Use of oxygen-ozone therapy in the treatment of panniculopathy edematous fibro sclerotic: a clinical case

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Abstract

Oxygen-ozone therapy represents an effective therapeutic reality for panniculopathy, particularly in the edematous phase, as well as for the treatment of localized adiposity.

Our case report is a 38 years-old patient with Edematous Fibrosclerotic Panniculopathy (EFP) diagnosed through physical examination.

We compared the anthropometric measurements at the beginning of the treatment and after 18 sessions of oxygen-ozone therapy and it was seen that despite the study was conducted only on one patient, comparing our clinical case with the clinical cases of studies whose number was significant, we can say that EFP therapy with oxygen ozone produces satisfactory results both from a subjective point of view and from an objective point of view.

Introduction

EFP (Edematous Fibrosclerotic Panniculopathy) is a disorder of the lymphatic vein microcirculation and subcutaneous white adipose tissue, which mainly affects women of childbearing age.

The term EFP was proposed by Bizzarri in 1974, although commonly this disease is known as "cellulite" and is often considered only as a blemish, despite being a real pathology.

This pathology is characterized by the presence of adipocyte hypertrophy and lymphatic vein stasis which causes stagnation of excess fluids and catabolites: all this leads the adipocyte to find itself in a state of general suffering, unable to eliminate the waste substances with consequent accumulation of the same. This makes the arrival of oxygen and nutrients to the adipocyte difficult with a consequent reduction in tissue trophism, which can lead to fibrosis and sclerosis of the tissue over time, thus laying the foundations for the progression of the damage.

Oxygen ozone therapy represents an effective therapeutic reality both for EFP and for other diseases of the adipose tissue such as localized adiposities.

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Key words: oxygen-ozone therapy; panniculopathy edematous fibro sclerotic; cellulite.

Conflict of interest: the author declares no potential conflict of interests.

Received for publication: 7 September 2020.
Accepted for publication: 24 September 2020.

Licensee PAGEPress, Italy
Ozone Therapy 2020; 5:9368
doi:10.4081/ozone.2020.9368

Edematous Fibrosclerotic Panniculopathy (EFP)

Pathopathogenesis and etiological aspects

The Edemato Fibrosclerotic Panniculopathy (EFP) indicates a disease of the adipose tissue, the term of which was coined by Bizzarri in 1974. The term "Panniculus" indicates that the problem is located at the level of the subcutaneous adipose panniculus, "Edemato" indicates that there is a situation of edema (stagnation of liquids) probably due to poor circulation in the hypodermis and then in the dermis; "Fibrosclerotic" indicates that the tissue undergoes fibrous and sclerotic modifications (as if delimiting the edema).[1-3]

EFP affects about half of the population: in 95% of cases it affects women and in 5% men.

There are many causes that give rise to EFP, often dependent on various factors that add up to each other.

"Primary" factors, those that cannot be eliminated: i) familiarity (inheritance, predisposition); ii) Caucasian race; iii) female, because in women the action of estrogen on specific receptors prevails. The problem begins with adolescence, a period in which there is a real hormonal storm that marks the transition from childhood to adulthood.

"Secondary" factors, that is, those potentially eliminable: i) vascular diseases; ii) endocrine pathologies; iii) use of birth control drugs; iv) the menstrual cycle (the orange peel appearance in the premenstrual period is accentuated); v) pregnancy: during which an increase in estrogen occurs, responsible for increased appetite, stagnation of fluids and aggravation of blood circulation.

"Aggravating" factors, that is due to our lifestyle that could certainly be controlled: i) sedentary life or excessively rapid weight loss: the muscle tissue gives way and therefore the visual situation of the EFP worsens. Practicing daily movement helps maintain efficient muscle, circulation and metabolism by helping to burn fat and prevent circulatory stasis; ii) Eating disorders: a wrong diet, high calorie, rich in fats, abuse of salt, alcohol and coffee causes accumulation of localized fat and fluid retention; iii) Wrong posture, contributes to aggravate blood circulation and

therefore EFP because it compresses the vessels; iv) Prolonged standing position: too long standing causes poor blood circulation since the blood struggles to rise from the lower limbs, resulting in circulatory stasis; v) Too tight clothing which causes poor circulation due to compression of the vessels; vi) Shoes that are too tight or with too high heels hinder the venous and lymphatic return and prevent the correct functioning of the "venous pump"; vii) Constipation and overweight; viii) Stress and smoking are other factors that aggravate the state of EFP, as stress increases free radicals that worsen microcirculation and help accelerate skin aging.

