



HEAT SHOCK PROTEIN HSP70: PREREQUISITES FOR USE AS A MEDICINAL PRODUCT

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Heat shock protein Hsp70 is one of the main cytoprotection components under the action of various external stimuli. The analysis of the literature data shows that nowadays, the researchers' overwhelming evidence has proven the role of Hsp70 as a biological target for the drug development; however, the ideas about its use as a drug are often multidirectional.

The aim of the article is to analyze and generalize the literature data on the features of the physiological functions of heat shock protein Hsp 70, and indicate the possibilities of its use for the pharmacological correction of various pathological conditions.

Materials and methods. In the process of selecting material for writing this review article, such databases as Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG, etc. were used. The following words and word combinations were selected as markers for identifying the literature: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.

Results. In this review, the pharmacology of one of the key members of this family, Hsp70, was focused on. The literary analysis confirms that this molecule is an endogenous regulator of many physiological processes and demonstrates tissue protective effects in modeling ischemic, neurodegenerative and inflammatory processes. The use of recombinant exogenous Hsp70 mimics the endogenous function of the protein, indicating the absence of a number of typical limitations characteristic of pharmacotherapy with high molecular weight compounds, such as immunogenicity, a rapid degradation by proteases, or a low penetration of histohematogenous barriers.

Conclusion. Thus, Hsp70 may become a promising agent for clinical trials as a drug for the treatment of patients with neurological, immunological, and cardiovascular profiles.

Keywords: Hsp 70; cytoprotection; chaperone; neuroprotection; recombinant drugs

Abbreviations: MPs – medicinal products; ALS – amyotrophic lateral sclerosis; Hsp – heat shock protein; HSF1 – heat shock factor 1 / heat shock factor 1; HSEs – heat shock elements; TNF – tumor necrosis factor; PRRs – pattern recognition receptor; SBDa – sphingolipid binding domain alpha; NBD – nucleotide binding domain; NEF – nucleotide exchange factor; DISC – DISC-death-inducing signaling complex; BAG-1 – BAG family molecular chaperone regulator 1; CHIP – carboxyl terminus of Hsc70-interacting protein; E3 – ubiquitin-protein isopeptide ligase; TRAIL – TNF-related apoptosis-inducing ligand; BID – pro-apoptotic member of the Bcl-2 family; FANCC – Fanconianemia complementation group C; PKR – protein kinase R; MCA – middle cerebral artery; 17-DMAG – 17-demethoxygeldanamycin; NF- κ B – nuclear factor-kappa B; AIF – apoptosis inducing factor; UPS – ubiquitin-proteasome system; JNK – Jun N-terminal kinases; Hip – hunting interacting protein; Hop – hunting interacting protein; Hsp 70-1 – Heat shock 70 kDa protein 1; DR4 – death receptor 4; DR5 – death receptor 5; p53 – protein p53; rhHsp70 – recombinant human heat shock protein 70; NMDA – N-methyl-D-aspartate receptor; IL-6 – Interleukin 6; TNF- α – Tumor necrosis factor-alpha; IL-1 β – Interleukin 1 β ; MCP-1 – monocyte chemotactic protein; TLRs – Toll-like receptors; DAMP – damage-associated molecular pattern; FasR – Fas-receptor; SMAC – the second mitochondrial activator of caspase; MAPK mitogen-activated protein kinase; ICAD – inhibitor of caspase-activated DNase; IKK – kappa B inhibitor kinase; Apaf 1 – apoptosis protease activating factor-1; CCP – cellular cytosolic protein; MMPs – matrix metalloproteinases; Mcl-1 – myeloid Cell Leukemia differentiation protein 1; ASK1 – apoptosis signal-regulating kinase 1; BBB – blood-brain barrier; Casp 9 – caspase 9; FADD – Fas-associated death domain.

