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The use of medical ozone in dentistry

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Abstract

Background: Lately, due to a higher incidence rate of infections, as a result of a decreased immuno-inflammatory response capacity of the human body and an expanded role of viral agents in triggering inflammatory diseases, the interest for the use of medical ozone has increased significantly. The reason for this growth of popularity lies in the fact that medical ozone has antimicrobial, antiviral, anti-inflammatory, immunomodulatory, antioxidant and other properties; it is not a pharmacological product, but rather a "hormetic stress" [18], a pure ecologic physico-chemical factor with multiple biological effects [19].

Conclusions: The action of medical ozone on the human body is diverse and multidirectional. The pharmacological effect of medical ozone represents the so-called "hormesis" phenomenon, and this effect is triggered by short chains of hydroxides – hydro-peroxides, also defined as ozone peroxides. Medical ozone possesses antalgic, anti-inflammatory, anti-edematous, antioxidant, myorelaxant, detoxing, immunomodulatory, antimicrobial, antiviral, antifungal effects, it activates the cellular metabolic processes, stimulates oxygen metabolism and improves rheological properties of blood. In dentistry, medical ozone is used on its own or in combination with other treatment methods. At the same time, the aspects of the use of medical ozone in clinical periodontology requires additional research.

Key words: medical ozone, dentistry.

Introduction

The action of medical ozone on the human body is diverse and multifaceted. In various medical fields ozone therapy has been applied for several decades, however the use of ozone in dentistry is relatively recent, since the middle of the 90s. Medical ozone has antalgic, anti-inflammatory, anti-edematous, antioxidant, myorelaxant, detoxing, immunomodulatory [1, 2], antimicrobian, antiviral and antifungal properties; it activates cellular metabolic processes, stimulates oxygen metabolism and improves the rheological properties of the blood [4, 17].

The efficiency of the use of medical ozone has been proven and debated in the treatment of a series of systemic diseases, mainly of inflammatory nature, both in surgical and therapeutic fields [5, 6]. In comparison with the therapy with antibiotics, ozone therapy has a larger spectrum of therapeutic actions, it does not result in microbial resistance and does not trigger mutagenic and carcinogenic effects [7, 8]. Moreover, in case of the existence of microflora resistant to antibiotic treatment, supplementing antibiotic treatment with ozone therapy leads to the neutralization of antibiotic resistance and intensification of the overall effects of antibiotics [9, 10]. In

dentistry, medical ozone is used on its own or in combination with other treatment methods [11, 12, 13].

Historical background

Ozone was mentioned for the first time by the Dutch physicist M. van Marum in 1785, who observed the formation of an oxidizing gas with a characteristic smell during some experiments with electrical discharges. The name ozone, though, comes from the German chemist Friedrich Schonbein in 1840, who discovered for the first time the capacity of ozone to react with biological substrates. In 1857 the German engineer and inventor Werner von Siemens develops the first technical unit of ozone, yet in 1880, the first medical publications appear in America, attesting its therapeutic efficacy. The first generator of ozone was patented in 1896 by the physicist Nicola Tesla and commercialized from 1900. Subsequently, he was the first to ozonize olive oil for medical use. During the First World War, ozone was used for disinfection and treatment of plagues and the treatment of wounds and gangrene. In 1935 the surgeon Erwin Payr presents his publication of 290 pages about the application of ozone in surgery, while the Swiss dentist E. A. Fisch was the first to use ozone in dentistry and is the author of many publications about the use of ozone, defended his thesis on this theme in 1950.

In the following years, due to Dr. J. Hansler, the first medical ozone generator was created. Together with H. Wolff, the author of the book "Medical Ozone", Hansler sets up the Medical Society for Ozone Therapy [14, 14, 16]. In 1973 the International Ozone Association (IOA) was created, and in 2005 the World Federation of Ozone Therapy (WFOT).

Today, after 160 years of use, ozone therapy is recognized and applied in 50 countries [17].

Lately, due to the growth of the number of cases of infections associated with the background of reduction of the immuno-inflammatory response of the body and the increase of the role of viral agents in the outbreak of inflammatory diseases, the interest in the medical use of ozone has considerably increased. The reason for this growth of popularity is that ozone, despite having antimicrobial, antiviral, anti-inflammatory, immuno-modulatory, antioxidant etc. characteristics, is not a pharmacologic substance, but a "hormetic stress" [18], an ecologically pure physico-chemical factor with multiple biological effects [19].

The physicochemical properties and biological effects of ozone

Ozone (derived from the Greek *ozein*, meaning "to smell"), is the second allotrope (active) form of oxygen – and is a gas of a bluish color, with a specific odor; in small quantities it has a pleasant "smell of purified and fresh air", which becomes stingy and intensely irritating when in large proportions.

Its molecule is made of three oxygen atoms (O3), has a strong affinity for the electron (1,9 eV) and has a molecular weight of 48.00 g/mol. In normal temperature and pressure conditions (0°C; 1atm=101.3kPa), the weight of one liter of ozone equals to 2.143g/l. One liter of air weighs 1.429g/l, respectively ozone is heavier than air. The melting point of ozone is minus 192.5°C and it acquires a bluish-dark violet color. The boiling point is minus 119.9°C. The rate of dissolving of ozone in water is 49.4 ml/100ml (0°C; 1atm=101.3kPa), which is 10 times higher than the rate for oxygen [49]. The reaction for the production of ozone is reversible:

$$3O2 + 68kcal \rightarrow 2O3$$

The ozone molecule is unstable and self-decomposes to oxygen, with emission of heat. Additionally, the life of an ozone molecule depends on the temperature, thus at a temperature above 20°C the concentration of ozone decreases by half within 40 min., at 30°C in 25 min., at minus 50°C the concentration of ozone diminishes by half within 3 months [20], and at minus 78°C, in a glass recipient and certain pure plastic and metal recipients, ozone practically does not decompose [21].

The main quality which defines the specifics of the physicochemical properties is as follows:

· A high level of excess energy of the ozone molecule and $O3 \rightarrow {}^{3}/2O2 + 34$ kcal/mol

· the powerful oxidation action; the ozone is the 2-nd powerful oxidant, next to fluorine and persulfate [49, 22].

The ozone oxidizes all types of metals, except for gold and platinum. It is capable to enter in reaction with the majority of organic and inorganic substances. At the thermodynamic level, these reactions may develop up to complete oxidation, i.e. up to the formation of water, carbon oxides and oxides of other elements.

