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BIOPHYSICS OF COMPLEX SYSTEMS

Experimental Investigation of Some Systemic Effects of Nitric Oxide Inhalation

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Abstract—The goal of this work was to study the influence of nitric oxide inhalation on parameters of blood pro- and antioxidant systems in rats under both an intact condition and experimental thermal injury. We studied 40 Wistar rats that were divided into four equal groups. The intact group was subjected to no manipulation exñept a single blood sampling, main group I was subjected to inhalation of a air mixture containing 20 ppm of nitric oxide for 10 days, the control group was subjected to thermal injury and conventional treatment, and main group II was subjected to thermal injury and daily inhalation of nitric oxide (20 ppm) for 10 days. We studied the intensity of lipid peroxidation in the blood plasma, the total antioxidant activity, the peroxide resistance of erythrocytes, the level of malondialdehyde in the blood plasma and erythrocytes, and the activity of superoxide dismutase. It was shown that daily inhalations of a mixture containing a low concentration of nitric oxide (20 ppm) modified blood oxidative metabolism in healthy and burned rats. We hypothesized that the activation of lipid peroxidation in erythrocytes accompanied by a pronounced increase in the catalytic activity of superoxide dismutase is a unified response of healthy and burned rats to exogenous nitric oxide exposure. We also observed a moderate prooxidant effect in the blood plasma of healthy animals comparable to that in the erythrocytes of these rats. In the case of thermal injury, oxidative stress tended to be corrected after the end of the course of inhalation.

Keywords: nitric oxide, inhalation, oxidative metabolism, lipid peroxidation, antioxidant activity, malondialdehyde

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INTRODUCTION

Inhalation exposure is one of the most affordable noninvasive methods for studying the systemic effects of different compounds [1]. This approach is most suitable for gaseous compounds. Therefore, inhalation may be a component of a model of the systemic effect that is caused by different physico-chemical agents [1–4].

In recent years, our team has been carrying out comprehensive studies of the biological effects of various oxygen and nitrogen reactive forms in the free and bound state (in the latter case, in iron-containing and cytochrome nitrosyl complexes [5, 6]). In particular, we showed that these agents have different effects on the balance of pro- and antioxidant systems in the blood in vitro [7]. We also found that this modification of homeostasis occurred under intraperitoneal injection of aqueous dinitrosyl complexes with glutathione ligands in healthy and burned rats [8]. At the same time, systemic effects of nitric oxide inhalation have been studied insufficiently in few experimental studies [2]. It should be noted that in recent decades this impact is a component of the treatment algorithm for various acute and chronic lung pathologies [9–11]. The efficacy of gaseous nitric oxide (NO) in these cases is still a matter of debate [9].

Special attention should be paid to the strong correlation between the metabolic roles of nitric oxide and reactive oxygen species, which are involved in common cellular regulatory cascades [12, 13]. The features of this interaction acquire great importance when an imbalance in the pro- and antioxidant systems is formed, including oxidative stress, which is a universal component of different pathologies [12, 14, 15]. In this respect, it is interesting to assess the effect of exogenous administration of nitric oxide on the oxidative metabolism.

The goal of this work was to study the effects of nitric oxide inhalation on the parameters of the blood pro- and antioxidant systems of healthy and burned rats.

MATERIALS AND METHODS

The study was performed using 40 sexually mature male Wistar rats (220–250 g) divided into four equal groups. The intact group was subjected to no manipulation except a single blood sampling, main group I

was subjected to inhalation of an air mixture containing 20 ppm of nitric oxide, the control group was subjected to thermal injury and conventional treatment, and main group II was subjected to thermal injury and daily inhalation of nitric oxide (20 ppm).

In the case of the control group and main II group, the combined injury was simulated according to the previously developed method [16], which consisted of contact thermal burns to the skin of the back (20% of the body surface) in combination with thermal-inhalation injury. The keeping of animals and the experimental intervention was performed according to the order of Ministry of Health of the Soviet Union no. 775 from August 12, 1977. The injury was performed under combined anesthesia (oletyl + xyla). The rats were then randomly divided into two equal groups. Starting on the first day after the thermal injury, the animals of the control group were subjected to daily intraperitoneal injections of physiological solution (3 mL). The animals of the main group II received treatment vie inhalation of an air mixture containing nitric monoxide (20 ppm) in addition to physiological solution, as in the case of the control group. The duration of the therapy for the animals of both groups was 10 days.

