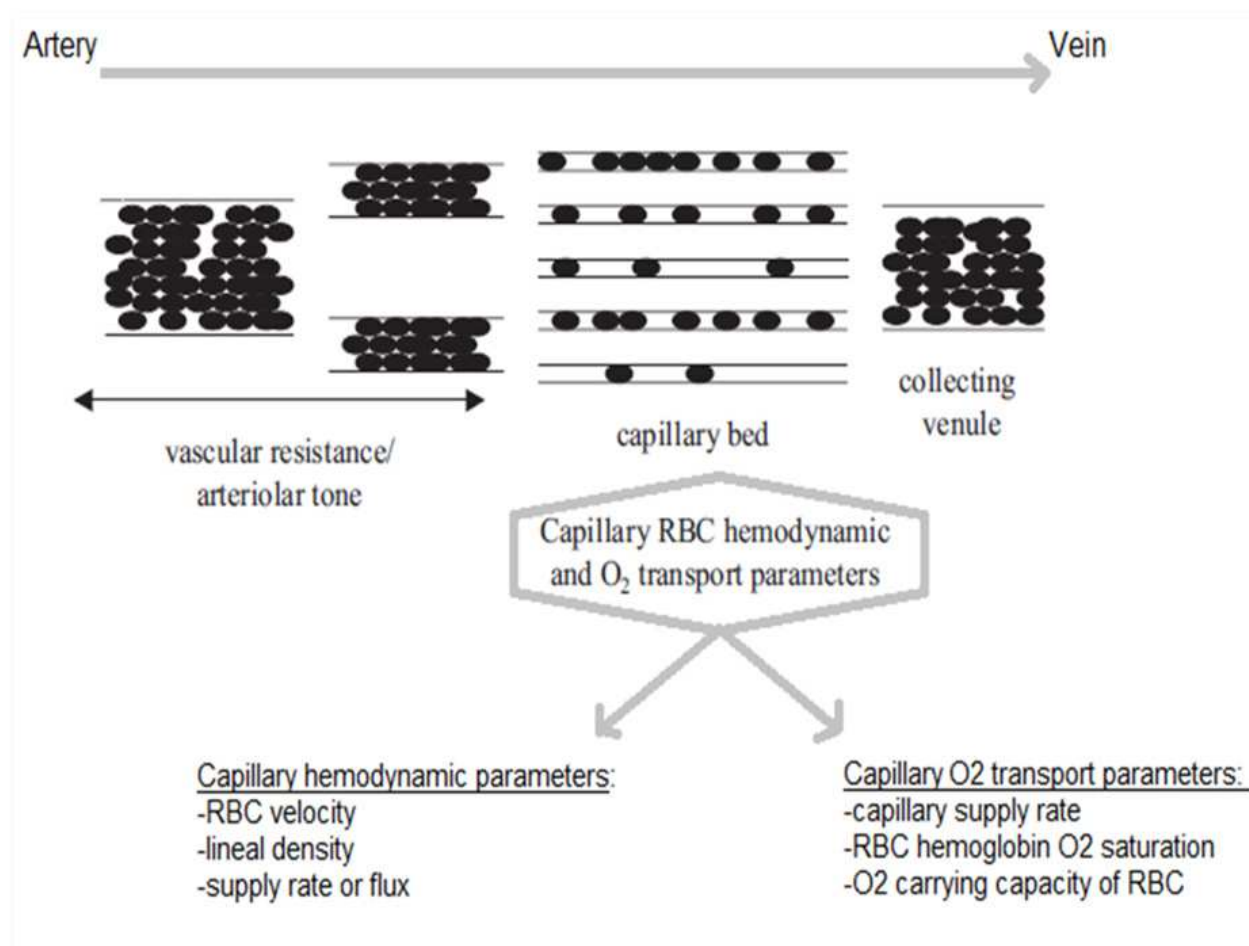


Fig. 1. The above figure, adapted from work of Bateman, clearly shows the interaction and importance of deformability of RBCs for microcirculation. Arteriolar tone establishes the blood flow into an organ, and capillary resistance and rheology factors determine RBC heterogeneity within the capillary bed (Bateman et al., 2003).

dynamic phenomenon can be observed both in vitro and in vivo and is found to be responsible for much of the increase in viscosity at low shear rates. The aggregation combined with yield stress of blood is expected to reduce blood flow compared to that of non-aggregating system (Antonova et al., 2008; Rampling, 1988). The in vivo erythrocyte aggregation observed in certain species at low shear rates is mediated by fibrinogen and other large plasma proteins (Lee & Smith, 2008; Merrill et al., 1966). The term aggregability has been used to express the intrinsic aggregation behavior of RBCs regardless of the properties of the suspending medium (Baskurt & Meiselman, 2003). Although earlier studies suggested that the macromolecular composition of the suspending medium was the only determinant of RBC aggregation, more recent studies have shown that RBC cellular properties also play a very important role in the aggregation process (Armstrong et al., 2001; Baskurt & Meiselman, 2003; Kobuchi et al., 1988; Meiselman, 1993). The process of RBC aggregation can be considered the result of a balance between aggregating and disaggregating forces. Disaggregating forces include fluid shear forces, electrostatic repulsion between cells, and the elastic properties of the cell membrane (Baskurt & Meiselman, 2003; Kobuchi et al., 1988; Mohandas & Chasis, 1993). During red cell aggregation, resistance to flow may decrease with hematocrit reduction or increase due to