Despite its high potential for oxidation, the interaction pattern of ozone is very selective. The cause of this selectivity is the polar structure of the bond angles of the ozone molecule ($\mu = 0,49$ D) [24], more specifically the positive polarization of the oxygen atom, which confers electrophilic characteristics to the entire molecule, and due to these molecules with a high electronic density become its preferred reactive elements. The components with simple double bonds C=C enter into an immediate reaction, phenols and free amines are oxidized within a few seconds, meanwhile other substances, for example alcohols, become oxidized within several hours.

Of special interest are the reactions of ozone with organic molecules possessing double or triple bonds, which result in understanding the bio-chemical traits of the interaction of ozone with biological objects. The contact with these molecules leads to the creation of complexes and intermediate compounds which are less studied (zwitterione/amphyone, malozonyde, cyclic ozonides), which may be hydrolyzed, oxidized, reduced or thermally disintegrated into multiple substances, and mainly aldehyde, ketone, acids or alcohols (tab. 2) [25].

Ozone reacts with unsaturated hydrocarbons, amines, sulfhydryl groups, organic compounds containing sulfur and nitrogen, and aromatic compounds. Besides unsaturated fatty acids, aromatic amino acids are also sensible to the action of ozone, and mainly the ones containing SH groups. The product of the interaction of the ozone molecule and the bio-organic substrates is the molecule – ozonide [26]. As a result of its dipolar structure, the ozone molecule may lead to

a 1,3-dipolar cyclo-addition of unsaturated bonds, resulting in the formation of a primary ozonide (I), in conformity with the reaction presented in fig. 1, known also as the Crigée mechanism (1953, 1975). Ozonation of aromatic compounds is similar to ozonation of olephyns with creation of polymeric ozonides [26].

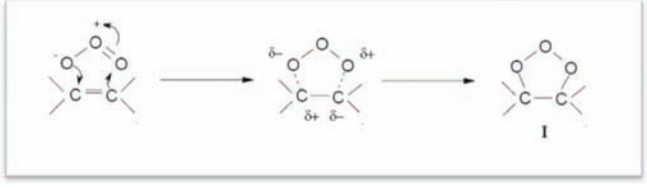

Fig. 1. Formation of primary ozonide.

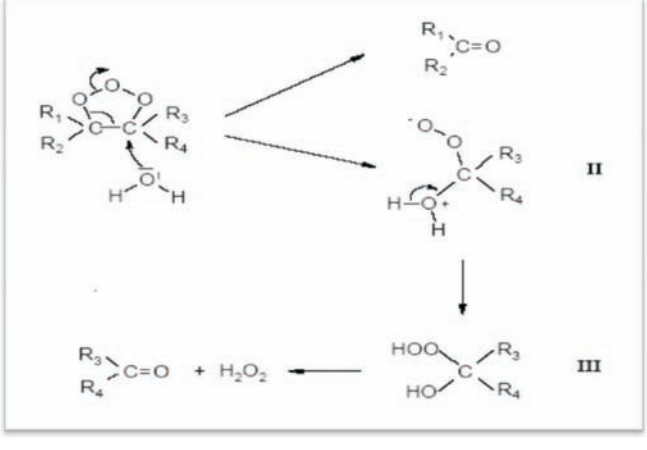

Fig. 2. Primary ozonide is decomposed into a carbonyl compound (aldehyde or ketone) and a zwitterion (II), leading to formation of a hydroperoxide hydroxide (III), the stage which results further in decomposing into a carbonyl group[26].

Ozone may be toxic if inhaled and an irritant for the eyes. Inhaling by humans and mammals of ozone in higher concentrations may lead to its reaction with the mucous compounds of the airways and may result in damaging the substance which covers the interiors of the lungs (pulmonary surfactant), because it has a reduced anti-oxidation capacity. Within the anatomopathological investigations the pattern common in an ozone intoxication is described as follows: blood clotting is disturbed, lungs are affected by a large number of hematomas [27]. Due to this, the maximum allowed concentration of ozone in the air of a working room is about 0,1 ppmv inhaled within one hour, which is 10 times higher than the human olfactory threshold (0,01 ppmv) [28]. The World Health Organization allows for 8 hours of work in a room in which the ozone concentration is 0.06 ppmv, a threshold with an easily-recognizable and quite powerful ozone smell [28]. The conversion is as follows:

1ppmv = 1.0 mcg/ml or 1.0mg/l or 1.0g/m3

Medical ozone is a product of pure oxygen (min. 99,5 %), created in an ozone generator, an apparatus equipped with a tube, in which, through an endothermic process, take place high voltage electrical discharges between the electrodes. In fact, medical ozone is a compound of oxygen and ozone (95% O2 - 5% O3) [37]. Medical ozone cannot be generated from air, due to the presence of nitrogen, which generates toxic nitrogen oxides [29]. Medical ozone is administered externally (on the skin, on the injured surface), internally (per os et per rectum) and parenterally, within the therapeutically allowed concentrations, and does not have a toxic effect on the human body. If used in specific conditions and for the treatment of specific diseases, the therapeutic efficiency of medical ozone is identical or similar around the world. The use of unsuitable administration methods and amounts, is the main cause of the lack of efficiency and adverse effects. Due to this, the medical societies advocating for the use of ozone have established treatment protocols, as a basis for the methodological standards and guidelines, which have been revised and published in compliance with the recent results of research and based on the practice in this field acquired during the last thirty years [29]. These were used to standardize the application, indications, concentration, dosage and frequency of the treatment, based on the mechanism of action and pharmacological properties of medical ozone.

The pharmacological effect of medical ozone reflects the so-called hormesis phenomenon, expressed by the stimulatory or beneficial effect following the exposure to low concentration of a substance, otherwise toxic if used in high concentrations [30]. The concept of ozone applied in low doses (Low-Doze Ozone), with a moderate oxidative stress, becomes nowadays the ideal strategy in the field of ozone therapy [18].