The NO-containing air mixture was prepared using an experimental generator (Russian Federal Nuclear Center Research Institute of Experimental Physics, Sarov, Russia) [17]. On the next day after completing the full course of inhalation, the animals were decapitated under anesthesia.

The blood of the animals was stabilized by 3.8% aqueous sodium citrate at the ratio of 1 : 9. The red-blood-cell mass was obtained by centrifugation of the blood at 3000 rpm for 10 min. Erythrocytes were washed three times with an isotonic sodium chloride solution.

The activity of the pro- and antioxidant systems was studied in the rat blood plasma by Fe^{2+}-induced biochemiluminescence on a BCL-06 instrument (Nizhny Novgorod, Russia). The evaluation parameters were the biochemiluminescence light sum for 30 s, which was considered as a criterion of the intensity of lipid peroxidation and the slope of the kinetic curve of chemiluminescence $\tan 2\alpha$, which is traditionally identified with the total antioxidant activity of antioxidant systems. In addition, we evaluated lipoperoxidation in the erythrocytes, i.e., peroxide resistance in washed erythrocytes, similarly to the assessment of the value of lipid peroxidation in the blood plasma. Integrated data on the balance of the pro- and systems was obtained by the calculation of the oxidation potential, which is the ratio of the biochemiluminescence light sum to $\tan 2\alpha$.

The concentration of malondialdehyde in the blood plasma and erythrocytes was evaluated by the Sidorkin and Chuloshnikova method (1993).

The activity of superoxide dismutase was evaluated by the Sirota method (1999).

The statistical analysis of the results was performed by the Statistica 6.0 program. The normality of the distribution of the parameters were evaluated using the Shapiro-Wilk test. The Kruskal-Wallis test was used to assess the statistical significance taking the distribution of the parameter into account.

RESULTS

We studied the effect of inhalation of nitric oxide at low concentrations on the balance of the pro- and antioxidant blood systems in healthy animals. The results showed that this action resulted in a moderate but significant increase in the intensity of lipid peroxidation in the form of a change in the Fe-induced biochemiluminescence in the blood plasma samples by 19.2% as compared to healthy rats ($p < 0.05$). The prooxidant effect was also confirmed by a decrease in the total antioxidant activity of the biomedium by 25.7% relative to the physiological values ($p < 0.05$). At the same time, one of the most stable markers of the lipid peroxidation intensity, i.e., malondialdehyde concentration in the blood plasma, does not tend to an increase and is at the same level as in animals of the intact group.

The lipoperoxidation processes were also intensified in erythrocytes of healthy rats after the course of nitric oxide inhalation, which was identified by an increase in both peroxide resistance of erythrocytes by 28.4% and the concentration of malondialdehyde by 49.1% as compared to these values in the intact animals ($p < 0.05$ in both cases). The increase in the activity of superoxide dismutase by 56.1% relative to that in healthy rats ($p < 0.05$) indicates the adequacy of the metabolic response of red blood cells in this variant of the NO stimulation. Thus, a moderate prooxidant effect of NO inhalation on oxidative metabolism in blood occurred. In our opinion, however, this effect can be considered as training.

Simulation of burn disease as a result of a thermal injury promoted pronounced oxidative stress. This was confirmed by a distinct activation of lipid peroxidation in the blood plasma (by 67.5% as compared to the intact rats, $p < 0.05$) and an increase in malondialdehyde concentration in the plasma and erythrocytes (by 103.1 and 13.3%, respectively, $p < 0.05$ in both cases), which was accompanied by decreasing the catalytic activity of superoxide dismutase (by 53.4% relative compared to healthy rats, $p < 0.05$).

Unlike the intact animals, the rats that had the combined thermal injury showed a fundamentally different response to the inhalation effect. In particular, the intensification of lipid peroxidation after NO inhalation was found in healthy animals, whereas in rats of main group II, the studied impact led to a pronounced antioxidant effect. We observed the normal-

