On the Mechanisms of Correction of Systemic, Peripheral Circulatory and Microcirculatory Disorders by Ozone in Critical Conditions

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Abstract

In experimental-clinical conditions we studied efficiency of systemic ozone therapy and mechanisms of its therapeutic action in critical conditions: hemorrhagic shock, terminal condition, combined trauma – blood loss + shock; in clinical conditions: an early stage of burn disease.

In experimental conditions of postreanimation stage and in patients at an early stage of burn disease we investigated reactions of direct oxidation of main organic substances (carbohydrates, proteins and lipids) by ozone and influence of ozonolysis metabolites on systemic, peripheral circulation and microcirculation. The previous investigations have established that rate constants of reaction between ozone and carbohydrates are within $5,4 - 4,1^3$ $k \cdot l \cdot mol^{-1}$, moreover ozone mainly destroys glycoside bonds of polysaccharide, and oxidation of its pyranoside rings is insufficient [7]. A more important subject of our clinical conditions was the mechanism of glucose metabolism intensification [11] with accumulation of 2,3 DPG and restoration of the respiratory function of blood through ozonolysis. Besides, 5-carbohydrate sugars (riboses) resulted from ozonolysis are used for synthesis of nucleotides. We have established that through ozonization in the erythrocytes it comes to a significant increase in the level of energy substrates. The decreased level of ATP can be explained by involvement of energy capacities in work of membrane ATP.

It has been shown that through protein ozonolysis rate constants of reaction with aminoacids are quite high, and end products of metabolism of tryptophan, histidine and tyrosine are serotonin and histamine. According to our data [3,4,2] major ozonated autohaemotherapy or low-flow ozone-oxygen therapy for endotoxicosis at an early postreanimation stage is associated with an increase in the blood level of biologically active substances. A result of the increased level of biologically active substances is their positive influence on cardiovascular, respiratory and humoral systems of homeostasis, peripheral circulation and capillary flow (opening of capillary anastamosis, widening of capillary bed, increase in peripheral temperature). An increase in endogenic catecholamines facilitated an increase in systemic AD (through α-adrenoreceptors), an increase in stroke volume and cardiac output (through β-adrenoreceptors), a decrease in renal vascular resistance, an increase in renal flow and renal filtration (that finally resulted in an increase in diuresis).

Extracorporeal ozonization was associated with oxidation of unsaturated fatty acids and a reliable increase in the pool of saturated fatty acids. It came to an increase in arachidonic fatty acid by 21% (predecessor of prostaglandin E1) and linoleic fatty acid by 16%. Accumulation of fatty acids 20:4 and 18:3 presupposes further synthesis of prostaglandin E1 (PGE1) by cyclooxidation. We confirmed this supposition through detection of PGE1 in the patient's blood immediately after sessions of major autohaemotherapy with ozone by method of crystallography.

Stimulation of synthesis of endogenic PGE1 has also contributed to vasodilatation, an increase in cardiac output, a decrease in total peripheral vascular resistance through widening of arterioles, precapillary sphincters and can be considered as one of the mechanisms facilitating improvement of peripheral circulation.

Introduction

Ones of end products resulted from action of ozone on the organic substrates (sugars, amino acids and fatty acids) are numerous biologically active substances (prostaglandins) producing a regulatory effect on the functional state of the organism's vital organs and systems. In addition to the earlier studied bioregulatory effects of ozone realized through stimulation of regeneration of histamine, serotonin, catecholamines the given investigation demonstrates results of interaction between ozone and fatty acids as well as an attempt to explain influence of their metabolites on the state of the organism's vital systems in critical conditions.

Nowadays, the increased interest in prostaglandins can be explained by their high biological activity and a wide range of action which variety can be only compared with the effect of hormones. For today the ways of their synthesis and metabolism are investigated to a great extent, it has been shown involvement of prostaglandins in the functional regulation of different systems and organs, there have been proposed hypotheses on their involvement in the pathogenesis of some diseases and made attempts in using prostaglandins in different fields of medicine. At the same time, it appears important to continue with investigations into this biologically active substance, its properties, development of methods and factors of possible influence on it, their stimulation as well as development of new methods of determination of prostaglandins in biological liquid and tissues.

