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Heat Shock Proteins: Changes Related to Aging, Development of Thrombotic Complications, and Peptide Regulation of the Genome[1]

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Abstract—The present review of published data and the authors' own results addresses the role of heat shock proteins in the regulation of cell and tissue homeostasis and considers the decrease in their expression levels as one of the main factors of aging. Heat shock proteins are involved in the regulation of proliferation, apoptosis, and differentiation of cells, as well as in that of intracellular homeostasis, and, therefore, play a substantial role in maintaining the activity of the immune, cardiovascular, and other systems of the organism. These proteins are also implied in the development of atherosclerosis, myocardial infarction, ischemic stroke, and other diseases accompanied by thrombotic complications. The use of short peptides provides an opportunity to restore and normalize the expression of heat shock proteins, which probably accounts for the antistress and geroprotective activity of these peptides.

Keywords: heat shock proteins, homeostasis, hemostasis, aging, peptides.

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BASIC FEATURES OF HEAT SHOCK PROTEINS

Heat shock proteins (HSPs), which have been shown by A. Tissieres et al. [78] to be strongly induced by elevated temperatures, have recently attracted special attention from researchers. In addition to temperature elevation, the biosynthesis of HSPs is induced by other factors: toxins, anoxia, hypoxia, ischemia, chemotherapeutic agents, carcinogens, and even concomitantly the differentiation and development of cells and tissues. Intensive HSP synthesis occurs in infectious diseases, inflammation, fever, ultraviolet irradiation, exposure to electromagnetic fields and heavy metal salts, alkalosis and acidosis, exposure to lipopolysaccharides, ischemia, hypoxia, cytokine attack, and exposure to animal and plant toxins. Consequently, these proteins are also called stress proteins [79]. HSPs are found both inside the cell and on the cell membrane [13, 27].

According to the universally accepted classification, all HSPs are subdivided into six families depending on their molecular mass in kilodaltons. Heat shock proteins with molecular mass lower than 40 kDa are included into the small HSP (sHSP) family. Other HSP proteins belong to families called HSP70, HSP80, HSP90, HSP100, and HSP with molecular masses of 110 kDa and above. HSPs with molecular masses of 70 kDa are of special interest due to the high levels of these proteins in tissues under stressful conditions. These proteins have been studied in the greatest detail and their role in the functioning of organs and tissues has been established. HSP70 was shown to be the main molecular chaperone involved in the utilization of irreversibly damaged proteins, or folding [40, 43, 44].

What do chaperone (chaperone is a French word for an elderly lady accompanying a young girl to the ball) functions include? Newly synthesized proteins are known to have tertiary and quaternary structure. High temperature and stress to which the cell is exposed cause the formation of anomalous proteins due to aggregation. The functions of HSP in the regulation of correct folding (structure formation) of newly synthesized proteins and the destruction of anomalous protein aggregates are called chaperone functions, and the proteins themselves are called chaperones. Therefore, the main function of HSPs is the protection of the cell from damaging factors. Furthermore, HSPs are nonspecific adaptogens that protect the cells from various stressors.

In addition to HSP70, the chaperone group includes HSP22, HSP27, HSP60, and HSP 90. The propensity for chaperoning is determined by the structure of the chaperone proteins, which are able to engage in cyclic ADP/ATP-dependent binding to other proteins [34, 43, 45, 67, 87].

[1] A review of published data and the authors' own results.

The expression of the HSP70 gene was shown to be regulated by HSF1 (heat shock transcription factor). HSF1 and HSP70 together with the respective mechanisms of synthesis and activation constitute an intracellular stress-sensing system, which perceives and evaluates intra- and extracellular stress signals and initiates the appropriate response.

In unstressed cells, HSP70 is found in both the cytoplasm and the nucleus as an inactive monomer devoid of DNA-binding ability. The monomeric form of HSF1 is only stabilized by binding to HSP70.

Stress causes the formation of denatured proteins that replace HSF1 in the complex with HSP70; after this, liberated HSF1 molecules quickly form an active homotrimer structure, i.e., a complex of three HSF1 molecules, which migrates into the nucleus and binds to the regulatory fragment of the HSP70 gene and, consequently, induces the transcription and synthesis of HSP70. An increase in the intracellular concentration of HSP70 causes a decrease in the concentration of damaged proteins in the cells and these proteins can not replace HSF1 in the complex with HSP70 as efficiently as before. Therefore, HSP70 binds to HSF1 again and causes the latter to transition to an inactive monomeric form; this causes the HSP70 synthesis to stop [64].

HSPs are divided into two groups, namely, constitutive and inducible. The concentration of constitutive HSPs is rather high in the basal state and does not significantly increase in stress. Inducible HSPs are almost undetectable under normal conditions, but their synthesis is dramatically upregulated by stress. However, this division is highly conventional depending on the specialization and state of the cell.

ROLE OF HEAT SHOCK PROTEINS IN CELL FUNCTIONING AND LIFESPAN

The most important role of constitutive HSP70 is the restoration of the tertiary and quaternary structure of the protein. HSP70 proteins were shown to bind to the newly synthesized protein chain fragments, which are most likely involved in undesirable hydrophobic aggregation. Afterwards, HSP70 uses the energy of ATP hydrolysis to transport the protein chain to the endoplasmic reticulum or the mitochondria, or to the Golgi complex. The transmembrane transport of the protein chain to a different heat shock protein occurs in these structures. Consequently, the HSP of the respective organelle that received the protein chain regulates the final formation of the subunit structure of the protein [13, 15, 71].