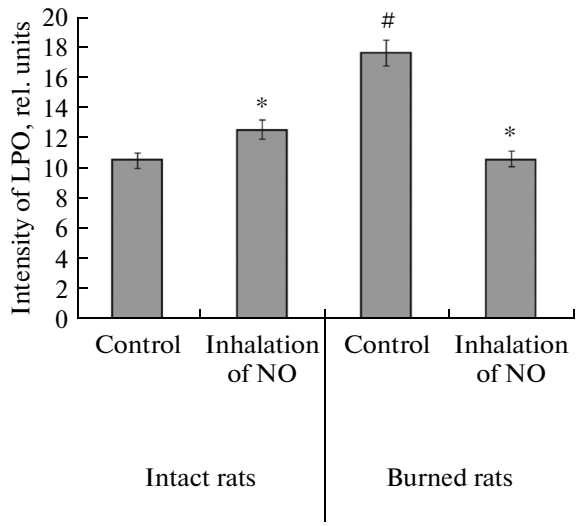

Fig. 1. The intensity of lipid peroxidation in the blood plasma of healthy and burned rats (* designates a statistically a significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

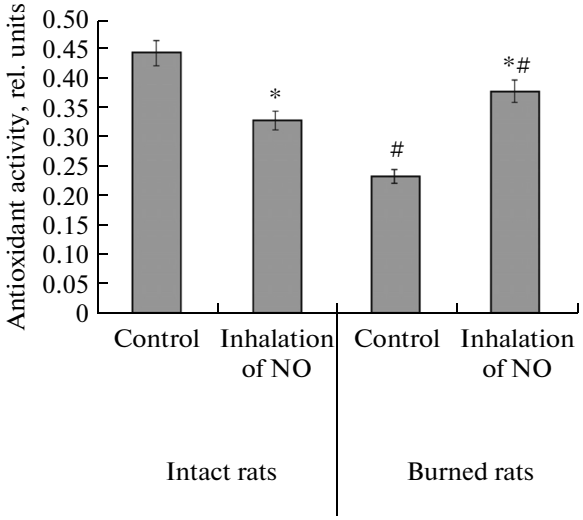

Fig. 2. Total antioxidant activity in blood plasma of healthy and burned animals (* designates a statistically significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

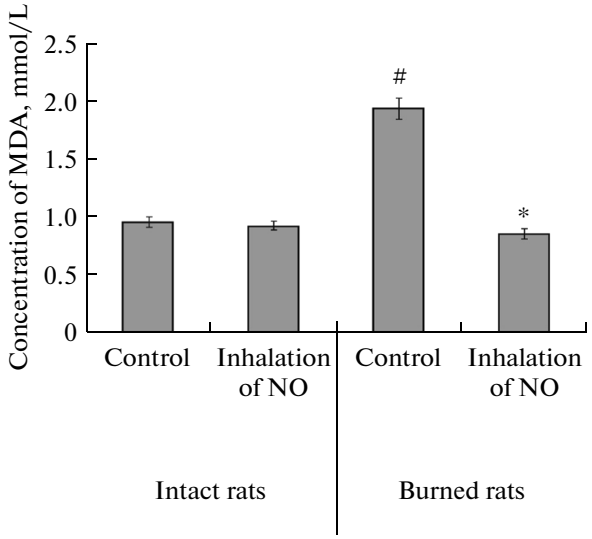

Fig. 3. The concentration of malondialdehyde in blood plasma of healthy and burned rats (* designates a statistically significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

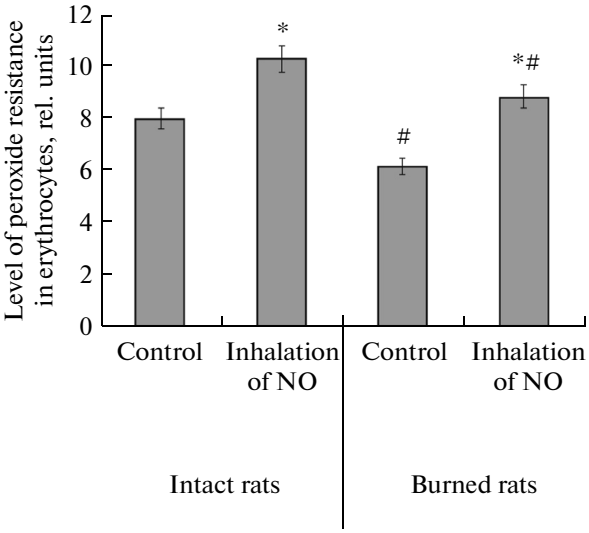

Fig. 4. The level of peroxide resistance in erythrocytes in healthy and burned rats (* designates a statistically significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

ization of lipid peroxidation in the blood plasma to physiological values (Fig. 1) and the pronounced increase in the total antioxidant activity in the blood plasma (Fig. 2). The combination of these two parameters indicates the corrective action of inhaled nitric oxide on oxidative stress that develops as a result of a severe thermal injury. A similar effect was identified for the malondialdehyde concentration during NO inhalation therapy (Fig. 3). Its concentration was found to decrease by a factor of 2.27 compared to rats that did not receive NO inhalation ($p < 0.01$), although this parameter did not differ from the values for the intact healthy animals at the end of the treatment ($p < 0.05$).

