Heat Shock Proteins in Inflammation

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Introduction

From roundworms to mammals, living organisms have evolved strategies to permit survival in divergent environments. Evidence shows that some of these adaptive biological features are evolutionarily conserved; among these is heat acclimation. This phenomenon was described first as inducing physiological and biochemical adaptations to protect against extreme changes in environmental temperature [1]. This “heat shock response” is now accepted widely as a key mechanism to protect cells from untoward environmental perturbations [2].

The heat shock response was first identified in Drosophila melanogaster [3]. Early experiments showed that exposure to heat led to “chromosomal puffing” that correlated with a dramatic increase in the synthesis of a previously unrecognized group of proteins [3]. This finding was later extended to other eukaryotic organisms. These ‘heat shock proteins’ (HSPs) appeared to mediate a molecular mechanism that protected living cells from the untoward effects of heat [3]. Of these, one of the most widely studied is the 70 kDa HSP (HSP70). The genes encoding members of the HSP70 family are a key evolutionary adaptation that is conserved across species. The HSP70 gene is genetically simple, with a single exon and no introns, which permits rapid transcription and translation [4, 5]. Of the 70 kDa subfamily members, the inducible HSP72 is highly expressed during stress while the constitutive heat shock cognate protein (HSC)70 (also known as HSP73) is constitutively expressed, with basal levels present in the cytosol at most times [6].

Within the cytosol of eukaryotic cells, members of the 70 to 78 kDa subfamily of HSPs bind to and release both non-native protein aggregates and native proteins with incomplete or damaged tertiary structures [6]. In this sense, HSP70 family members act as molecular chaperones to ‘guide’ proteins to their ultimate fate–degradation, elimination, repair, or completion of the synthetic process. The chaperone’s ‘guiding’ mechanism relies on recognition of hydrophobic regions of non-native proteins or unstructured back-bone regions of proteins. They promote the correct protein folding through cycles of substrate binding and release. This is regulated through a catalytic site by an energy-requiring ATPase dependent mechanism [3, 5, 7, 8].

Under environmental stress conditions, misfolded protein intermediates may accumulate. [9]. The self-association of non-native protein intermediates to nearby
proteins may induce the formation of protein aggregates [10]. In contrast to misfolding, aggregation is a highly cooperative inter-molecular process that strongly depends on the concentration of misfolded monomers. Aggregates may be composed of different oligomers over a wide distribution of sizes. The presence of these aggregates is common in a number of disease processes, including neurodegenerative disorders such as Alzheimer’s, Parkinson’s, and Huntington’s diseases. The exposure of hydrophobic protein domains to unaffected proteins or membranes may disrupt normal activity. For example, association of the hydrophobic region of a damaged protein with a neuronal cell membrane may change ion flux and alter function. HSP70 may prevent this and this may be a key mechanism by which HSPs limit or prevent intra-cellular pathological processes. This underscores the fundamental importance of the HSPs to normal living cells [11].

While this review will focus on HSP70, other subclasses among the HSPs play important roles. These are organized by their molecular size: HSP100, HSP90, HSP60, HSP40 (J-domain proteins) and small HSP families, such as HSP22/27 [12,13]. Most HSPs are constitutively and ubiquitously expressed molecular chaperones that guide the normal folding, intracellular disposition, and proteolytic turnover of many of the key regulators of cell growth and survival [14]. Thus, the protective process involves the interaction of many different HSPs. For example, HSP90, which comprises 1–2% of total cellular protein in non-stress conditions [15], supports meta-stable protein conformations and expresses a high affinity binding state to hormone receptors. This involves both HSP70, which participates in assembly of multiprotein complexes, and HSP40, a co-chaperone that stimulates HSP70 ATPase activity [14].

At the transcription level, HSPs, such as HSP70 and HSP90, are regulated by the activities of a family of heat shock transcription factors (HSF). One of these, HSF-1, normally is expressed in a negatively regulated state as an inert monomer in either the cytoplasm or nuclear compartments [16]. Upon exposure to a variety of stresses, HSF-1 trimerizes and accumulates in the nucleus. HSF-1 trimers bind DNA regions called heat shock elements (HSEs) with high affinity. Some small HSPs are transcribed constitutively due to multiple binding of low levels of HSF1 [16].