Unfortunately, the native structure of some proteins is not restored after disaggregation and, thus, part of the proteins remain irreversibly damaged; this waste must be utilized to prevent it from interfering with cell functioning. This is accomplished either by lysosomal enzymes or by ubiquitin-dependent proteolysis. HSP70 proteins are involved in both of these processes as transporters; they either facilitate the transfer of the denatured protein to the lysosomes or transport the proteolytic enzyme complex directly to the denatured protein [13, 64].

After the cell is exposed to damaging factors, HSP translocation inside the cell occurs, in addition to the activation of HSP70 synthesis. As a result of stress, HSPs accumulate in the most vulnerable parts of the cell, i.e., the nucleus (during the first 4–5 h) and, later, in the perinuclear and perisarcolemmal zones and along actin filaments [79].

The role of HSP accumulation in the nucleus after damage to the cell is the protection of the genetic material, limitation of preribosome degradation, restoration of nucleolar structure and function, and the shielding of DNA fragments accessible for nucleases. Therefore, HSPs play an important role in increasing the resistance of the cell machinery of protein synthesis to damage.

HSP accumulation in the perisarcolemmal zone occurs at the same time as a large amount of functionally active ribosomes and mRNA molecules available for translation appear in the same region of the cell. Thus, HSPs enable the stress-induced migration of ribosomes. HSP and ribosome accumulation in the perisarcolemmal region is supposedly necessary for the quick replenishment of membrane proteins, for instance, membrane channel proteins, receptor proteins, enzymes, and the tissue factor (**TF**). Therefore, the role of this phenomenon is to compensate for the damage to membrane proteins in the quickest and most efficient way [79]. Therefore, HSPs can be considered the markers of cell destruction [76].

Physical activity is known to be accompanied by the acceleration of blood clotting and an increase in the fibrinolytic activity of the blood. The concentration of HSP70 mRNA in leukocytes and the content of this protein in serum also increase in most subjects engaged in demanding physical activity. HSP70 is evidently released into the blood plasma by both leukocytes and other cells. However, cell death does not occur, since the content of creatine phosphate, ALT, and AST in the serum does not change. Therefore, increased HSP70 concentration in the serum is a consequence of adaptation to stress [19].

However, one should note that HSP is necessary not only for the recovery of the structure and function of damaged cells, but also for the normal functioning of the cells, since these proteins are involved in maintaining homeostasis, growth, and differentiation of cells; furthermore, they possess direct antiapoptotic action [69]. Pronounced antiapoptotic activity is characteristic of constitutive HSP70 and HSP90β, which prevent the degenerative changes in the cells exposed to different stressors [47].

An increase in the HSP32 content in human monocytes and lymphocytes upon aging has been demonstrated. An especially sharp increase in the

concentration of this protein in the leukocytes occurs in acute infections when hypercoagulation develops, and fibrinolysis is often downregulated. The monocyte content of HSP32 significantly increases and the lymphocyte content of this protein decreases in response to heat shock. Direct correlations between the content of C-reactive protein and interleukin-6 (*IL*-6) in the plasma and HSP32 in blood cells are detected in humans suffering from infectious diseases [66].

One of the hypotheses that explain the occurrence of death is cell damage by free radicals, which accumulate during the lifetime of the cell and cause structural alterations in the cytoplasm. The insufficient chaperone function of HSPs in aging causes the death of cells and the whole organism. The aging-related decrease in the chaperone function of HSPs has been demonstrated in various model objects (drosophila, nematodes, daphnia, and others) [38, 55, 58, 59, 63]. If the above hypothesis is correct, the enhancement of the chaperone function of the HSPs must contribute to an increase in lifespan. For instance, experiments with nematodes (*Caenorhabditis elegans*) have shown that the mutation of a single gene that contributes to high-temperature tolerance increases the lifespan of the animals. An increase in transient temperature causes the accumulation of HSP70 in Drosophila and an increase in the lifespan of flies [77, 80]. Finally, food restriction decelerates the accumulation of acid radicals (prevents lipid peroxidation) in rodents, which contributes to the enhancement of chaperone functions and increase in the lifespan of the organism [61].

HSPs play an important role in the functioning of the immune system. Microbial pathogens or autologous inflammation spots (damaged tissues) can cause the release of HSPs which are subsequently recognized by the surface receptors of the immune system—*TLR*-2, *TLR*-4, *CD14*, *CD91*, *CD94*, *LOX*-1, and others. As a result, information concerning the development of a pathological process is transmitted and the immune system becomes involved in the defense reaction [15].

THE ROLE OF HEAT SHOCK PROTEINS IN THE DEVELOPMENT OF ATHEROSCLEROSIS AND THROMBOTIC COMPLICATIONS

Under normal conditions, HSPs are predominantly localized inside the cell, and therefore immune tolerance to these proteins does not develop. This property is the reason for the involvement of HSPs in the pathogenesis of autoimmune and systemic vascular diseases, such as atherosclerosis. Virtually any massive tissue damage or infection cause the release of HSPs into the extracellular space which is followed by the formation of anti-HSP antibodies. HSPs released into the extracellular liquid and expressed on the cell surface stimulate macrophages and dendrite cells, which leads to the synthesis of proinflammatory cytokines, as well as membrane-associated adhesion and co-stimulatory molecules [83].

The infection hypothesis of atherosclerosis and cardiovascular pathology development is currently gaining importance. Data confirming the involvement of *Chlamydia pneumoniae* in the development of atherosclerosis is the most extensive [53, 56, 65].

