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Short Communication

Features of changes in molecular markers to assess their participation in the mechanisms of patho- and sanogenesis in cerebral ischemia. Current state of the problem

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Abstract

The relevance of the work is due to the widespread prevalence of cerebrovascular pathology and ischemic strokes among the population. As is known, the key links in the pathogenesis of cerebral ischemia are an acute lack of oxygen supply to the brain, inhibition of the aerobic and activation of the anaerobic pathway of glucose utilization, changes in the acid-base state, electrolyte imbalances, oxidative stress, inflammation, excitotoxicity, and apoptosis.

Most studies are devoted to studying the mechanisms of brain damage during ischemia, while insufficient attention is paid to the mechanisms of sanogenesis. There is a need to search for new data on the molecular and cellular mechanisms of the development of damage and compensatory processes in brain neurons during cerebral ischemia. Thus, there is a need to search for new data on the molecular and cellular mechanisms of the development of degenerative and compensatory processes in the brain during ischemia of varying severity, while developing effective ways of neuroprotection.

Keywords: molecular markers; pathogenesis; sanogenesis; cerebral ischemia

Introduction

The relevance of the work is due to the widespread prevalence of cerebrovascular pathology and ischemic strokes among the population [1]. As is known, the key links in the pathogenesis of cerebral ischemia are an acute lack of oxygen supply to the brain, inhibition of the aerobic and activation of the anaerobic pathway of glucose utilization, changes in the acid-base state, electrolyte imbalances, oxidative stress, inflammation, excitotoxicity, and apoptosis [1-7].

Most studies are devoted to studying the mechanisms of brain damage during ischemia, while insufficient attention is paid to the mechanisms of sanogenesis. There is a need to search for new data on the molecular and cellular mechanisms of the development of damage and compensatory processes in brain neurons during cerebral ischemia. It is assumed that activation of compensatory mechanisms will reduce the severity of neurodegenerative disorders in the brain and increase the effectiveness of treatment [4-6].

To do this, there is a need to detail the processes of patho- and sanogenesis during cerebral ischemia of varying severity, which is possible by studying them at the cellular and molecular level using a number of molecular markers.

In the context of protection against damage during cerebral ischemia, chaperones or heat shock proteins (HSPs) play an important role.

One of the key elements of the chaperone system is heat shock proteins (HSP, Heat Shock Proteins) - this is a class of functionally similar proteins, the expression of which increases with increasing temperature or other stress effects on the cell [11].

Most enzymatic systems have optimal activity in a fairly narrow temperature range, and when body temperature deviates even by a small amount, their activity can change significantly. Heat shock is the reaction of cells and systems to temperatures exceeding normal values for the body. Heat shock proteins are the main molecular markers of both heat shock itself and almost any exogenous stress [6,7].

Increased expression of genes encoding HSPs is regulated at the transcription stage. Heat shock proteins are synthesized in various cells, in many intracellular structures (in the cytoplasm, nucleus, endoplasmic reticulum and mitochondria) in all multicellular organisms under the influence of stress factors: the action of heavy metals, amino acids, viral, bacterial and parasitic infections, inflammation, malignant transformation, autoimmune reactions, as well as in response to growth factors, cellular differentiation and hormonal stimulation. Even in resting cells, up to 2% of all proteins can be representatives of this family. HSP synthesis is a universal nonspecific cell

response to stress. In some cases, the content of HSP in cells can reach 20% of all soluble cytoplasmic proteins [5].

Functions of heat shock proteins

Heat shock proteins are universal molecular chaperones (from the English chaperon - to accompany), i.e., proteins that bind to other molecules and in such a complex perform certain functions [11].

The main function of HSP is considered to control the formation of new proteins and the formation of their tertiary structure (folding). By binding to growing peptide chains on the ribosome, HSPs prevent their nonspecific aggregation, protect against premature proteolytic degradation, and promote correct and timely folding of the polypeptide into the tertiary structure. HSPs also bind altered proteins or proteins whose tertiary structure has already formed incorrectly, protecting the cell from their effects.

When exposed to stress factors, HSP activity increases sharply. They intensively bind to denatured proteins and maintain them in a state capable of subsequent restoration. HSPs are present in the cytoplasm in combination with a special transcription factor HSF (heat shock factor). When exposed to stress, HSF is separated from HSP, acquires DNA-binding activity and accumulates in the nucleus, where it activates the transcription of new chaperones and suppresses the transcription of other genes. At the end of the stressor, the released HSPs bind HSF and return to their original state [5,11].

Heat shock proteins take part in the transport of protein molecules through mitochondrial membranes and the nuclear envelope, in the processing of proteins into antigenic peptides and their binding to molecules of the Major histocompatibility complex (MHC class I) [4].

