ФАГОЦИТАРНАЯ АКТИВНОСТЬ МОНОЦИТОВ ПЕРИФЕРИЧЕСКОЙ КРОВИ В УСЛОВИЯХ *IN VIVO* И *IN VITRO* ГИПОКСИИ У ВЫСОКОУСТОЙЧИВЫХ И НИЗКОУСТОЙЧИВЫХ К НЕДОСТАТКУ КИСЛОРОДА КРЫС

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Резюме. Известно, что существуют индивидуальные различия устойчивости к гипоксии, которые могут определять предрасположенность к развитию и тяжесть течения различных заболеваний, в том числе инфекционно-воспалительных и опухолевых. Стандартизованных способов оценки устойчивости к гипоксии экспериментальных животных и людей без гипоксического воздействия не существует. Поиск молекулярно-биологических маркеров, позволяющих выявить людей с различной устойчивостью к дефициту кислорода в условиях нормоксии или при умеренном гипоксическом воздействии, несомненно целесообразен. Возможно, что оценка исходной устойчивости к гипоксии позволит прогнозировать развитие и тяжесть течения заболеваний, механизмы которых связаны с кислородной недостаточностью. Одним из способов оценки устойчивости организма к гипоксии без воздействия в барокамере или в условиях гор может быть моделирование гипоксии *in vitro*. Цель исследования – охарактеризовать фагоцитарную активность моноцитов периферической крови у высокоустойчивых и низкоустойчивых к гипоксии крыс Wistar в условиях нормоксии, а также после гипоксического воздействия *in vitro* и *in vivo*. Устойчивость крыс к гипоксии определяли по «времени жизни» животных «на высоте» 11 500 м в барокамере. Через месяц после определения устойчивости к гипоксии одну группу крыс помещали в барокамеру на высоту 5000 м на 1 час для моделирования гипоксического состояния *in vivo*, а у другой группы крыс получали кровь из хвостовой вены для моделирования гипоксического состояния *in vitro* в условиях 1% кислорода в течение 1 часа. Проводили оценку фагоцитарной активности моноцитов периферической крови методом проточной цитофлуориметрии. Показано, что в условиях нормоксии у исходно высокоустойчивых и низкоустойчивых к гипоксии крыс фагоцитарная активность моноцитов не различалась. Фагоцитарная активность моноцитов после *in vitro* и *in vivo* гипоксического воздействия была выше у высокоустойчивых к гипоксии животных по сравнению с низкоустойчивыми. Увеличение фагоцитарной активности моноцитов по сравнению с условиями нормоксии наблюдалось только у высокоустойчивых крыс в условиях *in vitro* гипоксического воздействия. Полученные результаты свидетельствуют о том, что высокоустойчивые

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и низкоустойчивые к гипоксии организмы различаются по фагоцитарной активности моноцитов в условиях недостатка кислорода, что может определять течение воспалительных и опухолевых заболеваний. При поиске маркеров устойчивости организма к гипоксии целесообразно использовать моделирование гипоксии *in vitro*.

Ключевые слова: моноциты, фагоцитоз, in vitro, устойчивость к гипоксии, фагоцитарная активность, крысы

PHAGOCYTIC ACTIVITY OF PERIPHERAL BLOOD MONOCYTES UNDER *IN VIVO* AND *IN VITRO* HYPOXIA CONDITIONS IN TOLERANT AND SUSCEPTIBLE TO OXYGEN DEFICIENCY RATS

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Abstract. It is known that there are individual differences in resistance to hypoxia, which can determine the predisposition to the development and severity of various diseases, including infectious, inflammatory and tumor. There are no standardized methods for assessing resistance to hypoxia in experimental animals and humans without hypoxic exposure. The search for molecular-biological markers, identifying people with different resistance to oxygen deficiency under normoxic conditions or under moderate hypoxic exposure is undoubtedly efficient. It is possible that the assessment of the basic resistance to hypoxia can help to predict the development and severity of the course of diseases, the mechanisms of which are associated with oxygen deficiency. One of the methods to assess organism resistance to hypoxia without exposure in a decompression chamber or in highland conditions can be modeling hypoxia *in vitro*. The aim of the study was to characterize the phagocytic activity of peripheral blood monocytes in tolerant and susceptible to hypoxia Wistar rats under normoxic conditions, as well as after hypoxic exposure *in vitro* and *in vivo*. The resistance of rats to hypoxia was determined by the gasping time at an altitude of 11.500 m in a decompression chamber. A month after determining the resistance to hypoxia, one group of rats was placed in a decompression chamber at an altitude of 5,000 m for 1 hour to simulate the hypoxic state *in vivo*. Blood from the tail vein of the other group of rats was placed in 1% oxygen for 1 hour to simulate the hypoxic state *in vitro*. The phagocytic activity of peripheral blood monocytes was assessed by flow cytometry. It was demonstrated that phagocytic activity of monocytes did not differ in tolerant and susceptible to hypoxia rats under normoxic conditions. The phagocytic activity of monocytes after *in vitro* and *in vivo* hypoxic exposure was higher in tolerant to hypoxia animals in comparison to susceptible ones. An increase in the phagocytic activity of monocytes compared to normoxia conditions was observed only in tolerant rats under *in vitro* conditions of hypoxic exposure. The obtained results indicate that tolerant and susceptible to hypoxia organisms differ in the phagocytic activity of monocytes under conditions of oxygen deficiency, which can determine the course of inflammatory and tumor diseases. The data obtained will be the basis for further experimental investigations organism hypoxia resistance markers.

