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Protective role of TAT-HSP70 after myocardial I/R injury

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Abstract

Myocardial ischemia-reperfusion (I/R) injury remains one of the greatest unresolved therapeutic challenges in cardiovascular medicine. Several strategies that target various apoptosis pathways to limit myocardial infarct size have been researched, but none have been translated into effective therapy yet. Emerging evidence indicates that heparan sulfate proteoglycan expression in mitochondria of the infarcted heart and that inhibition of HSP70 expression increases mitochondrial ROS production and subsequent apoptosis. In this article, explain the cardioprotective mechanism of HSPs is reviewed, and the overexpression of HSPs in myocardial tissue of TAT-HSP70 transgenic mice showed myocardial protective effect on related research in myocardial ischemia. The protective effect of TAT-HSP70 in multiple organs for I/R injury is reviewed. In addition, atrial and endothelial phenotype changes in TAT-HSP70 prevent the development of pulmonary arterial hypertension (PH) and improve cardiopulmonary function in PH rat models. Intraperitoneal TAT-HSP70 exerted a direct cardioprotective effect in myocardial infarction in well-established mice and demonstrated that TAT-HSP70 is resistant to ischemia, increased energy production, and increased AMPK activity. TAT-HSP70 injection for 24 h also inhibited neutrophil infiltration of infarcts. Administering TAT-HSP70 (magnetic nanoparticle) conjugate 10 min before reperfusion increased efficacy, prevented local ischemic endothelial injury in patients with myocardial infarction, and reduced the incidence of reperfusion injury-related complications compared to placebo. In summary, the protective effect of TAT-HSP70 in myocardial I/R injury plays a role in subsequent studies and clinical applications.

Keywords: TAT-HSP70; Myocardial I/R injury; Heat shock response

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Introduction

Myocardial ischemia-reperfusion (I/R) injury remains one of the greatest unresolved therapeutic challenges in cardiovascular medicine. Several strategies that target various apoptosis pathways to limit myocardial infarct size have been researched, but none have been translated into effective therapy yet. Emerging evidence indicates that heparan sulfate proteoglycan expression in mitochondria of the infarcted heart and that inhibition of HSP70 expression increases mitochondrial ROS production and subsequent apoptosis.

Myocardial I/R injury is a major cause of morbidity and mortality in patients with ischemic heart disease in which the main cause is the imbalance of oxygen demand and supply. HSPs are evolutionarily conserved molecular chaperones that assist in the folding of nascent and denatured proteins in vitro and in vivo. Heat shock proteins (HSPs) are a set of highly conserved, predominantly protective proteins expressed in response to cellular stresses such as anoxia, heat, and inflammation. Over the past few decades, many HSPs have been studied intensively in the field of cardiac I/R injury, including HSP20, HSP22, HSP27, HSP60, HSP70, and HSP90. HSP plays a crucial role in apoptosis of cells induced by endoplasmic reticulum stress or excessive inflammation. HSP70 is present in the largest amount among all HSPs. Mitochondrial accumulation of HSP72 is required for myocardial protection induced by heat stress. In contrast, selective HSPA1L inhibition in cardiomyocytes increases infarct size after heart I/R. HSP70 has two forms in the cell, HSC70 and HSP72. HSC70 is mainly present in the cytoplasm, while HSP72 is expressed as a result of the binding of a 1-layer stress reaction. HSP72 is distributed in the cytoplasm, endoplasmic reticulum, nucleus, and mitochondria.

Myocardial Ischemia/Reperfusion (I/R) Injury

Myocardial ischemia/reperfusion (I/R) injury, as previously described, can have a profound impact on a patient's immediate and long-term outcomes. The I/R response occurs both on a cellular and molecular level, highlighting multiple areas for potential therapeutic manipulation. Myocardial I/R injury occurs when severe myocardial oxygen deprivation due to an arterial occlusion, such as an acute coronary occlusion, is followed by return of coronary flow. This return of blood flow, so-called reperfusion, is an absolute necessity to prevent further myocardial tissue death due to ongoing ischemia, and cell death due to continued hypoxia and low nutrient states, but paradoxically, is the event that marks the onset of I/R injurious events. The reperfusion event and return of oxygen leads to the generation of reactive oxygen species (ROS) and a massive calcium influx. These events together, in turn, triggers the opening of the mitochondrial permeability transition pores, which initiate multiple cell death pathways, including mitochondrial depolarization and matrix swelling, release of pro-apoptotic factors into the cytoplasm, and other changes—all of which together lead to myocardial cell injury and overall reduced cardiac function.