Heat shock proteins are involved in protecting cells from stress-induced apoptosis by inhibiting the pathways of its activation and stabilizing y cell structures. It is assumed that HSP is involved in the processes of necrosis and cleansing the body of necrotic cells. The release of intracellular HSPs occurs only in the event of cell death by necrosis [3,11].

By interacting with steroid hormone receptors, HSPs prevent the activation of cellular stress programs before the onset of stress. Under the conditions of its development, excessive stimulation by these hormones is mitigated. Blockade of steroid hormone receptors reduces the apoptotic effect of the latter, due to which some cells under stress (for example, lymphocytes) increase life expectancy. Thus, HSP is a kind of link between stress at the level of the whole organism and the stress response of individual cells. Hence their other name – “stress proteins”.

HSPs support the native conformation of proteins and accompany them after synthesis into various compartments of the cell, thereby protecting proteins from aggregation and denaturation.

By migrating into the nucleus and binding to chromatin and the nucleolus, HSPs thereby prevent the occurrence of mutations and provide conditions for the repair of DNA damage.

By interacting with microtubules and microfilaments, HSPs stabilize the cytoskeleton, which increases the cell's resistance to mechanical damage, denaturation and aggregation of cell proteins [1,11].

An important role in the body’s adaptation to hypoxia belongs to a specific regulatory protein – hypoxia-induced factor (HIF, abbreviated from English Hypoxia-inducible factors), the activity of which increases with a decrease in oxygen tension in the blood. Hypoxia-inducible factors are a group of transcription factors that respond to a decrease in oxygen content in cells or to hypoxia.

It has been shown that HIF plays a major role in the systemic response of the body to hypoxia and is synthesized in many tissues of the body, including nervous tissue, where its expression is maximum in neurons. HIF-1 functions as a master transcriptional regulator of the adaptive response to hypoxia. When expressed, more than 40 genes are activated, the protein products of which increase oxygen delivery or promote metabolic adaptation to hypoxia.

In humans and animals, the highly conserved transcription complex HIF-1, which belongs to the PER-ARNT-SIM (PAS) subfamily of the basic family of transcription factors, is expressed [2,12].

In the presence of oxygen (normoxia), prolyl hydroxylase (PHD) hydroxylates HIF-1α, which subsequently binds to the adapter protein pHVL, is ubiquitinated and degraded in the S26 proteasome. During hypoxia, prolyl hydroxylase loses activity, and HIF-1α dimerizes in the cell nucleus with constantly active HIF-1β, thus activating the transcription of a number of genes that ensure cell adaptation to hypoxia. With a significant deficiency of ATP, the cell may undergo apoptosis (via the mitochondrial pathway), and in the case of ineffective adaptation mechanisms, necrosis [3,12].

HIF-1 is a heterodimeric protein, the beta subunit of which is constantly expressed, and the synthesis of the alpha subunit is regulated by oxygen. HIF-1 subunits contain three domains: the N-terminus is the bHLH domain for DNA binding, the central region is the Per-ARNT-Sim (PAS) domain, which facilitates heterodimerization, and the C-terminus is responsible for the spatial orientation of transcriptional coregulatory proteins [12].

At normal oxygen concentrations, hydroxylation of the proline amino acid residues of the HIF-1 alpha molecule occurs as a result of the activity of O2 and/or the Fe-dependent enzyme prolyl hydroxylase, which is a molecular oxygen sensor. The altered HIF-1 alpha subunit undergoes proteasomal degradation through a series of stages. In a state of hypoxia, the HIF-1 alpha protein molecule is not hydroxylated, remains stable and accumulates. The HIF-1 alpha and HIF-1 beta subunits combine. The resulting transcription protein HIF-1 in the cell nucleus binds to specific DNA sequences in genes whose expression is induced by hypoxia [5,12].

HIF-1 synthesis can occur through oxygen-independent mechanisms. Thus, HIF-1 is synthesized in reactions controlled by signaling systems such as MAPK (mitogen activated protein kinase - activated by signals that promote proliferation) and PI3K (phosphatidylinositol 3 kinase - a regulatory protein located at the intersection of various signaling pathways and controlling key cell functions). It is of particular importance in the regulation of functions such as growth, survival, aging, and tumor transformation. It should be noted that PI3K belongs to a group of enzymes collectively called reperfusion injury rescue kinases (RISK). These kinases are believed to be potential targets for pharmacological intervention in reperfusion injury. Activation of this group of enzymes leads to inhibition of the opening of mitochondrial pores, resulting in a cytoprotective effect. MAPK and PI3K signaling systems are activated through the tyrosine kine receptor zy, specific succinate-dependent receptor GPR-91, etc. Receptor agonists are tyrosine hydroxylase, cytokines, growth factors (for example, insulin-like growth factor), succinate.

