Heat shock protein 70 and the acute respiratory distress syndrome

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Introduction

Sepsis and 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 [1,2]. The lung is the organ most often affected in MODS, with pulmonary dysfunction taking the form of the acute respiratory distress syndrome (ARDS), an often lethal inflammatory disorder. Recent data indicate that, at best, the mortality rate associated with ARDS is 29% [2–5]. Unfortunately, although some pathophysiologic mechanisms underlying ARDS have been identified, most have defied elucidation and treatment remains largely supportive.

Although the pathophysiology of ARDS remains obscure, the disease is known to involve unchecked inflammation that ultimately damages and perhaps destroys type I and type II alveolar epithelial cells [6]. This has important ramifications. Type I cells are highly differentiated, are flat, appear to be quiescent, and facilitate gas exchange [6]. Recent work demonstrates that these cells can respond to inflammatory stimuli by producing chemoattractant molecules (chemokines) and expressing key adhesion molecules [7–9]. Similarly, metabolically active type II cells produce surfactant and other products essential to pulmonary function [6]. Damage to type I cells stimulates type II cells to un-dergo mitosis, differentiate into type I cells, and spread [3,6]. Thus, injury to type II cells impairs gas exchange and other essential pulmonary functions by reducing synthesis of surfactant and other key proteins and by limiting regeneration of type I cells [10–12]. It therefore is likely that preservation of functional type II cells is essential for recovery from lung injury.

The heat shock response represents a mechanism of cellular protection [13] that has evolved to protect cells from untoward environmental perturbations. Activation of this pathway by any of a number of noxious stimuli—heat, hypoxia, hypoglycemia, transition metal intoxication, ischemia/reperfusion, endotoxemia, shock—results in the elaboration of a series of heat shock proteins with specific cytoprotective activity [14–18]. Of these, the most widely studied is the 70-kDa heat shock protein 70 molecule (HSP-70). Stress-induced increases in the expression of HSP-70 have been demonstrated in a number of tissues, including lung, kidney, heart, and liver [13–18]. The lung, however, is unique in that there is HSP-70 expression in the absence of insult [13]. Notably, the stress—inducible form of HSP70 (HSP72) has been detected in normal rat colon [19]. This article explores the data on ARDS and the HSP-70 molecule.

Pathways and mechanisms contributing to cell damage in ARDS

Inflammatory pathways in ARDS

Cell loss related to ARDS is complex. The excessive inflammation characteristic of the early phase of the disorder leads to accumulation of neutrophils in the perivascular, interstitial, and alveolar spaces [3]. Neutrophil accumulation is mediated by a number of factors. Two are of key importance: (1) elaboration and release of neutrophil chemokines such as interleukin-8

(IL-8), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-2 (MIP-2) and (2) expression, on the surface of pulmonary endothelial and epithelial cells, of adhesion molecules capable of binding neutrophils. Among these is intracellular adhesion molecule-1 (ICAM-1) [7–9]. Neutrophils can cause damage up to and including cellular necrosis. Cell loss in ARDS also proceeds via activation of pathways leading to programmed cell death or apoptosis [20–22]. It is known that the cytokines tumor necrosis factor-α (TNFα) and IL-1β are responsible, in part, for neutrophil-mediated necrosis and apoptosis [20–25]. This reflects TNFα/IL-1β-stimulated expression and release of MCP-1, MIP-2, and ICAM-1. Elements of this enhanced elaboration of key chemoattractant/adhesion molecules is modulated by a cytoplasmic signal transduction that culminates in activation of the nuclear protein transcription factor NF-κB [25–31]. NF-κB also initiates apoptosis via the caspase-8 pathway [23–27]. Therefore, each step in the proinflammatory cascade might modulate cell injury in ARDS.

Alteration in gene expression

Alveolar cell damage may be initiated during sepsis/ARDS by an alteration in gene expression. This can take two forms. Expression of some genes, such as those encoding cytokines and cell-surface antigens, is increased [20–23,29–31]. Of equal importance are recent studies indicating inappropriate transcriptional down-regulation of certain genes encoding key cellular proteins. For example, using a model of sepsis that leads to ARDS—cecal ligation and double puncture (2CLP) in rats and mice—we have 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 [32–34]. Furthermore, we have demonstrated inappropriate down-regulation of expression of several key genes in the lung following 2CLP, including surfactant proteins (SP)-A and (SP)-B and, most importantly, HSP-70 [35–37]. Using Northern blot hybridization and immunoblotting, we examined the temporal expression of HSP-70 in lungs of animals surviving 2CLP [36]. HSP-70 mRNA increased after a sham operation but failed to increase after 2CLP. Immunoblotting and immunohistochemistry demonstrated that HSP-70 levels were unchanged after either 2CLP or the sham operation. Therefore, HSP-70 mRNA does not increase after 2CLP despite damage to alveolar cells. The failure of 2CLP to increase mRNA levels in the face of the severe damage caused by 2CLP implies profound pulmonary epithelial dysfunction, similar to findings in the liver. Importantly, several recent studies indicate that 2CLP, sepsis, and endotoxemia impair HSP-70 expression [36,38–40]. These experiments led us to investigate in depth the role of HSP-70 in ARDS and inflammation.