Chlamydia pneumoniae which is often found in atherosclerotic plaques [53, 55, 56] contains HSP60/65 which elicit the formation of antibodies in the human and animal organism. Antibodies against the bacterial HSP65 (as well as those targeting HSP60) cross-react with human HSP60. Immunization of normocholesteric rabbits by HSP is accompanied by a relatively rapid development of inflammatory atherosclerotic damage in the aortal intima. If the rabbits are fed a cholesterol-enriched diet, typical atherosclerotic plaques similar to the human ones are formed [64]. HSP60/65 are capable of stimulating human monocytes which release proinflammatory cytokines and aggravate the inflammatory process; besides, they stimulate *TF* expression on endothelial cells. These factors play a major role in the development of thrombosis, as well as in the development of atherosclerosis.

The degree of homology between microbial and viral HSP60 and the corresponding human protein is high, and therefore the emerging antibodies directed against microbial heat shock proteins can interact with human HSPs which are expressed by endothelial cells in response to stress (including the classical factors promoting the development of atherosclerosis) [39, 81]. The resulting immune complexes display complement-dependent and antibody-dependent cytotoxicity towards endothelial cells and damage their membranes, this playing a significant role in the formation of atherosclerotic plaques [72].

Chlamydial and human HSP60/65 are detected even in plaques from young people and adolescents. Besides, soluble forms of human and bacterial HSPs were detected in the blood of patients suffering from atherosclerosis. Due to the immune molecular mimicry between bacterial and human HSPs, the latter can become autoantigens causing the formation of antibodies which ultimately lead to the damage to endothelial cells and the development of atherosclerosis [85]. These cross-reacting antibodies are recognized by the epitopes of the respective HSPs which are the autoimmune targets on the early stages of atherosclerosis development [68].

Focal expression of HSP60 in endotheliocytes, endothelium-associated mononuclear cells and smooth muscle cells of aortal intima is observed already at the initial stages of atherogenesis. Besides, this chaperone was detected in the surface layers of atherosclerotic plaques [15]. The binding of HSP to receptors of monocytes and lymphocytes attached to the endothelial surface cannot be excluded [84]. On one hand, this mechanism may ensure the interaction of *T*-lymphocytes and antibodies with the antigens,

and on the other hand, the proinflammatory pathway and the migration of *T*-reactive lymphocytes directed against HSP into the arterial wall can be triggered [86].

J.A. Berliner and coauthors [1990] have demonstrated that the expression of the chaperone HSP70 is increased in human macrophages and smooth-muscle arterial cells, and the degree of this increase is proportional to the extent of the atherosclerotic damage [35]. HSP70 expressed by the macrophages is concentrated in the central parts of the atheromas surrounding necrotic sites and lipid deposits, and the protein expressed in smooth muscle vascular cells is concentrated on the surface of the uncomplicated plaques.

It has recently been shown that the low molecular-weight HSP27 is also involved in the pathogenesis of atherosclerosis. Its content in atherosclerotic arteries, including the sclerotic plaques themselves, is high. However, HSP27 undergoes plasmin-mediated proteolysis. Fragments of this protein, as well as aggregates and proteolysis products, were detected in the atherosclerotic arteries of different animal species. Incubation of myocytes from the human aortal wall with plasmin leads to overexpression and phosphorylation of HSP27 with subsequent exit from the cytoskeleton into the cytosol, cell nucleus, and plasmatic membrane [62].

Besides, damage to the endothelium is accompanied by the release of HSP20 from the blood vessel walls into the plasma where this protein prevents the aggregation of thrombocytes and the formation of new atherosclerotic plaques. However, only native, non-aggregated HSPs can exert this action. The concentration of HSP20 in the vascular wall decreases accordingly. Specific sites interacting with HSP20 were detected in human thrombocytes. Besides, HSP20 decreases the ability of thrombin to activate phospholipase C and therefore prevents the release of phosphoinositol from the membrane of blood platelets [52]. This prevents the development of thrombotic complications and therefore is a part of the protective action of heat shock proteins.

HSPs and their complexes with peptides are efficiently captured by antigen-presenting cells (APC) by endocytosis mediated by the following receptors: *CD91* (a multipotent receptor binding 32 different ligands including α_2-macroglobulin, HSP60, HSP90, *G*ρ96 and many others), *CD40* (a member of the *TNF* receptor family), *CD36* (a scavenger receptor expressed by macrophages and immature dendritic cells), as well as by *TLR2* and *TLR4* [20, 37]. The scavenger receptor family includes also *LOX*-1 which promotes the exocytosis of HSP70 in human dendritic cells [37]. The above named receptors are involved in the immune response to HSPs, as well as in the excretion and removal of the degradation products of these proteins.

The data presented prove that heat shock proteins represent the connecting link between infection and atherosclerosis.

The interaction of HSPs with macrophage *TLR2* and *TLR4* was shown to induce an increase in the intracellular Ca^{2+} concentration and the activation of the nuclear factor *NF*κ*B* leading to increased production of *NO*, *IL*-1b, *IL*-6, *IL*-12, *IL*-18, *TNF*α, chemokines and adhesion molecules promoting inflammatory reactions, endothelial damage and atherosclerosis development [21, 82]. An increase in the levels of *IL*-1, *IL*-12, *IL*-18, and IFNγ was shown to promote the progression of atherosclerosis in experimental animals, while blocking the above named cytokines decreased the degree of atherosclerotic changes by 15–69% [51]. Furthermore, proinflammatory cytokines *IL*-1 and *TNF*-α stimulate the production of MCP-1 (monocyte chemoattractant protein-1), which causes monocyte migration into the intima and is therefore a powerful activator of atherosclerosis development [57]. Moreover, all of the aforementioned cytokines activate the expression of *TF* and von Willebrand factor (*v*WF) and inhibit fibrinolysis [7–9, 30, 46], which promotes the development of thrombotic complications.