Material and methods

We investigated critical conditions: hemorrhagic shock, terminal condition, combined trauma – blood loss + shock; in clinical conditions: an early stage of burn disease. Under our observation there were 126 adult non-pedigreed dogs of the weight of 8 to 22 kg, and 172 patients at the age of 18 to 73 years in the Russian Burn Center Clinic.

In experimental conditions of a postreanimation stage and in patients at an early stage of burn disease we investigated reactions of direct oxidation of main organic substances (carbohydrates, proteins and lipids) by ozone and influence of ozonolysis metabolites on systemic, peripheral circulation and microcirculation. For diagnostics of ozonolysis products (Prostaglandin E1) in blood serum we conducted a crystallography investigation of blood serum.

The subject investigated was blood serum of 30 patients; they were divided into 3 groups:

A. Control group of 10 patients treated neither with Vazaprostan (a synthetic analog to Prostaglandin E1) nor ozone therapy;
B. Experimental group No. 1 of 10 patients treated with ozone therapy;
C. Experimental group No. 2 of 10 patients treated with Vazaprostan (a synthetic analog to Prostaglandin E1).

The patients of group B received ozone therapy in the form of treatment of 100-150 ml of the patient's autoblood with an ozone/oxygen gas mixture at ozone concentration of 5000 mcg/L. The patients of group C received intravenous infusions of Vazaprostan solution.

Results and discussions

Our investigations into the oxidation of mono- and polysaccharides by ozone have established that rate constants of reaction between ozone and carbohydrates are within 5,4 - 4,1^3 k·l·mol^{-1}, moreover ozone mainly destroys glycoside bonds of polysaccharide, and oxidation of its pyranoside rings is insignificant [7]. Therefore, clinically more important is indirect effect of ozone, for example, intensification of glucose metabolism in the erythrocytes. This "turbocharged" effect of glucose utilization in the erythrocytes leads to accumulation of 2,3 DPG and restoration of the respiratory function of blood [11]. Besides, 5-carbohydrate sugars (riboses) resulted from oxidation are used for synthesis of nucleotides. This fact was confirmed by our investigations [4] when through ozonolysis of autoblood it came to a significant increase in energy substrates in the erythrocytes – ADP up to 69%, AMP – up to 47%. At the same time, the level of ATP in the erythrocytes decreased by 17-40% that was explained by involvement of energy capacities in work

of membrane ATPases providing oxidative phosphorylation in cell (respiratory chain). According to H. Wervej (1981) the activity of galactose-oxidase enzyme in the erythrocytes through small doses of ozone increases up to 800%.
It has been also shown that through protein ozonolysis rate constants of reaction with amino acids are quite high being within the range of $10^1 – 10^5$ $l \cdot mol^{-1} \cdot s^{-1}$, so for tryptophan KO3 = $5,6 \cdot 10^4$ $mol^{-1}s^{-1}$, for tyrosine KO3 = $6,6 \cdot 10^3$ $mol^{-1}s^{-1}$, for histidine KO3 = $5,7 \cdot 10^3$ $mol^{-1}s^{-1}$ [7]. In blood plasma albumins are for the most part available as complexes with tryptophan (Tr) [6]. It is known that products of metabolic transformations Tr BAS (biologically active substances) perform a key role in intracellular regulation of metabolic and genetic processes. One of end products of oxidative transformation Tr is serotonin. This is a trophotropic metabolite (involved in restoration of energy expenditures by cells and organs, intensification of assimilation processes), it activates parasympathetic nervous system.

According to our data [4] the use of low-flow ozone-oxygen therapy of endotoxicosis at an early postreanimation stage is associated with an increase in the blood level of serotonin by 30% as compared with the control ($p<0,01$). A final result of the increased level of endogenic serotonin is its positive effect on cardiovascular, respiratory and humoral systems of homeostasis; its antioxidant effect can be of particular interest.

The blood level of histidine is quite high (up to 10% in Hb). As a product of its biotransformation is histamine. The biosynthesis of histamine through ozonolysis is simpler. It only requires one process of decarboxylation. One of the most important clinical effects of free histamine is its effect on capillary flow (opening of capillary anastamosis, widening of capillary bed, increase in peripheral temperature).