Heat shock protein 70

The heat shock response is a phylogenetically conserved endogenous mechanism that has evolved to protect cells from untoward environmental perturbations [13]. The response was first identified in *Drosophila melanogaster*, and the findings were later extended to other eukaryotic tissues. Exposure to heat led to synthesis of a previously unrecognized group of proteins that appeared to mediate a molecular mechanism to protect living cells from the untoward effects of heat. Therefore, the proteins became known as "heat shock proteins" (HSPs) and the response as the "heat shock response." Additional studies revealed two key facts. First, noxious stimuli other than heat led to elaboration of HSPs. Second, preliminary exposure to heat conveyed tolerance to both subsequent heat shock and to additional noxious stimuli. This "thermotolerance" phenomenon protected cells from hypoxia, ischemia, inflammation, and exposure to heavy toxic metals, endotoxin, and reactive oxygen species [41].

Of the proteins produced during the heat shock response, the most widely studied is the 70-kDa HSP-70. HSP-70 subspecies have been observed in many organs after diverse insults. The genes encoding members of the HSP-70 family are a key evolutionary adaptation. They are conserved across species (from single-cell organisms to humans), are genetically simple (a single exon and no introns, permitting rapid transcription), and have a long protein half-life. A number of noxious stimuli have been shown to induce HSP-70 expression in the lung, kidney, heart, pancreas, and liver in vivo [14–18,38]. Importantly, prior elaboration of HSP-70, like heat pretreatment, protects cells, reduces inflammation, and alters transcriptional activation in vivo and in vitro [42–51]. Thus, altered HSP-70 expression might be of importance in the modulation of ARDS.

Within the cytosol of the eukaryotic cells, members of the 70- to 78-kDa family of HSPs act as molecular chaperons. This involves facilitating folding/refolding of cellular proteins as well as preserving and stabilizing the tertiary structure. The 70- to 78-kDa family of HSPs includes the inducible HSP72, which is highly expressed during stress, and constitutive HSC70 (also called HSP73), which is constantly present at basal levels in the cytosol. All HSP-70 family members with nucleotide sequences of 72, 73, 75, and 78 kDa are highly evolutionarily conserved. Furthermore, there is 60%–70% homology between eukaryotic organisms [52].

All HSP-70 molecules include one major peptide binding site and an enzymatic catalytic binding site. The

peptide-binding carboxyl-terminal domain is less conserved than the amino-terminal 44-kDa catalytic site. This catalytic site has ATPase activity, which is vital for binding and releasing peptides during stress [41,53,54]. The purpose of the intracellular chaperone HSP machinery is to identify nonnative protein aggregates and to participate in de novo protein folding. Chaperones recognize hydrophobic residues and unstructured backbone regions in proteins, and they promote folding through cycles of substrate binding and release. This process is regulated by ATPase activity and is aided by other cofactors [53,55]. Chaperone binding may not only block intermolecular aggregation directly by shielding the interactive surfaces of nonnative polypeptides, it may prevent intramolecular misfolding.

Expression of HSPs is modulated by an intracellular signal transduction pathway that activates heat shock factors (HSFs). When stimulated by an appropriate signal, HSF-1, a 75-kDa cytosolic protein, translocates to the nucleus, binds to the heat shock responsive element (HSRE), and initiates HSP-70 transcription [56].

Although the protective role of HSPs is highly conserved across species, the profile of HSP transcription and the time of appearance can be expressed uniquely in various tissues. The great divergence in HSP expression explains the plasticity with which these proteins function [57,58]. Elevated levels of HSPs following diverse inciting causes have led researchers to conclude that HSPs are involved in cellular protection in normothermic environments as well as in response to heat. For example, Marber et al. demonstrated cardioprotection against ischemic injury using transgenic mice overexpressing HSP-70 [16].

Heat shock response in inflammation and acute lung injury

Data show that HSP-70 can limit inflammation. Heat pretreatment before a variety of insults protects cells, inhibits proinflammatory cytokine release, alters activation of transcriptional pathways, and prevents apoptosis in vivo and in vitro [13–18,41–51]. Indeed, studies have demonstrated that heat treatment significantly improves the outcome from phospholipase A_1–mediated acute lung injury or systemically induced ARDS [38–40,42,44]. We hypothesized that restitution of an appropriate HSP-70 response might be protective. To test this hypothesis we used adenovirus-mediated gene enhancement to treat the impaired pulmonary heat shock response following 2CLP in rats. Previous studies had revealed that this insult resulted in an ARDS-like state characterized by neutrophil accumulation and protein-rich interstitial edema formation [36,40,42,59–75]. In our experiments we administered an adenovirus designed to express porcine HSP-70 (AdHSP) into the