Keywords: monocytes, phagocytosis, in vitro, resistance to hypoxia, phagocytic activity, rats

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Introduction

Hypoxia, or oxygen deficiency, can develop in any pathological condition of the body, including infectious diseases, systemic inflammatory response, sepsis, acute respiratory distress syndrome, and the new coronavirus infection [4]. The typical cell response to oxygen deficiency is based on the activation of the signaling pathway of the hypoxia inducible transcription factor HIF (Hypoxia-Inducible Factor). According to literature, both animals and humans differ in individual sensitivity to oxygen deficiency, as well as in the content of HIF-1 [3, 8, 15]. Individual resistance to hypoxia can determine the severity of infectious and inflammatory diseases. We have previously demonstrated that the systemic inflammatory response is more severe in susceptible to hypoxia rats than in tolerant animals [3].

Currently, there are no standardized methods for assessing resistance to hypoxia in experimental animals and people without hypoxic exposure. One of the most common methods for determining resistance to oxygen deficiency is a model that reproduces the conditions of hypobaric hypoxia in decompression chambers in which volunteers, pilots, astronauts or experimental animals are placed [3, 6, 7, 9, 12]. The common way to assess the human resistance to hypoxia is the height exposure of several thousand meters and the division of subjects into resistant and sensitive to hypoxia, depending on their susceptibility to acute mountain sickness and high altitude pulmonary edema [9, 12].

Due to the fact that sensitivity to O_2 deficiency determines physiological reactions, adaptive processes, and, obviously, the course of a number of diseases, the search for markers for screening the body for resistance to oxygen deficiency without hypoxic exposure, placement in a decompression chamber or in highland conditions, is an urgent task. Determination of molecules that provide resistance or sensitivity to stress factors such as hypoxia plays an important role in assessing adaptive capabilities under hypoxic exposure of varying severity. Taking this into consideration, the search for molecular biological markers that make it possible to determine individual resistance to oxygen deficiency in humans under normoxic conditions or under moderate hypoxic exposure is undoubtedly expedient. One of the ways to assess resistance to hypoxia without exposure in a decompression chamber or in highlands can be modeling hypoxia *in vitro*.

Oxygen deficiency and activation of HIF-1 regulates glycolysis and energy metabolism, which affects the functioning of innate and adaptive immunity cells, in particular, promotes the inhibition of apoptosis, an increase in phagocytic activity and the migration of neutrophils and macrophages [11, 13].

Since, as mentioned above, organisms with different resistance to hypoxia are characterized by different levels of HIF-1 activation, it is likely that the functional activity of neutrophils and monocytes also differs. Features of the functional activity of monocytes in animals with different resistance to hypoxia can affect the severity of inflammatory and alterative damage in infectious and inflammatory diseases, in particular, in a systemic inflammatory response. However, data on the phagocytic activity of monocytes in tolerant and susceptible to oxygen deficiency rats under conditions of normoxia, as well as hypoxia, are not presented in the literature.

The aim of the study was to characterize the phagocytic activity of peripheral blood monocytes in tolerant and susceptible to hypoxia rats under normoxic conditions and after hypoxic exposure *in vivo* and *in vitro*.

Materials and methods

The study enrolled adult male Wistar rats (3 months old, body weight 220-250 g, n = 50) and was approved by Bioethics Committee at the Avtsyn Research Institute of Human Morphology (Protocol No. 36 (12), March 28, 2022). All efforts were made to decrease suffering and possible stress for the animals and performed in accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The reporting in the manuscript follows the ARRIVE guidelines.

Hypoxia resistance was assessed through response to oxygen deprivation using decompression chamber test as described previously [3, 8]. The animals were exposed, one at a time, to simulated hypobaric hypoxia equivalent to 11,500 m altitude (180 mmHg) using a mercury barometer-coupled decompression chamber. All decompressions and recompressions were achieved gradually at a rate of 600 m (≈ 40 mmHg)/min to exclude effects of sharp changes, at 2 L/min airflow and 40-50% relative humidity. Time length till the first sign of characteristic hyperventilatory response ("gasping time") was recorded using an electronic stopwatch. Based on gasping time, the animals were assigned to three groups: "susceptible" (< 80 s, n = 15), "normal" (80-240 s, n = 23) and "tolerant" (> 240 s, n = 12). The "normal" group was excluded from subsequent experiments. All rats survived the decompression chamber test and resumed their normal activity without signs of trauma.