The effect of ozonolysis products of two above-mentioned amine acids (tryptophan and histidine) on the organism could be one-sided and even undesirable, if the rate of biotransformation of another amine acid – tyrosine were not practically the same. As a result of oxidative metabolism is synthesis of catecholamines (dopamine, noradrenaline, adrenaline). The effect of these BAS, in particular on cardiovascular system, is manifested as an increase in systemic AD (alpha-adrenoreceptors), an increase in stroke volume and cardiac output (beta-adrenoreceptors), a decrease in renal vascular resistance, an increase in renal flow and renal filtration. The endogenic catecholamines ensure an ergotropic effect of ozone on the organism – a humoral sympathetic influence on nervous system. Our observations [4] confirm the fact of release of accumulated endogenic catecholamines, their biotransformation from tyrosine. So, all the session (for 60 min) of low-flow ozone-oxygen therapy the level of AD in patients was reliably ($p<0,01$) higher than the control at a background of the twice decreased indirect effect of serotonin, histamine and other BAS ($p<0,01$).

The results of ozonolysis of the organic substrates (carbohydrates, amine acids and fatty acids) in the organism are demonstrated in Figure 1.

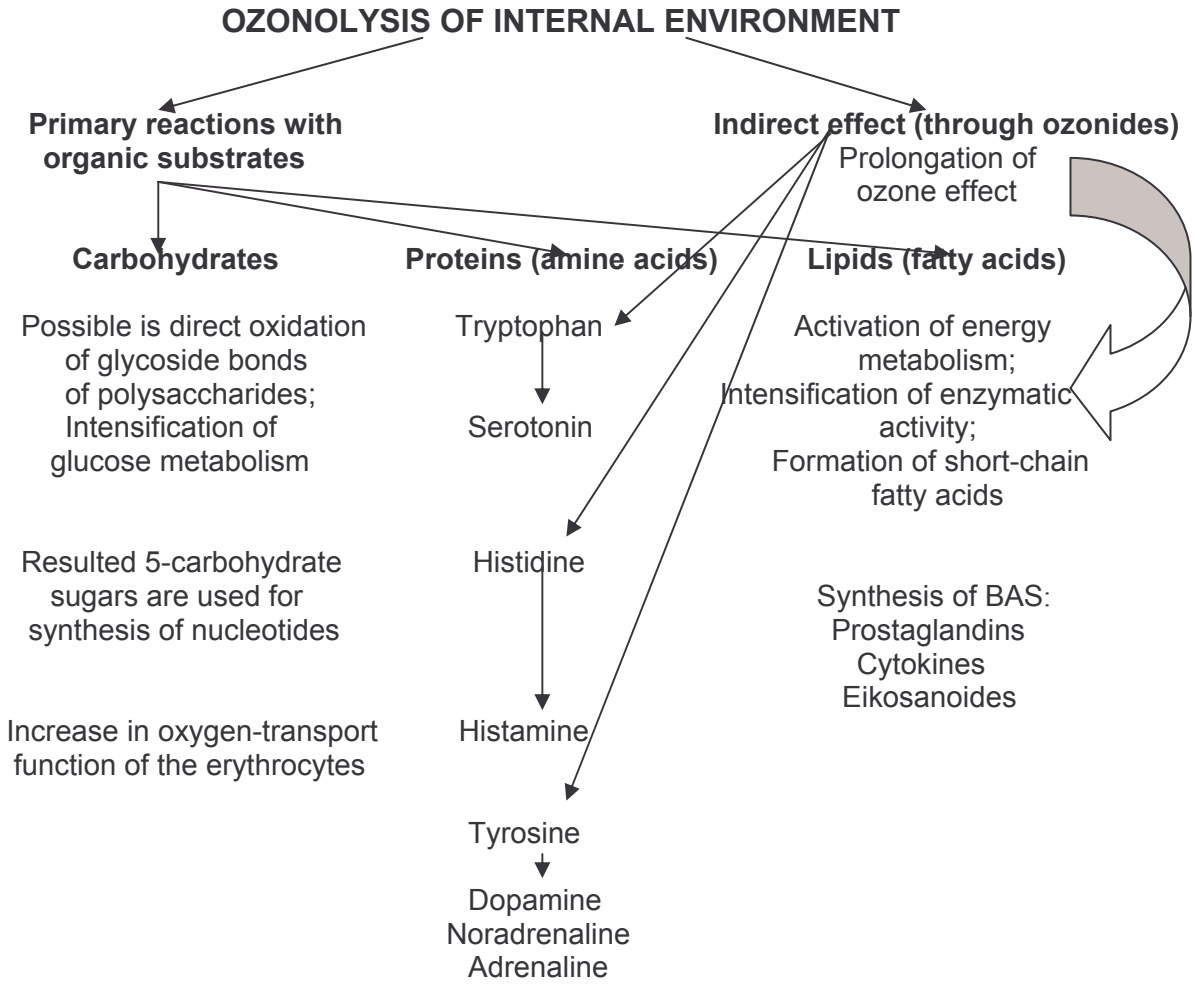

Figure 1. Ozonolysis of internal environment

In compensation-adaptation processes at a background of ozone therapy an important role is performed by products of oxidation of polyunsaturated fatty acids. When ozone is administered via the parenteral route, thanks to its selective interaction with unsaturated compounds on >C=C< bonds resulted in formation of fatty acids' ozonides and their follow-up decomposition to Kriege's zwitter-ion, formation of hydroperoxides and their follow-up transformation to hydroxyl and alcoxyl free radicals, processes of autooxidation are initiated and supported [10].

In our work we investigated the effect of ozone (O3) = 50 mcg/L on the fatty-acidic level of total plasma lipids in dogs at an early postreanimation stage. The level of fatty acids was determined after extraction of total lipids, production of thermostable fatty acids by method of gas chromatography.