Mechanism of action of medical ozone

Depending on the method of application, the mechanism of action of medical ozone may be included into two major categories:

- topical use (bactericidal action, virucidal, fungicidal);
- systemic use (regulation of the anti-oxidized cellular system, improved emission of oxygen by the RBC and immunomodulation by activating white blood cells (WBC);

Topical use of medical ozone in gaseous state or in the form of ozonized solutions, possesses anti-microbial, antiviral, anti-fungal effect, and the effect of cleansing and treatment of wounds. At the same time, it has a higher efficiency in the aqueous medium, because after decomposing of the ozone in water, a highly reactive hydroxyl radical is generated. In compliance with microbiological studies, ozone has the capacity to destroy all Gram-positive and Gram-negative known bacteria, including *P. aeruginosa*, *Legionella spp*, all lipid and hydrophilic viruses including the hepatic viruses A, B, C, HIV, spores and all known vegetative forms of pathogenic fungi [31, 32]. Among the causes of the bactericidal effect of ozone, most frequently mentioned is the disrupting of cellular membrane activity of the bacteria, by oxidizing the phospholipids and lipoproteins, directly affecting cytoplasmic integrity. Gram-positive bacteria are more sensitive to the action of ozone in comparison to Gram-negative bacteria, perhaps due to the differences in the membrane structure [34].

The attention of researchers regarding the virucidal effect of ozone is centered on the property of the ozone to disrupt the multiple configurations of the lipid molecules. Indeed, if the external lipid membrane of the virus is dislodged, the nuclear DNA or RNA cannot survive. Viruses have no protection against the oxidizing stress, on the other hand, the normal cells of a mammal have the structure of complex enzymes (i.e, SOD, catalase, peroxidase), which neutralizes the negative effects triggered by the reactive oxygen species (ROS) and oxidation disruptions. Viruses which possess, besides nucleocapsid, also an external lipoproteic shell derived from the membrane system of the host-cell (peplos or envelope), are defined as enveloped viruses [34]. The enveloped viruses are much more sensitive to the action of the ozone, when compared to the non-enveloped viruses, namely due to presence of the external glico- and lipoprotein membrane, which interacts easily with ozone [35].

Although the effects of ozone on unsaturated fats represent one of the most documented biochemical actions, ozone is also known to interact with proteins, carbohydrates and nucleic acids. This becomes especially relevant when considering the effect of deactivation of non-enveloped viruses by ozone [36].

In order to explore the utility of ozone therapy in viral diseases, researchers [23] considered the possibility of action of ozone in vivo, because the activity of virucidal ozone becomes insecure when viruses are in biological fluids or, even worse, when they are placed intracellularly (hepatocytes, CD4 + lymphocytes, monocytes, glial and neuronal cells), because, ironically, the powerful antioxidant system of the host cell protects the viral integrity.

The following mechanisms may have some importance:

- A prolonged ozone treatment seems to be able to induce an adaptation to chronic oxidative stress (SCO), and therefore, a balancing of the cellular redox status, which is a fundamental process for inhibiting the replication of HIV, HBV and HCV [35, 37];
- The induction of cytokine synthesis such as interferon (IFN) and interleukine (IL) in ozonized blood has been proved possible. Although ozone is a weak inducer, reinfused lymphocytes and monocytes migrating through the lymphoid system, can activate other cells, which in time will lead to a stimulation of the immune system [38];
- Blood ozone in minor authohemotherapy (AHTm), can induce the oxidation of free virus components, which could become an inactivated and immunogen vaccine [38].

Certainly, ozone improves the oxygenation and the hepatic metabolism and it has always been confirmed that fibrinogen and plasma prothrombin levels have a tendency to normalize infected patients, thus suggesting an improvement of the hepatic protein synthesis. In HIV infection, ozone therapy corrects acquired lipodystrophy and hyperlipidemia that accompany metabolic and cardiovascular complications. Cells with low enzymatic coverage are vulnerable to viral invasions and also susceptible to oxidation and elimination from the body, which allows their replacement with healthy cells [40].

In fungi, ozone prevents the cell growth in certain stages. Fungi called dermatophytes and families of fungi *Candida, Aspergilus, Histoplasma*, *Actinomycoses, Cryptococcus* are destroyed by ozone exposure. The cell walls of the fungi are multilayered and are composed of approximately 80% carbohydrates and 10% proteins and glycoproteins. The presence of many disulfide connections creates the possibility of oxidative inactivation of fungi with ozone. Additionally, ozone is able to diffuse through the fungal wall into the cytoplasm and disturb the cell organelles. The protozoa organisms affected by ozone include *Giardia, Cryptosporidium, Acanthamoeba, Hartmonella* and *Negleria.* The exact mechanism by which ozone exerts its antiprotozoal action has not been discovered yet.

The systemic application of medical ozone through major authohemotherapy (AHTM) and minor authohemotherapy (AHTm), in the form of saline intravenous infusions or as of gaseous mixtures of O2/O3 in rectal or vaginal insufflations, or subcutaneous, intramuscular, para/intra articular injections possess analgesic, antihypoxic, antiiinflammatory, antiedematous, antioxidant, myorelaxant, detoxification and immunomodulator effects.

The following processes are activated after systemic applications of ozone to the human body:

- The activation and induction of biological antioxidants (scavengers) of free radicals;
- The activation of the immuno-competent cells;
- The activation of the metabolism of the red blood cells (RBC).

Antioxidant action. Under normal conditions of temperature and atmospheric pressure, due to its high instability and solubility, ozone is dissolved instantly in water and in biological fluids such as: plasma (90% water from the composition of blood plasma), the same in extracellular fluid, or the thin layer of water covering the skin and the respiratory tract, intestinal, vaginal etc. mucus. Being a strong oxidant, ozone reacts immediately with a number of molecules, that are present in biological fluids and also with antioxidants, proteins and carbohydrates, especially with polyunsaturated fatty acids (PUFAs).

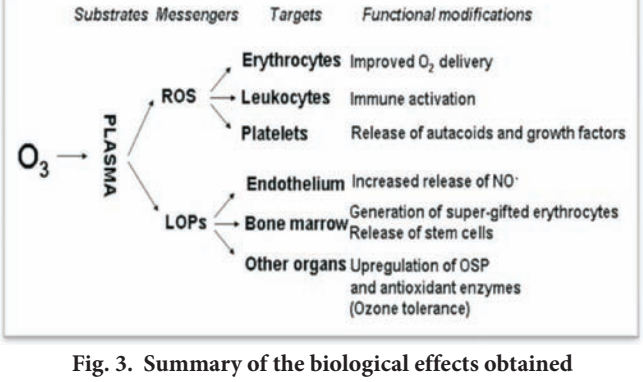

Fig. 3. Summary of the biological effects obtained during AHTM [26].