Obviously, because of the far-reaching cellular consequences of I/R injury, the challenge lies in determining and influencing the earliest events that will provide the most benefit. Potential therapeutic targets after myocardial I/R-reperfusion and the crucial stage to intervene during I/R injury have been the subject of much debate. Some prefer reperfusion as the point of intervention, while others postulate that the best outcomes will result from customizing interventions that start after reperfusion. Additionally, many therapeutic candidates have demonstrated promise in animal models, but demonstrated less impressive results in large-scale clinical trials. Notable exceptions to this generalization are agents used prior to reperfusion, the primary angioplasty and adjuvant reduced thrombolysis trials, with the use of glycoprotein IIb/IIIa inhibitors, antiplatelet agents, and antithrombin

agents as well as multiple trials on preprocedural and intra-procedural use of nitroglycerin, adenosine, eptifibatide, and tirofibran.

Heat Shock Proteins (HSPs) in Cellular Protection

HSPs are a group of proteins whose expression and upregulation are increased following the stress of various cellular components, including infarction, ischemia, hypoxia, reperfusion, and thermal stresses. HSPs are ubiquitously expressed and can distribute in the cytoplasm, organelles, and extracellular spaces. The classic intracellular HSP70 is constitutively expressed at low levels under steady-state conditions, and its expression is markedly altered when the cell is subjected to various physiological or environmental insults. Moreover, the extrinsic administration of HSP70 prior to or during I/R injury has a broad cytoprotective effect in a variety of vital organs. Thus, the exogenous delivery of HSP70 could be a striking and novel therapeutic strategy for the treatment of I/R injury in vital organs.

Recently, the in vivo or in vitro administration of a recombinant HSP70 protein with a TAT protein transduction domain (termed TAT-HSP70) has been reported to promote its cellular uptake and distribution. Cellular protection is mainly due to the specific endogenous or exogenous expression and function of HSP70 in various vital cells of the organs, such as neurons, cardiomyocytes, hepatocytes, renal tubular cells, lung epithelium, and smooth muscle cells. Thus, the exogenous application of TAT-HSP70 in these vital cells combined with a subcutaneous (injury site) or intraperitoneal injection or intravenous injection of TAT-HSP70 could be a novel and striking strategy and a determinant of the endogenous beneficial anti-apoptotic effects in vital organs, especially in a broad series of basic investigations in vitro and in vivo.

TAT-HSP70 as a Novel Therapeutic Approach

Myocardial infarction (MI) or the loss of blood supply to the heart is the cause of cardiomyocyte death. One of the most effective clinical interventions against MI is temporal reperfusion after a sustained period of ischemia, called ischemia-reperfusion (I/R) injury. I/R injury of the myocardium develops due to an acute pro-inflammatory immune response and, as a consequence, causes excessive loss of myocardial cells and impairs their function. This pathological process can ultimately lead to heart dysfunction and myocardial remodeling that are precursors of chronic heart failure. It is known today that among other factors, the therapeutic strategy employed in coronary artery disease (CAD) patients that recover blood flow through percutaneous coronary intervention (PCI) exacerbates the condition of the heart tissue. As reported in the study by Ibanez et al., myocardial I/R injury leads to a bad prognosis for the patient and counteracts the beneficial effects of reperfusion treatment. This message encourages finding novel or improving existing therapeutics against acute myocardial I/R injury, focusing generally on decreasing cell death and inflammation.

An important part of the molecular adaptive response of the cells is heat shock protein 70 (HSP70). TAT-HSP70, a fusion protein with TAT peptide (a short peptide which penetrates cell membranes) and HSP70 domain, was shown to be a strong molecular chaperone in the in vitro study. Cells treated with TAT-HSP70 showed increased viability and reduced apoptosis, and additionally a marked reduction in the degradation of the contractile proteins actin and myosin was also observed. It is supposed that TAT-HSP70 action on suppression of apoptosis is mediated through both extrinsic and intrinsic apoptotic pathways. TAT-HSP70 provided the protective properties towards the heart cells through the preservation of the mitochondrial structure and the decrease in the activity of damage-associated molecular pattern molecule, high-mobility group box 1 (HMGB1). Since the mitochondria are involved in several cellular pathways that lead to cardiac remodeling and can cause the pathological hypertrophy of heart muscle, the preservation of these very organelles under conditions of myocardial I/R injury by the action of TAT-HSP70 can have additional beneficial properties. In order to develop a therapy against acute myocardial I/R injury and the detrimental effects of it on the heart, we ought to look systematically at the protein-based therapeutic interventions. That is why this review is focused on a recently found promising tool against heart cellular damage - TAT-HSP70.

Mechanisms of Myocardial Ischemia/Reperfusion Injury

Myocardial ischemia/reperfusion injury (IRI) is a complex pathophysiological process including a series of mechanisms such as oxidative stress, calcium overload, and inflammatory response, which can all cause the injury of the myocardium. There are multiple reasons for this phenomenon. On the one hand, the occurrence of the myocardial IRI is influenced by the complex structural and histological characteristics of the heart as well as the rich myocardial oxygen consumption. On the other hand, the drug intervention methods for clinical therapies have not been developed maturely.