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БЕЛОК ТЕПЛОВОГО ШОКА HSP70 – ПРЕДПОСЫЛКИ ИСПОЛЬЗОВАНИЯ В КАЧЕСТВЕ ЛЕКАРСТВЕННОГО СРЕДСТВА

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Белок теплового шока Hsp70 является одним из основных компонентов цитопroteкции при действии различных внешних раздражителей. Анализ литературных данных показывает, что на сегодняшний день исследователями сформированы многочисленные доказательства роли HSP70 в качестве биологической мишени для разработки лекарственных средств, однако, представления о его использовании в качестве лекарственного средства зачастую разнонаправлены. **Цель.** Обобщить и проанализировать данные литературы об особенностях физиологических функций белка теплового шока Hsp 70 и обозначить возможности его применения с целью фармакологической коррекции различных патологических состояний.

Материалы и методы. В процессе подбора материала для написания обзорной статьи использовали такие базы данных, как: Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG и др. Параметрами для отбора литературы были выбраны следующие слова и словосочетания: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.

Результаты. В данном обзоре мы сфокусировались на фармакологии одного из ключевых представителей данного семейства – Hsp70. Литературный анализ подтверждает, что данная молекула является эндогенным регулятором многих физиологических процессов и демонстрирует тканезащитные эффекты при моделировании ишемических, нейродегенеративных и воспалительных процессов. Применение рекомбинантного экзогенного Hsp70 имитирует эндогенную функцию белка, свидетельствуя об отсутствии ряда типичных ограничений, характерных для фармакотерапии высокомолекулярными соединениями, таких как иммуногенность, быстрое разрушение протеазами или низкая степень прохождения через гистогематические барьеры.

Заключение. Таким образом, Hsp70 может стать перспективным агентом для клинических испытаний в качестве препарата для лечения пациентов неврологического, иммунологического и кардиоваскулярного профилей.

Ключевые слова: Hsp 70; цитопroteкция; шаперон; нейропroteкция; рекомбинантные препараты

Список сокращений: ЛС – лекарственные средства; БАС – боковой амиотрофический склероз; Hsp – белки теплового шока; HSF1 – фактор транскрипции – фактора теплового шока 1; HSEs – элементы теплового шока; TNF – фактор некроза опухоли; PRRs – рецепторы распознавания образов; SBDa – сфинголипид связывающий домен альфа; NBD – нуклеотидсвязывающий домен; NEF – фактор обмена нуклеотидов; DISC – комплекс, индуцирующий смерть; BAG-1 – Регулятор семейства молекулярных шаперонов BAG 1; CHIP – карбоксильный конец Hsp70 – взаимодействующего белка; E3 – убиквитин-протеин-изолепептидная лигаза; TRAIL – TNF-связанный лиганд, индуцирующий апоптоз; BID – проапоптотический член семейства Bcl-2; FANCC – группа комплементации фанконианемии; PKR – протеинкиназа R; MCA – средняя мозговая артерия; 17-DMAG – 17-деметоксигельданамицин; NF-kB – ядерный фактор-каппа B; AIF – фактор вызывающий апоптоз; UPS – убиквитин-протеасомная система; JNK – N-концевая киназа Jun; Hsp – хантинг-взаимодействующий белок; Нор – хантинг организующий белок; Hsp70-1t – белок теплового шока 70 кДа белок 1; DR4 – рецептор смерти 4; DR5 – рецептор смерти 5; p53 – белок p53; rhHsp70 – рекомбинантный человеческий белок теплового шока 70; NMDA – N-метил-D аспартат рецептор; IL-6 – интерлейкин 6; FNO-α – фактор некроза опухоли-альфа; IL-1β – интерлейкин 1β; MCP-1 – хемотаксический белок моноцитов; TLR – толл-подобные рецепторы; DAMP – молекулярная структура, связанная с повреждением; FasR – Fas рецептор; SMAC – второй митохондриальный активатор каспазы; MAPK – активируемая митогеном протеинкиназа; ICAD – ингибитор каспазо-активируемой ДНКазы; IKK – киназа ингибитора κB; Araf 1 – клеточный цитозольный белок; MMPs – матриксные металлопротеиназы; Mcl-1 – белок дифференцировки миелоидноклеточного лейкоза 1; ASK-1 – киназа, регулирующая сигнал к апоптозу; BBB – гематоэнцефалический барьер; Casp 9 – каспаза 9; FADD – белок, взаимодействующий с доменом смерти Fas-рецептора.