Extracorporeal ozonization of blood resulted in a reliable increase in the pool of saturated fatty acids such as 14:0 – by 360%, 15:0 – by 59%, 16:0 – by 17%, 17:0 – by 50%, 18:0 – by 19%. The total level of saturated fatty acids increased by 19%.

The unsaturated fatty acids are characterized by oxidative reactions at a place of double bonds. In this case it can come to both preliminary formation of ozonides with their follow-up degradation and direct breakdown of double bonds resulting in shortening of long-chain fatty acids. So, the level of fatty acids 20:5 decreased through ozonolysis by 2 times, at the same time it came to a reliable increase in 20:4 (arachidonic fatty acid) – by 21% and 18:3 (linoleic fatty acid) – by 16%.

The investigations have shown that direct interaction of ozone with fatty acids in the blood is associated with a well-manifested influence on the correlation between the pools of saturated and unsaturated fatty acids towards a decrease in the degree of unsaturation. An increase in the quantity of more saturated fatty acids with lower number of carbon atoms in the chain is very important for intensification of citric acid cycle activity. Such fatty acids easily pass through mitochondrion's membrane involving in beta-oxidation with formation of acetyl-coenzyme A. On this way ozonolysis of fatty acids activates one of the most powerful mechanisms of supply of substrates for Krebs' cycle.

A particular attention is paid to the fact of an increase in the blood level of arachidonic (predecessor of prostaglandins) and linoleic fatty acids. Oxidized metabolite of polyunsaturated fatty acids – dihomo-gamma-linoleic acid being a component part of cell membrane phospholipids represents Prostaglandin E1 (PGE1). Accumulation of fatty acids 20:4 and 18:3 presupposes further synthesis of PGE1 by cyclooxidation.

The biosynthesis of PGE1 under influence of ozonolysis of fatty acids facilitates vasodilatation, an increase in cardiac output, a decrease in peripheral vascular resistance through widening of arterioles, precapillary sphincters and postcapillary venules and can be one of the mechanisms improving peripheral circulation (Figure 2).

