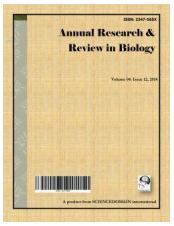

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The Response of Human Erythrocytes to NO-Stimulation

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Authors' contributions

This work was carried out in collaboration between all authors. Author AKM carried out the experimental model, participated in the design of the study, performed the statistical analysis, sequence alignment and drafted the manuscript. Authors AGS and AVD carried out the experimental model and biochemical investigations. Authors AAE and AVR participated in the results analysis and translation of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: The research of recent decades has demonstrated the participation of nitrogen monoxide in various metabolic and regulatory intracellular cascades. However, the majority of works in this field seeks to detect the functional activity of endogenous NO. In opposite, the biological effects of the exogenous nitrogen monoxide have been insufficiently looked into. That is why the aim of the present study was a comprehensive assessment of the effect of various NO-stimulation options on the state of human erythrocytes *in vitro.*
Methods: This study used 15 healthy subjects's (20-45 years old) blood samples divided into five portions. The first portion was allocated as the control; the second portion was treated with a flow from the Plazon apparatus (800 ppm NO); the third portion was processed in a stream tenfold diluted with air (80 ppm NO), fourth portion – with a gas mixture containing 75 ppm NO and fifth portion was introduced with a solution of dinitrosyl iron complexes (DNIC, 3 mM). In all blood

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samples we estimated the peroxide resistance of erythrocyte, levels of malonic dialdehyde and lactate, superoxide dismutase activity, activity of lactate dehydrogenase and aldehyde dehydrogenase.
Results: We stated that blood processing with high NO dose (800 ppm) causes elevation of peroxide resistance of erythrocyte, levels of malonic dialdehyde and lactate with inhibition of activity of superoxide dismutase, lactate dehydrogenase (in forward reaction) and aldehyde dehydrogenase. In opposite, low NO dose (75 ppm) and DNIC induced the decreasing of peroxide resistance of erythrocyte and stimulation of enzymes catalytic activity.
Conclusion: The study has revealed that low doses of gaseous NO and a solution of DNIC produce the most favorable effect on the oxidative and energy metabolism, as well as on the activity of aldehyde dehydrogenase of erythrocytes.

Keywords: Erythrocytes; nitrogen oxide; dinitrosyl iron complexes; lipid peroxidation; energy metabolism.

1. INTRODUCTION

The erythrocyte can be considered as the most available and fairly informative biomodel of the cellular level organization, from which a wide range of effects can be evaluated [1,2]. At the same time, scientific attention is not only directed to the structural features of the erythrocyte membrane and its gas transport function [2], but also towards its metabolic activity [1]. It is known that, despite the absence of a nucleus, these blood cells are characterized by a high metabolic rate, the metabolic processes being modified by numerous bioregulators [2,3].

The research of recent decades has demonstrated the participation of nitrogen monoxide in various metabolic and regulatory intracellular cascades. However, the majority of works in this field seeks to detect the functional activity of endogenous NO [4-7] and are essentially concerned with its effect on the catalytic properties of NO-synthase (NOS) and its individual fractions [8,9]. Commonly used for this purpose are either NOS-specific inhibitors (eg, L-NAME [4]), or L-arginine, the substrate of the enzyme studied [5,7].

At the same time, the biological effects of the exogenous nitrogen monoxide have been insufficiently looked into. Also, the studies related to the application of this effect have been mainly carried out at the organismic level [5,10]. There are only few studies that make use of cell cultures [5,6,11]. On the other hand, Russian and foreign intensive care physicians are actively considering the possibility of NO-inhalation therapy, and they seek to investigate the pharmacological efficacy of the repository form of the nitrogen monoxide – dinitrosyl iron complexes (DNIC) with glutathione and sulfur-containing ligands [12-14]. It should be noted that the results of these experimental and clinical studies indicate versatile action of exogenous NO [13]. However, in most cases the specific physiological and/or biochemical mechanisms have not been revealed yet. Furthermore, researchers tend to leave out any analysis of the baseline level of nitrogen oxide in biological fluids and tissues, or its dynamics after exposure [15].