redistribution of red cells. Blood cell adhesion to the microvessel wall may initiate flow reductions, as, for example, in the case of red cell adhesion to the endothelium in sickle cell disease, or leukocyte adhesion in inflammation (Antonova et al., 2008; Lipowsky, 2005). As strength of aggregation increases, RBCs form rouleaux and then clumps. These clumps frequently become lodged at the capillary entrance and resisted disruption by hydrodynamic forces (Lipowsky, 2005). RBC aggregation is dependent on the magnitude of shearing forces acting on the cells. Increased shear disrupts the aggregates, whereas reduced shear favors aggregation (Baskurt & Meiselman, 2003). RBC aggregation is thus the major determinant of blood viscosity under low shear conditions. Osmotic force arises from the depletion of molecules in the intercellular space where aggregation forces arise naturally as the density of the fluid particles between cells is less than in the surrounding region (Lee & Smith, 2008). So RBC aggregation is accepted as a significant determinant of resistance in venules. However, blood cellular elements other than RBCs have no significant effect on the macroscopic flow properties of blood but may contribute distinctly to blood flow resistance and flow dynamics in the microcirculation (Baskurt & Meiselman, 2003; Kaliviotis et al., 2010; Meiselman & Baskurt, 2006).

The relationship of the RBC's surface area to its volume is evaluated by the osmotic fragility test. The erythrocyte is characterized by a biconcave shape giving it an excess of surface area in relationship to its volume. When a RBC is placed in a hypotonic sodium chloride solution, a net influx of water into the cell will occur. When there is a decrease in the surface area to the cell volume, the osmotic fragility is increased, meaning decreased resistance to hypotonic solutions (and vice versa) (Fernandez-Alberti & Fink, 2000; Mariani et al., 2008).

The rheologic characteristics of blood and its formed elements continue to be of basic science and clinical interest, with numerous publications dealing with topics such as blood and plasma viscosity, RBC aggregation and cell deformability. Alterations of blood's rheologic behavior in pathologic states have been extensively studied, with the findings usually indicating changes assumed to be detrimental to tissue perfusion (e.g., increased RBC aggregation). However, the current literature contains relatively few studies dealing with two important areas: (1) relations between altered rheologic behavior and in vivo hemodynamics; (2) the effects of therapy in those clinical states associated with altered rheologic behavior (Meiselman & Baskurt, 2006).

## 2. Rheological parameters and pathological conditions

Continuation of the blood flow is essential for the preservation of health and life. Disorders of hemorheology and microcirculatory blood flow play an important role in the pathophysiology and clinical manifestations of a wide range of disease states. There is a large list of causes of microvascular failure (Isbister, 2007). Recent clinical observations have reported that reduced RBC deformability is a common risk factor for circulatory disorders including mainly diabetes, sepsis, malaria, hypertension, sickle cell anemia, ischemic conditions and stroke (Lipowsky, 2007).

In patients with diabetes mellitus there is increased viscosity and enhanced RBC aggregation, which is especially prominent in patients with poor glycemic control (Lacombe et al., 1989; Le Devehat et al., 2001; McKay & Meiselman, 1988). For arterial hypertension the association between blood pressure and blood viscosity should be mentioned. High blood pressure causes elevated hematocrit, plasma viscosity and fibrinogen (Ajmani, 1997; Bogar,

2002; Cicco et al., 1999; Hoiegggen et al., 2003; Klein et al., 1995). Severe septic events include misdistribution of blood flow and marked disturbances of microvascular flow leading to tissue hypoperfusion and impaired aggregation and deformability of RBC (Baskurt et al., 1997; Bateman et al., 2003; Condon et al., 2007; Kirschenbaum et al., 2000; Moutzouri et al., 2008; Piagnerelli et al., 2003a; Piagnerelli et al., 2003b; Voerman et al., 1989). Increased aggregation and deformability of RBC is also seen in cerebral and myocardial ischemic conditions as well (Bhavsar & Rosenson, 2010; Bolokadze et al., 2006; Francis, 1991; Huang et al., 1998a; Huang et al., 1998b; Kuke et al., 2001; McHedlishvili et al., 2004).

### 3. Ozone: Its general effects and use in medicine

Ozone is an inactivated, trivalent ( $O_3$ ) form of oxygen ( $O_2$ ). It is considered one of the most potent oxidants in nature. Ozone was first discovered by chemist Christian Frederick Schonbein in 1840 as a disinfectant (Bocci, 2011).

Ozone was used for the first time to disinfect operating rooms in 1856 and subsequently for water treatment in 1860. It is used to treat battle wounds and other infections during World War I (Bocci, 1996; Bocci, 2006). After the turn of the century, interest began to focus on the uses of ozone in medical therapy. However, it was not until 1932 that ozone was seriously studied by the scientific community, when ozonated water was used as a disinfectant by dentist E.A. Fisch. One of his patients, surgeon Erwin Payr, along with physician P. Aubourg, was the first medical doctor to apply ozone gas through rectal insufflations to treat mucous colitis and fistulae (Bocci, 2006). Major ozonated autohemotherapy was first described in 1954 and consist of ex vivo exposing human blood to a gas mixture composed of therapeutic oxygen and ozone for a short time followed by reinfusion in the donor (Bocci, 2011).