The protective role of HSPs is most evident in thromboses, myocardial infarction, stroke, and other thrombotic diseases. W. H. Dillman and R. Mestril (1995) showed that the size of the necrotic zone in the myocardium after the 20-min occlusion of the coronal artery, as well as the level of creatine phosphokinase during reperfusion, were much lower in genetically modified mice that overexpress HSP70 than in wild-type mice [41]. Furthermore, the recovery of heart function upon reperfusion occurred faster in genetically modified mice. The more resistant rats are to acute myocardial infarction, the higher the accumulation of HSP in their heart muscles [18]. Moreover, the concentration of HSP70 in blood plasma is increased more than 20-fold in disseminated intravascular coagulation (DIC) and thromboses, which is largely due to the release of protein from decaying cells [7–9, 29].

V. T. Ivashkin and O. M. Drapkina have shown [4] that human lymphocytes react to myocardial infarction by activating a protective protein system. On the first day after infarction, all patients can be divided into three groups, i.e., patients in which the system of synthesizing the inducible from of $HSP70_i$ is nonreactive, both under normal conditions and after heat shock, make up the first group; in the second group, the system of $HSP70_i$ is nonreactive, but is considerably induced by heat shock; and, in the third group, $HSP70_i$ synthesis occurs at normal temperature and is upregulated after heat shock. It is necessary to note that the parameters of the synthesis of $HSP70_i$ protein were highly correlated to the extent of myocardial injury during the first and second days of the disease. Extensive damage caused an increase in the levels of $HSP70_i$ in the lymphocytes at physiologically optimal temperature, but the inducibility of heat shock proteins by heating decreased. Moreover, the higher the difference in the $HSP70_i$ content in lymphocytes

before and after heat shock, the more favorable the prognosis for recovery from myocardial infarction.

O. M. Drapkina [2] showed that both an increase and a dramatic decrease in $HSP72_i$ production in the lymphocytes can occur in patients that suffer from postinfarction cardiosclerosis. Therapy can elicit either an increase or decrease in $HSP72_i$ production; however, in some cases, the concentration of this protein does not change. The author referred to the two former types of reaction as the exhaustion of the stress-modulating and adaptive effect of $HSP72_i$. Chronic cardiac insufficiency is especially grave in these patients and resistance to therapy, as well as an unfavorable clinical outcome, is often observed.

HSP70 was shown to be localized in cardiomyocytes and function as cardioprotective agents that prevent the unfavorable consequences of ischemia and, in particular, reperfusion. However, during myocardial ischemia, these proteins are capable of exiting cardiomyocytes and exerting a direct influence on immune system cells that function as proinflammatory mediators. HSP70 activates monocytes and macrophages and, as a result, the concentration of *IL*-1, *IL*-6, *IL*-12, and *TNF*-α in blood increases and these proteins affect hepatocytes, endothelial cells, and monocytes and increase the risk of myocardial infarction.

In the case of the significant activation of stress-limiting systems, i.e., HSP in the leukocytes and NO in blood plasma, the defensive reaction to stressors is more pronounced [18].

However, HSP70 is capable of stimulating thrombocyte aggregation and even inducing thrombosis. HSP70 was shown to increase the production and activity of soluble guanylate cyclase that catalyzes the formation of cGMP which is known to cause the aggregation and secretion of platelet granules at high concentrations [33]. Therefore, an increase in the HSP70 concentration can induce thromboembolic complications, in addition to increasing the risk of DIC. In this case, the risk of thromboses is especially high if HSP70 cannot fulfill its primary chaperone function.

It is necessary to note that the level of HSP70 in lymphocytes is especially low in patients who are in a grave postinfarction state; sometimes, the level of this protein is below the detection limit. The clinical course of the disease in these patients was aggravated by the development of acute dysfunction of the left ventricle and symptoms of early postinfarction stenocardia.

The degree of oxidative stress in these patients was very high and the endothelial dysfunction was very pronounced. The use of ACE inhibitors containing a sulfhydryl group did not ameliorate the state of these patients. After 6 months, the HSP70 level in lymphocytes remained as low as during the first day postinfarction. The data mentioned are indicative of the exhaustion of the organism's defensive system, which is an unfavourable prognostic factor for the progression and outcome of the disease [3].

Young rabbits aged 2–3 weeks were subjected to myocardial ischemia for 45 min, then to 45-min reperfusion [9]. Noradrenalin, which accelerates the processes of blood clotting, was injected intraperitoneally after 24 h. A dramatic increase in the level of HSP70, oxyproline, and ATP, as well as an increase in SOD activity and a decrease in MDA and the endothelin level in the myocardium, were observed. Structural changes in the reperfused muscle were restricted.

The facts mentioned are indicative of the restriction of damage to cell structures upon an increase in HSP70 concentration, which prevents the release of procoagulants from cells and the development of thromboses.

In the case of ischemia, estrogen injections cause vasodilation, a reduction in the aggregative activity of thrombocytes, and the deceleration of lipid peroxidation processes. At the same time, the activation of heat shock proteins is observed. For example, in a Mongolian gerbil global ischemia model, intraperitoneal estradiol injection leads to a significant activation of HSP25/27 and HSP70 in the arteries. If estradiol was injected 20 minutes before the induction of ischemia, the activation of these proteins was more pronounced [26].

The regulation of NO synthesis is one of the most important functions of HSP70 and HSP90 [67]; thus, they promote vasodilation, prevent platelet aggregation, and enhance the fibrinolytic activity of blood. The prolonged use of statins to treat ischemic heart disease and hypertonia leads to an increase in the levels of HSP70 and HSP90, which promotes NO synthesis [75].