The cellulite process favors the female sex, above all because of the diversity of the hormonal situation of the woman that creates particular receptivity conditions.[4-8]

Hormonal factors, in particular the production of 17 B-estradiol, determine alterations of the adipocyte. The adipocyte undergoes hyperplasia and hypertrophy, moreover, always under the ormanal thrust, changes are made at the level of the interstice producing changes at the microcirculatory level that aggravate the pre-existing pathology.

Adipocyte hypertrophy in the subcutaneous tissue causes an accumulation of excess fluids (residues of the biochemical processes of the organism) at the level of the intercellular spaces. There is also an imbalance of the venous and lymphatic system, with a slowing of blood flow and a retention of fluids by the tissues.

Patients with EFP have alterations in the venous-lymphatic microcirculation associated with hypo-oxygenation of the tissue, which over time leads to circulatory stasis and an accumulation of fluids and catabolites at the interstitium level. The fat cell is no longer able to eliminate the waste substances that accumulate making it difficult for the arrival of oxygen and nutrients in the cell itself. All this causes the formation of new collagen fibers that bind the adipocytes to each other, creating real groups of nodules (micronodules). Over time the damage increases leading to an increase and growth of the nodules, which are palpable on the skin.

In parallel, vascular damage also progresses; in fact, the inability to drain cellular catabolites increases and consequently cellular suffering increases, which will lead to fibrosis and sclerosis of the tissue over time. These microscopic modifications correspond to alterations of the external aspect such as modifications of the skin profile, "orange peel" skin and dry skin.

The characteristic pathogenetic element is therefore represented by an interadipocyte edema and by an increased capillary-venular permeability. Permeability is due to a slowing of the flow in the precapillary metarterioles: in fact, the lamellar flow typical of normal circulation disappears and is replaced by a tortuous flow which favors the cellular adhesion of the red cells to the endothelial cells, with consequent lesion of the endothelium. We also find alterations in the capillary circulation, such as the presence of capillaries congested by hyper-

inflow, which causes serious endothelial lesions with complete alteration of the vascular permeability that progressively leads to the formation of a transudate, which infiltrates the interstice between the adipocytes creating small but significant "gaps" that dissociate the fat cells. This leads to a hyperplastic and hypertrophic reaction of the adipocytes and the formation of new collagen fibers at the pericapillary and periadipocyte level with the formation of micronodules. Starting from micro nodules, therefore, by confluence, the disease evolves towards macronodular liposclerosis.[4,7,8]

This ethiopathogenetic hypothesis derives from Curri's observations, according to which the final phase of the process, characterized by diffuse sclerosis and the appearance of the painful lump, is consequent to an altered capillary-venular permeability, with a slowing of the speed and volume of blood flow at the level district microcirculatory, excluding any inflammatory process. In fact, the regressive abiotrophic process of the microvascular-tissue unit is considered to be the ethiopathogenetic basis, consequent to a condition sometimes framed in the preclinical phase of venous insufficiency, that is to say consequent to a primitive defect of the arteriolar devices of flow modulation with chronic maldistribution of the microcirculation. So, for Curri the EFP is due to a slowdown in the blood circulation with erythrocyte sludge, therefore compromising the capillary hydrostatic balance, with reduced parietal and tissue oxygenation, endothelial damage, increase in the permeability of the vessel walls, increase of interstitial fluids with relative protein content, interadipocyte edema, and breakage of the adipocyte membranes. Based on this ethiopathogenetic hypothesis, Curri identifies four evolutionary stages of EFP, still today a reference in the diagnosis: i) edema; ii) sclerosis; iii) localized fibrosclerosis with micronodules; iv) diffuse sclerosis with macronodules.

