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Effect of cold helium plasma on the catalytic activity of certain erythrocyte dehydrogenases of rat blood

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Abstract: *The work is aimed at clarifying the effect of cold helium plasma on the catalytic properties of lactate- and aldehyde dehydrogenase in rat blood erythrocytes. The effect was studied in 20 white Wistar rats. Upon completion of the full course of exposures (1 exposure per day for 5 days), blood samples were taken from all animals with subsequent erythrocyte isolation performed by standard differential centrifugation for assessing the activity of lactate dehydrogenase (LDH) and aldehyde dehydrogenase (AlDH). When assessing LDH activity, both direct and reverse reactions were considered. Gas flow microwave ionisation was applied to the synthesis of cold plasma using a special device developed at the Institute of Applied Physics, Russian Academy of Sciences. The plasma treatment was established to provide stimulation of LDH activity in both direct and reverse reactions. In the direct reaction, erythrocytic LDH activity in rats with plasma-treated skin almost doubled (94 %) against 48 % of activity growth in the reverse reaction. Rat blood erythrocyte AlDH tends to moderate inactivation, with catalytic properties observed to decrease by 13 %. Thus, treating the skin of healthy rats with cold helium plasma was demonstrated to stimulate the energy metabolism of blood cells, with moderate activity inhibition for AlDH presenting one of the detoxification enzymes. The nature of the observed shifts indicates their adaptability. In general, according to the obtained data, the modulation of free radical processes was confirmed to play a key role in the molecular-cellular mechanisms of the cold helium plasma action on the biological system.*

Keywords: *cold helium plasma, metabolic effects, lactate dehydrogenase, aldehyde dehydrogenase*

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Влияние гелиевой холодной плазмы на каталитическую активность некоторых дегидрогеназ эритроцитов крови крыс

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Резюме: *Цель работы – уточнение влияния гелиевой холодной плазмы на каталитические свойства лактатдегидрогеназы и альдегиддегидрогеназы эритроцитов крови крыс. На 20 белых крысах линии Wistar было изучено влияние гелиевой холодной плазмы на состояние эритроцитов. По* *завершении полного курса воздействий (по 1 сеансу в течение 5 дней) у всех животных брали образцы крови и дифференциальным центрифугированием по стандартной методике выделяли из них взвесь эритроцитов для оценки активности дегидрогеназ – лактатдегидрогеназы в прямой и обратной реакциях, а также альдегиддегидрогеназы. Холодную плазму синтезировали с применением разработанного в Институте прикладной физики РАН специального устройства, работающего по принципу СВЧ-ионизации газового потока. Установлено, что изучаемый фактор обеспечивает стимуляцию активности лактатдегидрогеназы как в прямой, так и в обратной реакциях. У крыс, чьи кожные покровы были обработаны холодной плазмой, активность эрит-роцитарной лактатдегидрогеназы в прямой реакции практически удвоилась, продемонстрировав прирост в 94 %, а в обратной – повысилась лишь на 48 %. Альдегиддегидрогеназа эритроцитов крови крыс демонстрирует тенденцию к умеренной инактивации, что проявляется в снижении каталитических свойств на 13 %. Таким образом, показано, что обработка кожных покровов здоровых крыс гелиевой холодной плазмой обеспечивает стимуляцию энергетического обмена клеток крови, а также умеренно угнетает активность альдегиддегидрогеназы, одного из детоксикационных энзимов. Характер наблюдаемых сдвигов указывает на их адаптивность. В целом полученные в результате проведенного исследования данные подтверждают ведущую роль модуляции свободнорадикальных процессов в молекулярно-клеточных механизмах действия гелиевой холодной плазмы на биологическую систему.*

Ключевые слова: *гелиевая холодная плазма, метаболические эффекты, лактатдегидрогеназа, альдегиддегидрогеназа*

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INTRODUCTION

In recent decades, the use of cold plasma in various therapeutic treatments has kindled the interest of biomedical specialists[1] [1–4]. The effectiveness of this treatment approach is confirmed by *in vitro* (on colonies of various microorganisms [1, 5, 6]) and *in vivo* (on infected skin abrasion models [5–7]) studies on the antibacterial activity of cold plasma-based therapies. The relevance of the antibacterial cold plasma effect is predetermined by the urgent need to find an alternative to antibiotics in the context of rapidly forming resistance in existing strains of pathogenic microorganisms [3, 5–7], though this has tended to eclipse research into other aspects of its effect on biological systems [1, 8, 9]. It should be noted here that both direct (at the place of treatment [4, 6, 9, 10]) and indirect (including systemic [1, 5]) realisation of these effects is possible. These particular aspects of the issue under discussion are discussed in less detail in the literature.