INTRODUCTION

Protein homeostasis in mammals has been maintained by a multicomponent system of proteins that regulate metabolic processes. That system depends on environmental conditions. The hypothesis of the existence of a heat shock proteins family, the first mention of which dates back to 1962, was put forward on the basis of the discovery of mammalian tissues' tolerance phenomenon to high temperatures after a sharp heating of the same tissue site to sublethal temperatures [1].

Currently, many studies aimed at studying the spatial form, molecular interactions and physiological functions of heat shock proteins, have been carried out [2, 3]. The proteomics of a large family of chaperones, the function of which is traditionally associated with the folding and assembly of proteins, has been described. Molecular chaperones play an important role in proteostasis. In particular, Hsp70 means a lot in protein coagulation, disaggregation, and degradation [4]. Through substrate-binding domains, Hsp70 interacts with a wide range of molecules, providing cytoprotective properties against cellular stresses of various etiologies. The functions variety of heat shock proteins prompts the need to study their behavior in various pathological conditions. In eukaryotic cells, in physiological and pathological terms, there are four main pathways of protein degradation: the ubiquitin-proteasome system and three types of autophagy: macroautophagy, microautophagy, and chaperone-mediated autophagy [5]. Hsp70 provides specificity in the choice of substrate for all types of the above listed processes. In the literature, different variants of this protein name can be found: heat shock protein 70 kDa, Hsp70, chaperone Hsp70, and Hsp73.

The multitude of Hsp70 physiological functions determine the researchers' interest in studying the possibilities of its use in various pathological conditions. Heat shock proteins occupy one of the important positions in all the variety of folding proteins in mammalian bodies. At the same time, the use of these chaperones is hard due to the high cost of their production using producers' bacterial strains. Recombinant drugs are the substances obtained by artificial means based on genetic engineering. At the moment, in the classification of recombinant drugs, pharmacologists distinguish 2 main groups: protein recombinant drugs and hormonal recombinant drugs. With the help of recombinant DNA, more than 400 genes (mainly in the form of cDNA) of various human proteins that are or can become drugs have been cloned. The analysis of the biotechnology market shows that the annual volume of the world drug market based

on human proteins is about \$ 150 billion and is constantly growing [6]. The advantage of biopharmaceuticals lies in their high targeting action, which is associated with a reduced risk of side effects in comparison with conventional low molecular weight drugs [7]. Biotechnological drugs have found application in the treatment of patients with pronounced adverse reactions to traditional synthetic drugs [8]. Modern methods of creating transgenic animal producers of recombinant proteins open up new prospects for their use aimed at the pharmacological correction of pathological conditions associated with a violation of the structural organization of protein molecules. This mini-review reflects the main mechanisms of functioning and molecular interaction of Hsp70 with effector molecules known to science in various pathological cascades. Taking into account the available literature data, the prospects of using this substance as a drug with neuroprotective and cytoprotective activities, are discussed.

THE AIM of the article is to analyze and generalize the literature data on the features of the physiological functions of heat shock protein Hsp 70, and indicate the possibilities of its use for the pharmacological correction of various pathological conditions.

MATERIALS AND METHODS

In the process of selecting material for writing this review article, the databases of Google Patents, Science Research Portal, Google Scholar, ScienceDirect, CiteSeer, Publications, ResearchIndex, Ingenta, PubMed, KEGG, etc. were used. The following words and phrases were selected for the selection of literature: Hsp70, Hsp70 stroke, Hsp70 neuroprotection, Hsp70 cytoprotection, recombinant drugs.