Given the above-mentioned facts, the aim of the present study was to comprehensively assess the effect of various NO-stimulation options on the state of human erythrocytes *in vitro*.

2. MATERIALS AND METHODS

This study used 15 healthy subjects's (20-45 years old) blood samples delivered from the Nizhny Novgorod Regional Blood Transfusion Station. Each sample contained 25 ml of blood to be divided into five equal portions by volume. The first portion in each experiment was the control sample (it underwent no manipulations); the second portion was treated with NO-containing gas flow from the Plazon apparatus (100 ml, with the nitrogen oxide concentration of 800 ppm); the third portion was treated with a similar flow but ten times diluted with atmospheric air (80 ppm NO; 100 ml). 100 ml of a gas mixture containing 75 ppm of nitrogen monoxide was injected into the fourth blood portion. The mixture was produced by an experimental generator developed at the Russian Federal Nuclear Center – RFNC [16]. 0.1 ml of a freshly prepared water solution of DNIC with glutathione ligands was introduced into the fifth portion. The concentrations of the compounds were measured by a spectrophotometer, with the known molecular extinctions at wavelengths of 310 and 360 nm – 3 mmol/l). The synthesis of

DNIC was performed according to the method of Vanin et al. [5,17]. The duration of the administration of the gas mixtures was three minutes in all the cases, the subsequent exposure being five minutes.

In the samples thus obtained, we studied the peroxide resistance of erythrocytes by Fe-induced biochemiluminescence on BHL-06 apparatus. The levels of malonic dialdehyde (MDA) in the erythrocytes were assessed according to the method of Sidorkin and Chuloshnikova (1993) [18]. The superoxide dismutase activity was studied using Sirota (1993) technique [19]. In the hemolysate of washed red blood cells (1:40), we measured the activity of lactate dehydrogenase (LDH) in forward and reverse reactions in accordance with Kochetov method (1980) [20] revised for the study. The presence of protein was established using modified Lowry method. The level of lactate in erythrocytes was determined using the SuperGL Ambulance analyzer.

To assess the direction of changes in the blood energy metabolism under the chosen physicochemical factors, certain specialized coefficients were used: the coefficient of balance of energy reactions (CBER) [20], the coefficient of substrate presence (CSP), and the normalized coefficient of substrate presence (CSPnorm) [21,22].

The activity of aldehyde dehydrogenase (ADG) in the donor blood was measured using Kershengolts and Serkina (1981) technique.

The results were analyzed with the aid of STATISTICA software package (version 10.0, StatSoft Inc.). Normality of the parameters distribution was evaluated using the Shapiro-Wilk test. Relying on the nature of distribution of characteristics for assessing the statistical significance of differences N-test Kruskal-Wallis was used. The data which showed $p \leq 0.05$ was considered to have statistically significant.

3. RESULTS

Assessing the intensity of lipid peroxidation processes in erythrocyte membranes was conducted as the first component within a complex analysis of the effects produced by various forms of nitrogen oxide in erythrocyte membranes. The level of erythrocyte peroxide resistance in the initial flow from the Plazon apparatus increased 1.36 times as compared with the control sample ($p=0.021$). This fact demonstrates the membrane-destructive effect or a decrease in the resistance of red blood cell membranes to oxidative actions and the intensified stimulation of their lipoperoxidation processes (Fig. 1). The study has shown that such effect is significantly levelled if the concentration of the nitrogen oxide in the gas

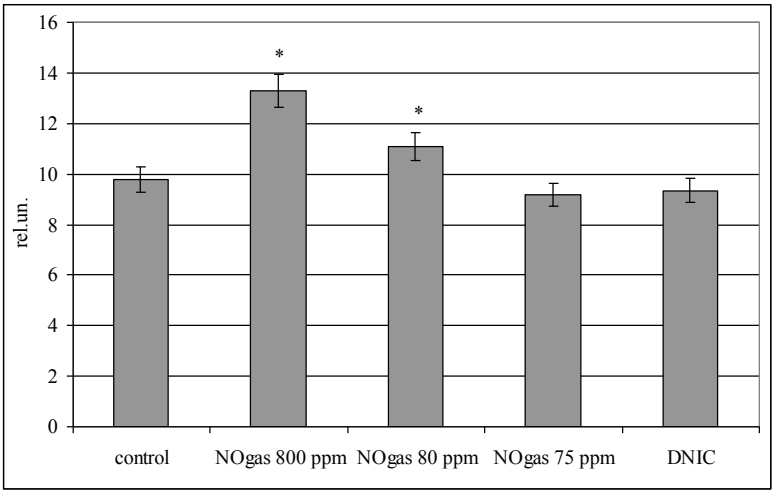


Fig. 1. Erythrocyte peroxide resistance under the action of free and bound nitric oxide