Recent studies have shown new evidence on the ethiopathogenesis of EFP

From the histochemical point of view, in EFP an anomalous evolution of the connective response leads to an abnormal production of new collagen fibers. By examining the dermis of the skin overlying the adipose tissue affected by EFP, by electron microscopy, an increase in glycosaminoglycans, an increase in the activity of fibroblasts, alterations of the wall of the microvessels, rarefaction of collagen and subelastic epidermal fibers and greater presence of interstitial fluids. In light of current knowledge we can indicate that the process presents in its evolutionary sequence: i) edema due to excessive hydrophilicity of the intercellular matrix; ii) microcirculatory alteration, followed by fibrosclerotic evolution; ii) intradermal adipocytic hernias, typical of the female subcutaneous; iv) unequal response of interlobular connective branches, independent of the degree of overweight; v) proteolysis of the above shoots, produced by metalloproteinases and other endogenous enzymes; vi) a chronic, subclinical, inflammatory condition.[4,9]

So, the new hypotheses lead to the identification of a state of chronic inflammation of the adipose tissue as an ethiopathogenetic motive.

EFP would therefore be due not to stasis, with the relative sclerotic involution of the microvascular-tissue unit, but to the alteration of the interlobular connective branches, which become thinner and looser with the progress of the disease, leading to fibrosclerosis associated with partial stretching and lacerations, or reactive thickening, involving the mechanical retraction of the dermal plane, which is macroscopically visible with crater-like skin. These lacerations of the interlobular branches seem to be favored by endogenous elastases and linkenases, related to the activity of estrogens, and by the action of other enzymes such as metalloproteinases, related to the inflammatory process. The pathogenetic evolution would be increased by the presence of glycosaminoglycans, which due to their chemical characteristic would trap the water released by the enzymes, preventing their binding with collagen: the dehydrated, rigid and fragile collagen fibers are better predisposed to the enzymatic attack and, therefore, contribute to the abiotrophic process of the matrix. The process, however, cannot be limited to the morphoistochemical evolution of some tissue components. We must return to the adipocytes and other cellular elements of the matrix, in their intertwining of systemic relationships with organs and vessels, to understand the overall ethiopathogenesis of panniculopathy. The alteration in endocrine and paracrine activity of adipocytes, the release of atherogenic molecules, such as the release of proteolytic enzymes and phlo-

gogenic cytokines, the increased macrophage infiltrate, the reduction of growth factors (endothelial) and defense are events that would lead to a chronic condition of low inflammatory level. In this context, cellulite would be worthy of be called this.[2,9,10]

Stadiation and variants of EFP

EFP can be classified into 5 progressive stages due to the worsening of vascular tissue damage, but in the same person we find skin areas with different stages of EFP at the same time: i) stage I (edema): there is an initial alteration of the capillary permeability that creates edema, that is, accumulation of liquids in the adipose tissue, especially around the ankles, calves and thighs. In this stage the patient feels only an initial heaviness in the lower limbs and if the skin is compressed there are no "fingerprints", therefore it is difficult to recognize it with the naked eye; ii) stage II (modification of the adipocyte): there is a progressive accumulation of metabolic waste that is eliminated in an increasingly difficult way. The appearance of the "orange peel" skin begins to become evident, a small adipose accumulation and the skin loses part of its elasticity; iii) stage III (fibrosis): fibrosis begins to appear, that is, the connective tissue that hardens the adipose tissue increases; and also, the first telangiectasias. The skin takes on an orange peel appearance, on palpation it can be cold and painful, moreover, the presence of small nodules can be felt. These are all symptoms of poor oxygenation, vascular fragility and difficulty in disposing of fats. Due to poor drainage of accumulated waste and insufficient oxygenation of the tissues, the fat cells remain trapped in the surrounding tissues and the reactive fibrosis process begins with the formation of fine nodulations which, if not contrasted, can evolve to the next stage; iv) stage IV (advanced fibrosis): in this stage the fibrosis of the tissues begins to become evident; palpation arouses pain and the lower limb shows signs and symptoms related to vascular insufficiency; v) stage V (sclerosis): in this stage (irreversible) there is a real fibrosis and sclerosis in her tissues, so that the tissue becomes hard-elastic to palpation and nodules of great painful dimensions arise. There is a sharp slowdown in blood and lymphatic flow, with increased nodules and painful to the touch. The number of dilated capillaries, almost always present, increases. Here and there hematomas appear, a sign of capillary fragility; the skin surface is no longer homogeneous and smooth but has the typical "mattress" appearance, is cold to the touch due to circulatory insufficiency, is painful if it is touched even with modest pressure and, if compressed, leaves a depression that disappears only after a few seconds.