RESULTS AND DISCUSSION

Basic Hsp 70 Biology

The human Hsp70 family of proteins includes 13 molecules that differ from each other in the expression level, a subcellular arrangement, and an amino acid composition. They are encoded by a polygenic family consisting of up to 17 genes and 30 pseudogenes [9]. The functional genes encoding human Hsp70 proteins are associated with several chromosomes. Major stress-induced Hsp70s chaperones include Hsp70-1 (HspA1A) and Hsp70-2 (HspA1B) proteins, referred to as Hsp70 or Hsp70-1 as a whole, differ from each other in only two amino acids. The expression of basal HSPA1A / B mRNA varies in most tissues and exceeds the expression levels of other Hsp70 isoforms in humans. Hsp70-1t (Heat shock 70 kDa protein 1) is a constitutive, non-inducible

chaperone that is 90% identical to Hsp70-1 [10].

Hsp70 is known to consist of two main domains: the N-terminal nucleotide binding domain (NBD) (45 kDa) and the substrate binding domain (SBD) (25 kDa). The first is a V-shaped structure consisting of two subdomains (lobes) surrounding the ATP binding site. The second one also consists of two: a substrate binding domain beta (SBD β) and a substrate binding domain alpha – (SBD α) [11].

Later data showed that chaperones perform a dual function in proteostasis, contributing to the implementation of the main stages of protein degradation [12]. The interaction of a particular chaperone with other chaperones or cochaperones determines the fate of the former through one of the common pathways of protein degradation, the ubiquitin-proteasome system (UPS), or autophagy. In eukaryotes, Hsp70 interacts with two cochaperones: J, the domain cochaperone, Hsp40, and the nucleotide exchange factor NEF. Hsp40 is known to stimulate ATP Hsp70 hydrolysis and can participate in the presentation of Hsp70 substrates [13, 14]. NEF promotes the exchange of nucleotides by Hsp70, inducing the release of ADP (Fig. 1) [15].

It has been proven that normally, Hsp70 plays several roles in signaling cascades involved in the cell growth and differentiation. The molecular mechanism of Hsp70 induction regulation depends on the activity of a unique heat shock transcription factor 1 (HSF1), which binds to the 5'-promoter regions of all Hsp genes and triggers the transcription. Under homeostatic conditions, Hsp70 is intracellular and associated with HSF1 [16]. High temperature, ischemia, and other causes for the accumulation of unfolded proteins lead to Hsp70 dissociation from HSF, leaving it free for target proteins to bind. In the stressed cell, dissociated HSF is transported to the nucleus, where it is phosphorylated, possibly by protein kinase C, to form activated trimers. The resulting trimers bind to the highly conservative regulatory sequences of the heat shock gene known as heat shock elements (HSEs). HSEs bind to the promoter region of the inducible gene Hsp70, which leads to an increase in the Hsp70 generation [17]. Through binding to HSF1, Hsp90 can also affect Hsp70: when Hsp90 dissociates with HSF1, the latter is released to bind HSEs and leads to an even greater Hsp70 induction [18].

The newly generated Hsp70 in combination with ATP, Hsp40 and Hsp90 binds to denatured proteins and acts as a molecular chaperone, promoting the repair, clotting and transport of damaged peptides within the cell. Subsequently, a complex is formed, which includes the Hip (hunting-interacting protein) and Hop (hunt-

ing-organizing protein) associated with the N and C terminal domains, respectively, due to which clotting and then refolding of denatured structures occurs [19]. If no clotting occurs, BAG-1 binds to the N-terminus of Hsp70, and CHIP E3 ubiquitin ligase binds to the C-terminus of Hsp70. This complex then interacts with the denatured protein and recruits it into proteasome [20]. Thus, Hsp70 is involved in the damaged proteins refolding.