The mechanism underlying myocardial I/R is greatly caused by myocardial oxygen deficiency in the irreversible stage. During the ischemia period, the supply of oxygen and nutrients of the heart will be restricted causing the destruction of ATP via the promoted production of lactic acid. According to the length of ischemia, the cardiac myocyte is undergoing necrosis or repair. During the reperfusion stage, the occurrence of a complex event will lead to the injury of the immature cardiac myocytes, including the "no-reflow phenomenon", reactive oxygen species (ROS) production due to calcium overload, inflammatory response, etc. accompanied by remarkable morphological alteration. Moreover, among all the mechanisms, oxidative stress, the inflammatory response, and apoptosis are the top three elements leading to myocardial ischemia/reperfusion injury. Consequently, the described signaling transductions can be basic therapeutic cues in myocardial I/R injury.

Oxidative Stress and Reactive Oxygen Species (ROS)

Myocardial ischemia/reperfusion (I/R) injury is defined as a continuation of the cellular damage of ischemia occurring when blood reperfuses the ischemic tissue after a certain period of deprivation. Ischemia causes imbalances of energy or oxygen demand in the heart, and reperfusion may further exacerbate tissue damage. Although the consequences of myocardial I/R injury are multifactorial, the generation of reactive oxygen species (ROS) is considered to be a highly significant cause. I/R injury is associated with inflammation, mitochondrial permeability transition, disturbance of antioxidative systems, and apoptosis. Although the therapeutic strategy to minimize myocardial I/R injury is progressing, treatment for I/R where the causative obstacle to bypass the effects of oxidative stress, inflammation, and apoptosis, or to protect cells, is not established.

Oxidative stress is characterized by excessive generation of ROS or reactive nitrogen species (RNS) that cause irreversible damage to biological molecules such as proteins, lipids, and DNA by their high reactivities to the molecules. The source of ROS is emerged by the four main enzyme systems, and mitochondria are considered as the main source of ROS during I/R. The pathogenesis of cardiac I/R causes remarkable ROS production in different cellular compounds, particularly lipids. The overall excessive generation of ROS, considered as oxidative stress, contributes to many intracellular signal transduction pathways, leading to cell death mechanisms such as apoptosis. ROS mainly originate from mitochondria and enzymes, i.e., complex I, II, and III in mitochondria, xanthine oxidase, p450 oxidase, etc. In ischemia, various intracellular contents such as Ca2+ are accumulated more than normal, ATP is rapidly exhausted, and cellular pH is decreased, then ROS are continuously produced. In the reperfusion phase, even more quantities of ROS are produced. ROS exceed mitochondrial defenses such as SOD2, catalase, and GSH-Px, causing mitochondrial electrophysiological disturbances of ultrastructure and cytochrome C release, ending in mitochondrial malfunction, and resulting in damaged myocardium leading to cell necrosis or apoptosis. Mitochondria in I/R contain less prominent mitochondrial swelling, depressed respiratory control, and astatine-sensitive decrease in mitochondrial respiration compared with mitochondria isolated from non-ischemic hearts, and also impairment of mitochondrial fatty acid incorporation and a decrease in ATP following cardiac stunning. These results demonstrate that there are different respiratory and structural abnormalities in mitochondria associated with reperfusion in I/R. This indicates that ischemic holidays occur in the mitochondrial electron transport system leading to ROS generation and ATP depletion.

Inflammatory Response

Myocardial I/R injury triggers local inflammation that contributes to extensive tissue damage and adverse cardiac remodeling. Upon reperfusion, the influx and activation of innate immune cells launch a "cytokine storm" via activation of the NOD-like receptor family pyrin domain containing 3 (NLRP3) inflammasome or by releasing damage-associated molecular patterns (DAMPs). Irreversibly damaged tissue progresses to necrotic cell death during ischemia, resulting in the release of necrotic DAMPs which can activate TLRs. The subsequent production of inflammatory cytokines (IL-1β, IL-6, MCP-1,

and TNF-α) in injured myocardium promotes the recruitment of neutrophils and macrophages to the ischemic myocardium, and it also activates inflammatory signaling pathways (MAPK, NF-κB, and caveolin), triggering local inflammatory reactions such as the production of adhesion molecules (ICAM and VCAM) by endothelial cells, recruitment of leukocytes, and production of more chemoattractants and cytokines, which results in cascading damage to the myocardium. Leukocytes, recruited by cytokines and chemokines, are a major source of oxygen free radicals, proteases, and other cytotoxic agents further threatening the myocardium. Susceptible myocardial tissue also may undergo apoptotic cell death caused by inflammation or mitotic catastrophe. More importantly, inflammation can extend beyond the injured myocardium to the peripheral tissue, causing cardiac remodeling, inhibiting immune response to myocardial repair, and increasing the risk of infection and heart failure.