BIOSYNTHESIS OF PROSTAGLANDIN E1
UNDER INFLUENCE OF OZONOLYSIS OF FATTY ACIDS

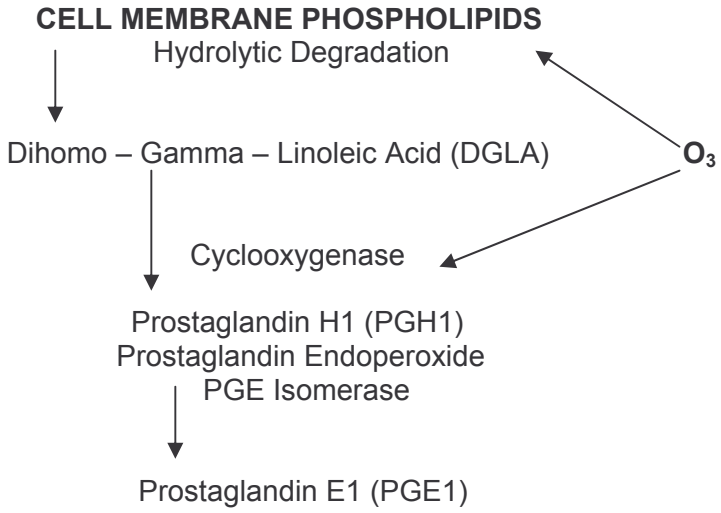

Figure 2. Biosynthesis of prostaglandin E1 under influence of ozonolysis of fatty acids

Nowadays, the increased interest in prostaglandins can be explained by their everywhere availability (they are found in all organs and tissues in man and animal), high biological activity (they take effect in extremely small dosages measured in nanograms), a wide range of action which variety can be only compared with the effect of hormones. The level of prostaglandins in the organism's tissues is quite low, and their synthesis in the organism's tissues occurs in small quantities. The human body synthesizes about 100 mcg of main prostaglandins a day, and already within one blood circulation 90% of prostaglandins come to destruction. For today the ways of their synthesis and metabolism are investigated to a great extent, it has been shown involvement of prostaglandins in the functional regulation of different systems and organs, there have been proposed hypotheses on their involvement in the pathogenesis of some diseases and made attempts in using prostaglandins in different fields of medicine. At the same time, it appears important to continue with investigations into this biologically active substance, its properties, development of methods and factors of possible influence on it, their stimulation as well as development of new methods of determination of prostaglandins in biological liquid and tissues.

Nowadays, determination of prostaglandins in biological liquid is carried out by physical-chemical methods [9] including: absorption spectrophotometry, gas-liquid chromatography, fluorescence analysis as well as radioimmunological and biological methods. Among the known methods the radioimmunological one is most sensitive and specific. It is also known the immunoenzymatic method of determination of prostaglandins in biological liquid which is based on enzymatic marker. However, the known methods are difficult in performance, and expensive equipment required for their performance is a rarity in most of practical clinics.

For the first time using the method of crystallography we made a qualitative determination of prostaglandins in blood serum at a background of ozonization of internal environment. The subject investigated was blood serum of 30 patients.

The common practice of crystallography investigations into blood serum has shown that in health there are three morphotypes of crystal structures: thread-form, spheroliths of large and moderate size. In pathological conditions it comes to changes in size, form, brightness and color of crystal structures. There are established three main streams of formation of pathological crystal morphotypes: appearance of secondary form-producing structures, appearance of atypical crystal forms and amorphous condition. Large secondary structures can look like a rectangle, rhombus, needle and bush. Atopic structures are differentiated according to crystal form defects and insertions of other structures into the main form. In contrast to monochromatic secondary and basic forms atopic structures of blood serum have different color scale. Besides, blood samples of some patients can't form any crystals at all and after drying represent an area covered by non-structured mass – amorphous condition. For convenience in using crystallography data it was introduced a "crystallography formula" including three parameters B, S, A, where B – basic structures of 1st, 2nd or 3rd thread-form type. Spheroliths of large and moderate size, in brackets there is indicated the percentage content of the registered morphotypes against the total crystal surface. S – secondary structures looking like a rectangle, rhombus, needle and bush, in serum – rhombus-form, dendritic, round with secondary growth and polymorphous, A – atopic structures. A positive effect of treatment is associated with a decrease in the number of crystals with atopic forms and secondary structures, and in case amorphia before the treatment it comes to restoration of crystallization through positive effects of treatment. The investigation of crystallogram also covers availability of crystallization centers according to their quantity, observation of breakdown, thickening of crystal rays, quantity of crosses as well as separately located crystals in the form of cross figures, availability of crystals with thick and bushy rays.