The medical generator of ozone produces it from pure oxygen passing through a high voltage gradient (Bocci, 2006; Li et al., 2007). A gas mixture comprising no less than 95% oxygen and no more than 5% ozone used in medicine is known as medical ozone therapy (Bocci, 2006; Guven et al., 2009). When the gas mixture composed of 95% oxygen and of no more than 5% ozone, is mixed ex vivo, a complex series of physical and chemical processes occur: oxygen dissolved in plasmatic water, almost instantaneously saturates hemoglobin to form oxyhemoglobin and the  $pO_2$  increases far higher than physiological level while  $pCO_2$  and pH remains fairly constant (Bocci, 1996). But there is not a general consensus regarding the biological effects and possible damage induced by ozone in blood.

It is well known by its toxic effects. Ozone therapy is still a controversial form of alternative therapy. Young children and adults with lung problems are told to stay indoors, because ozone can aggravate allergies, bronchitis, asthma and other health problems. On the other hands, many believe that ozone will help heal them of cancer, heart disease, candida, HIV related problems and a host of other diseases including autoimmune disease, rheumatoid arthritis and low back pain. There are few elements that have been as controversial as ozone, and none that have created such a medical paradox: how can a gas be both dangerous to health as a pollutant, yet can also be used to effectively treat some of humanity's most threatening diseases?

Ozone, when given in lower doses, exerts beneficial effects acting on the oxidant mechanisms (Ajamieh et al., 2002; Chen et al., 2008). In spite of encouraging results obtained with ozone therapy, its clinical use remains controversial due to the scarce knowledge of the



mechanisms underlying its therapeutic action and the efficacy in heterogeneous diseases (Ajamieh et al., 2002; Gornicki & Gutsze, 2000). Currently, with reappraisal of ozone therapy, ozone has been utilized worldwide in research and clinical field. It has been used as a therapeutical agent for the treatment of different diseases (Bocci, 2011; Gornicki & Gutsze, 2000; Viebahn-Haensler, 2007).

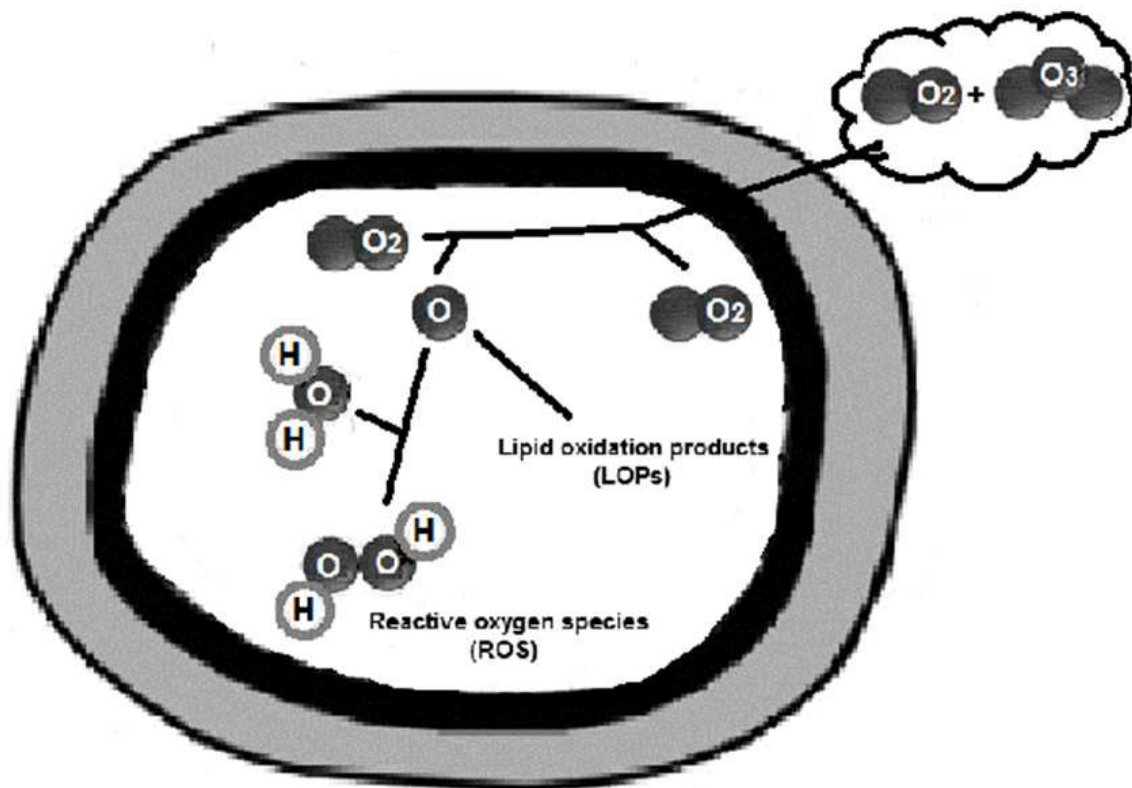


Fig. 2. The fate of oxygen-ozone in the circulation.