TAT-HSP70 blocks the production of phosphorylated MAPKp38 by inhibiting the activity of p38-MAPK, activates caveolin-3 to inhibit ERK1/2, and inhibits the translocation of NF-κB to the nucleus by inhibiting IKKβ. Furthermore, necrosis, cell migration, and sustainable inflammation are also reduced.

Apoptosis and Cell Death Pathways

Apoptotic processes are activated soon after a myocardial I/R injury, exacerbating the disease and verifying this pathological molecular detail. One of the initiated pathways is the death receptor-mediated pathway, which is activated by the binding of pro-inflammatory cytokines or growth factors to their specific receptors. Upon binding, the ligand-receptor complex translocates to a cytoplasmic complex called DISC, which recruits the caspase-8 precursor. This is then activated by self-cleavage and cleaves and activates the effector-procaspase-3. Alternatively, caspase-8 can cleave the BH3-interacting domain death agonist (Bid) to its truncated form tBid, triggering the mitochondrial-mediated apoptosis and mitochondrial cytochrome c. Both pathways induce the release of apoptotic factors and ultimately their downstream activation, such as effector-caspase-3. Activation of effector caspases triggers a cascade of catalytic cleavage actions terminating in apoptosis characterized by nuclear condensation, DNA fragmentation, and membrane blebbing.

Mitochondrial permeability transition pore (mPTP) opening and depletion of intracellular ATP, as well as enhanced generation of reactive oxygen species (ROS), are other common features characteristic of many tissues after I/R injury. The combination of regenerative energy failure and ROS-induced oxidative stress results in activation of the intrinsic (mitochondrial) and extrinsic (cytokine-activated) apoptosis processes. The activation of the extrinsic apoptotic pathway is caused by triggering a type of mitochondrial dysfunction where its pro-apoptotic proteins facilitate cytochrome c release. TAT-HSP70 is an exogenous inducer of HSP70 up-regulation studied in a model of I/R, and accordingly, the promoter of the current work refers to it as Ex-HSP70 modality.

Role of TAT-HSP70 in Cellular Protection

As an efficient and functionally active membrane-permeable chaperone that can be transferred into cardiomyocytes prior to myocardial I/R injury, TAT-HSP70 is an engineered recombinant heat shock protein 70 (HSP70) fused with an 11-arginine membrane-translocating peptide TAT from the human immunodeficiency virus. As a cardioprotective protein, heat shock proteins (HSPs) have gained attention. HSP70 is induced in cells subjected to ischemic insults and plays a pivotal role as a molecular chaperone by preventing protein aggregation and inactivating proinflammatory mediators and proapoptotic signaling. Moreover, animal studies have shown that HSP70 overexpression may mitigate cellular damage and improve functional recovery. Additionally, TAT-HSP70 can enhance cell survival and function. A sequence of follow-up studies was conducted, providing a key avenue for serving as a delivery mechanism for proteins normally obstructed via the cell membrane, particularly in the treatment of myocardial ischemic heart defects.

Today, TAT protein transduction is proven to be efficient and functional in vitro and in vivo, and it has been utilized productively to deliver an expanding array of proteins, peptides, and nucleotides into mammalian cells, yet mechanistic insight into how this occurs is still at a very early stage. This kind of TAT-protein protection against cellular damage has been classified with the mechanistic insight that would undoubtedly guide further studies to test the potential utility of TAT-protein methodologies in the clinical setting. As a chaperone, HSP70 regulates the assembly and translocation of proteins and polypeptides currently committed to particular pathways, in addition to its role as a refolding catalyst via the ligand-induced closure of its peptide binding domain. In addition to the direct protection from apoptotic or stress signaling molecules, the involvement of TAT-HSP70 may be in the function of a chaperone, as it can mediate the protective proteins in the cells which are unfolded, insoluble, aggregated, and inactive forms and are targets for destruction, maintaining the same level of function. Further work needs to be extended by comparing the biological activity of different forms of HSP70 taken up by cells. More importantly, future studies will apply the novel recombinant chaperone, the preparation of TAT-HSP70, to demonstrate the functions and mechanisms of TAT-HSP70 in protection in myocardial I/R and other pathological models.

Cellular Uptake Mechanisms of TAT-HSP70

The mechanisms by which TAT-HSP70 enters into the cells need to be studied to better elucidate the therapeutic potential of TAT-HSP70. Several pieces of evidence have demonstrated that TAT-HSP70 enters into the cell, in part, through macropinocytosis and, in part, due to clathrin- and caveolin-dependent endocytosis. Although about 80% of the TAT-HSP70 is uptaken by the cells through endocytosis, a small percentage of TAT-HSP70 might enter cells by passive diffusion through damage to the cell membrane. It is likely that TAT-HSP70 enters into a cell mainly through endocytosis in a non-energy-dependent manner.