However, HSPs also play a significant role in the development of cardiovascular pathology, which is often accompanied by the development of thromboembolic conditions. The concentration of autoantibodies to HSP27 dramatically increases in stable stenocardia patients during angionotic attacks accompanied by chest pain. The content of autoantibodies increases dramatically in elderly people, as well as in hypertensive and diabetic patients [73].

The titer of autoantibodies to HSP27 increases dramatically in patients suffering from unstable stenocardia; during the first 12 h after myocardial infarction, the titer is on average 5 times higher than in healthy subjects and decreases concomitantly until the patient's condition is ameliorated. An increase in the titer of HSP27 antibodies is believed to be an early marker of myocardial infarction [73].

The chaperone function of heat shock proteins, in particular HSP70, is believed to decrease with aging and especially during the development of atherosclerosis, when ischemic heart disease, stroke, and thromboembolic diseases threaten the subject's health [74].

ROLE OF HEAT SHOCK PROTEINS IN DEVELOPMENT OF DIC

The expression of *TF*, a powerful trigger of disseminated intravascular coagulation, is undoubtedly the most intensive at a pathological site of tissue destruction. Leukocytes, including monocytes and neutrophils that carry *TF*, are transported to the site of injury. Lymphocytes that form aggregates with thrombocytes are accumulated at the same site. Proinflammatory cytokines were shown to have a predominantly local effect on immunocompetent cells and macrophages [5]. This process must be accompanied by the formation of *TF*-carrying microvesicles and the secretion of procoagulants by macrophages with the subsequent transport of procoagulants into the tissue fluid, then into the lymph, then into blood. However, both the tissue fluid and lymph contain all blood-clotting factors. Our observations [7, 9, 10], as well as the studies carried out by Yu. M. Levin, show that, in addition to blood, both interstitial liquid and lymphs are clotted in DIC. Moreover, in many cases, disturbances of lymph circulation in DIC must precede accelerated blood clotting within the vessels.

The vast majority of diseases starts with local injury to cells and tissues and is often accompanied by fever, which causes the synthesis of HSPs or stress proteins.

It is obvious that protein aggregation occurs in cells undergoing pathological changes during a disease. If this process becomes irreversible, the cell receives a signal to start a death program, or apoptosis. If aggregation is followed by the recovery of the cytoplasm structure, the normal functioning of the cell is gradually restored.

How does the disturbance of the cytoplasm structure occur? This process involves inducible HSP70 proteins, which are dramatically upregulated by pathogenic stimulants.

Unfortunately, some proteins are incapable of restoring native structure after disaggregation; some of them remain irreversibly damaged. This "waste" must be utilized or removed so that it interferes with the cell's functioning; as was already mentioned, this task is accomplished by HSP70.

HSP70 induces the aggregation of macrophages, neutrophils, and mast cells, which enhances the synthesis of proinflammatory cytokines, such as *IL*-1, *TNF*-α, and others, which in turn promote thrombocyte aggregation, accelerated blood clotting, and fibrinolysis inhibition, which are observed in DIC [9, 10, 36, 54, 75].

Original data obtained by V. A. Nazarov et al. [16] show that lipopolysaccharide (LPS) caused the activation of the stress response in the proinflammatory phenotype of macrophages that did not initially contain HSP70, but did not cause the development of anti-inflammatory reactions. Macrophages that initially contained HSP70, or those in which the proinflammatory phenotype was induced in advance by LPS, did not develop a stress response, while the anti-inflammatory phenotype showed a pronounced stress response. The proinflammatory phenotype was characterized by increased production of proinflammatory cytokines, such as *IL*-1, *IL*-6, *IL*-8, *IL*-12, *TNF*α, and others, which accelerated the process of blood clotting. On the contrary, the anti-inflammatory phenotype was accompanied by decreased synthesis of proinflammatory cytokines and an increase in the concentrations of *IL*-4 and *IL*-10, which decelerate blood clotting. Therefore, the presence of HSP70 in macrophages causes stress response to be reprogrammed, namely via the transformation of macrophage phenotype. Furthermore, it has been shown that LPS stimulation was accompanied by accelerated NO production in macrophages lacking HSP70, while in macrophages containing this type of HSP70, this reaction was not observed [16].

The facts mentioned here are clearly indicative of a protective role of HSP 70, which promotes the spatial restriction of the site of injury and liquidation of the DIC syndrome that always occur upon the injection of LPS to humans or animals.

Furthermore, our speculations are indirectly confirmed by the results of E. A. Alekperov et al. [1], which show that stressors (bacterial LPS or phorbol-12-myristate-13-acetate injection, or treatment of thymocytes by catecholamines, the physiological mediators of stress) first cause a decrease in HSP70 in various cultured cells. Strong oxidative stress (H_2O_2 treatment) induces a significant decrease in the HSP70 content followed by a quick increase, followed by a slow decrease in the cytoplasmic pool of this protein in lymphoid cells of the *EL*-4 line. The authors suppose this reaction to be universal and caused by the release of a part of the cytoplasmic pool of the above named protein into the environment. Since HSP70 is a long-lived protein, it cannot be quickly eliminated, which confirms the hypothesis mentioned above.

Stressor agents initially cause the exhaustion of the cytoplasmic pool of HSP70, which is inevitably accompanied by protein aggregation in the cell and, consequently, by the structuring of the cytoplasm. It is beyond doubt that some cells cannot resist the action of the stressor and already receives an apoptosis-inducing signal at this stage. However, this stage of the process of cytoplasm structuring is reversible in most cells, since the HSP70 content in the cell increases dramatically, becoming many times higher than the initial level. However, the intracellular pool of HSP70 gradually becomes exhausted, and the reaction becomes irreversible. This is either followed by an increase in constant intravascular blood clotting or the development of a typical acute or chronic DIC syndrome.