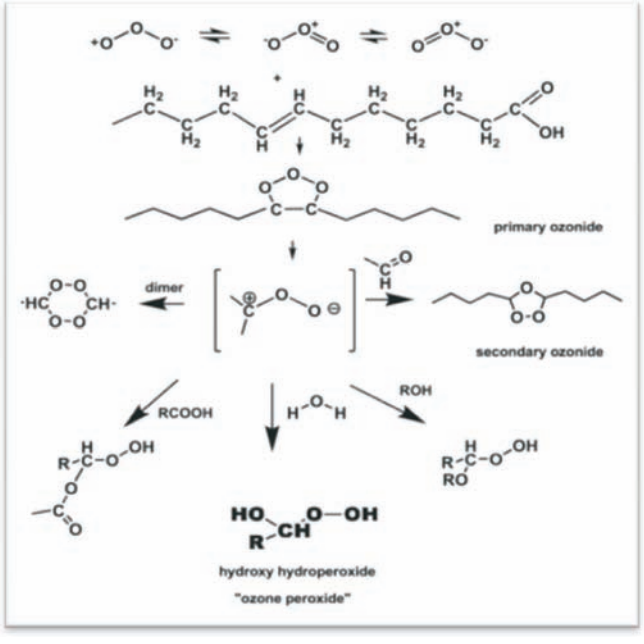

Fig. 4. The reaction of ozone with isolated double bonds (Crigée mechanism). Hydroperoxides, in this case "ozone peroxides", are perceived as active pharmacological substances [18].

The reaction of ozone with so many molecules involves several main processes taking place simultaneously:

· On the one hand, the ozone is inevitably consumed during oxidation of ascorbic and uric acids, sulfhydryl groups (SH) of a reduced glutathione (GSH), the proteins and glycoproteins present in the plasmatic water [43], an important reaction because it generates the reactive oxygen species (ROS), which in turn triggers a chain of biochemical reactions in the blood. ROS are neutralized in the first 30-60 seconds of the antioxidant system (SA).

· On the other hand, the reaction of lipid peroxidation takes place [44]. In the hydrophilic environment of the plasma, one mole of unsaturated olefin and one mole of ozone, lead to the formation of two moles of aldehyde and a mole of hydrogen peroxide (H_2O_2), [26]:

- **o O3 + antioxidants = ROS;**
- **o O3 + arachidonic acid** (or triglycerides and chylomicrons) = **H_2O_2 + LOPs;**

These reactions, consummated within a few seconds, use the entire doze of ozone and generate hydrogen peroxide, which is an oxidant and not a radical molecule (usually included in the ROS group) and a variety of aldehydes, known also as lipid oxidation products (LOPs).

Immediately after the ozone is diluted in the plasmatic water and reacts with the polyunsaturated fatty acids (PUFAs), the concentration of H_2O_2 starts to increase, but decreases equally rapidly, because this non-ionized molecule quickly penetrates the erythrocytes, leukocytes and platelets, activating a series of biochemical reactions and simultaneously is reduced by water, due to the intracellular anti-oxidizing enzymes: glutathione peroxidase (GSH-Px), catalase and GSH. This important moment corresponds to an acute and

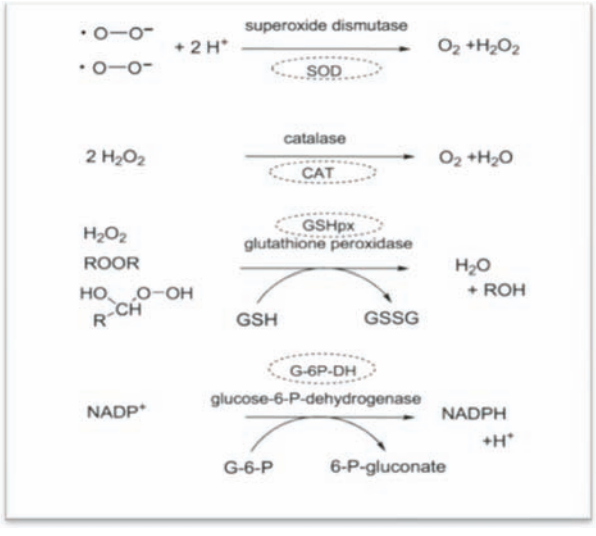

Fig. 5. Situation of oxidation stress, in which the biological anti-oxidation system reduces the ROS. "Ozone peroxides " are controlled by glutathione system, and not by catalase [18].

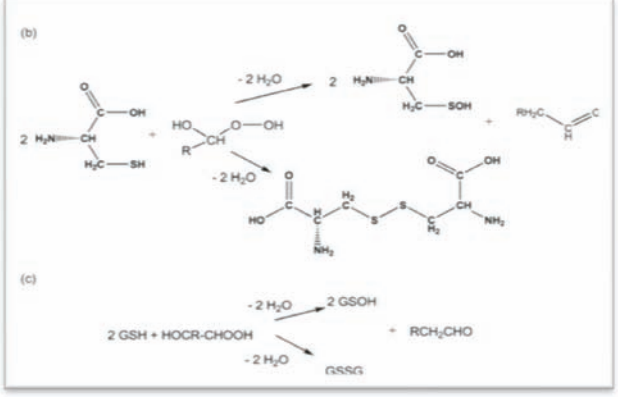

Fig. 6. Reaction of "ozone peroxides" with cystein (residuum) and (c) Glutathione [18].

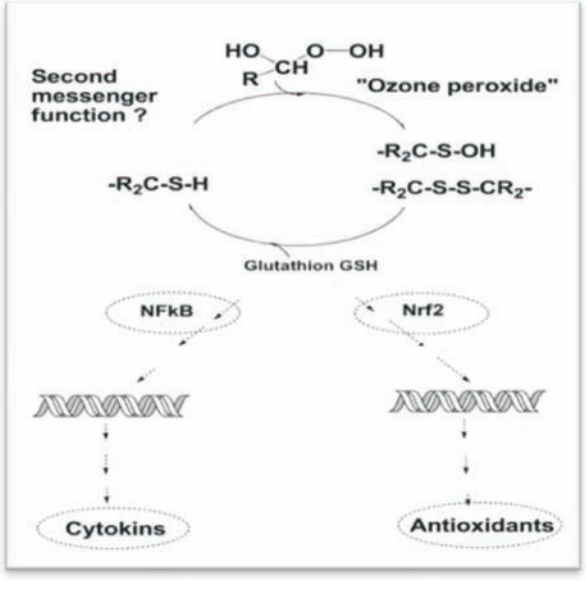

Fig. 7. "Ozone peroxides" as secondary messengers for transmission of an intracellular signal.

transitory controlled oxidation stress, necessary for the biological activation, which excludes any toxicity, provided that the used dose of ozone is compatible with the anti-oxidizing capacity of blood [45]. While the ROS are responsible for the immediate biological effects (fig. 3), LOPs are important due to tardive effectors, which may reach any organ, especially the bone marrow, where after binding to receptors in submicromolar concentration, determines adaptation to repeated acute oxidation stress, a typical feature of the ozone autohaemotherapy [38]. Given its transitory presence in the cytoplasm, H_2O_2 (generated by ozone) acts as a chemical messenger of the ozone. Currently, the H_2O_2 is largely recognized as an intracellular signaling molecule, capable of activating the tyrosine-kinase, which phosphorylates the nuclear transcription factor - kB (NF-kB), allowing for the synthesis of a various number of proteins [46].