Interaction of Hsp70 with some of the pro- and anti-apoptotic proteins

A stress-induced expression of Hsp70 allows cells to cope with a large number of unfolded and / or denatured proteins resulting from the external stress. Traditionally, such typical pathological processes include inflammation, hypoxia, apoptosis, and tumor growth [21].

Apoptosis, as the body's response to pathological changes, is involved in the pathogenetic links of many diseases, such as strokes, neonatal hypoxia, degenerative retinal diseases, graft rejection, Alzheimer's disease and other neurodegenerative diseases [21, 22].

Caspase-independent and caspase-dependent apoptosis pathways are distinguished. The caspase-dependent pathway of apoptosis is divided into internal and external. Complex signaling pathways occur in the cell from the initiation to the start of a signaling molecules cascade, including many proteins. It is obvious that the impact on any element of this cascade can be a therapeutic target for a pharmacological correction of apoptosis processes. For example, nerve growth factors inhibit apoptosis and appear to meet therapeutic needs in diseases with extensive autolysis. An increase in Bcl-2 expression can inhibit pathological neuronal apoptosis in response to neurotoxic factors. In addition, it has been proven that low molecular weight caspase inhibitors, for example, Z-VAD-FMK, are effective in the treatment of amyotrophic lateral sclerosis in animals [23].

Apoptosis is required to maintain cellular homeostasis. The Hsp70 expression increases the cell survival under stress. Hsp70 knockdown cells are sensitive to autolysis [24], while the Hsp70 overexpression inhibits apoptosis, acting either through the internal Akt / PKB mitochondria-dependent or the external receptor-dependent pathway [25].

External apoptosis is initiated by plasma membrane-bound proteins of the TNF receptor family, which lead to the activation of caspase-8/10 in the death-inducing signaling complex (DISC) [26]. Hsp70 can also inhibit the assembly of the DISC signaling complex, in-

hibiting apoptosis induced by Fas, TRAIL, and TNF. After TNF-induced DISC formation and activation of caspase 8, Hsp70 can inhibit BID activation [27]. When interacting with an extracellular ligand, membrane receptors transmit death signals to the intracellular space through their cytoplasmic domains. The membrane receptors involved in apoptosis belong to the superfamily of tumor necrosis factor (TNF) receptors, the activation of which depends on two main ligands: TNF and Fas. TNF and its receptors, namely TNFR-1 and TNFR-2, are responsible for initiating the main apoptosis pathway, i.e. the TNF pathway. The interaction between TNF and its receptors has been shown to signal death by recruiting two adaptive proteins: the TNF receptor-associated death domain (TRADD) and the Fas-associated death domain (FADD) protein. A cascade of these processes affects programmed cell death through the action of caspases. FNO ligands form homotrimers that bind to FNO receptors on the membrane [28]. In TNF- α -induced apoptosis, Hsp70 interacts with the FANCC protein (Fanconianemia complementation group C, PKR inhibitor) through its ATP domain and forms a triple complex with FANCC and PKR [29, 30]. It also resists TRAIL-induced apoptosis and the formation of a death-causing signaling complex with death receptors DR4 and DR5 [31]. The Hsp70 function in Fas-induced apoptosis is under-explored, but the adverse effects depend on the cellular context [32].

The internal apoptotic pathway is initiated by the release of various factors from the cell mitochondria. In response to the brain damage and the resulting oxidative stress, a transitional pore of permeability is formed in mitochondria. That leads to the release of cytochrome C into the cytosol, where a number of pro-apoptotic molecules ultimately cause the activation of effector caspases. Among these molecules, there are Bcl-2 family proteins, some of which are pro-apoptotic. These molecules are the main regulators of the mitochondrial membrane. Bcl2 and Bax are targets for the suppressor protein of p53 tumor. In response to DNA damage, Bcl2 transcription is repressed, and Bax is induced [33, 34]. Tumor cells often have mutated p53 that forms a stable complex with Hsp70 / Hsc70. A stress-mediated expression of Hsp70 inhibits nuclear import of p53 [35]. However, the Hsp70 regulation of the NF- κ B function is still under-explored. Cytosolic Hsp70 can inhibit the expression of NF- κ B, and membrane-bound Hsp70 can induce this transcription factor [36]. In neuronal stem cells, the Hsp70 induction by the recombination plasmid pEGFP-C2-HSP70 significantly blocks caspase-3 and reduces neuronal cytotoxicity, including a neuronal loss and a