Research done in our laboratory [8, 9] showed that the concentration of autoantibodies to HSP70 increase dramatically in the serum and blood plasma of patients suffering from complicated appendicitis, acute lung abscess, and the aggravation of chronic osteomyelitis.

Thus, the normal content of antibodies to HSP70 in the serum equals 32.2 ± 1.2 ng/ml, while in aggravated appendicitis, it amounts to 540.3 ± 30.2 ng/ml in the serum and 860.3 ± 50.3ng/ml blood plasma. In lung abscesses, the concentration of antibodies amounts to 330.2 ± 20.1 ng/ml in the serum and to 640.2 ± 50.3 ng/ml in blood plasma. In the aggravation of chronic osteomyelitis, the corresponding concentrations amount to 450.5 ± 24.7 ng/ml in the serum and 720.3 ± 47.6 ng/ml in blood plasma. It is necessary to note that the level of antibodies in plasma was 1.5–2 times higher than in the serum. The additional expression of heat shock proteins, which are capable of binding autoantibodies in the serum, by leukocytes and thrombocytes during the formation of a fibrin clot can partially account for this phenomenon. The above-mentioned fact explains the need to assay HSP antibodies in blood plasma rather than in the serum.

It is beyond all doubt that cytoplasmic protein coagulation occurs in surgical purulent infection, which is a powerful stimulus of HSP gene activation and a dramatic increase in the production of heat shock protein by nucleated cells of the host organism. In the case of especially grave pathology, the synthesis of the cell's proteins nearly stops and the chaperone levels reach 15–20% of the total protein content in the cytoplasm [60]. However, this is often not sufficient to revive the cells affected by pathology.

However, these facts cannot fully explain the significant increase in the content of HSP autoantibodies. Purulent inflammation suppresses the so-called oral tolerance and the saprophytic microorganism starts a new stage of destructive inflammation pathogenesis that promotes the transition of the process into a chronic form. Factors of innate resistance (antibodies, sensitized lymphocytes, activated neutrophils, stimulation of the complement system, bactericidal activity of the serum, lysozyme, and others), as well as antibiotics, stress the saprophytic microflora and stimulate the hyperproduction of heat shock proteins in it. The latter proteins are expressed both on the surface of the microbial cells and in host-organism cells affected by the pathological process. Heat shock proteins possess pronounced antigenicity and, therefore, induce the production of antibodies and sensitize lymphocytes, which closes the vicious circle that strengthens and prolongs the inflammation process. HSPs are also produced by microbes that cause surgical purulent infections. Therefore, the synthesis of heat shock proteins, including HSP70, is a complex reaction caused both by the host response to the invasion by pathogenic microbes and HSP production by the microorganisms themselves.

The higher the HSP concentration, the more intensive the formation of autoantibodies. Therefore, it is clear that our data may be indicative of a dramatic increase in HSP70 concentration in the blood during surgical purulent infection. One should also add that exogenous HSP70 released upon the destruction of bacterial cells can be internalized by host cells and, therefore, form an additional protein pool that contributes to its protective action. This fact is especially important because, during grave diseases, the production of HSP70 by the cells is suppressed, which makes the cells more vulnerable to multiple stress factors.

The protective role of heat shock proteins in intravascular blood coagulation can also be illustrated by the following example. It is beyond doubt that sepsis is always accompanied by DIC syndrome, which leads to the occurrence of multiple organ failure [8, 9, 28, 54]. On the other hand, the pretreatment of rats with HSP70 prior to LPS injection prevents the consumption of blood-clotting factors (except fibrinogen) for 5 h or more and promotes the normalization of fibrinolysis. Furthermore, the structure of cells subjected to LPS treatment remains unaltered. The data obtained allow for the assumption that HSP70 can be used in the future as a medication to prevent the development of gram-negative infections [17].

Even more convincing results were obtained by M. Dieude and coauthors [42], who injected IgG antibodies that target HSP60 to *BALB/c* mice with trauma of the common carotid artery. The formation of thrombi in these mice occurred much faster and the stability of thrombi was much higher than in the control group injected by immunoglobulin G (IgG) incapable of binding HSPs. Moreover, occlusion proceeded to the final stage without reperfusion in mice injected with anti-HSP60 IgG. In the control group, occlusion proceeded to the final stage in 64% mice and reperfusion was observed in 65% of animals. Undamaged contralateral arteries in anti-HSP60 IgG injected mice were also found to be altered; the level of expression of *P*-selectin in endothelial cells was increased and the concentration of νWF in the blood was increased as well. The authors assume that endothelial cells treated by anti-HSP60 IgG antibodies overactivate νWF. The latter protein is a transporter of factor VIII; it promotes blood clotting and the intravascular aggregation of thrombocytes.

However, a different mechanism of thrombosis enhancement exists. A dramatic increase in *P*-selectin expression level is accompanied by the adhesion of leucocytes and thrombocytes to the endothelium, which finally leads to the enhancement of intravascular coagulation. Stimulated leukocytes can also express *TF*, which leads to DIC, in addition to the formation of a local thrombus. For example, a direct correlation between the content of HSP60 targeting antibodies and thrombosis is observed in systemic lupus erythrematosis patients.

It is necessary to note that antibodies targeting HSP60 and other heat shock proteins can be generated by the organism suffering from various infectious and inflammatory diseases, since all disease-causing agents regardless of their nature contain various HSPs. Undoubtedly, in this case, favorable conditions are

created for the increase in constant intravascular blood coagulation and the development of DIC.