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections $p<0.05$)*

stream is reduced by diluting the latter with atmospheric air (+14% as compared with the initial level, p=0.039).

Blood bubbling with a gas mixture from the experimental NO-generator minimally reduced the studied parameter as compared with the intact sample (by 7%; p=0.063), which indicates a membrane-stabilizing effect. The concentration of the gas mixture was equivalent to a tenfold dilution of the flow from the Plazon apparatus without admixtures of active oxygen [16]. At the same time, the introduction of DNIC solution into the biological fluid had virtually no effect on the peroxide resistance of erythrocytes.

With regard to the level of MDA in erythrocytes (Fig. 2), which also indicates the intensity of peroxidation in their membranes, we should emphasize that using NO in a concentration of 800 ppm leads to a steep MDA increase (up to 48%, p=0.012 as compared with the control values), which confirms the activation of lipid peroxidation processes in the presence of NO. If the gas mixture is diluted with air, the severity of this effect moderately decreases. In this case, the concentration of MDA rises up to 31% comparing with the control sample (p=0.027). The flow from the experimental NO generator, initially producing a concentration of 75 ppm, significantly increases the level of the metabolite (by 20% as compared with the control values, p<0.05). An increase in the MDA concentration has been observed for the water solution of DNIC, which is comparable with the MDA of the tenfold diluted gas stream from the Plazon apparatus (up by 32%, p=0.020).

For the studying of lipid peroxidation processes, we have evaluated the activity of a basic component of enzyme antioxidant protection, the superoxide dismutase system, which utilizes the superoxide anoin radical (Fig. 3). It has been revealed that all the options of bubbling the whole blood with NO-containing gas streams inhibit the activity of this system. The effect, however, varies significantly. Thus, treating the biological fluid with the strongest flow from the Plazon apparatus (concentration of nitrogen oxide – 800 ppm) caused the suppression of superoxide dismutase by 1.64 times as compared with the control sample (p=0.017), whereas decreasing the NO level to 80 ppm leveled these changes only moderately (the decrease in the activity of superoxide dismutase by 29% comparing with the control values of the intact blood, p=0.033). This effect was least observable when applying the flow from the experimental NO-generator as the gaseous phase. In this case, the activity of superoxide dismutase only decreased by 19% as compared with the control sample (p=0.041).

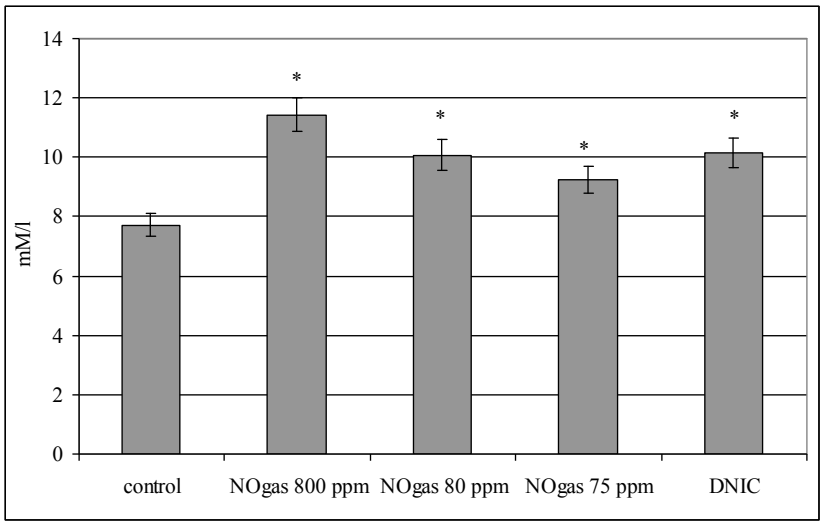


Fig. 2. Malonic dialdehyde level in erythrocyte under the action of free and bound nitric oxide

