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The influence of ozonated saline on some physical and chemical parameters of blood serum: in vitro study

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Abstract

This study aims to study the features of the initiated crystallogenesis of ozonated blood of healthy people, depending on the value of the saturating concentration of ozone. We studied the nature of the initiated crystallization of the "blood – ozonated saline solution" system (n=15) with an ozone concentration of 0; 1000 mcg/l; 3000 mcg/l; 6000 mcg/l; 10000 mcg/l; 20,000 mcg/l and 40,000 mcg/l. For the analysis, blood serum was mixed with aliquot amounts of 0.9% ozonated or controlled sodium chloride solution (component ratio 1: 1). The crystal formation result was evaluated using a set of semi-quantitative indicators. It has been established that human blood reacts most optimally to low doses of ozone (1000-6000 micrograms/l). The range of 6000-10000 micrograms/l is "borderline", and higher doses of ozone have an adverse effect on the biological fluid being studied.

Keywords: ozone, crystallogenic properties, blood, biocrystallomics

1. Introduction

One of the approaches to the study of the physical and chemical properties of biofluids is to assess their ability to self-crystallize and initiate crystallogenesis of different basic substances (1-3). According to T. A. Yakhno et al. (2015), changes in the dynamic parameters of the biomaterial self-organization process can be used as criteria for modeling various impacts under in vitro conditions (4). An assumption is made about blood serum as a substrate model for predicting the nature of the body's response (5).

From a practical standpoint, it is of interest to study the reactivity of human functional systems during ozone therapy (6-8). In this regard, the prevailing approach is based on the evaluation of the effectiveness of the procedure after its completion based on the analysis of the dynamics of individual indicators (6), which does not allow the selection of optimal treatment regimens. In this regard, it is reasonable to search for ways to preventatively assess the need and effectiveness of ozone therapy, which requires the study of the possibilities of crystalloscopic methods in the study of blood response to metered ozonation.

The purpose of this study is to study the features of the initiated crystallogenesis of ozonated blood of healthy people, depending on the value of the saturating concentration of ozone.

2. Materials and Methods

2.1. Experiments description

Venous blood sampling (4 ml) was performed on 15 practically healthy volunteers. Next, the samples were saturated with a saline solution treated with an ozone-oxygen mixture with an ozone concentration; without ozonation (control); 1000 mcg/l; 3000 mcg/l; 6000 mcg/l; 10000 mcg/l; 20,000 mcg/l and 40,000 mcg/l (saturation rate of 30 mcg/l*min; duration – 3-5 minutes). We studied the nature of the initiated crystal formation in the "blood–ozonated saline solution" system with the concentration of the latter increasing in the above range, for which freshly prepared blood serum was mixed with an aliquot amount of 0.9% ozonated or control sodium chloride solution (the ratio of components is 1: 1). It should be emphasized that with such an experiment it is possible to conduct only a teziographic test

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(1, 2), because its mandatory conditions are the introduction of a solution of sodium chloride into the biological fluid, and this substance is a good crystal-forming agent acting as a base substance.

Evaluation of the results of the initiated crystallogenesis of blood serum was carried out visuametrically according to the traditional algorithm using a system of basic (the main teziographic coefficient Q, the coefficient of zoning P) and a number of additional criteria [uniformity of the distribution of elements in the micropreparation R; the severity of cellular C; zones of the dried sample (Z), including the marginal (Kz); clarity of texture (T)]. The application of visuometry to the description of the result of dehydration structuring allows us to quantitatively study the changes in both the initiating potential of the biological medium and the correctness of the formation of crystalline and amorphous facies elements.

In the blood plasma samples, the Fe-induced biochemiluminescence method was used to determine the maximum chemiluminescence flash (LPO), considered as a criterion for the intensity of lipid peroxidation, and the parameter inverse to the chemiluminescence light sum (AOA) and depending on the number of free radicals in the analyzed medium and the total antioxidant activity of the blood plasma. The measurements were performed on a BHL-06 device (Medozons, Nizhny Novgorod).

2.2. Statistical Analysis

The results were processed using the program Statistica 6.1 for Windows. The normality of the distribution of parameter values was evaluated using the Shapiro-Wilk criterion. Taking into account the nature of the distribution of the trait, the Kraskal-Wallis H-test was used to assess the statistical significance of the differences. The differences were considered significant at a significance level of $p<0.05$.