In essence, aesthetic cellulite can be divided into 3 essential growth stages: i) edematous; ii) fibrous; iii) sclerotic. The sooner action is taken against EFP, the better the results will be. EFP in the first stage, when only stagnant liquids are the protagonists of the picture, is certainly more manageable than a EFP with the presence of fibrous nodules, therefore it is important to recognize its characteristics.

There are several types of EFP: i) compact: it is the EFP of the young person, it mainly affects people in good physical shape with a slightly mobile toned muscle. It is located in particular on the knees, thighs and buttocks, the most affected area is often painful on palpation and stretch marks appear on the skin. It is the most common form, it is hard, it tends the epidermis, it does not undergo modifications by rising from the supine to the erect position, and by pinching the skin with the fingers, the tissue seems to adhere to the underlying planes. Rarely painful spontaneously, it is almost always accompanied by signs of venous or lymphatic fatigue returning to the lower limbs. We note an ease to hematoma and stretch marks, the latter expression of an uneasiness of the elastic fibers of the dermis; ii) Soft EFP: occurs mainly in middle-aged women with hypotonic skin tissue, or in people who have undergone many changes in body weight. Sclerotic nodules are present and the tissues are rich in liquids, this causes the ballottament during movements and in the transition from supine to orthostatic. It is located inside the thighs and arms; iii) EFP edematous: it is the consequence of circulatory diseases of the lower limbs. There is always a lot of water retention, therefore it is characterized by the presence of water stagnation especially in level of the buttocks and pelvis, giving the tissues a swollen and spongy appearance.[4,11,12] It is very painful to the touch and often spontaneously. It is always associated with poor venous and lymphatic circulation of the lower limbs: initially only appears a sense of heaviness and tension in the legs and feet; over the years more marked signs of venous insufficiency can be added up to the presence of swellings such that the acupressure leaves a persistent hollow on the skin (fovea +). This form constitutes the final stage of degeneration and is characterized by spongy tissue falling in an upright position and oscillating during walking. On palpation the muscle tissue is practically inconsistent hip. Therapy is extremely difficult and often disappointing; targeted gymnastic exercises play a fundamental role. It mainly affects the lower part of the legs, feet and ankles giving rise to the so-called "column legs".

Clinical aspects and diagnosis

The diagnosis of EFP is purely clinical and is based on the history and physical examination, to which instrumental investigations such as ultrasound of the subcutaneous tissue and echo color doppler of the vessels of the lower limbs can be added.

The anamnestic investigation is aimed at investigating eating habits, weight history, smoking practice, taking medications, on sports practices, on the psychic sphere and socio-environmental factors (mood, sleep), on the possible presence of hyperestrogenisms that induce activation of vasoactive quinines causing an increase in vessel permeability which favors the passage of electrolytes and water in the interstitial spaces, on the possible hereditary nature of venous and lymphatic insufficiency of the lower limbs.

The morpho-anthropometric evaluations take into consideration the existence of possible absolute weight excesses, adipose excesses, muscular deficiencies, also evaluating the relationships and the bone structures in order to establish the weight targets or the localized treatments. The BMI (Body Mass Index = Weight/Height squared) is calculated starting from the measurement of body weight and height. We then move on to the evaluation of the somatotype, the habitus (android or gynoid) and of the muscular trophism of the subject to arrive at the evaluation of the plicometry or impedancemetry which allow to evaluate the relationships between lean mass, fat mass, total water content, basal metabolism, the desirable weight and many other parameters.