tracheas of rats subjected to 2CLP in the hope that it would reverse these abnormalities and improve the outcome. Our approach was unique because other studies on sepsis and ARDS activated the entire heat shock response, with its attendant production of a number of peptides. In addition, the other investigations provoked an enhanced response in the entire organism. In contrast, our studies were designed to increase only the expression of HSP-70, a single peptide, in one organ, the lung. Our preliminary studies showed that AdHSP did indeed increase HSP-70 expression in the lung. Virus uptake following 2CLP occurred primarily in pulmonary epithelial cells, especially type II pneumocytes. There was some additional uptake in alveolar macrophages, a finding that could affect neutrophil accumulation by altering chemokine production [40]. Next, we administered AdHSP to a cohort of animals subjected to 2CLP. Unoperated and sham-operated animals served as controls, as did a cohort of rats given a different adenovirus that did not contain the HSP-70 gene. Our studies revealed that treatment with AdHSP attenuated neutrophil accumulation, septal thickening, interstitial fluid accumulation, and alveolar protein exudatation—changes characteristic of ARDS 48h after 2CLP. Furthermore, AdHSP administration significantly decreased 48-h mortality [76].

Possible mechanisms to explain the protective effect of HSP-70

There are a number of potential mechanisms to explain the cytoprotective effects of HSP-70. Three have been investigated: preservation of protein structure and configuration [13,77,78]; attenuation of cytokine-induced inflammatory mediator production [49,79]; and blockade of apoptosis [13,50,80]. Each of these processes, which appear to occur as a result of HSP-70 binding to hydrophobic domains of proteins involved in inflammation or apoptosis, may be important in the pathogenesis of ARDS. In a sense, the heat shock response is counterregulatory, protecting cells from excessive inflammation by limiting some of the potentially harmful effects (unlimited tissue damage, necrosis, apoptosis, altered protein expression, impaired or overexuberant regeneration) of an unchecked inflammatory response.

HSP-70 induction inhibits proinflammatory cytokine induction, gene expression, and apoptosis in many cells, including human and murine lung epithelial cells [45,48,49,81,82]. These findings suggest that one mechanism of protection may be the ability of HSP-70 to inhibit proinflammatory and apoptotic responses via modulation of NF-κB activity [49,83]. The actual point of inhibition in the NF-κB pathway has not been completely elucidated. This represents an important gap in our understanding of HSP-70 biology. For NF-κB to

translocate into the nucleus, its inhibitor molecule, IκBα, must undergo phosphorylation, ubiquitination, and proteosomal degradation [84–89]. Yoo et al. indicated that inhibition of IκB phosphorylation by HSP-70 induction is most likely related to inhibition of the IκBα kinase (IKK) complex [49]. Others, however, have disputed this [90–92]. Support for this conclusion can be found in the work by Ran et al. [93], who demonstrated that HSP-70 binds to the γ-subunit of the IKK complex, disrupting the IKK heterodimer [93]. Additional investigations involving the effects of HSP-70 on the entire cytokine/NF-κB pathway are of major importance.

HSP-70 may also attenuate ARDS via stabilization and preservation of damaged intracellular proteins [13,77,78]. This may result from the unique ability of HSP-70 to disaggregate and refold denatured proteins [78]. During this process, HSP-70 binds to hydrophobic protein domains of native proteins or peptides whose tertiary structure has been lost [77]. It appears that damaged protein is stabilized in a conformation that facilitates refolding. This allows reconstitution of the tertiary and quaternary structure when normal conditions are restored [13]. Although this property has been well demonstrated in vitro, currently there are no data to support protein stabilization as a direct protective mechanism in the lungs or any other organ of intact animals. This highlights another major gap in our understanding of HSP-70 biology. Because damage to or loss of proteins in pulmonary epithelial cells has been implicated in the pathogenesis of ARDS, an understanding of a mechanism to restore damaged cellular components may contribute to strategies designed to modulate ARDS or any other inflammatory disease [94–101].

Finally, a large body of evidence indicates that expression of HSP-70 contributes to blockade of apoptosis [23,50]. Saleh et al. demonstrated that HSP-70 forms a complex with the preliminary apoptotic factor Apaf-1, attenuating oligimerization and formation of the apoptosome [102]. Because loss of pulmonary epithelial cells is important in the pathogenesis of ARDS, a better understanding of the role played by this aspect of HSP-70 activity is important.

Conclusions

In summary, ARDS is a disorder that involves overwhelming inflammation, alterations in protein expression and function, and cell death by apoptosis and necrosis. Each of these abnormalities can be limited or controlled by an appropriate heat shock response, specifically involving induction of HSP-70. Previous studies have demonstrated failure to increase HSP-70 expression following 2CLP. HSP-70 deficiency contributes to inflammation, altered protein expression, and alveolar cell loss in ARDS. We and others have demonstrated that correcting this deficit may protect alveolar cells, reduce functional and morphologic abnormalities, and improve the outcome in experimental ARDS. Several mechanisms by which HSP-70 may exert its attenuating effects in ARDS have been identified. These findings may have important ramifications with regard to the pathogenesis of ARDS and can help direct further investigation and the development of novel therapeutic approaches.

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