Although ozone therapy has drawbacks (as it is intrinsically toxic cannot be breathed, cannot be stored, and must be used with caution and competence), it is used for a wide variety of health problems. There are different methods of ozone therapy in medical practice: direct intra-arterial and intravenous application, rectal insufflations, intramuscular injections, major and minor autohemotherapy, intra-articular injection, ozone bagging, ozonated water, and ozonated oil. Autohemotherapy calls for the removal of venous blood from the patient, and then the ozonated blood is re-introduced into a vein. It is probably the most commonly used type of ozone therapy today. Besides, rectal insufflation method is considered one of the safest (Bocci, 2011; Travagli et al., 2007).

Although ozone has been in use for many years, the dynamic of the events occurring during ozonation of whole human blood have been recently clarified (Bocci & Aldinucci, 2006; Travagli et al., 2006): At a normal gas pressure of 760 mmHg, only 2.0mL of oxygen dissolve in 100mL of water and allows complete oxygenation of hemoglobin. Ozone, one of the strongest oxidants, is 10-fold more soluble than oxygen and, even more important, it reacts immediately with hydrophilic antioxidants present in plasma (Travagli et al., 2007).

Still there is not a general consensus regarding the biological effects and possible damage induced by ozone in blood. The main controversy is due to ozone being an extremely

reactive and unstable molecule. However it is well known that the plasma contains a wealth of antioxidants (Travagli et al., 2007). So when erythrocytes are not suspended in saline and their membrane is not deprived of the albumin protection, the antioxidant defense systems of RBCs are activated immediately after the introduction of ozone to the blood.

Ozone also causes a significant reduction in NADH and helps to oxidize cytochrome C. There is a stimulation of production of enzymes which act as free radical scavengers and cell wall protectors: Glutathione peroxidase, catalase, and superoxide dismutase. Production of prostacycline, a vasodilator, is also induced by ozone.

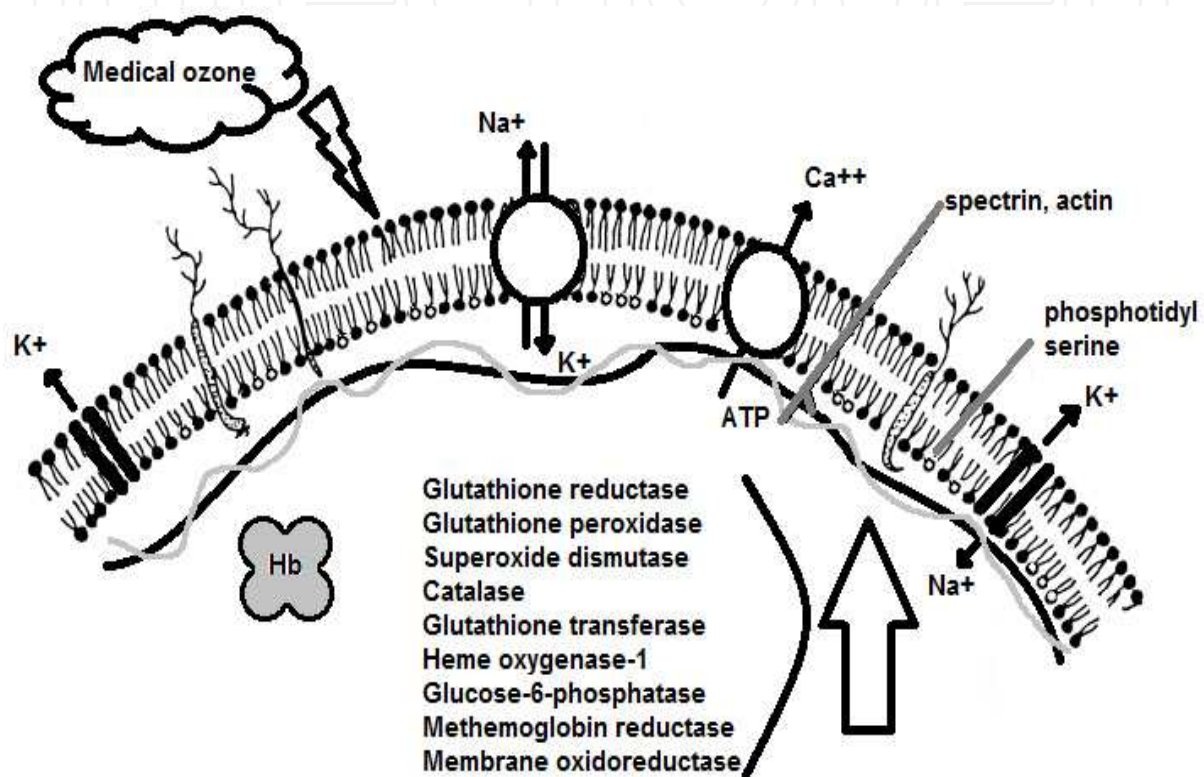


Fig. 3. Ozone possesses the property of stimulating certain antioxidant enzyme systems.