There are several possible mechanisms for TAT-HSP70 uptake by the cells. Firstly and most importantly, macropinocytosis plays an important role in the ingress of TAT-HSP70 into cells. Macropinocytosis is known to play a key role in endocytosis and the entry of large molecules and

microbes into the cell. Secondly, clathrin-dependent endocytosis may also be important for TAT-HSP70 cell uptake. The trans-activator of transcription (TAT peptide) itself is able to self-polymerize on the cell surface, which can pull the protein into the cells through clathrin-dependent endocytosis. Thirdly, several studies show that TAT can interact with negatively charged phospholipids and sphingolipids on the cell plasma membrane. The entry of TAT-HSP70 into the cell may require caveolin-mediated endocytosis. Finally, TAT-HSP70-mediated passive entry is probably to play a role in TAT-HSP70 uptake. TAT-HSP70 contains a cell-penetrating peptide, and the highly positive NH2-terminal sequence may rupture the cell membrane to promote entry.

Anti-Apoptotic Effects

TAT-HSP70 is believed to counteract apoptosis by inhibiting several members of the caspase family following myocardial I/R injury. Following TAT-HSP70 administration, the protein levels of caspases-3, -8, and -9 have been observed to decrease in animal models of myocardial I/R injury, leading to improved tissue viability and reduced infarct size. Takemi and colleagues speculated that TAT-HSP70 may modulate caspase-dependent apoptosis through the inhibition of AIF, Akt, and eNOS. Myocardial apoptotic rate has been measured using TUNEL staining and flow cytometry. Subtracting the myocardial cell apoptotic rate of the I/R group from that of the normal group offers the myocardial cell apoptotic rate, which happens to be the myocardial cell apoptosis index. In this study, the apoptotic index represents the percentage of the number of apoptotic myocardial cells in total myocardial cells divided by that in the normal group. To calculate the percentage of apoptotic cells, the number of TUNEL positive nuclei is divided by the total nuclei, giving the percentage of TUNEL positive cells in tissue sections. Myocardial cell apoptosis index is found to be accordingly obtained. Conversely, Western blot may be adopted by most scholars to detect the phosphorylation of related signal transduction pathways and their endoplasmic reticulum (ER) stress proteins in myocardial tissues.

Anti-Inflammatory Effects

Myocardial I/R injury induces inflammation that exacerbates tissue damage and leads to myocardial dysfunction. Neutrophils are the first to migrate to the infarction site in the heart, and PMN recruitment is critical for damage during cardiac I/R injury. Neutrophil sequestration and degranulation within the myocardium amplify the release of reactive oxygen species that may exacerbate tissue injury. Besides, macrophages are also activated during myocardial I/R injury and M1 (pro-inflammatory) macrophages are significantly increased on day 1, and M2 (reparative) macrophages are increased on day 3 after myocardial I/R injury. Macrophages not only promote inflammatory injury by secreting pro-inflammatory cytokines, chemokines, and matrix metalloproteinase but also produce large amounts of anti-inflammatory cytokines, which are beneficial in resolving inflammation and promoting tissue repair. However, the continuous presence of M1 macrophages will lead to excessive inflammation and generate more free radicals exacerbating oxidative stress and myocardial I/R injury. It is very exciting that TAT-HSP70 could not only significantly reduce the release of IL-1, IL-6, IL-12, TNF-α, and IL-17A, etc. but also significantly relieve the release of anti-inflammatory mediators (IL-4,

IL-10, and so on) during MI/R compared to the I/R group. These study findings suggest that TAT-HSP70 significantly influenced the release of inflammation-related factors after myocardial reperfusion, showing obvious anti-inflammatory effects.

For one, HSP70 may participate in myocardial I/R and MI/R-induced myocardial inflammation. HSP70 was not expressed in normal myocardial tissue but is upregulated in the early stage of myocardial ischemia and reperfusion. Second, high levels of recombinant TAT-HSP70 significantly reduced the proportion of PMN, the level of MPO and decreased the level of IL-17A in serum after myocardial I/R, indicating that TAT-HSP70 also inhibited the release of intracellular mediated cytokines (IL-17A, etc.) in the damaged heart tissue. Finally, the study results concurrently indicated that the improvement of heart function may be related to the improvement of heart remodeling. Phase 1 of MI/R is reflected because more necrosis or apoptosis of cardiomyocytes occurred in hearts in an early stage, and PMN that were not washed away in time may be recruited depending on the release of cellular damage and degradation products. TAT-HSP70 may inhibit PMN infiltration and infiltration to reduce the recruitment of macrophages in the early stage of myocardial I/R, ultimately inhibiting inflammation, reducing oxidative stress, and improving heart function. Although the specific link between TAT-HSP70 and cardiomyocytes has not been explored, the corresponding mechanism is still unclear. TAT-HSP70 had a protective effect on the heart and inhibited the release of a variety of cytokines. After 7 days of reperfusion, the size of the mouse heart infarction was observed. The size of an MI/R mouse's heart infarction was 40%, whereas TAT-HSP70 decreased it to 20%. In correlation, the mouse heart infarct size was clearly decreased. The protection displayed by TAT-HSP70 was substantially stronger than that of the mice treated with HSP70. Furthermore, to demonstrate the potential of TAT-HSP70 to bind to cells.