synapse damage in cocultured cells [37]. After an inflammatory stimulus, oligodendrocyte progenitor cells and mature oligodendrocytes from mice deficient in Hsp70 come into apoptosis caused by the caspase-3 activation [38]. Fig. 2 shows some of the apoptotic proteins that Hsp70 interacts with.

Experience in pharmacological use of recombinant Hsp70 Neuroprotective action

Studies confirming the neuroprotective role of endogenous heat shock proteins [39] have stimulated the development of pharmacological strategies based on the use of recombinant human Hsp70 [40]. Thus, Xinhua Zhan et al. demonstrated that the administration of Fv-Hsp70 2 and 3 hours after focal cerebral ischemia resulted in a 68% decrease in the volume of the infarction zone and significantly improved sensorimotor functions compared to the control group [41]. Similar results were presented in the publication by a Russian scientific group under the guidance of M.A. Shevtsov. The authors demonstrated that both preliminary and postischemic intravenous administration of rhHsp70 dose-dependently reduced the zone. Moreover, a long-term treatment of ischemic rats with rhHsp70 in the form of alginate granules with a sustained protein release further reduced the infarction volume and the apoptosis zone [42].

Similarly, the intranasal rhHsp70 administration resulted in a two-fold decrease in the local ischemia volume in the prefrontal cortex in the study of the mice with a photothrombotic stroke. In addition, the intranasal rhHsp70 administration reduced the level of apoptosis in the ischemic penumbra, stimulated axonogenesis, and increased the number of synaptophysin-producing neurons. In an isolated crayfish mechanoreceptor consisting of a single sensory neuron surrounded by a glial membrane, exogenous Hsp70 significantly reduced photoinduced apoptosis and necrosis of glial cells [43].

Moreover, as a therapeutic agent for slowing down neurodegenerative processes, rhHsp70 also demonstrates a high potential. In the study by David J. Gifford et al., recombinant human Hsp70 delayed the onset of paralysis in a murine model of amyotrophic lateral sclerosis caused by overexpression of the mutant SOD1 gene. When administered intraperitoneally three times a week, starting from the 50th day of life, rhHsp70 led to an increase in life expectancy, a delay in the onset of symptoms, preservation of motor function, and an increase in the number of innervated neuromuscular connections compared with the control tissue [44].

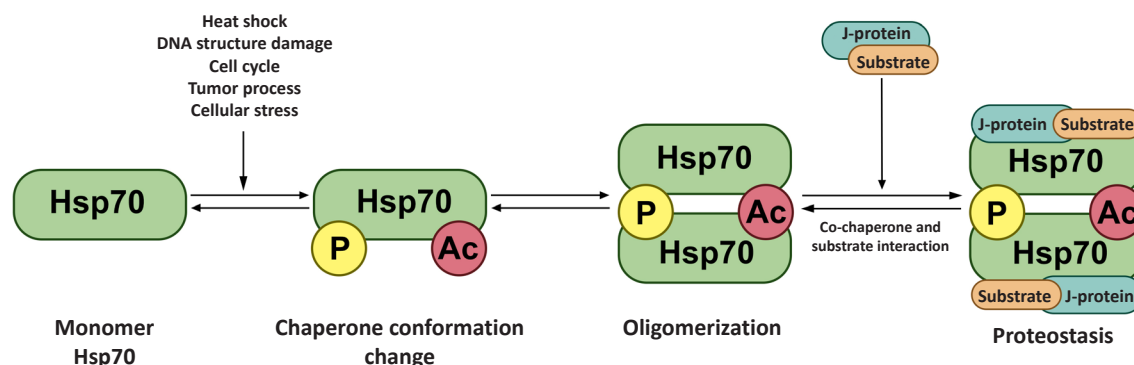


Figure 1 – Model of the Hsp70 oligomerization assembly line

Note: Cellular stress changes chaperone conformation, which facilitates Hsp 70 oligomerization. Co-chaperones and associated substrates bind to Hsp 70 oligomer, forming active chaperone complex