Practically, the H_2O_2 functions by oxidizing the cysteine [47], which on its turn influences the mononuclear blood cells [48], platelets, endothelial cells [50] and the erythrocytes [28, 51]. Once inside the cell, the H_2O_2 molecules are almost immediately reduced to water and/or lipoperoxides to hydro-peroxides.

Hydroperoxides or "Ozone peroxides", a term proposed by several authors [28] assume the role of physiologically active ozone metabolites. Due to selective reaction of ozone with the double bonds C = C of essential fatty acids, the classic ozonolysis described by Criegée (fig. 4) becomes the dominant reaction present in physiological conditions with the pH values ≤7,4.

The ozonolysis lasts only fractions of a second, and forms mainly short chains of hydroxi-hydroperoxides in a aqueous medium (ozone peroxides), which are obviously responsible for the pharmacological effect during the systemic treatment with medical ozone.

Ozone peroxides are reactive oxygen compounds, associated with the membranes, which act as second messengers through the residuum of cysteine and/or through reduced levels of glutathione (GSH), in a less aggressive manner compared to the superoxide radicals ·O-O– and the H_2O_2 and are taking over the anti-oxidating regulation, without the need of SOD and catalase, similar to the oxidation stress developed as a result of relevant pathological conditions (fig. 5, 6).

Short chains of Hydroxi-hydroperoxide with a tendency to low radical reactions may result in initiating regulation of the anti-oxidizing protective mechanisms, as a signal for redox (e. g., through the nuclear factor Nrf2 into stress and through the nuclear factor NFkB into inflammatory processes (fig. 7) [18].

Processes for oxidating the residuum of cysteine and glutathione induce regulation of cytokines and of the anti-oxidation system through NFkB, and respectively through Nrf2 [18].

During a lengthy treatment, the activity of LOPs shall culminate in the regulation of antioxidation enzymes, generation of oxidative stress proteins (OSP) (heme oxygenase 1, a typical marker) and probably the release of a stem cell, the essential factors which explain some of the extraordinary effects of ozone therapy.

The therapeutic response obtained after the repeated oxidative stresses suggests a preconditioned effect, eventually capable of restoring the balance of the oxido-reducing tissue system, which has been modified through pathogenic factors [28].

Immunomodulatory action. Presently, the immunomodulatory effects of ozone therapy are related to induction of cytokine by the lymphocytes and monocytes [38], such as interferons (IFN) B and gama, interleukins (IL) 1B, 2, 4, 6, 8 and 10, the tumor necrosis factor (TNFx) and growth factors GM-CSF (granulocyte-macrophage colony-stimulating factor), TGFβ1 (transforming growth factor beta 1) [52], PDGF (platelet-derived growth factors), bFGF (the basic fibroblast growth factor), EGF (epidermal growth factor), KGF (keratinocyte growth factor), thus promoting the synthesis of intracellular matrix and the healing process (fig. 9) [53, 54, 55, 56]. It is possible that these cytokines also activate other lymphoid cells, which leads to immunostimulation without adverse effects [57, 58].

At the same time, the influence of ozone on the phagocyte activity of leukocytes is being studied. Therapy with medical ozone modulates all the dislodged stages of phagocytosis and contributes to rapid elimination of inflammatory processes [59]. Firstly, the time of adherence to the surface of a phagocyte is reduced (PMN, macrophage*)* and secondly, the stage of respiratory explosion becomes specifically pronounced, being determined by the generation of peroxides. Another possible modality for phagocyte activation is the increased synthesis of the phagocyte stimulation factor [60].

Hemorheological action. Erythrocytes contain a large reservoir of GSH (approximately 1 mmol / l), thioredoxin with two available cysteines and powerful antioxidant enzymes (catalase, GSH-Rd, GSH-Px, GSH-Tr and SOD). They may neutralize quickly large quantities of oxidants, such as OH, H_2O_2, OCl^-, $ONOO^-$ and at the same time may recycle back the oxidized compounds [61]. In the erythrocytes, the activation of the oxygen dependent processes are manifested through the increased activity of the glutathione system [4, 38]. Oxidation of sulfhydryl groups (–SH) results in accumulation of oxidized state of glutathione (GSSG) with the modification of the reduced and oxidized GSH [62], which leads to the use of nicotinamide adenine dinucleotide phosphate, in reduced form (NADPH), for recycling of oxidized glutathione (GSSG) at the initial GSH level. NADPH is a coenzyme which serves as an electron donor for various biochemical reactions; additionally the NADPH regenerates also other intracellular antioxidants, especially vitamin E and ascorbic acid [60]. In its turn, the NADP, in its oxidized state is reduced after activation of the pentose phosphate pathway, a reaction in which the glucose-6-phosphate dehydrogenase (G-6PD) is the key enzyme, leading to an increased glycolysis (fig. 8) [38].

Thus, ozone therapy stimulates oxygen metabolism by increasing glycolysis in red cells (fig. 9). On the one hand, this leads to activation of 2,3 diphosphoglycerate (2,3DPG), which in its turn results in an increased quantity of oxygen

released by the oxyhemoglobin to the tissues, which result in improved oxygen levels in tissues:

$$HbO_2 + 2,3 - DFG = Hb\ 2,3 - DFG + O_2.$$

Increased consumption of oxygen by the body has been proven by special measurement methods of the gases contained in blood. The clearest confirmation was the proof of an increased arteriovenous oxygen difference [63]. The partial pressure of oxygen contained in the venous blood, after following a session of ozone therapy, diminishes from 40 mm Hg to 20 mm Hg or even less [60, 64], which means that ozone therapy ensures an increased release of oxygen into the tissues with an insufficient blood supply – an effect impossible to obtain by medication [65, 66].