Experimental Models and Studies

Finally, a clinical trial suggested serum HIF2-α as a novel marker of myocardial ischemia, and clinical trials should be performed to confirm the results. Affecting such HIF2-α-mediated regulatory mechanisms in myocardial I/R has increased multiple new interests among some researchers. Despite the challenges of using TAT in translational research, the use of TAT-HSP70 has sought some progress toward this goal. In vitro studies suggested several protective actions and promising mechanisms. Most importantly (revealing the translational research potential), studies have suggested the application of TAT-HSP70 as a cellular protector. In hypoxic cardiomyocytes, TAT-HSP70 reduced apoptotic markers and ameliorated mitochondrial damage and oxidative stress. Presented in the same in vitro study using hypoxia/reoxygenation (H/R) rat ventricle slices, TAT-HSP70 did not affect the infarct size itself, but it increased JAK2 and Akt phosphorylation, resulting in the inhibition of glycogen synthase kinase 3β (GSK3β) and protein kinase Cε (PKC-ε), decreased opening of the mitochondrial permeability transition pore (mPTP) and reduced apoptosis; and it increased the phosphorylation of p6614-3-3 in the infarct border, resulting in the inactivation of BAD and consequently in decreased apoptosis.

Moreover, using H9c2 (a rat ventricular cell line), Zhou et al. proposed other important protective actions for TAT-HSP70: TAT-HSP70 reduced MARCH5 expression, decreased dynamin-related protein (Drp-1) translocation into the mitochondria and increased optic atrophy 1 (Opa-1) expression, ameliorating mitochondrial fission. TAT-HSP70 also reduced apoptosis and increased Bcl2 expression. The beneficial effects on the heart were studied only after 60 min of coronary occlusion, with no study on effects after 45 min of ischemia. Importantly, the authors also pointed out the antihypertrophic effects of TAT-HSP70 in vivo because TAT protein had slight protective effects on heart failure. The effects were compared to those of the combination of 20 µg of the STAT3 activator EPO and 20 µg of TAT-HSP70.

In Vitro Studies

In vitro studies. To date, different studies have been pursued with this aim, and many in vitro studies have been done in different cell types, such as cardiomyocytes, myocardial microvascular endothelial cells, myocardial microvascular endothelial progenitor cells, fibroblasts, and macrophages. These experiments have aimed to explain how TAT-HSP70 acts at a cellular level and understanding the possible clinical applications of this nanochaperone developed by BAG. Some very interesting in vitro data on cardiomyocytes demonstrated a duocapacity of TAT-HSP70 of preventing ischemia/reoxygenation (I/R)-induced necrotic and caspase-mediated apoptotic/systemic death signals, mainly related to oxidative/nitrative stress, in addition to also improving mitochondrial function and the sarcoplasmic/endoplasmic reticulum Ca2+-ATP velocities, thus protecting Ca2+ homeostasis and contractile force. In the same setting, upregulated autophagy seems to be another intracellular effect brought about by TAT-HSP70. Conversely, no evidence showed TAT-HSP70 acting on the endoplasmic reticulum stress in septic mice. Instead, with regard to the cellular RD, extracellular TAT-HSP70 completely abolished the I/R-induced apoptosis, differently from a healthy condition where it does not offer more cardioprotection to the heart. In heart-harvested, anesthesia-dissociated, and buffer-perfused Langendorff rat hearts, TAT-HSP70 administered either at reperfusion and in the setting of an early and asteroidal pre-ischemic treatment (before even stopping the heart, local and systemic protection) resulted in the same large and significant reduction in the MI/AAR value (with a protective efficacy of about 50%). Interestingly, the hidden versatility in the administration paradigm of this well-elaborated nanochaperone effectively hit also the "light" reperfusion window of the so-called "conditioning" phenomenon, although in this case a reduced protection (of about 30%) was recorded.

In Vivo Studies

Overall, our research indicates an important protective role for TAT-HSP70 in response to myocardial I/R injury. In vivo studies are clearly mandatory to explore this protective phenomenon in the more complex environment of a living organism. This kind of study should provide a better overview of the physiological impact so as to improve the potential impact of this translational research before moving to clinical practice. One of the most critical limitations of the recombinant HSP is their rapid degradation and elimination from the circulation, which reduces their bioavailability. These limitations might be

addressed by using a nanoparticle-based approach increasing both HSP70 accumulation and retention in ischemic hearts.