At normal temperature and atmospheric pressure, due to its high solubility and depending upon its relative pressure, some ozone dissolves into the water. But it does not equilibrate with the ozone remaining in the gas phase, unlike oxygen. The reason for this is the immediate interactions between ozone, a potent oxidant, and a variety of molecules present in biological fluids, namely antioxidants, proteins, carbohydrates and, preferentially, polyunsaturated fatty acids (Travagli et al., 2006; Viebahn-Haensler, 2007). Both mono- and polyunsaturated fatty acids and cholesterol, are present in lipoproteins and cellular membranes. Free and bound cysteine, methionine, tyrosine, tryptophane and histine as well as free and protein-bound carbohydrates are among the potential targets (Bocci, 1996).

The reaction of ozone with such a variety of molecular compounds involves: a) an initial stage of reaction in which, some of the ozone dose is unavoidably consumed during oxidation of ascorbic and uric acids, sulphhydryl (SH)-groups of proteins and glycoproteins. Although albumin, ascorbic and uric acids tame the harsh reactivity of ozone, they allow this first reaction that is important because it generates reactive oxygen species (ROS),

which triggers several biochemical pathways in blood. ROS are neutralized within 0.5-1 minute by the antioxidant system (Bocci, 2011); b) a late stage at which lipid oxidation products (LOPs), such as: peroxy radical complex mixtures of final products of low molecular weight aldehydes (maloniladdehyde) and alquenaes and also, hydrogen peroxide (H2O2). Actually hydrogen peroxide is not a radical oxidant, it is included within ROS. This and the LOPs are responsible for the therapeutic and biological late effects of ozone (Bocci, 1996; Bocci & Aldinucci, 2006). Therefore, the excessive production of oxygen metabolites or inadequate defense to counter their accumulation in the body, with consequent tissue injury, promotes or accelerates the development of multiple pathological processes, being the mechanism of signal transduction for activation or repression of transcription specific genes are the cornerstone of the mechanism of action for modulation of oxidative stress. So only the dose of ozone that is not is consumed by the antioxidants present in plasma stimulates the formation of ROS and LOPs which are responsible for the biological and therapeutic effects of ozone.

It seems obvious that erythrocytes ozonated ex vivo may be modified only for a brief period. Only repeated therapeutic sessions may allow to LOPs to reach the bone-marrow and activate a subtle development at the erythropoietic level, favoring the formation of new RBCs with improved biochemical characteristics, which provisionally were named “supergifted erythrocytes”. If this hypothesis is correct, every day, during prolonged ozone therapy, the bone marrow may release a cohort of new RBCs with improved biochemical characteristics (Bocci et al., 2011).

The proposed beneficial effects of ozone therapy does not abruptly stop with the cessation of the therapy but rather persists for 2-3 months, probably in relation to the life-span of the circulating supergifted erythrocytes (Bocci, 2011). During continuing ozone therapy it has been observed that very young RBCs have a significantly higher content of glucose 6-phosphate dehydrogenase (G6PD). This result increase the probability of the postulation that only a cycle of more than 15 treatments could improve an pathology (Bocci et al., 2011).

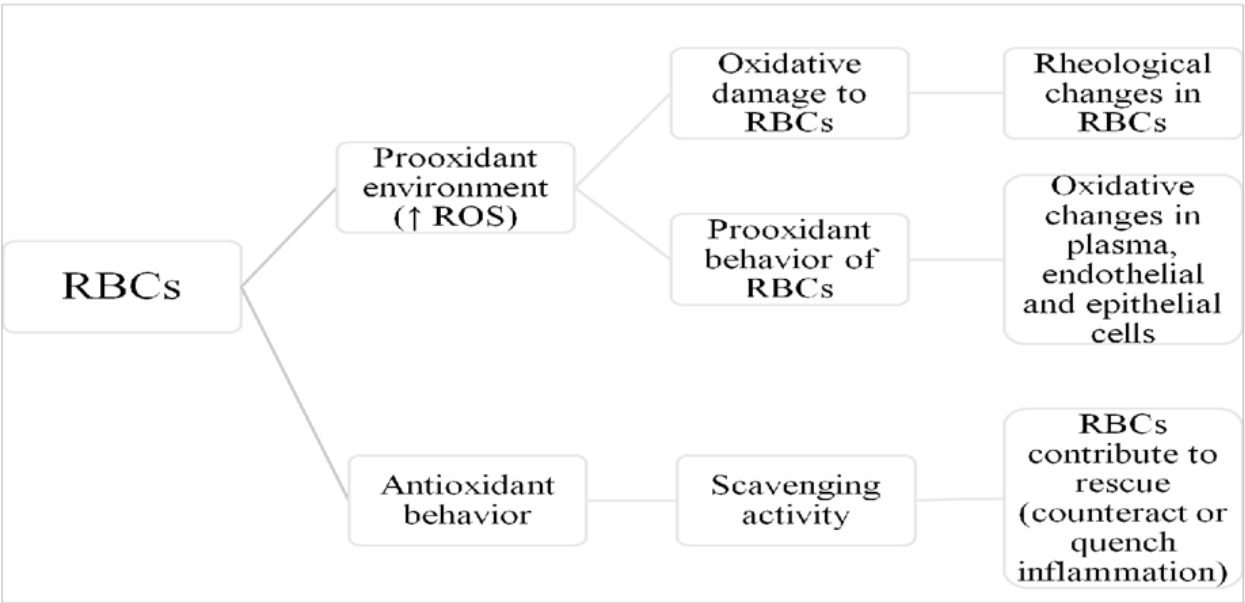


Fig. 4. The oxidant and antioxidant role of RBCs.