On the other hand, the increased levels of glycolysis result in activating the Krebs cycle (the aerobe pathway) by increasing the production of the adenosine triphosphate (ATP). The decreased level of the plasma glucose in vitro has been demonstrated by a series of studies [62], and this is especially clear in cases of patients with diabetes [67].

In the process of ozone therapy, the saturation with oxygen of both plasma and erythrocytes takes place. In the presence of ozone, blood is capable to absorb up to 2-10 times more oxygen than normally [68]. At the same time, it becomes possible to maintain the exchange of substances through the intracellular liquid, in conditions of a disturbed vascular tone. All patients who were treated with ozone in the form of AHTM presented a statistically significant increased level of partial oxygen pressure in arterial blood, a decreased partial pressure of carbon dioxide and a raised level of oxyhemoglobin [68]. After concluding the treatment, the maximum time for reduction of oxyhemoglobin decreases very slowly, more precisely within a period of several weeks and even months.

It seems obvious that erythrocytes may be modified by ozone therapy only for a short period of time. However, the repeated therapeutic administrations may allow for the LOPs compounds to permeate the bone marrow and activate the subtle development of an erythropoietic level, favoring generation of "super-gifted erythrocytes" – new erythrocytes with improved biochemical properties [61]. Based on this hypothesis, due to extended ozone therapy, the bone marrow may release a group (approximately 0,9% of the total fund) of new erythrocytes with improved biochemical properties. In fact, the therapeutic benefits do not cease once the treatment is stopped, but rather continue to exist for up to 2-3 months, perhaps in correlation with the life span of circulating super-gifted erythrocytes. It has been demonstrated that after an extended period of treatment with ozone, the fraction of young erythrocytes, isolated by the old erythrocytes [69] by separation in density degree, had a much higher content of G6PD (Glucose-6-phosphate dehydrogenase) [61]. Activation of metabolic processes in the erythrocytes contributes to accumulation of macro energetic compounds ATP [70]. As a result, the transportation function of the cellular membrane is intensified, by activation of ionic pumps ATP-ase Na+ - K+ and, subsequently, the concentration of intracellular cations (K +) and extracellular cations (Na +), the level of resting potential of the membrane and distribution of electrical charges are regulated, and thus the activity of adhesion and aggregation of cells is modified, which determines the rheological properties of blood. Additionally, the generation of a lipid bilayer of peroxides diminished the viscosity of the lipid bilayer membrane, leading in turn to an improved flexibility and elasticity (capacity to deform) of erythrocytes [60].

ROS and LOPs are responsible for the increase of the function of erythrocytes [26], and also for the activation of leucocytes [38], platelets and endothelial cells [50]. This

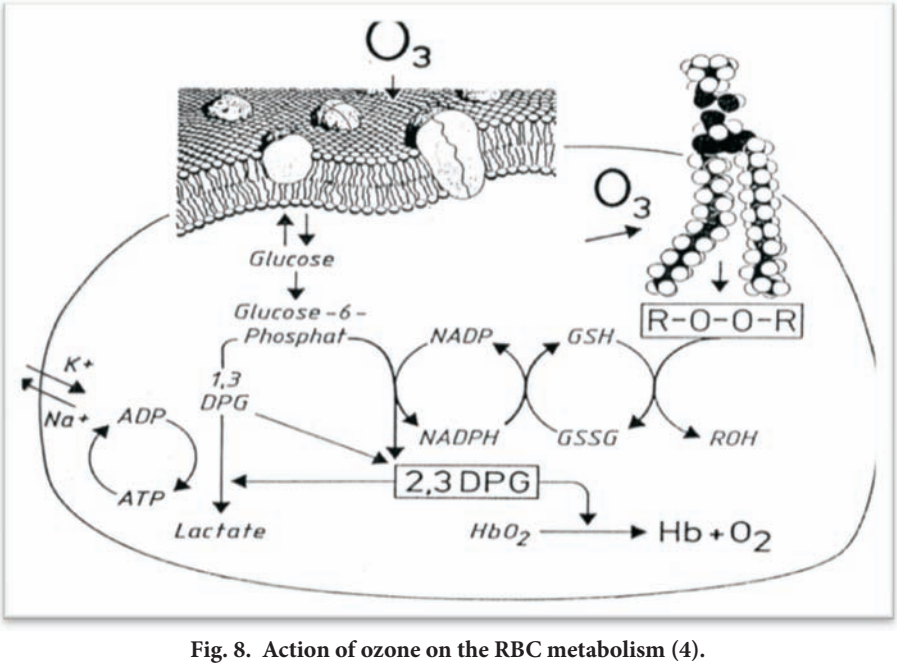

Fig. 8. Action of ozone on the RBC metabolism (4).

multi-faceted and simultaneous activation results in a boosted release of nitric oxide (NO), prostacyclin (through the selective reaction with the double bonds of arachidonic acid, by launching its metabolism), adenosine [71], autacoid and contributes to an improved tissue vascularization [72]. Indeed, through the interaction with the endothelium, the LOPs result in a boosted generation of NO and NO-tiol, which shall result in turn in an increased oxygen input, thus improving the microcirculation. Moreover, the phenomenon of adaptation to chronic oxidative stress implies the fact that repeated ozone treatments induce the synthesis of the oxidative stress proteins, of which the HO-1 (hemo-oxigenase) serves as a primary example. This enzyme induces a boosted level of bilirubin (a lipophilic antioxidant equally powerful to tocopherol) and CO. The enzyme reduces indirectly the vasoconstriction, due to suppression of the endotheline-1 genes and inhibits the proliferation of smooth muscle cells [73]. It is a known fact that the NO, the release of which is boosted by ozone therapy [72], is the most important physiological vasodilator and inhibitor of platelet and leukocyte aggregation and of adhesion to endothelium, which in cooperation with certain traces of CO increases vascular relaxation [38].

The performed studies on ozone therapy effects on hematologic/hemodynamic parameters attest that the treatment with medical ozone, practically, does not modify any levels of the methemoglobin, neither hematocrit levels, because of the absence of any changes in the RBC volume, caused by an edema or lysis, but actively influences the active blood clotting system. The diminished blood and plasmatic viscosity is due to the decreased fibrinogen levels [51].