3. Results

Based on the analysis of the dynamics of the initiating properties of the blood serum of practically healthy people when it is treated with various doses of ozone, it is established that there is a two-phase dependence of the nature of the structuring of the biological medium on the saturating concentration of ozone (Fig. 1, Table 1). Thus, the study of the main teziographic coefficient reflecting the degree of the initiating effect of the biomaterial in relation to the base substance used (sodium chloride solution of isoosmotic concentration) demonstrates dose-dependent stimulation of the crystallogenesis of the formed system when using saturating ozone concentrations of 1000-6000 mcg/l. The level of 6000 micrograms/l of ozone is the "boundary" value, the inflection point of the graph, and higher concentrations of the compound have the opposite effect, consistently increasing the inhibition of biosystem structuring. In particular, if the saturating concentration of 10000 micrograms/l still contributes to moderate activation of structure formation (the value of the main teziographic coefficient is lower than the one set for 6000 micrograms/l ($p<0.05$) but exceeds 1), then the use of ozone at a concentration of 20,000 micrograms/l and higher leads to persistent inhibition of crystallization even compared with the control sample pure basic substance (the level of the specified parameter is significantly lower than 1; $p<0.05$).

Table 1. The influence of ozone dose on the level of teziographic parameters

Ozone dose	Main teziographic coefficient (Q)	Crystallinity (C)	Facia destruction degree (FDD)	Clarity of marginal zone (Mz)
0 (control)	1,77±0,16	2,08±0,17	0,61±0,09	1,78±0,13
1000 mcg/l	2,21±0,19	2,24±0,19	0,94±0,10*	1,91±0,15
3000 mcg/l	2,57±0,23*	2,31±0,20	0,74±0,07^	2,06±0,12*
6000 mcg/l	2,76±0,24*^	2,48±0,21*	0,65±0,09	2,23±0,14*
10000 mcg/l	1,43±0,16*^	2,16±0,18^	0,82±0,07*^	1,65±0,16^
20000 mcg/l	0,72±0,12*^	1,62±0,14*^	1,56±0,12*^	1,42±0,16^
40000 mcg/l	0,51±0,12*	1,36±0,14*^	2,41±0,15*^	1,17±0,13*^

Note: * - the level of statistical significance of the differences relative to the control is $p<0.05$
^ - the level of statistical significance of the differences relative to a lower dose of ozone is $p<0.05$

Thus, low saturating concentrations of ozone (less than 6000 mcg/l) in isoosmotic conditions in vitro stimulate the initiating structuring of the basic substance activity of the blood serum of practically healthy people, while higher, exceeding this level, dose-dependently inhibit crystal formation, achieving at extremely high concentrations (40,000 mcg/l) practically reducing the initiating properties to double inhibition relative to the control sample of sodium chloride solution. The data on the two-phase dependence confirm the previously obtained results (9-14).

A similar but more complex dependence was registered for the degree of destruction of samples of biosystems formed by blood serum of practically healthy people and sodium chloride solution containing various doses of ozone (Fig. 2). First of all, it is important to emphasize that the essence of the parameter "facia destruction degree" is an assessment of the

correctness of crystallogenesis of the analyzed substrate (1, 2).

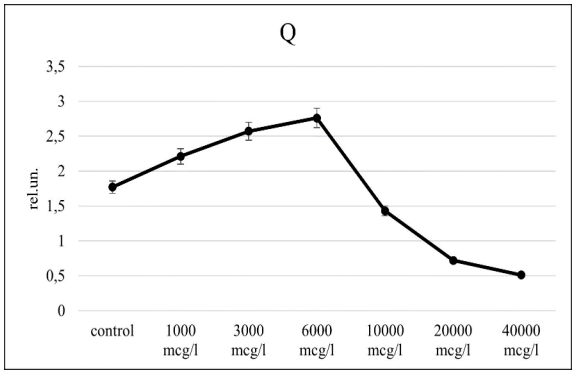

Fig. 1. The level of the main teziographic coefficient of the blood serum of healthy people during its ozonation

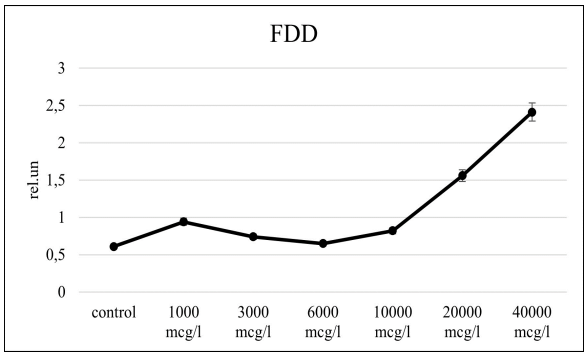

Fig. 2. Changes in the degree of destruction in blood serum samples of healthy people when it is saturated with ozone in different concentrations

Taking into account the fact that at low saturating doses of ozone (up to 10,000 mcg/l) there were no significant differences in the level of the indicator relative to blood serum samples into which an ozonized saline solution was injected (in Figure 2 it is designated as "control"; $p>0.05$ for all these concentrations), we can say that these doses of the compound do not cause or have a negative effect on the biological medium (in terms of crystallogenic activity). In this case, this parameter acts as an "indicator of toxicity" for the created biosystem.