To prove a possibility of stimulation of production and registration of the received effect we conducted experimental investigations. Since prostaglandins have never been determined by crystallography any time before, it was necessary first to get a crystallography picture with artificially synthesized prostaglandin E1 used as a test agent. Nowadays, it is known a medicinal agent Alprostadil (Vazaprostan) artificially synthesized and successfully used in the treatment of vascular diseases representing a stabilized synthetic analog to prostaglandin E1 [5].

The results of experiments are shown in Figures 3, 4, 5, 6.

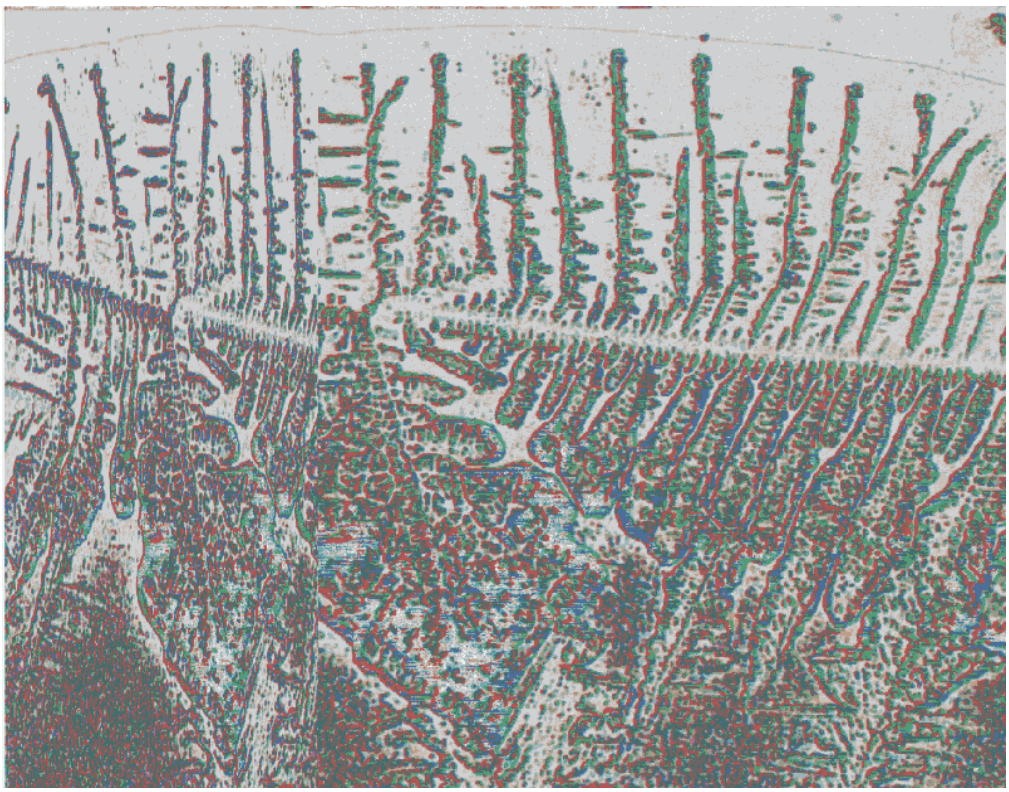

Figure 3. Crystallogram of therapeutic preparation Vazaprostan in vitro (physiological saline base)

In a visual field there are up to 80% of basic structures – thread-form crystals. Secondary structures are represented by characteristic rhombus-form crystals in a quantity of 2 pieces in a field of vision. Dendritic crystals cover up to 20% of total area.