In previous *in vitro* experiments conducted by the authors, cold plasma was demonstrated to affect the oxidative and energy metabolism of biological systems along with their physical and chemical properties, as well as leading to an increase in antibacterial activity [8, 11–13]. Moreover, shifts were recorded in a number of metabolic parameters for blood [12] and the state of systemic haemodynamics and microcirculation in animals with dermal administration of the studied treatment [14, 15]. Although one possible cause of these transformations involves the effect on the catalytic activity of blood enzymes, no such confirmation of this hypothesis is provided in the experimental literature.

Against this background, the present study was aimed at clarifying the effect of cold helium plasma on the catalytic properties of lactate dehydrogenase (LDH) and aldehyde dehydrogenase (AlDH) in rat blood erythrocytes.

EXPERIMENTAL PART

The study was performed on 20 healthy sexually-mature male Wistar rats. The animals were divided into two groups equal in number. The first group (*n* = 10) presented a control group with no manipulations performed other than a single blood sampling. Rats of the second group (*n* = 10) were treated daily over the course of 5 days with cold helium plasma applied to a pre-epilated dorsal surface. A single treatment time comprised 1 min.

Cold plasma was synthesised using a special device developed at the Institute of Applied Physics, Russian Academy of Sciences and based on the phenomenon of gas flow microwave ionisation [8, 11–15]. Grade A bottled helium was used as a source gas for obtaining the cold plasma.

[1] Aleinik A.N. Plasma medicine: textbook. Tomsk: Publishing house of Tomsk Polytechnic University, 2011. 45 pp.

Upon completion of the full course of exposure to cold helium plasma, blood samples were taken from all animals. Subsequent isolation of an erythrocyte suspension was carried out by differential centrifugation according to the standard method for evaluating the activity of lactate dehydrogenases (LDH) and aldehyde dehydrogenase (AlDH). The LDH activity was determined in the erythrocyte hemolysate admixed with distilled water (1:40 vol.) according to the method of G.A. Kochetov[2]. Both direct and reverse reactions were considered for evaluation of LDH activity.

AlDH activity was determined spectrophotometrically according to the method described by B.M. Kershengolts and E.V. Serkina (1981). The pro-tein content was specified using the modified Lowry method.

Statistical data processing was carried out using Microsoft Excel 2007 and the Primer of Biostatistics 4.03 software program.

RESULTS AND DISCUSSION

Cold helium plasma was shown to contribute to significant changes in the catalytic properties of erythrocyte enzymes. The studied treatment was observed to stimulate LDH activity in both direct and reverse reactions (see Fig. 1). However, the extent of these shifts is not the same: at the end of the course of exposure to cold plasma, the catalytic enzyme activity in both reactions is practically equalised, while, in the intact animals of the first group, a moderate predominance of the reverse reaction occurs. In this regard, in rats treated with cold plasma, the activity of erythrocyte LDH in the direct reaction almost doubled (94 %, $p < 0.05$ as compared to the intact animal level). Conversely, in the reverse reaction an increase of 48 % ($p < 0.05$) was observed. Indirectly, this can be seen as indicating a stimulating effect of the considered treatment on the intermediate element of energy metabolism with an increase in the production of pyruvate, the primary substrate of the Krebs cycle [16, 17].

A different aspect of the modification was revealed with respect to AlDH (see Fig. 2). The specified enzyme related to the enzyme deto-xification system tends to a moderate inactivation with catalytic properties decreased by 13 % ($p < 0.05$ relative to the level detected for intact rats of the first group). This may be due to increased production of free radicals induced by external exposure to cold plasma. Although these compounds are intensively utilised by the antioxidant system of blood and tissues, their secondary (malon-dialdehyde) and tertiary (Schiff's bases) products require the invo-lvement of appropriateenzymes in the detoxification process [18].