On the contrary, the introduction of a saline solution saturated with high concentrations of ozone (20,000-40,000 mcg/l) into a biological fluid leads to a statistically significant increase in the degree of destruction of facies ($p<0.05$) up to almost total destruction of all structural elements of the sample (at 40,000 mcg/l). In the latter case, the micropreparation of dried blood serum is an amorphous mass, among which it is almost impossible to distinguish individual structures.

Such a reaction of the crystallogenic properties of the biological fluid indirectly confirms the general concept proposed by the Association of Russian Ozone Therapy and is based on the preference for low doses of ozone compared to the high doses recommended by European ozone therapists. From these positions, it is important to note that the appointment of a low-dose ozonated saline solution is also clinically preferable (11, 12, 15, 16), which further confirms the interpretation of the results of the study, indicating in favor of low and medium doses of ozone, and serves as a justification for the potential use of the considered technology as a method of individualized selection of intravenous ozone therapy regimens.

In general, by modeling the biosystems "whole blood – ozonated saline solution", the nature of the response of this biofluid to ozonation under in vitro conditions has been studied. Based on the conducted studies, it is shown that with an increase in the saturation dose of ozone, there is a nonlinear dynamics of changes in the parameters of a teziographic test with an extremum corresponding to 10,000 mcg/l, which, taking into account the analysis of all applied criteria, is interpreted as optimum. Thus, low concentrations of ozone have a dose-dependent stabilizing effect on blood serum, and the optimal concentration is 10000 mcg/l, while high-dose exposure (20000-40000 mcg/l) has a negative, disorganizing effect on the studied bio-liquid.

It is known that the main "application point" of the action of medical ozone is the process of lipid peroxidation (7-9, 15, 17). Therefore, the study of the activity of pro- and antioxidant systems can act as a "gold standard" in assessing the blood response to ozonation (9, 15, 16, 18). With this in mind, we conducted a chemiluminescent analysis of the level of lipid peroxidation and the activity of the antioxidant system of the blood of practically healthy people when it was saturated with ozonated saline solution (Fig. 3 and 4).

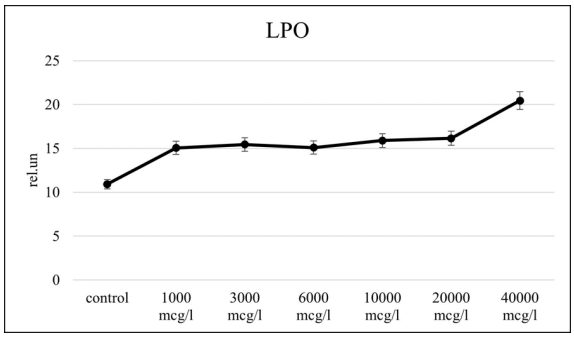

Fig. 3. The level of lipid peroxidation in the blood of healthy people during its ozonation

It was found that the introduction of a sodium chloride solution containing any of the applied ozone concentrations into the studied biological fluid leads to stimulation of lipoperoxidation (Fig. 3; $p<0.05$ – for all cases compared with the control, including the introduction of an ozonized saline solution). At the same time, the use of low saturating concentrations of ozone (1000, 3000 and 6000 mcg/l) causes moderate stimulation of lipid peroxidation (by 34.5, 39.8 and 35.4% relative to the blood into which an ozonized saline solution was injected; $p<0.05$). Even the use of a sodium chloride solution saturated with ozone at a concentration of 10,000 mcg/l does not cause a sharp "jump" in lipid

peroxidation, increasing its level by only 42.5%. In turn, a further increase in the amount of ozone injected (at a saturating concentration of 20,000 mcg/l) practically doubles the activity of lipid peroxidation (by 80.5%), which can be considered a negative trend.

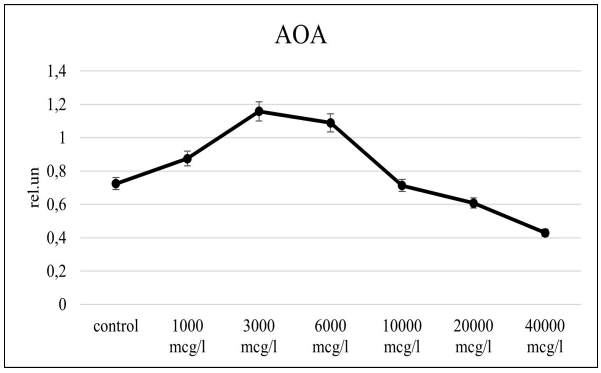

Fig. 4. Dependence of the activity of the antioxidant system of the blood of healthy people on the concentration of injected ozone

It should be noted that the assessment of only the dynamics of lipoperoxidation without taking into account the activity of the antioxidant system does not allow us to identify the direction of shifts in this balancing system; therefore, we also carried out a study of this indicator (Fig. 4).