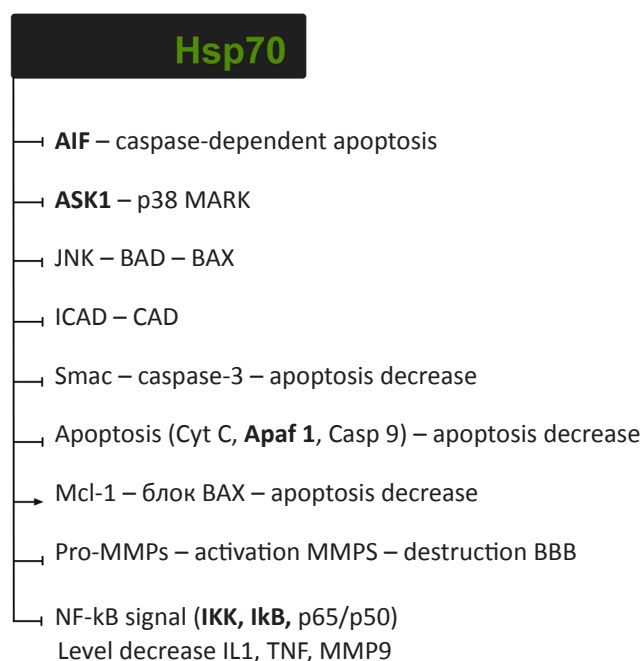


Figure 2 – Interaction of Hsp70 with apoptosis and inflammation regulating proteins

In the transgenic mouse model of Alzheimer's disease and in the mice with bullectomy, intranasally administered rhHsp70 quickly penetrates the affected areas of the brain and mitigates multiple morphological and cognitive anomalies, normalizing the density of neurons in the hippocampus and cortex of the brain and reducing the accumulation of amyloid- β and amyloid plaques [45, 46].

In addition to the direct cytoprotective activity against neurons, rhHsp70 demonstrates a GABA-ergic effect: a preliminary intracerebroventricular administration of Hsp70 reduces the severity of the seizures caused by NMDA- and pentylentetrazole. Moreover, traced Hsp70 in neurons was co-localized with NMDA receptors, synaptophysin, and L-glutamic acid decarboxylase [47].

Anti-inflammatory activity

A preventive Hsp70 administration reduced the toxic effect of *E. coli* endotoxin on the rat organism and significantly increased the survival rate of the animals during the experiment [48, 49]. In addition, in the models of sepsis caused by the administration of lipoteichoic acid, it was shown that the prophylactic administration of exogenous human Hsp70 significantly attenuates numerous homeostatic and hemodynamic disorders and partially normalizes the coagulation system disorders and many biochemical blood parameters, including the concentrations of albumin and bilirubin [50, 51].

It has been shown that both intracellular and extracellular Hsp70 modulates the activation of the key

pro-inflammatory factor NF- κ B [52]. Thus, overexpressed Hsp70 blocks the NF- κ B activation and p50/p65 nuclear translocation by inhibiting IKK-mediated phosphorylation of I κ B (NF- κ B inhibitor). It is of interest to note that the opposite effect occurs when Hsp70 is outside the cell. It is assumed that extracellular Hsp70 can act as a damage-associated molecular pattern (DAMP) through the innate immunity receptors TLR2 and TLR4 and thus trigger pro-inflammatory cascades. [53] An increase in the expression / secretion of NF- κ B-dependent pro-inflammatory cytokines, including interleukin IL-1 β , interleukin IL-6 and FNO- α , in response to extracellular Hsp70 in human lung cancer cells, dendritic cells and monocytes, was also notified. [54] However, other studies have shown that in the cultures of synoviocytes obtained from the patients with rheumatoid arthritis, extracellular Hsp70 inhibits the NF- κ B signaling pathway, decreasing the level of IL-6, IL-8, and MCP-1 [55]. In addition, it has been shown that extracellular Hsp70 reduces the production of proinflammatory cytokines such as FNO- α and IL-6 in monocytes exposed to TLR agonists and contributes to the attenuation of the inflammatory response [56].