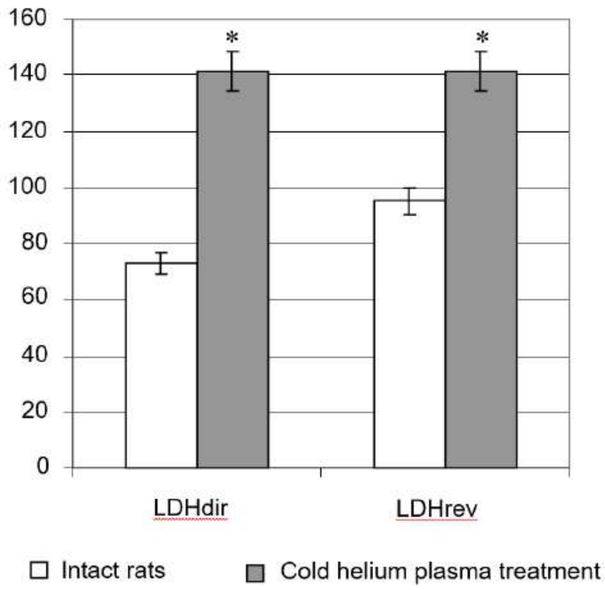

Fig. 1. *Lactate dehydrogenase activity in direct (LDHdir) and reverse (LDHrev) reactions in rat blood erythrocytes (nM NADH/min per mg protein; * – statistical significance of differences relative to the intact animal level)*

Рис. 1. *Активность лактатдегидрогеназы в прямой (ЛГпр) и обратной (ЛДГобр) реакциях в эритроцитах крови крыс (в нмольД НАДН/мин*мг белка; * – статистическая значимость различий по отношению к уровню, характерному для интактных животных)*

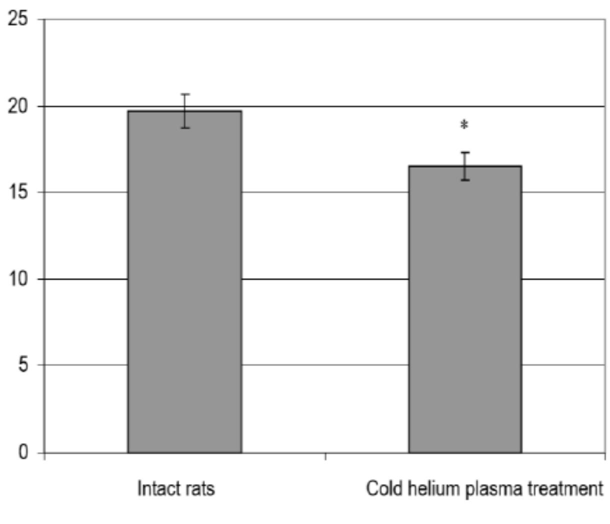

***Fig. 2**. Aldehyde dehydrogenase activity in rat blood erythrocytes (nM NADH/min per mg protein; * – statistical significance of differencesin relation to the intact animal level)*

Рис. 2. *Активность альдегиддегидрогеназы эритроцитов крови крыс (в нмоль НАДН/мин*мг белка; * – статистическая значимость различий по отношению к уровню, характерному для интактных животных)*

[2] Kochetov G.A. Enzymology practical guide: textbook for students of biological specialities. Moscow: Vysshaya Shkola, 1980. 272 pp.

This is confirmed by the increase in the concentration of malondialdehyde in rat blood erythrocytes exposed to the cold helium plasma demonstrated by the authors in previous studies [12, 13]. Moreover, no stimulating effect of cold plasma on the intensity of free radical reactions in the membranes of these blood cells was registered. For this reason, together with the results obtained in the framework of this study, the plasma effect can be characterised as training and pro-adaptive.

It is also important to emphasise that the presented data indirectly indicate the consistency of the working hypothesis on the induction of free radical processes as one of the main systemic effects caused by cold helium plasma to the body, since all detected metabolic transformations resulted from the studied treatment are directly associated with a short-term increase in the intensity of free radical oxidation in the blood and tissues.

CONCLUSION

Thus, according to the dynamics of the catalytic properties of erythrocyte lactate dehydrogenase in direct and reverse reactions, treating the skin of healthy rats with cold helium plasma was established to stimulate the energy exchange of blood cells with simultaneous moderate inhibiting AlDH activity – one of the detoxification enzymes. The nature of the observed shifts indicates their adaptability. In general, according to the obtained data, the modulation of free radical processes was confirmed to play a key role in the molecular-cellular mechanisms of the cold helium plasma action on the biological system.

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Contribution

Andrew K. Martusevich, Anna G. Soloveva, Svetlana Yu. Krasnova, Alexandr G. Galka, Alexandr V. Kostrov carried out the experimental work. The authors on the basis of the results summarized the material and wrote the manuscript. Andrew K. Martusevich, Anna G. Soloveva, Svetlana Yu. Krasnova, Alexandr G. Galka, Alexandr V. Kostrov have equal author’s rights and bear equal responsibility for plagiarism.

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