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

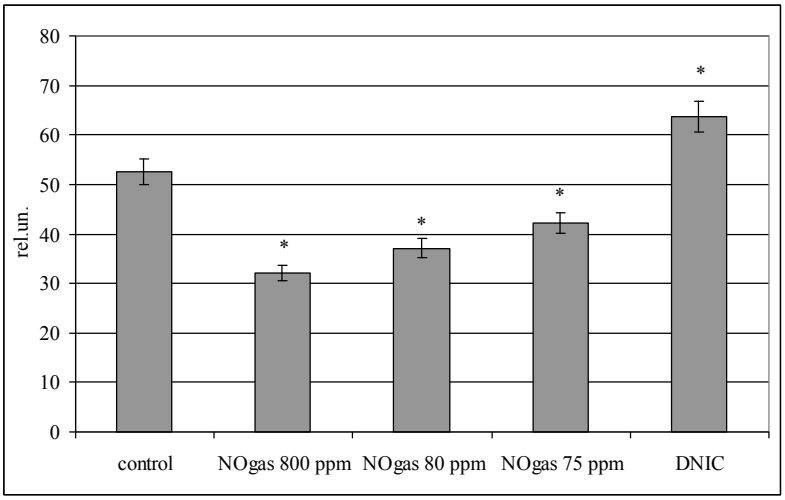


Fig. 3. Activity of erythrocyte superoxide dismutase under the action of free and bound nitric oxide

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

A fundamentally different type of changes in the functioning of the superoxide dismutase system was detected after administering the solution of DNIC into the blood. This effect caused a moderate activation of the enzyme up to 21% comparing with the intact level (p=0.038), which indicated improved conditions for the activity of superoxide dismutase system and could be due to the antioxidant properties of DNIC itself. As the authors have previously pointed out, nitrogen

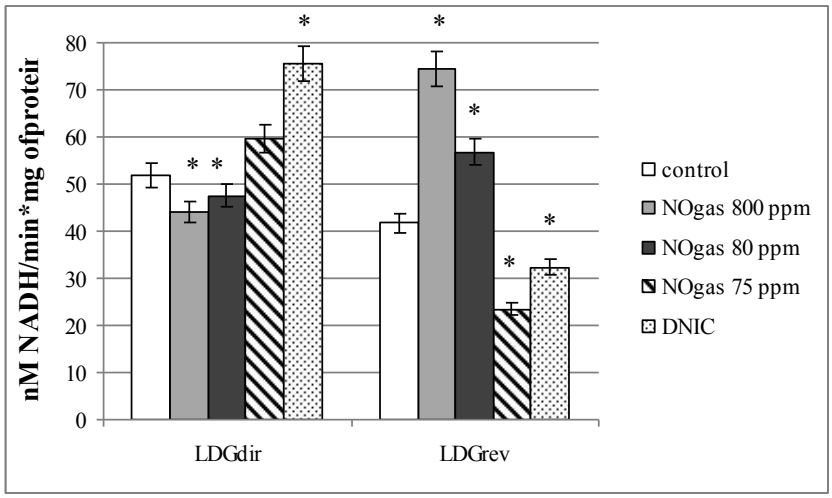


Fig. 4. Activity of erythrocyte lactate dehydrogenase in direct (LDGdir) and reverse (LDGrev) under the action of free and bound nitric oxide

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

oxide, which is part of these complexes, is able to react with superoxide anion to form peroxynitrite, persisting in the constitution of DNIC and not escaping into the environment [23]. Being part of DNIC, peroxynitrite can isomerize into nitrate, followed by its release outwards. This rules out the emergence of peroxynitrite in this environment, which in its autonomous state could produce cytotoxic agents after its protonation – the hydroxyl radical and the nitrogen dioxide.

With regard to the recovered glutathione, which was used in DNIC synthesis and could add to DNIC antioxidant potential [3,24], it appears from the method of synthesizing this compound [17] that during this procedure glutathione passes into its oxidized form, which does not have antioxidant effect.

