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Ultrastructural features of blood cells in HIF-1 α gene variations in specialists of extreme conditions

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Abstract

The peculiarities of changes in the ultrastructure of blood cells due to personalized variations of the HIF-1 α gene under hypoxic loading (breathing by gas mixture with reduced oxygen content up to 12% and achieving hypoxic state during heavy physical training on bicycle ergometer) were conducted.

It was shown that allelic polymorphism of the HIF-1 α gene influence on hypoxic-induced changes in the ultrastructure (and therefore function) of blood cells - the C / C genotype of winterers indicates their greater adaptive capacity compared to the C / T genotype that lies in the increasing the number of δ -granules in platelets, and reducing the number of α -granules and tubules of the inner skeleton. Such changes primarily indicate an increase in serotonin depot and therefore, indirectly, an increase in the contractile activity of the vascular wall, which may serve as a marker of the formation of adaptive reaction in response to hypoxic effects and stress. This is also indicated by the moderate swelling of mitochondria with an increase in their average diameter by 31%, which is considered to be a sign of an

increase in the energy capacity of the mitochondrial apparatus in order to replenish ADP and ATP reserves for the further inclusion of adaptive responses. Hypoxic loading in winterers with C / T-genotype led to oversteering of platelet function with decrease in contractile activity of the vascular wall.

The number of mitochondria in leukocytes significantly increased (by 24%) in winterers with C / C genotype; not only mitochondrial morphogenesis was activated, but also dynamic processes, in particular the association of organelles with pronounced longitudinal association and / or the formation of bridges between mitochondria. These facts are considered as an indicator of information exchange in the cell mitochondrial apparatus. In leukocytes of winterers with C / T genotype, changes in the mitochondrial apparatus were of a different nature: there were clear signs of hypoxic damage of organelles and the total percentage of structurally altered mitochondria exceeded 21%.

Key words: allelic polymorphism; the HIF-1 α gene; platelet and leukocyte ultrastructure; hypoxic load; winterers of Antarctic expeditions.

Introduction

Analysis of current literature data showed that the study of molecular and genetic mechanisms of the adaptation process to extreme effects, and in particular to hypoxia in humans is now a priority [1]. The molecular-genetic markers that influence the adaptation to hypoxia associated with the physiological and biochemical states of the human organism are highlighted. Among them, the main place is occupied the gene HIF-1 α , which is activated when hypoxia occurs. The product of this gene causes the expression of ACE, eNOS3, PAI-1, BDKRB2, EPO, VEGF, and ENDT1 genes, which play important roles in adaptation to hypoxia [2, 3]. The formation of adaptive responses is conditioned by a set of molecular genetic mechanisms that trigger the expression of the corresponding genes. In this case, in individuals homo- or heterozygous for a gene, stimulated by a certain influence, different responses are formed (or can be formed) in systems, organs, tissues and cells of the organism. In particular, the adaptation of winterers to the Antarctic conditions and its effectiveness, an important role belongs to the specified gene HIF-1 α - hypoxia-inducible factor, the expression of which can affect both the psychophysiological state and structural changes in the organism [4, 5].

Differences in response to hypoxic loading between winterers and individuals who have not been adversely affected by the environment may be largely due to genetic factors, in particular, the level of HIF-1 α gene expression, and more precisely by its allelic

polymorphism. This gene is considered to be the leading transcriptional regulator of genes responsible for the reaction on oxygen deficiency, as well as the leading transcriptional regulator of the expression of a large number of genes responsible for the development of adaptation. It is known that it is activated in physiologically important sites of regulation of oxygen pathways, providing fast and adequate responses to hypoxic stress, includes genes that regulate the process of angiogenesis, vasomotor control, energy metabolism, erythropoiesis and apoptosis. The HIF-1 α subunit is oxygen-sensitive, has a specific function in hypoxia-stimulated gene regulation, and is a target for oxygen-sensitive signaling pathways [6]. That is why, under hypoxic loading, there may be differences in changes in the ultrastructure of blood cells in individuals in the presence of allelic polymorphism of a given gene.