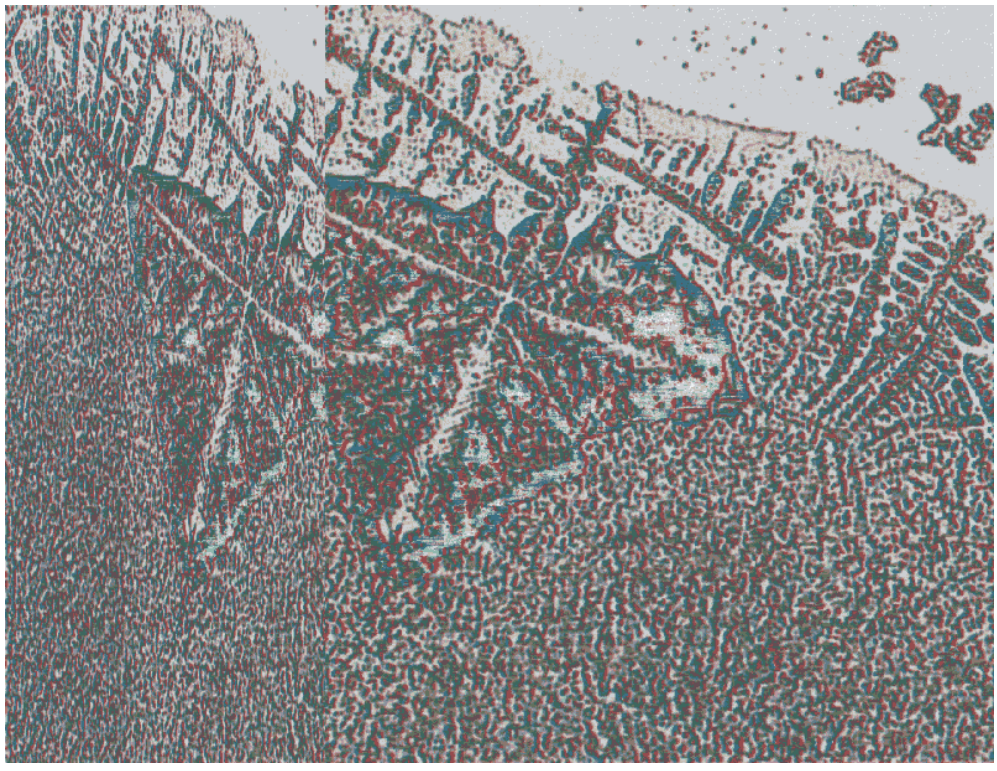

Figure 4. Crystallogram of plasma after administration of therapeutic preparation Vazaprostan (physiological saline base)

In a visual field there are up to 70% of basic structures – thread-form crystals. Secondary structures are represented by characteristic rhombus-form crystals in a quantity of 1 piece in a field of vision, dendritic crystals cover up to 20% of total area. Atopic crystals cover up to 10% of total area.

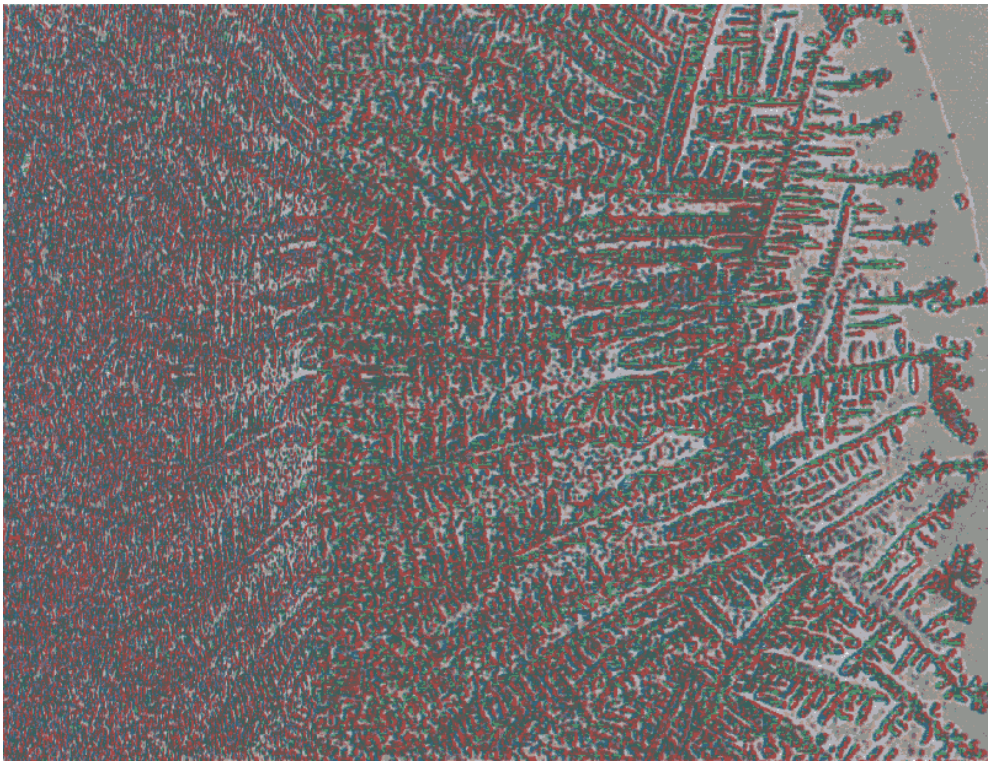

Figure 5. Crystallogram of plasma before ozone therapy

In a visual field there are up to 80% of basic structures. Secondary structures represented by dendritic crystals cover up to 20% of total area.