A month after determining the resistance to hypoxia [3, 8], in order to simulate the hypoxic state *in vivo*, one group of rats was placed in a decompression chamber at an altitude of 5,000 m for 1 hour, then blood was taken from the tail vein under Zoletil anesthesia (Virbac Sante Animale, France). Blood from the tail vein of the other group of rats was placed in 1% oxygen for 1 hour to simulate the hypoxic state *in vitro*. The phagocytic activity of peripheral blood monocytes was assessed after *in vivo* and *in vitro* exposures by flow cytometry using the IngoFlowEx kit (Exbio Diagnostics, Czech Republic).

Statistica 8.0 software was used for statistical processing of the obtained data. Data were expressed as median and interquartile range Me ($Q_{0.25}$-$Q_{0.75}$). Since the data were not normally distributed, the Mann–Whitney, Kruskal–Wallis, and Dunn non-parametric tests were used to establish the significance of differences between the indicators. Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Under normoxic conditions, the phagocytic activity of monocytes did not differ in tolerant and susceptible to hypoxia rats (Table 1). The phagocytic

TABLE 1. PHAGOCYTIC ACTIVITY OF MONOCYTES UNDER NORMOXIC CONDITIONS, AFTER *IN VIVO* AND *IN VITRO* HYPOXIC EXPOSURE IN ANIMALS WITH DIFFERENT OXYGEN DEFICIENCY RESISTANCE, Me ($Q_{0.25}$-$Q_{0.75}$)

Phagocytic activity of monocytes, %	Tolerant	Susceptible	p
Normoxia	4.4 (4.2-5.7)	4.5 (3.8-7.3)	0.89
***In vivo* hypoxic exposure**	7.0 (6.2-8.8)	2.7 (1.6-3.5)	0.02
***In vitro* hypoxic exposure**	17.7 (9.7-18.8)*	3.5 (1.6-8.0)	0.015

Note. p, statistical significance of differences between tolerant and susceptible to hypoxia rats, Mann–Whitney test; *, statistically significant differences compared with normoxia, Kruskal–Wallis and Dunn test.

activity of monocytes after *in vivo* and *in vitro* hypoxic exposure was higher in tolerant to hypoxia animals compared to susceptible ones. The functional activity of monocytes is largely determined by the activation of the HIF-1 [11, 13]. We have previously demonstrated that after acute hypoxic exposure, the content of HIF-1 in the blood serum and its expression in the liver are more pronounced in tolerant to hypoxia rats compared to susceptible ones, which ensures adaptation to hypoxia [2]. Probably, in monocytes of tolerant to hypoxia rats, under conditions of oxygen deficiency, a more pronounced activation of HIF-1 occurs, which leads to an increase in their phagocytic activity. Differences in the phagocytic activity of peripheral blood monocytes after hypoxic exposure can determine the course of the systemic inflammatory response, in which innate immune cells play a key role.

It should be noted that a statistically significant increase in the phagocytic activity of monocytes compared to normoxia conditions was observed only in tolerant rats under *in vitro* conditions of hypoxic exposure. Thus, *in vitro* hypoxic exposure using lower oxygen concentrations has a more pronounced effect on blood cells compared to *in vivo* hypoxia and avoids acute hypoxic effects on the body. The pronounced effect of hypoxic exposure *in vitro* is probably due to lower oxygen concentrations, as well as the absence of adaptive changes on the part of the whole organism, such as the release of hormones, activation of the antioxidant defense system, etc. The use of *in vitro* hypoxic exposure on blood cells can be a promising method for determining the organism's resistance to oxygen deficiency. Therefore, when searching for markers of resistance to hypoxia, it is advisable to use hypoxia modeling *in vitro*.

It was demonstrated that short-term (within 2 hours) exposure to systemic hypoxia (5.500 m) on the body of healthy volunteers increases the phagocytic activity of neutrophils, but not monocytes [5]. However, in this work, individual resistance to hypoxia and gender characteristics were not taken into account; both men and women were included in the studies. Later, it was demonstrated that exposure to hypoxia (0.5-3% oxygen) *in vitro* during 24 hours does not affect the phagocytic activity of neutrophils in healthy volunteers [14]. At the same time, an increase in the phagocytic activity of monocytes under hypoxic conditions was demonstrated by many researchers [1, 10].

However, these studies did not take into account individual resistance to hypoxia. The revealed differences in the functional activity of peripheral blood phagocytes in animals with different resistance to hypoxia should be taken into account when developing new approaches to the prevention and treatment of infectious and inflammatory diseases.

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