The dynamics of lipid peroxidation in erythrocyte membranes were evaluated by their peroxide resistance using the biochemiluminescence method. The resultant characteristics were somewhat different as

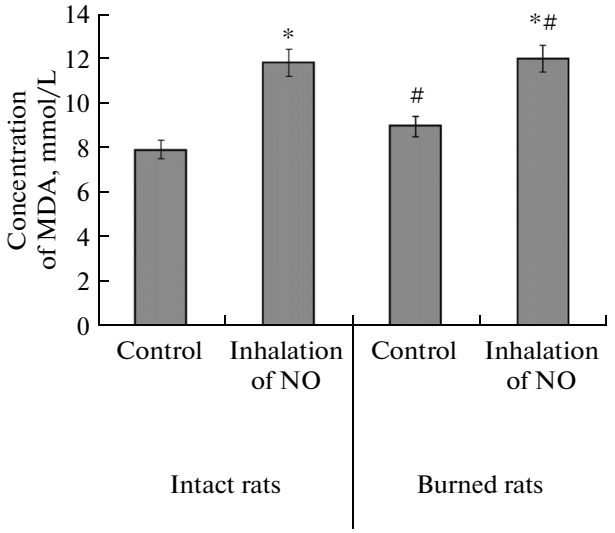

Fig. 5. The concentration of malondialdehyde in erythrocytes of healthy and burned rats (* designates a statistically significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

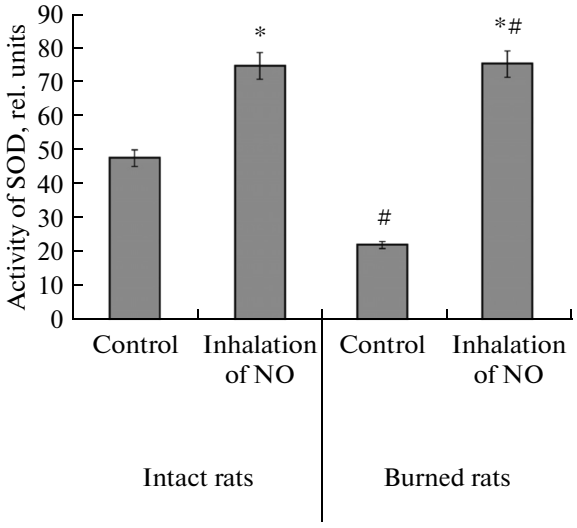

Fig. 6. The activity of superoxide dismutase in erythrocytes of healthy and burned rats (* designates a statistically significant difference relative to rats that were not treated with inhalation, $p < 0.05$, # designates a statistically significant difference relative to intact rats, $p < 0.05$).

compared to those in the blood plasma but similar to those in healthy rats after NO inhalation (Fig. 4). The value of this parameter was lower in animals of the control and main II groups as compared to that in intact rats before and after the course of NO inhalation, respectively.

The relationships for lipid peroxidation in erythrocytes are fully confirmed by the evaluation of the malondialdehyde concentration (Fig. 5). At the same time, the induction of lipid peroxidation in erythrocytes was compensated by the significant activation of antioxidant enzymes, particularly, superoxide dismutase (Fig. 6). Its catalytic activity was shown to be significantly reduced under severe thermal injury and to be recovered after the course of nitric oxide inhalation. In the latter case, this activity exceeds this value in burned animals that did not receive NO inhalation by many times (by a factor of 3.42, $p < 0.01$). This suggests that the effect of the inhalation NO therapy on oxidative metabolism erythrocytes in both burned and healthy rats is primarily stimulation their own antioxidant potential of the pool of blood cells.