Postural assessments are carried out: possible asymmetries of the trunk and spine are assessed, possible scoliosis is sought, the alignment and symmetry of the shoulder line, the lower corner of the shoulder blades, the iliac crests, the glute and popliteal folds, the possible valgus or varus of the knees, the dorsal hyperkyphosis and lumbar hyperlordosis. With the aid of the podoscope it is possible to verify the correct plantar support which, if altered (cavism or flatness), will cause a malfunction of the return circle in the dynamic phase of the movement. With these assessments, the possibility of the individual to carry out physical activity in order to establish the type and intensity of physical exercise useful for

improving physical performance (assessment of physical capacity) is noted. Another evaluation is the angiological evaluation of the lower limbs in which the functionality of the peripheral circulatory system is taken into consideration, mainly assessing the venous and lymphatic system of the lower limbs. After taking into consideration the referred clinic (sense of heaviness, fatigue, paraesthesia, swelling, cramps at rest and/or at night), we move on to inspection and palpation. The inspection allows to determine the presence of dyschromias, telangiectasias, microvarices or varices, dystrophies of the skin appendages, edemas, while palpation allows to determine the sense of pastiness, the positivity to the sign of the fovea and the painful reaction to the acupressure of the tissues. The Doppler exam completerd waves the investigation evaluating the continence of the venous circle. An ultrasound examination of the hypodermis is performed in standardized areas (trochanteric, subtrochanteric, gluteal, subgluteal, internal knee, external and internal premalleolar region), in order to distinguish the excess of localized District Adiposity (AD) from the EFP in its evolutionary stages, in order to allow the choice of targeted medical and/or physiotherapy corrective interventions.

Therefore, we proceed to the inspection of the skin surface which must be performed on the patient in an upright position, first frontally and then posteriorly and on both sides, with particular regard to the trochanteric, super-lateral, super-medial and posterior regions of the thighs and knees, abdominal, suprapubic, gluteal. The presence or absence of localized adiposity (so-called fat pads in the trochanteric region) is considered and the volume and shape of the thighs and buttocks are appreciated. The signs assessable at the inspection are in the order: i) generalized skin roughness (skin roughness or orange peel skin); ii) localized skin roughness, characterized by the presence of crateriform or irregular introflexions of the skin surface (madras skin or mattres phenomenon); or of the so-called quilt skin in which the linear ripples predominate, generally with a transversal trend with respect to the major axis of the limb; iii) the surface of the skin appears segmented into rectangular, trapezoidal or sectors rhomboid, irregular; iv) the pale skin (pale skin) which denotes the presence of hypothermic areas; v) telangiectasias, or microvaricosity, or intradermal venulo-ectasias, with particular emphasis on the location in correspondence of the super-middle and inferior-lateral regions of the thighs. We will appreciate the different anatomical and clinical manifestations, such as: laat red microvarices, rarely found; bleu-violet microvaric, star- shaped, arachniform, or branched (plate, grape cluster, point, arboriform, simple or complex sinuosity); skin stretch marks (striae atrophicae distensae cutis) whose length, shape, depth, color (red streaks or white streaks) are evaluated; skin atrophies; hyperpigmentation hyperchromia, dyschromia and hypochromia. Particular attention should be paid to hematomas from minor trauma, as a sign of abnormal capillary fragility. Finally, palpatory maneuvers can highlight the presence of macronodules or fine grains, or spontaneous or provoked pain, or even the so-called cellulite plaques, or hardened plates. In any case it is always to be kept in mind that in patients with EFP the clinically relevant aspects are related to the stage of the pathology.

Stage I is characterized by edema, which is due to stasis of liquids in the hypodermis and accumulation of adipose tissue. The symptomatology is represented only by the sense of heaviness in the lower limbs and there are no particularly evident clinical signs, so much so that it is difficult to recognize this stage with the naked eye. However, the skin begins to lose elasticity and, due to interstitial edema, the vessels present microectasis with increased permeability, consequently on palpation the skin is adhered to the underlying tissues and it looks pasty.