It has been shown [6–9, 28–30, 54] that all cellular structures have a pronounced procoagulant activity and many of them have *TF* exposed on their surface; i.e., they contain partial or complete thromboplastin. When these structures get into the vascular lumen or the extravasal space, they are capable of inducing the coagulation of interstitial fluid and lymphs, as well as blood.

This allows us to propose the following mechanism of DIC development in inflammatory, infectious, and other diseases; in addition to cell damage, invasion by a pathogenic microorganism causes cytoplasm structuring. Furthermore, the synthesis and expression of heat shock proteins (including HSP70) are increased and this should lead to the recovery of cytoplasmic structure and conservation of normal cell functioning. If the HSPs are capable of fulfilling this task, the pathological process acquires an abortive or mild character and the disease is soon terminated by recovery. In this case, constant intravascular coagulation may increase, but pronounced organ dysfunction does not develop. If HSPs cannot fulfill their function, the damaged cells receive a signal for starting a death (apoptosis) program. Cell damage or death cause the formation of microvesicles which often express *TF*, which promotes the coagulation of interstitial fluid, lymph, and blood. This also causes an increase in the concentration of proinflammatory cytokines (*IL*-1, *IL*-6, *IL*-12, *TNF*-α, and others) and a consequent increase in the expression of *TF*, as well as von Willebrand factor (vWF), and fibrinolysis inhibitors (including *PAI*-1 and *TAFI*). However, the content of proinflammatory cytokines increases both in the blood and at the infection site because all cytokines are known to exert a predominantly local action [5]. For example, the content of cytokines in fluids in contact with the pathological site (liquor in the case of brain diseases, saliva in oral cavity diseases, and tears in eye diseases) is much higher than their concentration in the blood [9]. Therefore, proinflammatory cytokines must primarily affect the clotting and fibrinolytic activity of the tissue liquid and lymph and only next to this, blood. Moreover, HSPs (mostly HSP70) are capable of stimulating proinflammatory cytokine formation; therefore, they are sometimes referred to as chaperokines [32]. This ultimately leads to the enhancement of constant intravascular blood clotting, the formation of sludge, the deceleration of fibrinolysis, and pronounced microcirculation disturbances, which can lead to the development of multiorgan failure with all of the consequences [7–11].

Why does this occur? The ability of HSP70 to protect damaged cells is actually not unlimited because the functioning of the chaperone mechanism is energy-dependent. Thus, 40 min after coronal artery occlusion, the deficit of macroergic compounds is about 90%, which is almost incompatible with cell survival. Electron microscopy of the ischemized cardiomyocyte reveals the condensation of intermediate filaments into perinuclear aggregates, the reorganization of the cytoplasmic network, the accumulation of active filaments around the nucleus, the vacuolization and disappearance of mitochondria, and certain features of nuclear chromatin aggregation and membrane destruction [4].

The action of thrombin, an essential participant in intravascular coagulation and thrombus formation, is known to be mediated by so-called proteinase-activated cell receptors (PARs). HSP90 was shown to be involved in *PAR1*-mediated morphological changes in astrocytes and other neuroglial cells caused by thrombin. Specific interaction of *PAR1* and HSP90 has been demonstrated in yeast cells; therefore, *PAR1* thrombin receptor was included into the list of client proteins interacting with the cytosolic form of HSP90. Geldanomycin, an inhibitor of HSP90, was shown to block the ATPase activity of HSP90 and prevent its interaction with client proteins. It is beyond all doubt that the data presented enable the implication of the coagulant protein thrombin in changes in the cytoplasm structure.

POSSIBILITIES OF RECOVERING HSP70 GENE EXPRESSION AS A RESULT OF TREATMENT BY SHORT PEPTIDES

The data presented above show that heat shock proteins play an important role in protecting the cell from damage caused by various stressors (increased physical load, myocardial infarction, and infectious diseases) and are involved in cell differentiation and immune-system activation. The expression of heat shock protein HSP70 was shown to decrease in aging. Therefore, it seems logical to assume that the overexpression of hSP70 can have geroprotective action in addition to stress-protective action.

Technology for synthesizing short peptides that possess a range of geroprotective effects (the ability to increase the lifespan of animals and induce cell differentiation and proliferation, as well as the ability to regulate gene expression) based on the analysis of the amino acid composition of various tissue extracts has been developed at St. Petersburg Institute of Bioregulation and Gerontology of the Northwest Branch of the Russian Academy of Medical Sciences [12, 23, 24, 25, 70]. Results of the recent study on the biological activity of short peptides showed that they are capable of stimulating the expression of heat shock protein genes, which largely accounts for the broad range of stress-protective and geroprotective effects characteristic of these peptides.

The regulation of the gene expression of HSP70 heat shock protein by *T*-34 tripeptide (Glu-Asp-Gly) was studied in a rat model of induced gastric ulcer. *T*-34 peptide was injected into animals subcutaneously (0.5 μg in 0.5 ml physiological saline solution) 5 days after the formation of the ulcer. Samples from the edge of the ulcer were taken on the seventh day and the

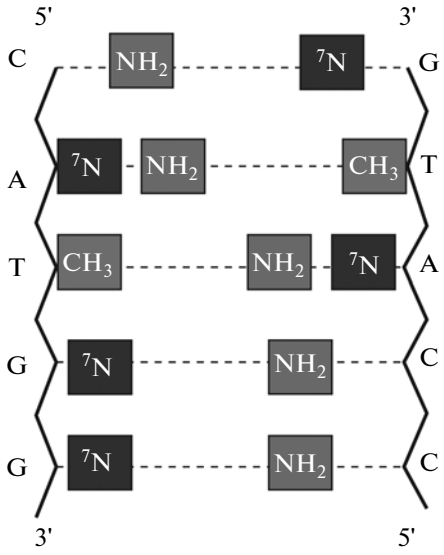

Fig. 1. Position of nucleotide pairs of *CATGG* block and functional groups of these nucleotides exposed on surfaces of major groove of DNA double helix: NH_2 is the donor of the hydrogen bond; ^7N is the acceptor of the hydrogen bond; CH_3 is the acceptor of the hydrophobic bond.