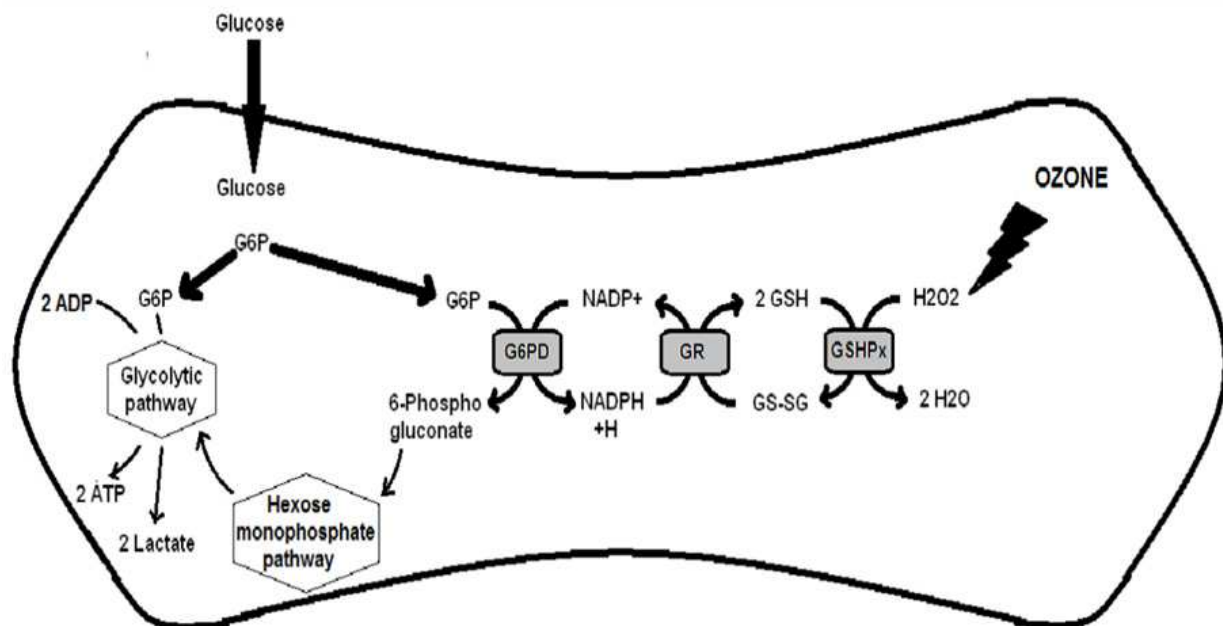


Fig. 5. The action of ozone on the metabolism of red blood cells (G6P: Glucose 6-phosphate; G6PD: Glucose 6-phosphate dehydrogenase; GR: Glutathione reductase; GSHPx: Glutathione peroxidase).

Ozone therapy causes an increase in the RBC glycolysis rate. This leads to the stimulation of 2,3-diphosphoglycerate (2,3-DPG) which leads to an increase in the amount of oxygen released to the tissues. Ozone therapy is avoided in G6PD enzyme deficiency as it acts through this enzyme. Ozone activates the hexose monophosphate pathway by enhancing oxidative carboxylation of pyruvate, stimulating production of ATP (Bateman et al., 2003). Functionally, the oxyhemoglobin sigmoid curve shifts to the right owing to the Bohr effect, i.e. a small pH reduction and a slight increase of 2,3-DPG. The shift to the right is advantageous for improving tissue oxygenation as the chemical bonding of oxygen to hemoglobin is attenuated, facilitating oxygen extraction from ischemic tissues (Bocci et al., 2011).

Above mentioned is the first and immediate reactions following introduction of oxygen-ozone to blood. The second reaction occurs in the bone marrow. When submicromolar amounts of LOPs present in the reinfused blood reach various organs, including the bone marrow, where they can influence the differentiation of the erythroblastic lineage (Bocci, 2011).

Besides, abnormal microvascular oxygen transport indicates that regulatory mechanisms have become dysfunctional and suggests that local cellular environments, as such, have been dramatically altered. The loss of capillary blood flow may potentiate the effects of proinflammatory mediators by increasing their residence time in the microcirculation and tissue (Bateman et al., 2003).

Downstream of the arterioles, microvascular RBC flow is passively distributed throughout the capillary networks and other vascular beds such as the liver sinusoids, according to local vessel resistance (diameter and length) and hemorheologic factors (blood viscosity and RBC deformability). RBCs are forced to deform and travel single file, often separated by plasma

gaps, as they pass through vessels that are of smaller diameter than their own. This distinctive microvascular flow behavior maximizes the surface area available for gas exchange between the RBC and the local environment (Bateman et al., 2003).