At stage II (fibrosis) the evolution of tissue edema consists of reactive fibrosis around the fat cells which, due to poor oxygenation, have poor metabolism and accumulation of waste. In this stage the cells are wrapped in a fine network of collagen fibers, which involves a constriction of the vessels and, therefore, alteration of metabolic exchanges. The skin appears pale, with an orange peel appearance and has telangiectasias. On palpation it is hypothermic and pasty.

Stage III (fibrosclerosis) is characterized by serious impairment of microcirculation with reduction of oxygenation and metabolic exchange of the tissue, micro nodules are present, on inspection the "orange peel" aspect is observed, on palpation and pinching of the tissues pain is found.

Stage IV (micro - macronodules) represents the evolution of the previous stage in which the micro nodules converge to form macronodules; during the inspection the so-called "mattress skin" is highlighted; on palpation, hard and painful macronodules are appreciated.

Blood chemistry tests are also useful to give a picture of the general state and influence of factors involved in the aging process and adaptation of the organism to stressful conditions, which usually precedes the most striking clinical manifestations of chronic stasis macroflebopathy over time. The instrumental diagnostic check is a must since it allows to make a presumptive diagnosis and can offer useful indications for the prognosis and for the possible success of the proposed treatments.

Faced with a first and second stage cellulite process, the question that must be resolved in the first instance is whether it is a "pure" form of localized adiposity or whether instead the first signs of a district microcirculatory "disturbance" already coexist stasis, with edema, lipoedema and initial modification of the volume and velocity of capillary-venular flow. While in the advanced stages of EFP, the instrumental investigation represents a confirmation of the diagnosis, it is instead a must in the initial or preclinical phases, when we are faced with an insufficiently probable situation on the clinical semeiological level. The instrumental investigation must be considered a completion of clinical semiotics, which confirms or questions the elements emerged from the objective examination.

Among the different methods in the EFP study, the following are highlighted: contact thermography, ultrasound and optical probe video capilloscopy.

Oxygen ozone therapy

Ozone is an allotropic form of oxygen; it is a gas with an acrid and pungent odor (the term ozone derives from the Greek "ὄζω" which means "to give off odor") and is in fact highly irritating to the respiratory mucous membranes. DRO Ozone is a molecule made up of three oxygen atoms (03).

It is an unstable gas (gaseous, at 20° C, has a half-life of 40 minutes, in an aqueous solution of 20 minutes) and in the liquid state it is explosive. Temperature has an influence on the half-life of ozone. The table below shows the half-life of ozone in air and water. Ozone decomposes faster in water and its solubility decreases at higher temperatures and is less stable. Ozone dissolved in water cannot be applied at temperatures above 40°C, because at this temperature the half-life of ozone is very short.

We have seen that ozone is highly irritating to the respiratory mucous membranes, for this reason if breathed it is toxic and therefore this route of administration is absolutely contraindicated. The first toxicity effects occur at concentrations of 0, 1 ppm with a burn-

ing sensation in the airways, at concentrations of 1 ppm, rhinitis, cough, vomiting, asthma occur, and at concentrations of 50 ppm death occurs in a few minutes. The risk of toxicity is in fact very high for the lung; direct inhalation is irritating even at low concentrations and can cause edema and bronchospasm. Ozone interacts with the hydrolipidic film interposed between the alveolar space and the alveolar wall. The lipid phase consists of the surfactant composed of 90% phospholipids and 10% proteins, the ozone reacting with these compounds causes the release of mediators such as prostaglandins and leukotrienes which cause bronchial constriction and increase of the reactivity of the airways. The toxicity is therefore due to the released mediators that trigger these events.