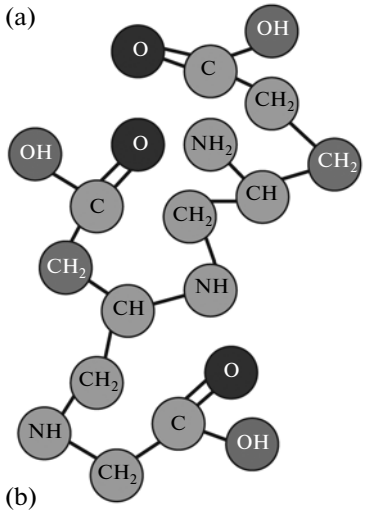

Fig. 2. Nucleotide sequence of promoter region of (a) heat shock peptide gene and (b) structure of biologically active Glu-Asp-Gly (EDG) tripeptide in stretched conformation.

expression of HSP70 heat shock protein gene was assayed by Western blotting. The expression of HSP70 heat shock protein gene in mucosa samples from the edge of a gastric ulcer on the seventh day after ulcer induction was shown to be 4.5 times higher than in normal mucosa of intact animals. *T*-34 peptide promoted mucosal repair and caused a decrease in HSP70 expression to control levels.

Research on HSPA1A heat shock protein gene expression in the case of increased physical load, which is a stress model, was performed in 20 female gymnasts. The subjects were divided into two groups of equal size; those in the first (experimental) group received the *T*-36 (Glu-Asp-Pro) peptide as a food additive (one capsule twice a day for 20 days) and those in the second (control) group were given a polyvitamin complex. The expression of the HSPA1A gene in the control group equaled 2.3 ± 0.08 at the beginning of the experiment and did not differ from the value obtained at the end of the experiment (2.0 ± 0.16). The expression of HSPA1A gene in the first group equaled 1.9 ± 0.13 at the beginning of the experiment and, after treatment by short peptides, it increased to 4.4 ± 0.15, which is almost twice as high than the initial value in the first group and the control group ($p < 0.05$). The data obtained are indicative of reliable upregulation of the gene expression of heat shock protein after the treatment by *T*-36 peptide, which implies the antistress effect of this peptide due to the peptide regulation of gene expression [22, 31, 48–50].

The data obtained allowed us to propose a hypothesis on the complementary interaction of the *T*-34 peptide and a promoter region of heat shock protein gene. The structure of the promoter of HSP70 protein gene (Fig. 2a) and the Glu-Asp-Gly (EDG) tripeptide (Fig. 2b) in stretched conformation are shown in Fig. 1. The tripeptide molecule has one terminal amino group and three carboxylic groups, two of which are on the side chains. The length of a molecule containing two peptide bonds is 14 Å. Thus, the complementary interaction of this tripeptide with double-stranded DNA requires an interaction site containing no less than five nucleotide pairs.

A statistical analysis of the nucleotide sequence showed that the promoter region of the gene of HSP70 protein contains four repeats of the pentanucleotide block *CATGG*. The distribution scheme of nucleobase functional groups on the surface of the major groove of the double helix of the block *CATGG* and the putative conformation of the nucleopeptide complex based on complementary hydrogen bonding of the tripeptide and the major groove of the DNA double helix is shown in Fig. 3. Therefore, the results of modeling the complementary interaction of the tripeptide under investigation and the promoter region of the HSP70 protein gene showed that their binding is possible, which probably accounts for changes in the expression of the aforementioned gene.

The concise data presented in our review are undoubtedly indicative of the significant role of heat shock proteins, which regulate proliferation, apoptosis, cell differentiation, and intracellular and extracellular homeostasis; in maintaining the activity of immune, cardiovascular, coagulative, and other systems of the organism; and the correlation between the decreasing expression of this protein and aging processes. The use of short synthetic peptides enables the restoration and normalization of the gene expression

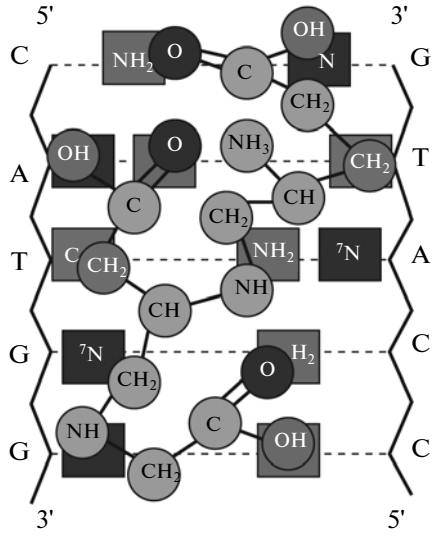

Fig. 3. Complementary interaction of Glu-Asp-Gly (EDG) tripeptide with *CATGG* binding site in promoter region of HSP70 heat shock protein. Seven hydrogen bonds and two hydrophobic bonds account for the stability of this complex.

of heat shock protein, which probably accounts for the antistress and geroprotective activity of these peptides.

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