An increase in the level of HIF-1 leads to an increase in the expression of genes that ensure cell adaptation to hypoxia and stimulate erythropoiesis (erythropoietin genes), angiogenesis (vascular endothelial growth factor gene), glycolytic enzymes (aldolase gene, lactate dehydrogenase, phosphofructokinase, pyruvate kinase, etc.). In addition, HIF-1 regulates the expression of genes involved in iron metabolism, regulation of vascular tone, cell proliferation, apoptosis, lipogenesis, the formation of carotid glomeruli, the development of B-lymphocytes, etc [12].

Muscle antagonizing A-kinase protein (mAKAP), through stimulation of ubiquitin E3 ligase, has been shown to influence the stability and nuclear positioning of HIF-1. Depletion of mAKAP or disruption of its effects on the perinuclear region in cardiomyocytes alters HIF-1 stability and transcriptional activation of hypoxia-associated genes. Thus, “compartmentalization” of oxygen-sensing signaling components may influence hypoxia sensitivity.

HIF synthesis is regulated by other transcription factors involved in protecting the cell from damage. transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells, NF-kB) is a universal transcription factor that controls the expression of genes of the immune response, apoptosis and cell cycle, is a direct modulator of HIF-1 expression under normoxia conditions [7].

Regulation of the HIF-1 α-subunit is carried out by two enzymatic reactions in which molecular oxygen reacts with two specific amino acid residues: in the presence of oxygen, the prolyl residue in the oxygen-dependent region is hydroxylated and oxygen is added to the aspartic residue near the C-terminal region of HIF-1. Both reactions are catalyzed by 2-oxoglutarate-dependent dioxygenases, which belong to a family of enzymes with diverse biological functions. These enzymes are classified as prolyl hydroxylase proteins and are identified as HIF prolyl hydroxylation (PHD) catalases. HIF-1 modified in this way binds to the VHL tumor suppressor protein gene, which has ubiquitin ligase activity, which leads to the degradation of HIF-1 α. When oxygen levels are low, HIF-1 α forms an active complex with the β subunit, as a result of which it becomes stable and binds to cytochrome P300 [12].

In addition to the main modification of HIF, there is also modulation of this protein by phosphorylation and acetylation. In addition, hypoxic cells exhibit increased expression of hydroxylase genes, leading to feedback inhibition of HIF proteins. In the presence of oxygen, the enzyme FIH (HIF inhibitory factor) also acts, which inhibits the transcriptional activity of HIF-1.

A critical role in the regulation of HIF-1 is played by the tumor suppressor protein VHL, consisting of 213 amino acid residues, since it is responsible for the ubiquitylation of HIF-α subunits in the presence of oxygen. In this process, VHL acts as the recognition component of the ubiquitin ligase complex. Cell culture experiments have shown that VHL is required for the regulation of HIF destruction in all cell types. Once hydroxylated, HIF-1α binds to VHL and is subsequently ubiquitylated and subsequently degraded by proteases. In cells where VHL is insufficient, HIF-1a chains remain active even at normal oxygen partial pressure [6].

Cell culture studies have shown that when prolyl hydroxylation of HIF-α is inactivated and, accordingly, degradation is inhibited, stabilization and accumulation of the α-chains of this protein occurs. HIF-α then moves to the nucleus, where it dimerizes with HIF-β and binds to hypoxia-responsible elements of the HIF genes, activating their transcription. At normal VHL levels, hydroxylation of the C-terminal aspartic residue occurs by enzymes of the FIH group, which leads to a decrease in the transcriptional activity of the HIF complex. Therefore, it is believed that the VHL–HIF chain can be considered a central regulator of oxygen homeostasis [5-7].

Inactivation of HIF-1α, HIF-2α, HIF-β, or VHL in mice during embryogenesis leads to embryonic death in utero or in the perinatal period. Mice homozygous for HIF-1α deficiency die in utero between days 8 and 11 due to neural tube defects or cardiovascular malformations. In mice deficient in HIF-2α, changes in catecholamine synthesis are observed, leading to cardiac damage, and disturbances in VEGF synthesis cause damage to the lungs, yolk protein, and are accompanied by mitochondrial abnormalities. Mice deficient in HIF-1β die from severe disturbances in yolk sac and bronchi vasculogenesis. Inactivation of VHL causes an increase in the transcriptional activity of HIF-1α and HIF-2α, so mice deficient in this phase who die during gestation due to abnormal placental vasculogenesis. These studies demonstrate the importance of the HIF system for fetal development [12].