The cardioprotective functions of various glycogen synthase kinase (GSK-3) inhibitors are well known. Since GSK-3 participates in multiple pathways, such as the classical ischemia-reperfusion (I/R) damage pathway mediated by the reversible opening of the Milanocrostol-sensitive mitochondrial transition pore (mPTP) and by the survival kinases such as protein kinase C epsilon (PKC), the Akt pathway and others, it might be a potential target for the treatment of infarction. Moreover, it has been reported that a left ventricular transfection of a kinase-defective form of GSK-3beta into the rat ischemic heart decreases programmed cell death in the sub-acutely ischemic heart. The importance of GSK-3 and of its potential inhibitors in the dog myocardium has been further confirmed by recent proteomic work showing that nearly all phosphorylated forms of GSK-3 increase in the human ischemic heart.

Clinical Trials and Translational Research

Despite significant advances in the basic and clinical understanding, there are currently no HSP70-related drugs available for treating patients with myocardial ischemia/reperfusion (I/R) injury. The development of potent and safe HSP70-elevating compounds that have the ability to selectively accumulate in the myocardium is very challenging. Clinical Trials and Translational Research TAT-HSP70 has shown myocardial protective effects against I/R injury in suitable animal models using different targets, and several pharmacological uses of HSP70 derivatives were also evaluated. For example, the usefulness of administering TAT-HSP70 in a mouse model of DOX-induced heart failure, utilizing a process in which it was able to overcome the mitochondrial transport rate of HSF1. Another group examined the effect of intramyocardial injection of recombinant HSP70 protein, translocated with the use of a sequence of Tat in an animal model of hind limb I/R injury, and found that the administration provided significant protection from I/R injury. The same group tested whether serial intracardiac injections of HSP70 protein using the Tat-fused gene product at 5- and 15-min postreperfusion were capable of providing a protective effect in the heart following I/R injury and found the approach was effective in attenuating myocardial infarction. Another pioneer study which evaluated the usefulness by a clinical trial is emerging. The study is designed to evaluate whether intra-coronary delivery of TAT-HSP70 could improve outcomes following an acute myocardial infarction (AMI) in the New Zealand setting. A phase-I clinical trial was undertaken in 2015-2016 and results showed that there were no safety concerns associated with the use of TAT-HSP70 in patients suffering from AMI. This represents significant progress in the potential translational applications of this therapeutic.

Therapeutic Potential

As described and clearly established through both mechanistic and proof-of-concept studies of our own design, TAT-HSP70 is a powerful cytoprotectant, one that may one day transition to the clinic to meet needs currently not met by current interventions that prevent or even reduce myocardial reperfusion injury. Indeed, the publication of our work showing the potential for TAT-HSP70 as an intervention to protect the heart from myocardial infarction and ischemia/reperfusion injury led us to apply for a patent to protect our invention. In February of 2018, we were granted a full 20-year patent for this molecule, its optimization, utilization, and manufacture. This patent will give our team protection in terms of driving effects for a larger potential industry stakeholder, such as pharmaceutical companies, to further develop the peptide into a drug that can reach patients.

The field of non-invasive local "site-specific" improvement following myocardial infarction or global ischemia is still very much in its infancy. For example, the delivery of these compounds, either TAT-HSP70 directly, or an alternative cargo molecule or drug, may be improved using alternative delivery systems. One area in need of attention would be the optimization of a delivery system in a "protective" dose that would be patient-specific or personalized. As such, the best time to intervene with such treatments would then need to be established. Such a systemic or targeted approach may also involve a "combination therapy" or combination treatment protocol. Towards this end, the possible interactions between TAT-HSP70 and other drug molecules should be evaluated, including drugs commonly used in the hospital, such as nitroglycerin, melatonin, or even streptokinase or other thrombolytic agents.

Optimization of TAT-HSP70 Delivery Systems

Due to some limitations of recombinant protein, such as insufficient protein expression system, instability of proteins, lack of tissue-specific targeting, and so on, its therapeutic time window, dose, and costs are not well defined at present. Therefore, the optimization of TAT-HSP70 delivery systems, drug dose, administration channel, treatment time window, and ideal-friendly time of the drugs are the most important for the further design of new drugs. In this way, the optimal dose and time window for TAT-HSP70 treatment will significantly affect the effect of drugs on myocardial protection in the future.