The great divergence in HSP70 expression explains the multiple function of these proteins. Elevated levels of HSP70 following diverse inciting causes have led researchers to conclude that HSP70 is involved in cellular protection in the normothermic environment [4, 17, 18]. A wide range of noxious stimuli, such as hypoxia, ischemia/reperfusion, hypoglycemia, endotoxemia, inflammation, and exposure to heavy toxic metals or reactive oxygen species (ROS), induce HSP70 expression in a large number of tissues. Since HSPs respond to environmental changes, expression in organs that are ‘outside’ the organism (for example, skin, lung, gastrointestinal epithelium) may occur in the absence of any apparent insult [17–24].

It has been demonstrated, both in vivo and in vitro, that exposure to a mild stress, such as heat pretreatment, induces high levels of HSP70. Increased HSP70 levels may confer protection from subsequent noxious stimuli and result in ‘cyto-
Heat Shock Proteins as ‘Disease Regulators’: Sepsis and Acute Respiratory Distress Syndrome (Fig. 1)

Sepsis, as well as the related systemic inflammatory response syndrome (SIRS), and multiple organ dysfunction syndrome (MODS), are the leading causes of death in patients in surgical intensive care units (ICUs) [24, 25]. The lung is the organ most affected in MODS, with pulmonary dysfunction taking the form of the acute respiratory distress syndrome (ARDS), an often lethal inflammatory disorder of the lung [26]. Recent data from the USA indicate that the mortality rate associated with ARDS is greater than 35% [26].

ARDS is characterized by an increased inflammatory process in the lungs. In this disorder, alveolar epithelial cells are damaged and ultimately may be destroyed [27,28]. While some contributory pathophysiologic mechanisms have been identified, most remain obscure. Therefore, a better understanding of the fundamental biological changes leading to ARDS would be of scientific and therapeutic value.

Several papers have explored the role of HSP70 in a model of lipopolysaccharide (LPS)-induced lung injury. These investigators concluded that heat pre-treatment...
induced HSP70 expression that protected the lungs against ventilator-induced lung injury (VILI) by decreasing cytokine transcription in the lung [29].

LPS stimulates the production and the release of many endogenous mediators of sepsis. These include tumor necrosis factor alpha (TNF-α), interleukin (IL)-1 and IL-6 [29]. A distinct profile in the expression of genes encoding members of the HSP70 family was demonstrated in leukocytes obtained from different phases of the disease course in septic patients [30]. These findings strongly suggest that HSP70 may play a role in the outcome of septic shock patients [30]. Further, studies proved that in an animal model of ARDS, heat pretreatment prevented mortality [31].

Previous studies had revealed that sepsis induced by cecal ligation and double puncture (CLP) resulted in an ARDS-like state characterized by neutrophil accumulation and protein-rich interstitial edema formation [27, 31, 32-38]. Using this model, we found impaired hepatic expression of several essential liver-specific genes, including those encoding proteins that catalyze gluconeogenesis, β-oxidation of fatty acids, ureagenesis, and bile acid transport [39–41]. Further, we have demonstrated inappropriate downregulation of the expression of several key genes within the lung. These include surfactant proteins (SP)-A and (SP)-B and, most importantly, HSP70 [27, 42, 43]. We found that HSP70 mRNA increased after a sham operation but failed to increase after CLP [27]. HSP70 protein levels were unchanged after either CLP or sham operation. Therefore, HSP70 mRNA fails to increase after CLP despite significant damage to alveolar cells. This lack of increase in HSP70 implies profound pulmonary epithelial dysfunction, similar to our findings in the liver, and is supported by several other studies indicating that sepsis and endotoxemia impair HSP70 expression [23, 27, 32, 44]. These experiments led us to investigate in depth the role of HSP70 in ARDS and inflammation, by using an adenovirus (AdHSP) to enhance HSP70 expression [38].