DISCUSSION

NO inhalation for the correction of respiratory distress syndrome in newborns and pulmonary hypertension in adults has been used empirically for the past 15–20 years [3, 9–11]. This treatment is based on the vasodilatory properties of nitric oxide [15, 18]. Nevertheless, the mechanism of the therapeutic effect is not well studied and has been described in only a few foreign publications [9]. It should also be noted that the efficacy of NO inhalation in the treatment of the above pathologies is periodically disputed and not always confirmed by large meta-analyses of the therapeutic results [9]. A more detailed disclosure of the mechanisms and effects of using gaseous nitric oxide for inhalation is needed. The dose dependence and the influence of the initial NO concentration on the therapeutic results should be taken into account [2, 5, 7]. It seems appropriate to study the effects of nitric oxide in physiological conditions based on the model of systemic pathology, which can be burn disease. This disease is known to be accompanied by severe functional and metabolic disorders involving endogenous intoxication and oxidative stress, which play a key role. In this work, we compared the effect of nitric oxide inhalation at low concentrations (20 ppm) on lipid peroxidation in the blood of healthy and burned Wistar rats.

The results demonstrated that the reaction of oxidative metabolism in the blood in response to NO inhalation has both common and specific characteristics when studying rats in physiological conditions and after a thermal injury. In both cases, we observed the same response to the external NO stimulation, i.e., a significant activation of lipid peroxidation in the erythrocyte membranes, which was seen as a pronounced increase in both the peroxide resistance of erythrocytes and malondialdehyde concentration. On the other hand, this reaction occurs on the background of the prevailing stimulation of antioxidant enzymes, mainly erythrocyte superoxide dismutase. Its activity after the course of NO inhalation exceeded that in the control group by a factor of 3.37 ($p < 0.01$). This suggests that the antioxidant properties of exogenous NO in erythrocytes are not the consequence of its

own antioxidant activity but are mediated through the activation of antioxidant enzymes in these blood cells.

A fundamentally different mechanism of nitric oxide action at low concentrations on the pro- and antioxidant balance occurs in the blood plasma. In this case, we observed specific features of the response to NO inhalation in healthy and burned rats. A moderate prooxidant effect was found in healthy rats, whereas in the case of the burn disease model, we observed the inhibition of oxidative stress, i.e., a significant reduction in the intensity of lipid peroxidation in combination with an increase in the antioxidant potential in the blood plasma. It is important to note that there is a distinct activation of the catalytic properties of superoxide dismutase in both cases. This suggests the possibility of direct action of increasing NO concentrations on the activity of this enzyme, which was confirmed by model experiments on endothelial cells [19] and experiments with the use of extracellular superoxide dismutase [20]. Stimulation of the gene expression of this enzyme is also possible during the course of treatment of animals with gaseous nitric oxide [20, 21].

Moreover, NO inhalation makes it possible to replenish the plasma pool of deposited forms of nitric oxide (mainly, dinitrosyl iron complexes [18]), whose concentration dramatically decreases during thermal injury [22]. Oxidative stress caused by burn disease is inhibited due to the pronounced antioxidant properties of nitric oxide and its complexes [23, 24]. Thus, we proposed a two-component mechanism of the action of inhaled nitric oxide on oxidative metabolism in the blood based on the influence on the activity of superoxide dismutase and the replenishment of the endogenous pool of nitric oxide.

CONCLUSIONS

We showed that inhalation of a gaseous mixture containing nitric oxide at low concentration (20 ppm) leads to modification of blood oxidative metabolism in healthy and experimentally burned rats. This effect has both unified and specific features. The common reaction to exogenous administration of NO in healthy and burned rats is the activation of lipid peroxidation in erythrocytes, which is accompanied by a significant increase in the activity of superoxide dismutase. We hypothesized that this enzyme might be the main molecular target for the action of exogenous nitric oxide in erythrocytes.

The specific response to inhaled NO is due to an imbalance in the pro- and antioxidant systems of the blood plasma. It was found that the NO treatment of healthy animals provides a moderate prooxidant effect (the intensification of lipid peroxidation, a decrease in the total antioxidant activity, and an increase in malondialdehyde concentration) in the blood plasma and erythrocytes. In contrast, the effects of oxidative stress were corrected in burned rats after the inhalation course. This was confirmed by biochemiluminescent analysis and by the evaluation of the malondialdehyde concentration. The observed effect may be due to the replenishment of the endogenous pool of nitric oxide (mainly, dinitrosyl iron complexes and S-nitrosothiols), which has antioxidant properties.

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