In the medical field, the great advantages of this gas are the limited contraindications and the absence of side effects if the indications for use and protocols are respected. The only contraindications to ozone therapy are: uncontrolled hyperthyroidism and the deficiency of glucose-6-phosphate dehydrogenase (favism). Pharmacological interactions are not described and the diffusion in the organism of the drugs is favored thanks to the improvement of the microcirculation. It cannot induce allergy as a gas and not a protein molecule. The beneficial effects are numerous, and affect the whole organism.

Ozone is naturally present also within all living beings, produced by neutrophil white blood cells 78, and performs several basic functions for life. The leukocytes in fact, in certain circumstances, produce peroxides and reactive oxygen species, such as ozone, which destroy the infectious agents by promoting immune and inflammatory reactions, which are useful in this case. Its use in the healing field dates back to the First World War, when it was used as an antibiotic. During the Second World War, it was accidentally observed that an atmosphere rich in ozone favored the healing of the wounds of the soldiers.

In medicine, for the production of ozone, pure oxygen is used as medical gas and electromedical equipment. The ozone generator contained in the electromedical equipment uses a voltage difference between 5.000 and 13.000 volts to split O2 into 2 atoms of 0, these combine with excess O2, forming 03. Then pure 02 enters the machine to produce it and a mixture of 02 and 03 comes out. There ozone concentration used in medicine is measured in pg / ml and varies from 2 to 110.

Biochemical mechanisms induced by the ozone

Ozone is an unstable molecule and its instability is precisely due to the presence of the third oxygen atom which gives rise to all its activities.

Ozone, although not a radical molecule, is a powerful oxidant that reacts instantly with other molecules in aqueous solution.

In practice, ozone, if properly administered, generates a calculated and transient oxidative stress that stimulates the intracellular antioxidant system, which in turn can intervene in preventing oxidation damage (typical of chronic degenerative diseases and pathologies inflammatory).

Ozone, although not a radical molecule, is a powerful oxidizer, very unstable, which reacts instantly with other molecules in aqueous solution.

In the gaseous state its decomposition is represented by the reaction: 203 = 302.

In biological liquids the substrates with which ozone preferably reacts are unsaturated fatty acids (the Anglo-Saxon acronym PUFA is used) and reducing compounds (*i.e.* electron donors), such as Reduced Glutathione (GSH) and proteins rich in cysteine.

When ozone is administered it reacts with PUFAs (Polyunsaturated Fatty Acids) bound to albumin or present in lipoproteins, and with GSH.

If the reaction takes place in a lipophilic environment, a cascade of products such as ozonides is produced and ozonides; if instead the reaction takes place in an aqueous medium, they develop hydrogen peroxide (H202 - oxidant) and aldehydes. The ozonation of 1 mole of PUFA generates 1 mole of hydrogen peroxide and 2 moles of aldehyde.

Plasma H202 is degraded by catalase and glutathione peroxidase (antioxidant enzymes). In plasma there are also vitamin C (ascorbic acid) and GSH, whose group-SH functions as a powerful reducing agent (electron donor).

Ozone reacts at the double bond level with unsaturated fatty acids and therefore not with saturated ones. By mixing ozone with blood, as happens in GAET, ozone reacts first with antioxidants, once these are exhausted, it reacts with PUFAs.

When the ozone is mixed with the blood in the bag, the reaction that takes place is this:

$$RC = CR \text{ '+ } O, + H20 - R - C = 0 + R' - C = 0 + H, O$$

This is the terminal compound of the oxidation of unsaturated fats (alkenal), which with hydrogen peroxide acts in the blood. The hydroperoxide enters the cells, platelets, red and white blood cells, causing various reactions. In red blood cells it causes the formation of 2-3 diphosphoglycerate which shifts the oxygen - hemoglobin dissociation curve to the right, *i.e.* it gives 02 more easily to the tissues. It also increases the production of ATP and consequently glycolysis.

With Rudolf Criegee 's reaction, ozone is added to the double bonds of unsaturated fatty chains. This electrophilic addition reaction allows to incorporate the 3 oxygen atoms of ozone in the double bond and, obviously, the higher the level of unsaturation, the greater the amount of oxygen that can be "stored".