We have demonstrated that intratracheal administration of AdHSP significantly attenuates lung injury in rats with sepsis-induced respiratory distress [38]. AdHSP, when compared to phosphate buffer saline (PBS) or a virus expressing a marker protein (AdGFP), attenuated CLP-induced neutrophil accumulation, septal thickening, interstitial fluid accumulation, and alveolar protein exudation [38]. More importantly, AdHSP treatment significantly decreased mortality in rats subjected to CLP [38]. In contrast to studies that provoked the entire heat shock response [31, 45, 46], our investigations present a unique approach to explore the effects of HSP70 on a single tissue, the lung [32]. We previously documented that AdHSP preferentially increases HSP70 expression in pulmonary epithelial cells [38]. An interesting finding was that 48 hours following CLP, virus uptake occurred primarily in pulmonary epithelial cells, especially type II pneumocytes [32].
HSP70 Inhibits Pro-inflammatory Cell Signaling Pathways in ARDS

The heat shock response is known to modulate inflammation [2]. The mechanisms that have been investigated involve the attenuation of both cytokine-induced inflammatory mediator production and apoptosis [2, 22, 31, 45]. Both processes are important in the pathogenesis of ARDS [48–50]. This involves cytokines such as TNF-α and IL-1β [48–50, 54].

HSP70 inhibits the apoptotic machinery including the apoptosome, the caspase activation complex, and apoptosis inducing factor [55–57]. HSP70 also participates in the proteasome-mediated degradation of apoptosis-regulatory proteins [58].

TNF-α and IL-1β exert their effects in part via cell signaling pathways involving the nuclear transcription factor, nuclear factor-κB (NF-κB) [59–61]. This important acute inflammatory pathway is modulated by HSP70. NF-κB is a dimeric protein, most often consisting of two subunits, p50 and p65 (Rel A). Normally, this dimer is retained in the cytoplasm by an inhibitory molecule, IκBα [62]. An essential step in NF-κB activation is IκBα degradation. This permits the migration of NF-κB into the nucleus where it can initiate transcription [61, 62]. Degradation of IκBα involves three sequential biochemical reactions. The first is phosphorylation of IκBα by IκB kinase (IKK). IKK is a complex molecule that contains two catalytic subunits, IKKα and IKKβ, an essential regulatory subunit IKKγgalso called NF-κB essential modulator (or NEMO) [63], and a recently identified co-modulator, the 105 kDa protein, ELKS [64–66]. The dominant catalytic subunit in inflammation is IKKβ [61]. Phosphorylation of IκBα is followed by poly-ubiquitination by SCFβ-TrCP ubiquitin ligase and, finally, proteolysis by the 26S proteasome [67–70].

Several in vitro models have proven that heat shock or elevated levels of HSP70 suppresses NF-κB activity and that this inhibition of NF-κB results in a general reduction in the inflammatory response [44, 46, 71, 73]. However, the exact molecular mechanism of the HSP70–NF-κB interaction is still unknown. Ran et al. [74] demonstrated that HSP70 promotes rather than inhibits TNF-mediated cell death, by binding to IKKβ. This resulted in inhibition of IKK activity and consequently inhibited NF-κB-dependent antiapoptotic gene induction [74]. Earlier, Yoo et al. demonstrated that HSP70 prevented phosphorylation of IκBα by IKKβ [71].

Both activation and modulation of inflammation require coupling of extracellular signals with intra-cellular events, processes involving a number of specific biochemical pathways. We investigated the hypothesis that AdHSP limits sepsis-induced acute inflammation within alveolar epithelial cells in part by suppressing NF-κB activation. In contrast to the observations of others [71, 74], we found that HSP70 reduced, but did not abolish, IKKβ activity. More importantly, we have uncovered a novel mechanism of IκBα stabilization that results from an association with HSP70 [75]. HSP70 binds to an incomplete protein degradative complex composed of phosphorylated-ubiquitinated IκBαsgF-κB, and partial IKK complexes that contain ELKS, IKKβs and/or IKKγg(NEMO). The association of HSP70 leads to stabilization of these intermediate complexes in a way that prevents proteasomal degradation of IκBα. Consequently, NF-κB is retained in the cytoplasm and is unable to induce inflammatory responses.
Conclusion

HSPs are important mediators of a number of key intracellular reactions. Of importance to the care of the critically ill are their involvement in protein repair and tertiary structure. HSP70 is known to modulate inflammation and apoptosis. In models of acute lung injury and ARDS, over-expression of HSP70 improves outcome, ameliorates lung injury and attenuates inflammation. The involvement of HSP70 in other aspects of lung injury and in other components of MODS is under investigation.

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