Flow heterogeneity within capillary beds may have two sources: unequal distribution of RBC supply among arterioles and unique properties of RBC flow in branching networks of capillaries. In determining capillary heterogeneity and functional capillary density, rheologic mechanisms appear to play a greater role than arteriolar heterogeneity, especially at low flow states. These mechanisms are also responsible for the Fahreaus effect, which is the drop in vessel hematocrit along the arteriolar tree to the capillary bed. In the skeletal muscle of septic rats stopped-flow capillaries have lower hematocrit, or lineal density (RBC/mm), than do neighboring flowing capillaries. Neither the implications nor the cause and effect relationship of this phenomenon is clearly understood (Bateman et al., 2003).

#### 4. Hemorheological effects of ozone treatment

In practice, autohemotherapy is the most commonly preferred route for ozone administration; although maybe the safest route to give ozone systemically is rectal insufflations, with almost no side effects. So the effects of ozone on blood cells actually should be investigated with priority. Minor autohaemotherapy involves removing a small amount (usually 10 ml) of the patient's blood from a vein with a hypodermic syringe. The blood is then treated with ozone and oxygen, and given back to the patient with an intramuscular injection. Thus the blood and ozone becomes a type of autovaccine given to the patient that is derived from their own cells, thus forming a unique vaccine that can be very specific and effective in treating the patient's health problem. Major autohemotherapy calls for the removal of 50-100 ml of the patient's blood. Ozone and oxygen are then bubbled into the blood for several minutes, and then the ozonated blood is reintroduced into a vein. Therefore besides giving it for treatment of any pathology involving blood or circulation, during the ozone therapy first blood is prone to the effects of ozone.

The studies investigating the effects of ozone therapy on hematologic/hemodynamic parameters are limited in number and controversial. This is partially because of that many studies on the effects of ozone on blood are conducted under conditions which are not physiologic and looking for the acute effects.

Medical ozone treatment does not practically change neither methemoglobin nor hematocrit levels. Unchanged hematocrit value means no change of the RBC volume due to swelling or lysis. Blood and plasma viscosity decreases due to reduced fibrinogen levels (Bocci, 2002). When high levels of ozone is given there is a minimal loss of K<sup>+</sup>, which rapidly returns to normal (Shinriki et al., 1998).

RBC flexibility in mice decreased when ozone given by inhalation (Morgan et al., 1985). Unsurprisingly, loss of deformability reported by the same investigators in a study conducted by ozonating saline-washed RBCs (Morgan et al., 1988). Ozonation of either human whole blood or saline-washed RBCs causes considerable damage. RBCs become quite sensitive to ozone when they are deprived of plasma antioxidants and their membrane has been totally deprived of the albumin protection (Travagli et al., 2007). What's more, in

an albumin deprived microenvironment RBCs become echinocytes, that initiates a strong decrease of the deformability (Mrowietz et al., 2008; Wong, 2005). This might be an additional effect to the possible ozone effect on saline-washed RBC. There are three major factors controlling RBC flexibility which should be kept in mind to explain the loss of deformability: Cellular viscosity, the ratio of surface area to volume, and the viscoelastic properties of cell membrane. At high shear rates the internal viscosity of RBC is the major determinant of the deformation; whereas at lower rates of shearing the membrane properties and cell geometry become more important (Baskurt et al., 2009; Bayer & Wasser, 1996).

Following high dose ozone application, Bayer et al. did not find altered deformability of human RBC. They proposed that a direct exposition of RBC (with an intact catalase and/or glutathion system) to ozone leads to an all or nothing effect either leading to no change on RBCs (no influence on elongation) or destroying them (hemolysis) (Bayer & Wasser, 1996). On the other hand, some other researchers have claimed that a slight peroxidation of the RBC membrane induces favorable consequences on cell functions such as increased fluidity of the membrane with enhanced cell deformability (Aydogan et al., 2008; Caglayan & Bayer, 1994; Coppola et al., 2002; Giunta et al., 2001; Rokitansky et al., 1981; Verrazzo et al., 1995). Studies demonstrated that ozone, within the therapeutic range, does not cause oxidation of the RBC membrane (Bocci & Aldinucci, 2006; Cataldo & Gentilini, 2005; Shinriki et al., 1998; Travagli et al., 2006; Travagli et al., 2007). As a consequence, the membrane of RBCs that is shielded by albumin molecules remains intact when we use the therapeutic concentrations of ozone (Travagli et al., 2006; Travagli et al., 2007). The results of our study, where low dose oxygen-ozone mixture rectally applied, showed an increase in RBC deformability proportional to the duration of treatment (Artis et al., 2010).

RBC repels each other because of their negative surface charge from sialic acid residues. At the same time they are attracted by the van der Waals forces, which are electrodynamic in nature. The balance of these two forces determines the most stable arrangement of red cells in an electrolyte solution in the absence of other forces (Fabry, 1987). The cellular properties of RBC determine the cell's intrinsic tendency to aggregate (Baskurt et al., 2009). Ozonation is known to increase the negative charge of the RBC membrane (Travagli et al., 2007). There are studies investigating the effects of ozone application on platelet aggregation, but to our knowledge there is not any study investigating changes in RBC aggregation with ozone treatment in the literature except ours. Following 15 days of treatment with ozone we observed a prominent decrease in RBC aggregation. However, the results were not different than the control group at longer periods of treatment (Artis et al., 2010).