The second component of the analysis consisted in assessing the intermediate stage of energy metabolism. We measured LDH activity in direct and indirect reactions (Fig. 4). It has been revealed that the gas flow generated by the Plazon apparatus contributes to the ferment activation of the reverse reaction with a simultaneous inhibition of the forward one. We view this trend as negative and confirming the data previously obtained [21,22,25]. At the same time, the initial gas flow causes much stronger changes in the activity of the enzyme than a tenfold dilution of the flow (p=0.040). It should be emphasized, however, that even in case of such dilution, the shift of the parameters studied remained significant as compared with the control sample (p=0.036).

On the contrary, processing the blood samples with a gas flow from the experimental generator with NO concentration comparable to the flow from Plazon diluted tenfold, contributes to an isolated activation of LDH forward reaction without any significant influence on the reverse, and increases the supply of pyruvate to the Krebs (tricarbonic acid) cycle.

The changes in the LDH activity accurately correspond to the results obtained when measuring the LDH substrate level in erythrocytes (Fig. 5). It should be pointed out that this parameter is more stable than the examined catalytic properties of the enzyme. Its maximum deviation (by 17.7% of the control values; p=0.044) was observed when the blood was being bubbled with a non-diluted gas flow from Plazon apparatus. The level of erythrocyte lactate increased to a lesser extent when processing the blood with a gas flow containing NO dose reduced tenfold (80 ppm). Still, this parameter remained significantly higher than the reference values (an increase of 14% as compared with the intact sample; p=0.039). When processing the blood with a flow from the NO experimental generator, we did not register any significant increase in the lactate concentration (by 9%; p=0.073). The parameter in question appeared lower as compared to

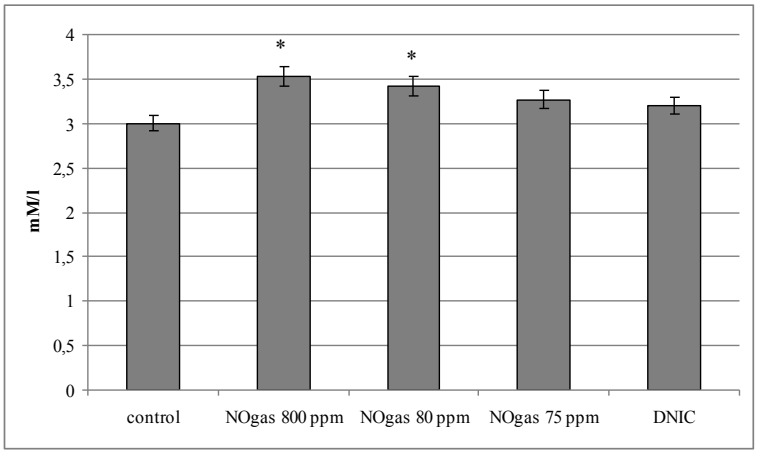
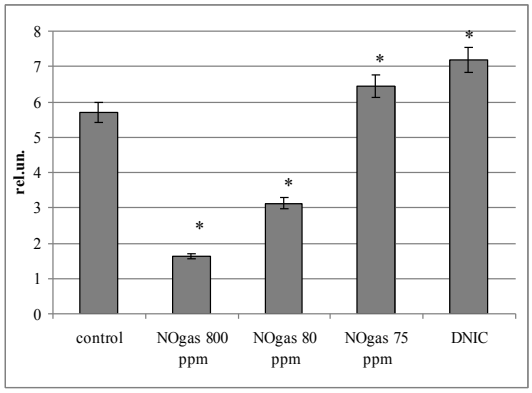


Fig. 5. Lactate level in erythrocyte under the action of free and bound nitric oxide