It was found that the dependence of the total activity of the antioxidant system on the concentration of ozone in the injected saline solution is also nonlinear (Fig. 4). Thus, low doses of the agent (1000, 3000 and 6000 mcg/l) significantly stimulate the activity of endogenous antioxidants compared to the control sample (by 21.7, 58.3 and 46.9%, respectively). At the same time, starting from the above-mentioned "borderline" ozone concentration of 6000 mcg/l for healthy people, a tendency to decrease this indicator begins to appear, although it is not statistically significant ($p>0.05$ relative to the ozone concentration of 3000 mcg/l).

This is due to an increase in the level of oxidant agents in the biosystem, which, at a given dose of ozone, are still stopped by the activation of antioxidant systems of the blood.

The use of medium and high saturating concentrations of the oxidant (10000 and 20,000 mcg/l) leads to further inhibition of antioxidant reserves, which indicates a high rate of their consumption when introducing large amounts of ozone (Fig. 4). At the same time, the activity of the antioxidant system is not only significantly less than the level detected for the saturating concentration of 6000 mcg/ l ($p<0.05$ for sodium chloride solutions with a saturating concentration of 10000 and 20,000 mcg/l), and relative to the control (83.8%; $p<0.05$ for a concentration of 20,000 mcg/l).

This indicates the depletion of the antioxidant potential of the blood when it is saturated with high doses of ozone.

4. Discussion

It is known that any biological system reacts sensitively to significant external influences, which can be various physico-chemical factors (5, 11, 15). In this regard, it is convenient to use experiments in vitro performed on blood samples to study biological effects (2-4, 15, 18). Within the framework of this study, an assessment was made of the transformation of the physico-chemical properties of blood under the conditions of exposure to a source of reactive oxygen species – a physiological solution saturated with an ozone-oxygen mixture with different concentrations of ozone. The integral criterion characterizing the physico-chemical properties of a biological liquid was its crystallogenic activity and the state of oxidative metabolism.

It was found that the studied serum parameters change non-linearly under the influence of ozonation. At the same time, low concentrations of ozone (up to 10,000 mcg/l) provide positive changes in the component composition and physico-chemical properties, as evidenced by the dose-dependent activation of crystallization of biological fluid without a significant increase in the degree of destruction of elements. In addition, in this range of ozone doses, moderate stimulation of free radical processes in blood serum was observed against the background of a pronounced increase in its antioxidant potential. This indicates proadaptive shifts in the oxidative metabolism of biological fluid and is consistent with the results of our previous studies on the antioxidant properties of low doses of ozone (1, 12).

Higher amounts of reactive oxygen species (20,000 mcg /l and above), providing the generation of increased concentrations of free radicals, lead to progressive oxidative stress. This is manifested in a dose-dependent increase in the intensity of lipoperoxidation in combination with a decrease in the total antioxidant activity of blood serum. According to the crystalloscopic test, in the specified range of ozone doses, inhibition of the crystallogenic properties of the biological fluid, a tendency to simplify the resulting crystal structures and a high degree of their destruction were recorded.

Thus, there is a two-phase dependence of the response of the physico-chemical and metabolic parameters of the blood serum on the acting doses of ozone contained in the saline solution during in vitro treatment.

In general, based on the study of crystallogenic properties and the state of pro- and antioxidant systems, it can be concluded that the blood of an almost healthy person reacts most optimally to low doses of ozone (saturation of saline solution with concentrations of 1000-6000 mcg/l). The range of 6000-10000 mcg/l is "borderline", and higher doses of ozone have an adverse effect on the studied biological fluid of a healthy person. Thus, blood can be considered as a micromodel for predicting the nature of the response of the human body and animals to the estimated impact.

Conflict of interest

None for each author.

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None to declare.

Authors' contributions
Concept: A.K.M., E.A.P, S.P.P., Design: L.K.K., A.V.D., Data Collection or Processing: L.K.K., A.V.D., S.P.P., Analysis or Interpretation: A.K.M., A.V.S., L.K.K., A.V.D., Literature Search: A.V.S., Writing: A.K.M., A.V.S.

Ethical Statement
This study was approved by the Local Ethic Committee of Lobachevsky University of Nizhny Novgorod (protocol N2 от 04.06.2014).

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