Under normal physiological conditions in the body of adult mammals, maintaining the HIF system at a certain level in all organs and tissues is also extremely important, especially in renal tissue. HIF-α subunits are identified in kidney cells - in the cortical and medullary layers, in S-bodies and glomerular cells. In the regulation of erythropoiesis, the kidneys play a very important role, since they serve as the main physiological oxygen sensor, responding to systemic hypoxia with a rapid increase in the production of erythropoietin (EPO) in the renal interstitial cells. The liver also participates in the production of EPO, but in a much smaller amount than the kidneys, and if EPO production in the kidneys is impaired, its extrarenal synthesis cannot compensate for renal losses. Naturally, the main regulator of EPO production is HIF-1α, which was discovered while studying the regulation of EPO. However, it has now been shown that HIF-2α also takes part in the regulation of erythropoiesis in both the liver and kidneys, but its formation in the liver is more pronounced [1-4].

It is known that HIF and iron interact through iron-regulated proteins (IRP's): IRP-1 and IRP-2. IRPs posttranscriptionally regulate the expression of iron metabolic proteins by binding them to messenger RNA (mRNA) of iron-regulated elements (IRE's). When cellular iron stores are depleted, the IRP-IRE complex prevents sequestration of the transferrin receptor and thereby enhances iron uptake by the cell; When there is a sufficient amount of iron in the cell, the IRP-IRE complex is inactivated, undergoes protosomal degradation, and iron is not absorbed. It is important to note that the degradation pathway of this complex involves PHD, the same enzyme that is involved in the hydroxylation of HIF. In addition, it has been established that HIF-2α is also post-translationally regulated by the IRP-IRE complex. It has been shown that in case of iron deficiency, HIF-2α is included in the feedback control mechanism to limit the production of EPO in order to prevent the development of even deeper iron deficiency [12].

Another important protein involved in the implementation of protective reactions is neuroglobin (Ngb), a representative of the family of globin proteins of the nervous system, involved in maintaining gas homeostasis of nerve cells. The similarity of the structure of Ngb with other globin proteins suggests similar

functions of ensuring oxygen homeostasis of cells. But since the affinity level of Ngb for oxygen is very high, and this is an obstacle to the release of oxygen, this metalloprotein is increasingly considered as an indicator of the level of oxygen in mitochondria, which oxidize organic substances and form ATP. At the cellular level, Ngb is found in the cytoplasm, vesicular structures, neurotubules, nucleus and mitochondria.

Analysis of the subcellular localization of Ngb shows that Ngb mRNA and protein are constantly detected in the pericarone and neuronal processes, indicating the active participation of Ngb in metabolic processes [8].

Neuroglobin regulates the functioning of neurons in pathology: suppresses oxidative stress, blocks mitochondrial apoptosis factors, binds free radicals. Neuroglobin synthesis is promoted by hypoxia inducible factor-1 alpha (HIF-1α), retinoid X receptor (RXR), zinc finger X-linked protein (ZFX), nuclear transcription factor Y alpha (NFYA) and transcriptional enhancer domain 1 (TEAD1) and SOX -4 [9].

The role of neuroglobin in hypoxia/ischemia is still unclear. Some studies indicate its neuroprotective effect in cerebral ischemia due to increased expression of endothelial NO synthase [8]. Other evidence argues against its importance for neuronal survival under oxygen-deprived conditions, as Ngb deficiency appears to increase HIF-1α expression [10].

Due to its ability to bind oxygen, Ngb enhances the supply of oxygen to the mitochondria of metabolically active neurons. This hypothesis is supported by the existence of Ngb in metabolically active cells and subcellular compartments.

In addition, neuroprotective effects can be realized through the following mechanisms:

1. Neuroglobin is a free radical scavenger and has chelating activity;
2. Capable of utilizing NO;
3. In mitochondria, it reduces the release of cytochrome C into the cytosol, inhibiting the caspase-9-dependent apoptotic pathway;
4. Acts as an inhibitor of the dissociation of guanine nucleotide of the heterotrimeric protein Gα, necessary for the normal functioning of intracellular signaling systems;
5. Inhibits Pak1 kinase and interacts with Rho GDP dissociation inhibitor (RhoGDI) and members of the RhoGTPase family to suppress the propagation of hypoxia or death signal induced by N-methyl-D-aspartic acid;
6. Acts as a metabolic regulator that increases cellular anabolism through inhibition of AMP-activated signaling [8-10].
7. Thus, there is a need to search for new data on the molecular and cellular mechanisms of the development of degenerative and compensatory processes in the brain during ischemia of varying severity, while developing effective ways of neuroprotection.

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