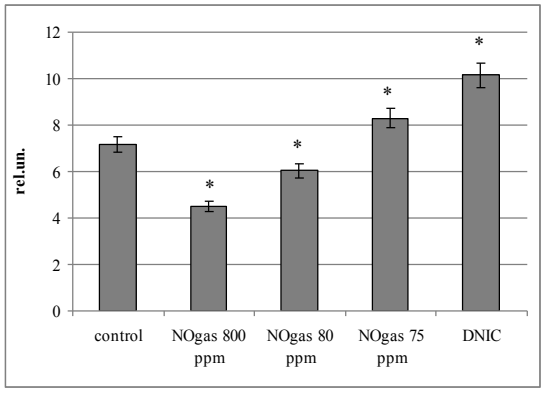
NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

bubbling the biological liquid with a non-diluted gas flow from Plazon (p=0.033). This confirms a better effect of the nitrogen oxide in low concentration (with no admixtures of oxygen in active forms) on the considered component of power metabolism.

To give a complex assessment of how power metabolism varies depending on the concentration of the agent studied, we used derivative coefficients describing both the LDH activity in both reactions and the current level of lactate (Fig. 6). It has been revealed that a gas flow containing 800 ppm NO (Fig. 6A) considerably reduces the CBER value (by 3.5 times; p=0.011), whereas a flow diluted ten times causes changes that are less manifest (reduction by 1.8 times; p=0,016 comparing to the



A. Coefficient of energy reaction balance



B. Coefficient of substrate support

Fig. 6. Coefficients of blood energy metabolism under the action of free and bound nitric oxide

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

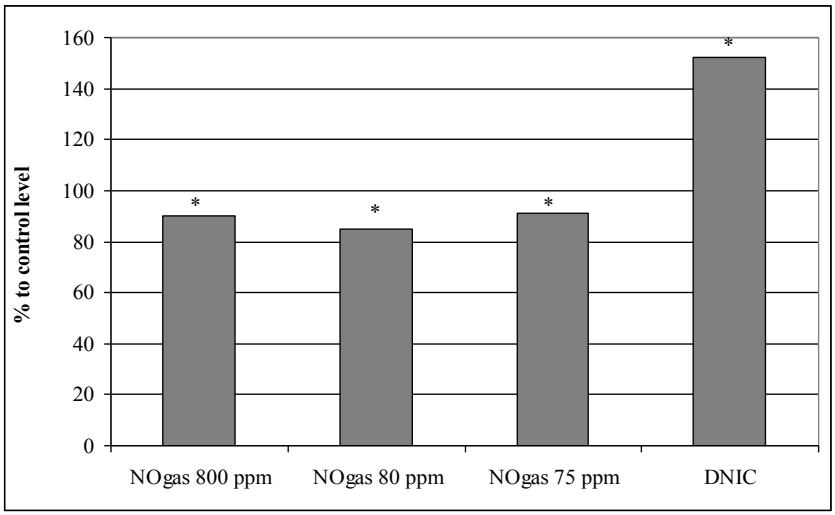


Fig. 7. Activity of erythrocyte aldehyde dehydrogenase under the action of free and bound nitric oxide (in percentage to the control sample taken as 100%)

NOgas 800 ppm – blood processing with air flow from «Plazon» (nitric oxide concentration - 800 ppm); NOgas 80 ppm – blood processing with tenfold diluted air flow from «Plazon» (nitric oxide concentration - 80 ppm); NOgas 75 ppm – blood processing with air flow from experimental NO-generator (nitric oxide concentration - 75 ppm); DNIC – injection of 3 mM glutathione-containing DNIC solution in blood sample («» - statistic value to burned rats without DNIC injections p<0.05)*

control sample and non-diluted flow). This trend indicates that the NO concentration which is achieved in the apparatus is too high for blood, resulting in a depressed energy metabolism of erythrocytes. If the concentration of the agent is reduced, it decreases the intensity of the negative effect.

An analysis of the CBER of blood processed with a gas flow from the experimental generator has revealed no depression but rather a moderate, yet significant, increase in the indicator considered (by 1.13 times; $p=0.048$). Similar changes were registered after introducing DNIC into the biological liquid, in which case we observed a 25.8% increase in CBER ($p=0.041$). This testifies to an optimizing effect of low-concentration gaseous nitrogen oxide and its repository form on the power metabolism of erythrocytes.

A similar trend was observed when considering the substrate presence coefficient, which characterizes the balance of LDH and one of its substrates (Fig. 6B).