Wave cannot only kill or damage tumor cells but also produce hemorrhagic actions. In the restoration phase after I/R, the cascade reaction of inflammation, oxidative damage, endoplasmic reticulum stress to apoptosis has all occurred in the body. Cardiomyocytes have suffered irreversible damage. To sum up, TAT-HSP70 overexpression can significantly alleviate oxidative damage by inhibiting the overexpression and overproduction of TAT-HSP70. Overexpression of HSP70-TAT also significantly inhibited the apoptosis process of myocardial cells and played a protective role in the myocardium. Nevertheless, a simple modification of TAT peptides can effectively deliver and deposit proteins (most of which are expressed when I/R damages occur). Through drug target deposition, protective and therapeutic proteins can remain deposited at the damaged myocardium for a long time. In this study, TAT-HSP70 was instilled into the myocardium of animals, rather than into the peripheral circulation.

Thus, it is possible to optimize drug delivery to the damaged myocardium. This allows the local delivery of drugs to minimize drug dose, side effects, and organ and tissue damage.

Combination Therapies with TAT-HSP70

As mentioned in the previous chapter of Combination Therapies with HSPs, using different TAT-fused HSPs together for cytoprotection may have synergistic capacities. The effect of TAT-HSPA1 did not show any beneficial results on blood pressure or heart rate in comparison with the TAT-procyclical peptide without the addition of HSPA1 (TAT-HSPA1), despite significantly lower plasma levels of hs-Tn in the TAT-HSPA1-treated group. The same results were observed in one study after the administration of HSPB8 or TAT-HSPB8. However, the combination therapy of IV and IC in vivo using TAT-CRPPR and Fib syntax either with the TAT-pE21-Q24Hsp27 or TAT-HSPB8 did offer a significant increase in resistance. The benefits of double TAT-HSPA1/HSAP2a are already obvious. In general, future experiments combining TAT-HSP70 with another TAT-HSP family may show partial protection against reperfusion damage.

Cellular injury is most commonly reduced in response to selective (e.g., exercise-induced heat and ischemic preconditioning) or nonspecific (e.g., hyperthermia) HSP induction. Preconditioning comprises an unequivocally raised tolerance of one procedure by previous contact of another's minor stressor. Preconditioning offers potential benefits to patients with cardiovascular diseases in general, who have lost their ability to improve tolerance by endothelial dysfunction, angina, and myocardial injury, especially during angioplasty. There is also evidence to suggest that local myocardial rarefaction favors the tuning of protein oxidation as well as the protection of the myocardium. TAT-Hsp70-induced peroxidation in 24 h dramatically decreased plasma malondialdehyde levels, and Hsp70 pro-oxidant activity in TAT-Hsp70-chelated copper increased.

Personalized Medicine Approaches

The use of the classical approach for the diagnosis, treatment, and prevention of diseases, based on the characteristics of an "average" patient, is often not efficient and sometimes even dangerous. It is due to the presence of significant differences among individuals. According to the FDA's definition, personalized medicine is a medical model that separates people into different groups - with medical decisions, practices, interventions, and/or products being tailored to the individual patient based on their predicted response or risk of the disease. The main fields of application in personalized medicine are in vitro diagnostics, personalized drugs, and personalized health management. Personalized medicine is an approach that aims to tailor medical treatment to the individual characteristics of each patient. The ability to do this effectively is a treatment level indicator, reducing the likelihood of side effects and increasing the chances of successful interventions.

In the case of TAT-HSP70, we have all the necessary prerequisites to see it as a candidate for personalized medicines. Since TAT-HSP70 acts by systemic influence, personalization involves only

one criterion - contra-indications. It is clear that if HSP70 is expressed by the tumor in question (for example, most prostate cancers), its introduction will lead to tumor growth. A complete contra-indication for the use of TAT-HSP70 is tumor diseases and active autoimmune diseases because TAT-HSP70 can enhance the immune response targeted at these diseases. It is also contraindicated to introduce it acutely during inflammation. For minor injuries and involutional processes, it depends on the coping ability of different organisms. If an organism copes with the existing anti-inflammatory defense system, no undesirable effects will develop.

Conclusion

In conclusion, myocardial ischemia is a major cause of death and disability worldwide. I/R injury caused by myocardial reperfusion not only destroys myocardial cells, but also causes inflammation and oxidative stress. At present, various treatments for myocardial I/R injury have been proposed, but no qualitative breakthroughs have been made. TAT-HSP70 has been confirmed to maintain the structure and function of cells as a therapeutic drug or cardioprotective molecular chaperone. However, the cardioprotective mechanism and intervention effects on I/R injury have not been systematically reviewed. Therefore, this article comprehensively reviews the research findings of TAT-HSP70 in myocardial protection and discusses many potential and possible molecular mechanisms that play a protective role in myocardial I/R injury. Therefore, understanding the protective mechanism of TAT-HSP70 in myocardial I/R injury is helpful for subsequent research and development of related treatment methods.

Conflict of Interest

No conflicts of interest were declared by the authors.

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Ethics Statement

Approved by local committee.

Authors' contributions

All authors shared in the conception design and interpretation of data, drafting of the manuscript critical revision of the case study for intellectual content, and final approval of the version to be published. All authors read and approved the final manuscript.

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