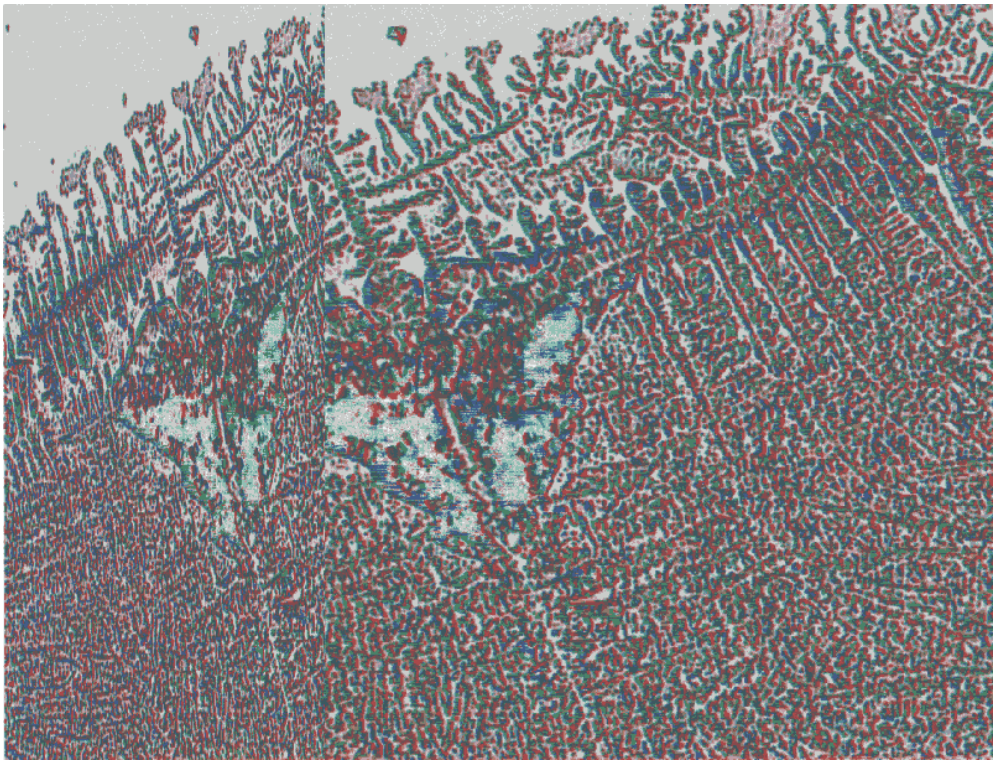

Figure 6. Crystallogram of plasma after ozone therapy

In a visual field there are up to 70% of basic structures – thread-form crystals. Secondary structures are represented by characteristic rhombus-form crystals in a quantity of 1 piece in a field of vision. Dendritic crystals cover up to 30% of total area.

The crystallograms of the patients from group B and group C allow visually determining an identical picture of secondary structures in the form of characteristic rhombus-form crystals that fully coincides with the crystallogram of therapeutic preparation Vazaprostan. The sessions of ozone therapy stimulate production of prostaglandins like intravenous infusions of therapeutic preparation Vazaprostan. The received data have shown that through stimulation of production of prostaglandins by means of ozone therapy it comes not only to an increase and production of "natural" short-life biologically active substances, but also prolongation of their active effect.

Conclusion

Thus, the present work has shown the role and significance of primary (direct) and secondary (indirect) contacts (reactions) of ozone with organic substrates of the patient's internal environment.

We have postulated that the occurred reactions are trigger, starting points in the processes of readaptation of peripheral haemodynamics and microcirculation.

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