The experiments conducted have also shown that various ways of blood processing have different impact on the activity of erythrocyte aldehyde dehydrogenase (AlDH) – the only enzyme of the human organism ensuring the biodegradation of nitrates (Fig. 7). In particular, if nitrogen oxide in its gaseous form is entered into the bioliquid samples, the catalytic properties of the enzyme become moderately inhibited (by 10-15% as compared with the level of native blood; $p<0.05$ for all cases), and this tendency does not depend on the dose of the influencing agent. It is worth noting that this effect is observed regardless of the NO generator specifications. It was observed both with the Plazon apparatus, which creates high concentrations of NO in combination with oxygen active forms, and with the experimental generator, which produces NO-containing air flow with significantly lower concentrations of the studied compound without admixtures.

Effects contrary to the described above were discovered for NO repository forms – dinitrosyl iron complexes serving as NO natural repositories (Fig. 7). When the human blood samples were impacted with DNIC solution, we observed activation of AlDH (by 53% of the level typical of the intact sample of biological liquid; $p=0.034$).

4. DISCUSSION

The present paper confirms our earlier suggestions as to the negative impact of the Plazon gas flow on the oxidizing and power metabolism of erythrocytes, and also on the function of detoxification enzymes in them. In our previous works we described the differentiated nature of how human whole blood responds to administering nitrogen oxide in various ways (in its gaseous or liquid phase) and from various sources (the Plazon certified apparatus for creating NO-containing cold plasma and the experimental NO generator developed in the Russian Federal Nuclear Centre) in terms of energy metabolism parameters [21,22,25]. These effects are due to the fact that, apart from the nitric oxide, the gaseous phase contains a considerable concentration of oxygen active forms, which produce highly toxic peroxynitrite when interacting. A number of supplementary studies testify to this fact [7,13,16,26]. With a view to this and given the need to find the optimum way of managing the NO level, which tends to change significantly in various morbid conditions, it is expedient to consider alternative paths of introducing the exogenous nitrogen oxide into the biosystems in vivo. Three basic methods are possible here: applying NO-containing gas flow purified of oxygen active forms to the greatest possible degree; using NO repository forms, which provide a gradual release of the compound in its free state; developing the potential of employing the physical or chemical stimulators of NO endogenous synthesis. The latter method has been realized, in particular, in Orbita device. However, despite the available data on its efficiency in various pathologies [27], it has proved difficult to dose the amount of nitrogen oxide, which is additionally formed under the influence of the electromagnetic field, and hence to match biological effects to it.

The first two methods specified appear to be more preferable, and they have been examined within the framework of the present research. A comparative analysis of the two NO generators, for which the concentrations of the active compound were synchronized, has permitted establishing the following: if the active forms of oxygen are removed from the gas mixture, its impact on the processes of lipid peroxidation in erythrocyte membranes improves. Despite the fact that bolus dosing of gaseous nitrogen oxide always stimulates free radical reactions in the biosystem (increasing the peroxide resistance of erythrocytes, boosting their MDA level), the effect

of the gas flow from the experimental apparatus developed in the Russian Federal Nuclear Centre can be recognized as beneficial, since its application does not result in an exhausted bioliquid antioxidant potential of the bioliquid and moderately stimulates lipid peroxide oxidation. On the contrary, high concentrations of NO (800 ppm) combined with active forms of oxygen (including ozone) in the air mixture, cause oxidizing stress in the blood plasma and erythrocyte membranes, and lead additionally to a depressed oxygen-transporting function of the latter, promoting the formation of nitrous hemoglobin [22,25].

The most positive response of the peroxide oxidation of lipids in the membranes of erythrocytes was observed when DNIC aqueous solution was entered into the biological liquid. In this case the amount of the active agent (in terms of NO) was similar to a tenfold dilution of the gas flows obtained from the Plazon apparatus and the experimental NO generator (9; 8 and 7.5 mcg respectively). Hence, having analysed the trends in the oxidizing metabolism of erythrocytes we can state the advisability of applying low concentrations of gaseous nitrogen oxide (<100 ppm) and the necessity to purify the gas flow from the admixture of oxygen-containing oxidizers. At the same time, the repository NO forms, including DNIC with glutathione ligands, boost the superoxide dismutase activity of erythrocytes, unlike gaseous airflow containing NO.