For example, allelic polymorphism of HIF-1 α , which consists in replacing cytosine (C) with thymine (T) at the 1744 gene position, has a significant effect on sport efficiency [7]. It was established that, depending on the polymorphism of the HIF-1 α gene, the oxygen regimes of the organism change [6, 8]. There is no consensus on the role of the allelic polymorphism of the gene. According to some researchers, this substitution increases the transcriptional activity of the gene allele, the stability of HIF-1 α protein, respectively, increases the resistance of cells to hypoxia [9], according to other results, the mRNA level is lower in Ser-allele carriers than in the Pro / Pro genotype [6]. Most current researchers consider the 582Ser-allele of this gene to be a velocity / force allele [5, 10]; other authors are of the opinion that this polymorphism influences on the development of endurance [7, 11]. It has also been shown that HIF-1 α dysfunction in heterozygous mice under hypoxia decreases the number of genes with increased expression [12]

There are a few data from our previous studies that indicate that in the Antarctic winterers with C / T-genotype HIF-1 α the circulatory system functioned with greater exertion, and in hypoxia resulting from heavy physical work, the efficiency of the hemodynamic links of regulation of the organism oxygen regimes was lower than that of winterers with C / C-genotype [13].

Thus, although the influence of such an important factor as hypoxia-inducible factor on adaptation processes, has long been studied, but there is no clear and unambiguous opinion on the role of gene polymorphism of this protein on the adaptation of the organism to extreme factors and hypoxia in researchers.

In connection with the above, **purpose of the work was** investigation of the peculiarities of changes in ultrastructure of blood cells due to personalized variations of the HIF-1 α gene under hypoxic loading after human adaptation to the Antarctic conditions.

Materials and methods of research

The investigation was conducted with the participation of winterers of Ukrainian expeditions (n = 24). Men aged 22-60 years (mean age 37 years) were surveyed. Genetic diagnostics included genotyping of the oxygen-dependent domain of HIF-1 α (hypoxia-inducible factor), which is a key transcription factor that regulates the expression of target genes in hypoxic and stress states, for the presence of the allelic polymorphism of the HIF-1 gene [14].

Hypoxic loading was carried out by breathing a gas mixture with reduced oxygen content up to 12% and achieving hypoxic condition during heavy physical work on the bicycle ergometer (up to 75% of the proper maximum oxygen consumption).

The study of the ultrastructure of platelets (P) and leukocytes (L) was performed in a cell mass obtained by double centrifugation from the blood of the subjects before and after hypoxic loading. Cell-enriched plasma was obtained by centrifugation of whole blood at room temperature for 15 min at 120g on a laboratory T-30 centrifuge (Ukraine). Plasma was separated from the axial cells and centrifuged at 2000g for 20 min using a Vortecs Combispin FVL-2400N mini-centrifuge (Latvia) [15.].

Sampling for electron microscopic examination was carried out in accordance with the conventional method for blood elements with double fixation by OsO₄ and glutaraldehyde, dehydration in alcohols of increasing concentration and filling in Epon-Araldit (reagents Fluka, Switzerland) [16]. Ultrathin sections 40-60 nm thick were counterstained with a 1% solution of uranyl acetate and lead citrate solution (Sigma reagents, USA) and viewed in an electron microscope TEM-124c (Ukraine). Morphometric calculations were performed using the Image Tool (USA) computer program in 130-150 fields for each group of subjects.

Statistical processing of the obtained data was performed using the program "Microsoft Excel" and "OriginPro" with the calculation of the mean values, the error of the mean, in assessing the reliability of the obtained results using the Fisher criterion and Student t tes, and conducting univariate analysis of variance. The level of statistical probability of intergroup differences was estimated by Student's t test. Such a statistical approach is legitimate because, due to the large array of data obtained, their values were within the normal distribution [17]. The differences between the mean values were considered statistically significant at $p < 0.05$.

Results and discussion

The results confirm the assumption that the HIF-1 α allelic polymorphism affects on the response to hypoxia in different ways.

Prior to the hypoxic loading in the surveyed winterers of C / C- and C / T-genotypes, the ultrastructure of P and their morphometric characteristics were not significantly different (Table 1).

Table 1 - Morphometric characteristics of platelets in winterers with C / C and C / T genotypes (M \pm ST)

Winterers groups	Quantity δ -granules, piece in cell	Quantity α -granules, piece in cell	Quantity tubules, piece in cell	Average diameter of mitochondria, μ m
C / C genotype - to hypoxia	6,5 \pm 0,9	5,1 \pm 1,3	7,3 \pm 0,7	0,56 \pm 0,02
C / T-genotype - to hypoxia	5,8 \pm 0,8	6,0 \pm 1,1	6,6 \pm 1,0	0,53 \pm 0,04
C / C genotype – after hypoxia	8,6 \pm 0,7*	2,8 \pm 1,5*	5,1 \pm 0,8*	0,73 \pm 0,07*
C / T genotype – after hypoxia	3,3 \pm 0,4*	8,1 \pm 1,2*	9,0 \pm 0,9*	0,61 \pm 0,04

Notes: * - differences were significant with respect to controls before hypoxia (p <0.05)

After hypoxic training, significant differences in the platelet ultrastructure were revealed. In individuals with C / C genotype, the number of δ -granules increased significantly, while the number of α -granules and tubules of the internal skeleton decreased. Such changes may indicate, first, an increase in the serotonin depot and, indirectly, an increase in the contractile activity of the vascular wall; secondly, the significant depletion of the ADP and ATP depot, the decrease of glycolytic enzymes activity and the significant effort of platelet functioning; third, about the decrease in the intensity of metabolic processes in the cell [18]. Such a set of sufficiently multidirectional changes may be the reason for including adaptive responses aimed at forming a balanced homeostasis.