The Criegee reaction is one of the fastest molecular chemical reactions in nature (speed: millions of moles/liters per second).[13-15]

The meeting of the ozone molecule with unsaturated fatty acids will determine the formation of ozonides which are intermediate forms (primary and secondary ozonides exist), from these aldehydes and alpha hydroxy peroxides are produced, and from these hydrogen peroxides and other aldehydes. Therefore, lipid oxidation products, called LOPs, are formed from PUFAs, such as the malonondialdehyde series and the 4-hydroxy - 2, 3 transnonenal (4 - HNE) which instead of being like the initially thought a toxic molecule, it proved to be a key element in the transduction of the signal, therefore we can say that ozone acts as a signal molecule, therefore as a second messenger. It has been seen that this molecule, at concentrations below physiological levels, can stimulate the expression of antioxidant and detoxifying enzymes inducing an adaptive response to stress.

11 4-HNE is an aldehyde which facilitates the activation of NRF2 and induces the expression of heme oxygenases, peroxiredoxin-1 and stress proteins A170. Hence the induction of these pud enzymes increases cell tolerance and thus protects cells from oxidative stress. Ozone is also able to stimulate the so-called HSP, that is, heat shock proteins, which are synthesized as a response to stress and are able to repair damaged proteins. Therefore, the Heat shock response determines a state of cytoprotection from inflammation, from neoplastic activities, aging and neurovegetative pathologies. The different isoforms of heme oxygenase act as sensors for oxidative stress but also as regulators of the homeostasis of redox systems.

Effects on microcirculation, erythropoiesis and tissue oxygenation

As mentioned, the use of ozone at therapeutic doses produces a calculated and transient oxidative stress, which stimulates the intracellular antioxidant system, preventing damage from oxidation. It has also been observed (experimentally) that the reintroduction of ozonated blood into the circulation produces an increase in the enzyme activity of NO synthetase by the endothelium. Nitrogen monoxide acts on smooth muscle cells inducing the release of guanylate cyclase with consequent production of cGMP (cyclic monofosted guanosine).

The cGMP seems to be involved in several mechanisms, among them, the mechanisms of learning, the suppression of apoptosis and protection against neuro degenerative phenomena. In addition to these mechanisms, cGMP plays a very important role in the modulation of the immune response and in neurohormonal transmission.

The presence of hydrogen peroxide also stimulates the production of VEGF (vascular growth factor), which represents a stimulus to neo-angiogenesis in the tissues during ble bord regeneration (granulation and scarring). These characteristics account for the positive effect of ozone in the presence of damage to the microcirculation. In these conditions, that is, in the presence of the inflammatory mechanism, there is an excess of oxygen consumption with all that follows (easier bacterial infection, delay in the mechanisms of protection, less production of tPA and in general prothrombotic condition).

The exposure of blood to ozone at therapeutic doses stimulates erythropoiesis and also induces, through biochemical mechanisms of the surface of the erythrocyte, its easier deformability and therefore a lower viscosity of the blood itself. This mechanism produces, in addition to a further reduced platelet aggregability, an easier stacking of red blood cells even in vessels of smaller dimensions such as those of the more distal microcirculation, with a better release of oxygen even to the most peripheral tissues.

Another important effect of ozone is to induce a greater production of ATP: ozone, in fact, by accelerating the process of aerobic glycolysis, ensures a constant oxidation-reducing mechanism of NADH, consequently an increase in ATP synthesis. This mechanism is useful in some particular conditions such as inflammation in general and therefore in many degenerative pathologies or in muscular effort, all conditions in which the demand for energy and therefore for ATP is increased.

The interaction of ozone with proteins rich in cysteine, with PUFA and GSH which is a reducing substance, produces LOP or lipid peroxidation products that stimulate erythrocyte glycolysis inducing an increase of 2,3 disphosphoglycerate.

Conclusions

In conclusion, although the study was conducted only on one patient, comparing our clinical case with the clinical cases of studies whose number was significant, we can say that EFP therapy with oxygen ozone produces satisfactory results both from a subjective point of view and from an objective point of view.[16-19]

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