Similar tendencies have been observed in the energy metabolism of erythrocytes. After administering high doses of gaseous nitrogen oxide from the Plazon apparatus, the activity of lactate dehydrogenase in the forward reaction becomes depressed, which occurs along with the initiation of the reverse reaction and with an increase in the erythrocyte level of the lactate. Conversely, the impact of low concentrations of NO and, especially, of its repository form promotes positive changes of the indicators in question. It was completely confirmed by the calculation of the power metabolism derivative coefficients, which rose in case the blood is processed with a flow from the NO experimental generator of nitrogen oxide, or if dinitrosyl iron complexes are introduced into it.

It is worth noting that various options of NO administration exert multidirectional impact on the catalytic properties of aldehyde dehydrogenase – an enzyme combining the detoxification function (utilization of aldehydes) and the ability to biologically transform nitro-compounds of the organic nature into nitrogen oxide. Our studies have proved that NO, the product of the second reaction, promotes in its gaseous form a moderate inhibition of the enzyme activity regardless of the agent dose, whereas introducing DNIC to the blood samples causes the opposite effect. Thus, after introducing 0.3 μmol of DNIC there occurred dose-related stimulation of the enzyme catalytic properties, which, in our opinion, is due to the need to utilize the exogenous substrate [28]. We can assume that this is additionally promoted by a potential similarity of organic nitrates as the main enzyme substrates to the exogenous nitrosyl iron complexes, whose destruction in the human organism can partly be accounted by AlDH functioning [29].

These data allow us to demonstrate the differentiated nature of the reaction of red blood cells to exogenous nitric oxide, taking into account its state (free or bound in repository form) and dose. This is significant from the standpoint of the great interest of clinicians to NO-therapy, implemented mainly by inhalation. This situation determines the importance of careful analysis of molecular and cellular mechanisms of action of this bioregulator to clarify the indications for the purpose of this type of treatment. At the same time, there is no consensus in the world literature on the effectiveness of NO-therapy and spectrum of indications for it. This fact, in our opinion, is connected with the lack of control of the current level of NO and its metabolites in biological fluids, reflecting its amount in organs and tissues. At the same time, is the concentration of nitric oxide in biological tissues is having a significant temporal variability, can modulate the nature of their response to NO-stimulation. This factor in combination with the dose and form of exogenous nitric oxide will determine the total response of the body to the studied effect. Therefore, the logical task of further research should be to improve the methods of the monitoring of current level of nitric oxide in biological fluids, especially rapid tests. The solution of this task may be a key to personification of NO-therapy.

5. CONCLUSION

The research conducted have for the first time permitted establishing the characteristic features of how the oxidizing metabolism of erythrocytes

responds in vitro to processing with nitrogen monoxide in its free and repository forms (the latter being part of DNIC, the natural carrier of the compound). These features include a strongly marked pro-oxidative action of the gas flow from the Plazon apparatus, which manifests itself in erythrocyte membranes and is moderately levelled in case of a tenfold dilution of the NO-containing mixture. The application of the NO experimental generator yields the minimum pro-oxidatic activity in the considered bio-object, while the administration of DNIC aqueous solution induces a moderate antioxidant action, which occurs in erythrocytes due to an increase in the activity of the superoxide dismutase system. The results of experiments are indicative of the membrane-protecting effect of the NO-containing air mixture from the experimental generator, and of the DNIC aqueous solution as well.

Processing blood with a gas flow containing nitrogen monoxide in a concentration of 800 ppm depresses the power metabolism of erythrocytes; however, diluting the flow ten times reduces the intensity of such impact, which suggests a dose-related effect. The presence of oxygen active forms in the flow is a no less important factor determining the manner, in which power production processes in a cell react to the influence of nitrogen oxide. Therefore, the application of a gas flow free from the admixture of oxygen active forms stimulates the function of LDH in erythrocytes.

CONSENT

As per international standard or university standard written patient consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

This study was approved by Local Ethical Committee of Privolzhsky Federal Medical Research Center (protocol №11 from 24/07/17).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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