Thus, the results of a number of studies indicate that Hsp70 exhibits a predominantly anti-inflammatory activity, but under certain conditions it can activate pro-inflammatory cascades.

Modern methods for producing recombinant forms of Hsp70

Currently, it is known about the creation of Hsp70A1 recombinant forms. In particular, two sources are isolated: its isolation from the biomass of the *E. coli* bacterial culture, expressing it in increased quantities, and from transgenic mice-producers. To analyze its activity, the following parameters are determined: a substrate-binding activity, the analysis of the restoring activity of proteins, the ability to displace endogenous substance from the cells, the ability to reduce endotoxin-induced ROS production, and the ability to stimulate the natural killer cell activity towards the cancer cells *in vitro*. It is known that a protein glycosylation during the production can complicate the result of its administration to patients, especially when the body contains cells expressing the native form, causing an autoimmune response. A modified version of the protein was named rhHsp70.128, which differs fundamentally from the wild-type protein (rhHsp70.135) at five putative N-glycosylation sites: QGDRTPSY, YFNDAQRQA, DLNKAINPD, KRNSAIPK, and ILNVAATDK. A chaperone activity of the recombinant Hsp70 was assessed using

carboxymethylated lactalbumin as a substrate protein. It was shown that the activity of the modified protein corresponds to the activity of the reference wild-type version and binds denatured lactalbumin with a similar efficiency. The next test consisted in measuring the activity of luciferase after its denaturation and recovery using the Hsp70 preparation in order to analyze its coagulability. The data show that all three tested samples were almost equally active. A series of experiments was also carried out to confirm the ability of the modified recombinant Hsp70 to displace its endogenous analogue from cells. Modified rhHsp70.128 as well as the wild-type probe, entered the cells and displaced endogenous Hsp70. An alternative way to obtain a recombinant Hsp protein, similar to that created in *E. coli*, was the creation of a female producers line expressing it in the mammary glands with a content of 1–2 mg/ml of protein in milk, depending on the animal. The study of its expression was carried out by the method of immunoblotting. It has been shown that the mutant protein can be efficiently isolated using ATP columns, as opposed to the wild type, by reacting to commercial antibodies. Based on the data obtained, it is obvious that the secretory production of the protein is technologically more advantageous in comparison with the cytoplasmic production due to the simplicity of its purification [57].

CONCLUSION

Chaperones are key regulators of cellular homeostasis that perform pleiotropic functions involving a wide range of signaling pathways. At the same time, heat shock proteins, the most studied family of chaperones, have a high pharmacotherapeutic potential for the treatment of a number of diseases associated with inflammation, apoptosis, and accumulation of misfolded proteins. This review was focused on the pharmacology of one of the key members of this family, Hsp70. The literature analysis confirms that this molecule is an endogenous regulator of many physiological processes and demonstrates tissue protective effects in modeling ischemic, neurodegenerative and inflammatory processes. The use of recombinant exogenous Hsp70 mimics the endogenous function of the protein, indicating the absence of a number of typical limitations characteristic of pharmacotherapy with high molecular weight compounds, such as immunogenicity, rapid degradation by proteases, or a low degree of passage through histohematogenous barriers. Thus, Hsp70 may become a promising agent for clinical trials as a drug for the treatment of patients with neurological, immunological, and cardiovascular profiles.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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