Fibrinogen is the primary factor for clotting, having the biological property to clot under the influence of the specific enzyme – thrombin. The product of this reaction - fibrin, forms the reticular basis of the thrombus, which clogs up the affected vessel. Fibrinogen plays an important role in the aggregation of erythrocytes and platelets [74]. The increase of the concentration of fibrinogen causes an increase of the viscosity of the blood [75]. Therefore, medical ozone, applied in low concentrations, by reducing the concentration of fibrinogen, lowers the aggregation of blood cells and ameliorates the rheological properties of the blood [76].

The activation of the fibrinolytic mechanism in the hemostasis system prevents the development of blood clots, resulting in partial or total thrombosis, leading in its turn to the fibrinolysis and ensures its elimination from the vascular bed. This is one of the primary mechanisms of revascularization and restoring of the blood flow to the organs and tissues [75].

In high concentration, medical ozone, used only for external applications, has a pronounced hemostatic effect [38].

Therefore, not the ozone itself, but ROS (largely H_2O_2) and LOPs are responsible for the succeeding and multiple biochemical reactions which take place in various cells throughout the body. [38].

Use of medical ozone in dentistry

Normally, the oral cavity hosts about 20g commensal bacteria which are better kept under control by the lymphoid tissue related to the mucous (MALT). However, they can become pathogenic and are largely responsible for tooth decay, which is reported by the Dr. E. Fisch (1899-1966), considered the first dentist to use ozone in his practice and to demonstrate

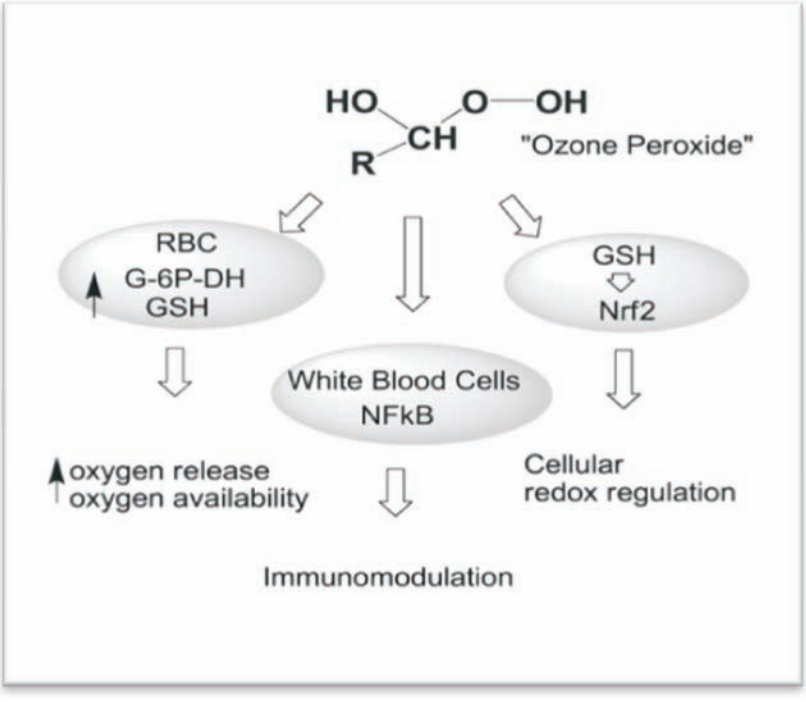

Fig. 9. The three pharmacological effects of medical ozone obtained through peroxides generated by ozone: (1) Improved release of oxygen to the red blood cells (RBC); (2) Immunomodulation through the activation of white blood cells (WBC) and (3) Regulation of the cellular anti-oxidation system [18].

the potential of ozone disinfection activity [38]. Nowadays, medical ozone in dentistry is applied as a complementary treatment or in combination with other methods of ozone therapy [13]. The most frequently used in dentistry and Oral & Maxillofacial surgery (OMS) are the ozonized solutions [77, 78, 79, 80, 81, 82]. At the 15th World Congress organized by the International Ozone Association (IOA, 2001) it was reported that application of ozonized water in oral cavity heals the wounds more speedily compared to placebo treatment. In dentistry the ozonized distilled water is used for cleaning up the oral cavity, antiseptic irrigation of canals and caries. The ozonized water jet fully removes the purulent material and disinfects the affected area [83]. The use of ozone in combination with the dental scaling and professional teeth brushing allows a considerable improvement of oral hygiene and periodontal indices [84, 85].

Although the direct use of ozone in the gaseous form is prohibited (toxic by inhalation), A. Baysan and E. Lynch successfully used a new system of delivering ozone to treat primary root carious lesions (PRCL), capable of avoiding any toxic risk. This system includes a source of medical ozone and a manual dental instrument, on the end of which is fixed a detachable silicone suction cup. Due to its elastic edge, it is sealed to the tooth surface and allows the exposure of the carious lesion to gas. The exposure to ozone for a period of 10-20 seconds, with a concentration of ozone (about 4 mcg / ml) and a gas flow rate of 600 ml / min is enough to destroy all the microorganisms present in the primary root carious lesions. In this case, the most important is to achieve the denaturation of protein and killing the lactobacilli which, following the metabolism of glucose, may result in generation of lactic acid, which in turn favors further demineralization of the dental enamel. The effect of rapid dental surface sterilization by ozone is maintained for about an hour, enough time for the enamel remineralization by calcium phosphate which is present in saliva, thus the tooth becoming stronger and more resistant to bacterial attacks for a period of three months, to the least [86].

The antimicrobial properties of medical ozone, when used for reducing the number of cariogenic bacteria, are also the cause of a significant reduction of microorganisms that are present in BP. From the prospective of a potential antibacterial agent, ozonized water, in most conditions presents less cytotoxicity than ozone gas or recognized antimicrobial agents such as chlorhexidine digluconate, sodium hypochlorite or hydrogen peroxide. As a result, the ozonized water meets the optimal cellular biological requirements regarding biocompatibility in oral application [87].

Some authors recommend complementing OMS methods with ozone therapy. The use of ozone in the pre- and post-surgery allowed for a considerable improvement in postoperative evolution, an acceleration of the operation wound epithelialization and a decreased time of antibiotics administration [88]. In OMS, ozonized solutions are used locally for treating purulent inflammatory processes (periostitis, alveolitis, phlegmon) [89, 78], in open fractures of the mandible and in posttraumatic prophylaxis of inflammatory complications. Ozone has a beneficial influence on metabolism and bone restorative processes [91]. It has been noticed that in patients with chronic osteomyelitis of the jaw, the use of medical ozone normalizes quickly and completely the non-specific resistance and T-cell immunity, thus accelerating the healing process and reducing the incidence of clinical complications [92].