The ultrastructural features of mitochondria (MC) in T of individuals with C / C genotype in response to hypoxia confirm this interpretation of the data obtained. Moderate swelling of MC was noted with the matrix clarifying, increasing their average diameter by 31% and, accordingly, their area (by 26%). Such changes indicate an increase in the energy

capacity of the mitochondrial apparatus to replenish ADP and ATP stores and ensuring the inclusion of adaptive reactions.

In individuals with C / T genotype P contained single dense granules, a significant number of large α -granules filled with protein debris, i.e. enzymes similar to lysosome enzymes, in addition, the cells also contained a large number of tubules of the inner skeleton of considerable diameter. Therefore, it can be assumed that hypoxic loading in winterers with C / T-genotype led to overstressing the functioning of P with a decrease in the contractile activity of the vascular wall, the development of the maximum possible number of macroergies. Such changes, to some extent, may indicate a decrease in cell backup capacity. The appearance of MC with small size and vesicular cristae (evidence of the energized state of the dense matrix organelles) also testifies the overstress of cell functions.

Different dynamics of changes in the mitochondrial apparatus in individuals with C / C and C / T-genotype in response to hypoxic load was observed in leukocytes too.

The initial morphometric characteristics of the mitochondrial apparatus L, as well as P, did not differ significantly (Table 2).

Table 2. Morphometric characteristics of the mitochondrial apparatus of leukocytes in winterers with C / C and C / T genotypes (M \pm ST)

Winterers groups	Number of mitochondria, units / 10 μm^2	Average diameter of mitochondria, μm
C / C genotype - to hypoxia	7,3 \pm 0,3	0,42 \pm 0,02
C / T-genotype - to hypoxia	7,7 \pm 0,5	0,47 \pm 0,04
C / C genotype – after hypoxia	9,1 \pm 0,5*	0,54 \pm 0,03*
C / T genotype – after hypoxia	9,7 \pm 0,6*	0,51 \pm 0,03

Notes: * - differences were significant with respect to controls before hypoxia (p <0.05)

Under the influence of hypoxia, the amount of MC significantly increased (by 24%) in the L of winterers with the C / C genotype. Not only the morphogenesis of MC was activated, but also dynamic processes, in particular the association of organelles with pronounced longitudinal association and / or the formation of "bridges" between MC, which are considered as an indicator of information exchange in the mitochondrial apparatus of the cell.

This is evidenced by the location of MC in the nucleus zone, which ensures the interaction of the nucleus and organelles. The average diameter of MC increased by 27.5% and the area by 21%, which characterizes the activation of macroenergy synthesis, probably due to the activation of the mitochondrial K^+ ATP-dependent channel [19, 20]. It should be emphasized that only a small percentage of structurally damaged MC ($10.8 \pm 0.7\%$ of the total organelles) were detected in leukocytes of the individuals in this group.

In leukocytes of winterers with C / T genotype, changes in the mitochondrial apparatus were somewhat different. The total number of MC also increased. However, their spatial location was on the periphery of the cell. In addition, the diameter and area of MC had only tended to increase, and other mechanisms, namely, the appearance of MC with vesicular cristae, were employed to maintain adequate cell energy supply.

At the same time, there were clear signs of hypoxic damage to MC - condensation (consolidation) of their matrix, expansion of intermembrane spaces, and, as it was found, the total volume of MC remained unchanged. Since the inner membrane of the MC was retained, these changes should be considered reversible. Partially or completely vacuolated MC was observed, and the overall percentage of structurally damaged organelles reached $21.3 \pm 1.8\%$.

CONCLUSIONS

The results obtained indicate that the allelic polymorphism of the HIF-1 α gene influences on hypoxic-induced changes of the blood cells ultrastructure (and, therefore, function) - the C / C genotype of winterers indicate their greater adaptive capacity compared to the C / T genotype. Therefore, the type of changes observed in blood cells may serve as a marker of the formation of adaptive reactions in response to hypoxic effects and stress.

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