The study of Zimran showed no damaging effects of ozone on red cell enzymes or intermediates. There was only minimal hemolysis that was not different from that caused by routine blood storage (Zimran et al., 2000). This hemolysis may be the result of the reaction of ozone molecules on the red cell membrane lipids, producing secondary ozonides. It is possible that subhemolytic damage to red cells shortens the life span of RBCs reinfusion. However, the number of cells destroyed would be no greater than those destroyed when red cells stored in the blood bank are infused. However as expected washed RBC yield higher values of hemolysis. The degree of hemolysis is lower if the anticoagulant is citrate instead of heparin. This enhanced hemolysis by heparin is probably favored by a concomitant  $\text{Ca}^{2+}$  influx (Bocci, 2002). On the other hand, exposure

of RBC to ozone can result in increased osmotic fragility of the cell membrane (Calabrese et al., 1985; Chan et al., 1977). In accordance with the previous results also we observed a significant increase in osmotic fragility which showed a decrement afterwards with continuing treatment (Artis et al., 2010).

It has recently been discovered that ozone is able to induce an adaptation to oxidative stress or promote an oxidative preconditioning through the increase and preservation of endogenous antioxidant systems (Ajamieh et al., 2002; Bocci, 2011). The adaptation develops after multiple ozone exposures (Ajamieh et al., 2002; Bocci et al., 2007). Prolonged exposure to ozone in aged subjects caused an increase of both ATP and 2,3-DPG in RBCs (Viebahn-Haensler, 2007). Even two weeks of treatment was found enough to induce the adaptation to ozone oxidative stress (Barber et al., 1999; Leon et al., 1998). This adaptation process may explain the changes in RBC aggregation and fragility with extended ozone application; but there was no adaptation in RBC deformability (Artis et al., 2010). It was also observed that 15 days after single dose of ozone application its effect on RBC deformability still persist but lessened (Buckley et al., 1975). Thus when talking about RBC deformability it can be said that there is either a later developing adaptation or it does not exist at all.

An increased negative charge of the membrane accompanied by a lower erythrocyte sedimentation rate together with decreased viscosity may explain an overall improvement of rheologic parameters of ozone therapy (Bocci, 2002).

## 5. Conclusion

The novelty of ozone therapy is that its functions are directed to restore and improve the metabolism of oxygen, together with sugars and fats to produce energy, through normal metabolic pathways of controlled combustion: Glycolysis, respiratory chain, fatty acid cycle, glucose 6 phosphate dehydrogenase, and oxidative decarboxylation of pyruvate.

Ozone therapy has been used for treatment of a variety of different pathological conditions including peripheral occlusive arterial disease, cerebral ischemia, gangrene, Reynaud's disease, senile dementia, thrombophlebitis, diabetes mellitus, ischemic heart disease, sepsis, etc. Of these, ozone application for superficial infection, burns, dental and intestinal conditions, and circulatory problems seem to have the best potential of cure.

Although ozone treatment is mainly applied as autohemotherapy; and its use is relatively and especially preferred in circulatory disorders (at which both local and systemic routes are preferred) with promising results. However neither its effects nor the mechanisms on blood cells are exactly known. Regarding to ozonation of blood, further research is indicated to delineate the nature of its dynamics and the extent of its effectiveness in (a) the identification of the compounds formed in this process dose and route of application dependently; (b) seeking scientific evidence for metabolic, immunological, endocrine and possibly neurological effects; (c) identification of the possible (and presently known) pathological conditions ozone therapy might be given; and (d) investigating to what extend ozone therapy might be applied in these conditions. With the increasing use of ozone in clinical practice, the RBCs could represent a useful tool to investigate in particular its vascular and hemodynamic effects. More in general, the improvement of clinical laboratory analyses aimed at the evaluation of RBC integrity and function, e.g. morphological/rheological



parameters, expression of surface antigens and, RBC redox state, could provide useful information in the clinical practice in the long term.

As a conclusion, ozone treatment seems to have a beneficial effect on RBCs. Nevertheless it should be kept in mind that ozone has different effects after acute or chronic applications on hemorheological properties of RBCs. New controlled studies are needed to be conducted on this promising treatment modality. Further studies on this subject are needed.

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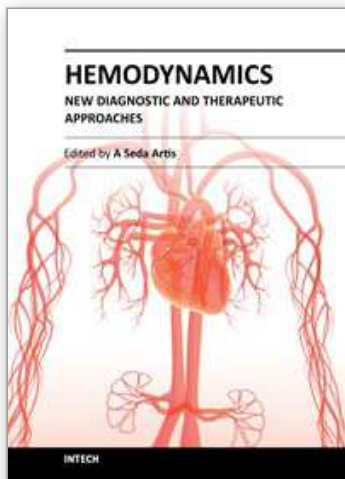
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