The relevancy of applying ozone therapy in inflammatory processes in the oral & maxillofacial region is determined by the spread of antibiotics resistant forms of microorganisms and the change of the patient's sensibility to this reaction. Several authors mention the necessity of systemic application of ozone therapy, by pleading the immunomodulatory action of medical ozone, which reduces the period of exudation from the wound, accelerates the emergence of the granulation and wounds epithelialization to the patients with phlegmons with torpid evolution in the oral & maxillofacial region [88].

Medical ozone is very effective in endodontics, except one must ensure the correct application of ozone and namely: sufficient concentration, suitable time and the correct method of application in the radicular channels, and certainly, after the procedures of preparation, irrigation and traditional endodontic cleaning have been completed. The studies have demonstrated the potential of using ozone as gas, ozonized water and oil in endodontic therapy. The ozonized water can be used as a final syringe in combination with a ultrasonic apparatus, that produces a very effective "streaming" sound, allowing ozonized water to penetrate many parts of the intra-radicular anatomy, much more efficient than conventional obturation techniques [93].The use of ozone in prevention of peri-implantitis is motivated by ensuring a proper diet and regular control of the bacterial plaque, and additionally, the use of ozone shows a positive healing of wounds due to an increased blood circulation in the tissue. Also, of interest are communications concerning the application in the CMF of ozonated oils, especially in cases of alveolitis [89]. The authors attest the reduction of the healing process in comparison with the traditional therapy duration. In cases of inflammatory diseases of the treatment resistant mucous tissues (i.e. relapsed herpetic stomatitis etc), some authors recommend the combination of the local ozone therapy with the systemic one. Such a treatment scheme allows for the achievement of a stable remission in short term for most patients [94].

The possibility of using ozone as a local antiseptic in treatment of odontogenic purulent sinusitis is of interest likewise. According to studies, ozone therapy is the most effective method when compared to other treatment methods. In case of emphasized intoxication syndrome, which complicates the development of sinusitis, the combination of systemic and local ozone therapy is recommended. This allows for rapid normalization of biochemical blood indicators [95].

Thus, ozone therapy experienced a boosted application in dentistry, including in periodontal dentistry [11, 12, 13].

The ozone acts on several pathogenic links of the periodontal disease, firstly by regulating the system of antioxidants (SA), and secondly by improved oxygen supply and reduction of healing time of periodontal tissues. Certainly, its bactericidal and immunomodulatory abilities are of major importance. In treatment of the periodontal disease, the medical ozone is locally applied into periodontal pockets (PPr), in the form of instillations, solutions or oil drips, and possess a proven anti-inflammatory potential, based on the analysis of objective diagnostic criteria [3, 90, 96, 97, 98].

Conclusions

The ozone molecule is unstable and breaks down into oxygen, succeeded by an exothermic reaction. The basic quality which defines the specifics of the physicochemical properties is the high level of excess energy of the ozone molecule and its strong oxidizing action. The pharmacological effect of medical ozone is represented by the so-called hormesis phenomenon, expressed through the stimulatory or beneficial effect following the exposure to low concentrations of a substance, otherwise toxic in higher concentrations, and this effect takes place due to the short chains of hydroxyl-hydro-peroxide, the so-called ozone peroxide. The mechanism of action of medical ozone is divided into two major categories, depending on the application method: topical or systemic application. The action of medical ozone action on the human body is diverse and multidirectional. The medical ozone possesses analgesic, anti-inflammatory, anti-edematous, antioxidant, muscle relaxant, detoxing, immunomodulatory, antimicrobial, antiviral, antifungal effects, it activates cellular metabolic processes, stimulates oxygen metabolism and improves the rheological properties of the blood. Ozone therapy is a highly effective method for treating many diseases, of which the pathogenesis of the inflammatory syndrome is based on the bacterial etiology and immuno-inflammatory response of the host. At the same time, unlike antibiotic therapy, ozone has a much broader spectrum of therapeutic action, does not create any microbial resistance and has no adverse effects. Medical ozone in dentistry is used in monotherapy or combined with other treatment methods. Meanwhile, the other aspects of the use of medical ozone in clinical periodontology requires additional research.

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The latest developments in cutaneous homeostasis

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Abstract

Background: The skin is a major actor of human homeostasis mainly due to its important role in body temperature regulation but also through its role of barrier against any external aggression, and as a transmitter of a lot of information to the brain. It is very important that this vital organ can regulate its own homeostasis to be able to assume its role for the rest of human body. It is commonly admitted that cutaneous homeostasis is more or less the barrier effect but the last discovery for the last decade opens new interesting fields of investigation. Degradation of tight junctions with age are well-known. In rosacea, the water permeation in epidermis sever the cells and break the junctions, it is an open door for microbial infections and dramatic dryness. On atopic mice skin model, Yokushi and al. showed in 2015 that tight junctions of atopic skin are more permeable and this is correlated with the filaggrin protein depletion. If junctions still stop microbials and big molecules penetration, they let small molecules under 30 KDalton to penetrate the epidermis. This could be one of the causes of the inflammatory status of atopic skins and of dryness as water permeation is increased as well.
Conclusions: In conclusion, skin homeostasis becomes more and more complex with the last discoveries about skin microbiota. Interactions between sebum, epidermal lipids, epidermal peptides and microbiota are huge. We have an open field to innovate in new treatment taking into account the capability of billions of living cells on our skin surface which talk with our cells all the time and work together to help our skin assume its defense role of the human body.

Key words: cutaneous homeostasis development.

Introduction

The skin is a major actor of human homeostasis mainly due to its important role in body temperature regulation but also through its role of barrier against any external aggression, and as a transmitter of a lot of information to the brain.

It is very important that this vital organ can regulate its own homeostasis to be able to assume its role for the rest of human body. It is commonly admitted that cutaneous homeostasis is more or less the barrier effect but the last discovery for the last decade opens new interesting fields of investigation.

We will study some of the last developments about 4 skin homeostasis mechanisms:

1. Cell cohesion.
2. The stratum corneum lipids and peptides.
3. The hydrolipidic film.
4. Skin microbiota.

Reminder on cell cohesion

From the basal layer to the stratum corneum, keratinocytes are hung together through tight junctions. Hemi-desmosomes hang cells to the dermis, desmosomes hang keratinocytes together, corneo-desmosomes give sealing to corneocytes until desquamation.

Physiology status

By hanging cells together very tightly, junctions filter the entry of external agents and avoid infections and irritation. In