The role of heat shock proteins in spinal cord injury

Review article

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Heat shock proteins (HSPs) are normal intracellular proteins that are produced in greater amounts when cells are subjected to stress or injury. These proteins have been shown to play a key role in the modulation of the secondary injury that occurs after the initial spinal cord injury (SCI). Heat shock proteins normally act as molecular chaperones and are called protein guardians because they act to repair partially damaged proteins. Normally intracellular, HSPs can also be liberated into the systemic circulation to act as important inflammatory mediators. In the setting of SCI, HSP induction has been shown to be beneficial. These proteins are liberated primarily by acutely stressed microglial, endothelial, and ependymal cells. Heat shock proteins have also been shown to assist in the protection of motor neurons and to prevent chronic inflammation after SCI. In animal models, several experimental drugs have shown neuroprotective effects in the spinal cord and appear to function by modulating HSPs.

Methods

Using the search words “heat shock proteins” and “spinal cord injury” we searched the PubMed database. All articles in English were retrieved from 1964 to 2008. Relevant articles were identified and reviewed, and the bibliographies of these articles were also analyzed to find other pertinent studies. All articles were reviewed with the primary goal of determining the role of HSPs in SCI.

Results

Sixty-one articles were identified, only 22 of which dealt with HSPs in SCI. The articles could be broadly classified into 3 types: 1) articles examining the role of HSPs in SCI;5,8,11,15,17,27,28,41,43,44,48–50 2) articles examining the manipulation of HSPs to alter outcome after SCI;10,25,32,38–42 and 3) review articles.3 All articles involved only animal models; there were no studies in humans.

Discussion

Historical Background

In 1962, Ritossa originally discovered HSPs in isolated heat-shocked Drosophila melanogaster salivary glands where their appearance coincided with chromosomal puffs. In this laboratory, the incubator thermostat was inadvertently set at a high temperature. The following day it was found that the salivary gland chromosomes of the fruit flies exhibited odd puffing patterns on microscopic examination, indicating a novel pattern of gene expression. These chromosomal puffs were specific transcription sites for HSP synthesis. It was not until 1986 that the important functions of HSPs were finally...
understood. At this time, Pelham et al. elucidated the role of HSPs as the cellular chaperone. However, it was Ellis et al. in 1987 that formally named this function and advanced the concept.

**Protein Structure**

As a class, HSPs are among the most highly expressed cellular proteins across all species. They account for 1–2% of total protein in unstressed cells. However, when cells are heated or subjected to other stressors, the fraction of HSPs increases to 4–6% of all cellular proteins. The HSPs are named according to their molecular weights; for example Hsp60, Hsp70, and Hsp90 (the most widely studied HSPs) refer to families of HSPs that are 60, 70, and 90 kD in size, respectively. Overall, their molecular weights can range 10–150 kD. Heat shock protein 90 is the most abundant nonribosomal protein (cytosolic version) and also the most abundant protein in endoplasmic reticulum. The structures of HSPs of various species have been described in several studies. Heat shock protein 70 is the most widely studied. It is also the most temperature-sensitive and highly conserved HSP. Heat shock protein 70 has 2 domains; an N-terminal NBD and a substrate binding domain. The 44-kD N-terminal NBD has adenosine triphosphatase activity and associates with the Hsp70 cochaperone DnaJ. These domains are connected by a conserved linker recently shown to be critical for interdomain communication. Heat shock protein 70 has a single peptide binding site with a hydrophobic channel flanked by an arch allowing peptide access. In contrast, Hsp90 has 3 structural domains; 1) an NBD that binds Hsp90 inhibitors and potentially peptides; 2) a middle segment that interacts with client proteins; and 3) the C-terminus, implicated in homodimerization. The characteristics of the important HSPs are listed in Table 1.

**Protein Guardians**

The most important function of HSPs is to act as molecular chaperones. Chaperones aid in the transport of proteins throughout the cell’s various compartments. Heat shock proteins are present in cells under normal conditions but are expressed at high levels when exposed to a sudden increase in temperature or other stress. Heat shock proteins stabilize proteins and are involved in the folding of denatured proteins. High temperatures and other stressors such as altered pH and oxygen deprivation make it more difficult for proteins to form their proper structures and cause already structured proteins to unfold. Left uncorrected, misfolded proteins form aggregates that may eventually kill the cell. Heat shock proteins are induced rapidly in high concentrations to deal with this problem. Any injury that contributes to protein denaturation, including ischemia, heat shock, heavy metals, hypoglycemia, low pH, and disease states appears to produce transcriptional activation of Hsp70. Heat shock protein 70 then acts in concert with Hsp90 and other HSP chaperones to renature the denatured proteins.

Increased expression of HSPs is mediated at multiple levels: mRNA synthesis, mRNA stability, and translation efficiency. The induction of HSPs is regulated by transacting heat shock factors and a cis-acting heat shock element present at the promoter region of heat shock genes. Once synthesized, these HSPs bind to the denatured protein and prevent aggregation. Some HSPs, such as Hsp104, are able to rescue already aggregated proteins. Because of these functions, HSPs are aptly called “guardians of proteins.” Although these proteins are normally intracellular, HSPs can be liberated into the systemic circulation after cell necrosis. Systemic HSPs are some of the most effective inflammatory mediators and are called chaperokines or extracellular HSPs.

**Heat Shock Proteins and SCI**

Significant advances have been made in understanding the action of HSPs in SCI in animal models. In these studies, the most common model used for evaluation was an ischemic SCI model produced by clamping the thoracic aorta. The most common HSP studied was Hsp70. Based on these animal studies, it is clear that HSPs are important modulators of SCI. In SCI the primary injury is known to produce a state of hyperexcitability due to intracellular entry of calcium, liberation of free radicals, and production of excitatory neurotransmitters including glutamate, lactate, pyruvate, and aspartate. All together, these events trigger what is known as the secondary injury cascade. This cascade is further magnified by the liberation of nitric oxide and adenosine in a delayed fashion. Excitotoxicity triggers a glial reactive response characterized by the release of proinflammatory cytokines, such as tumor necrosis factor–α and interleukin–1β that are mainly produced by microglial and endothelial cells. The production of cytokines by microglial cells leads to concomitant upregulation of Hsp70. As a chaperone, Hsp70 protects cells from proteotoxic stress through a variety of “holding and folding” pathways that prevent protein denaturation and the progression of lethal aggregation cascades in cells, thus preventing cell death both through necrotic and apoptotic pathways. However, the liberated extracellular HSPs can trigger a systemic inflammatory response.

### TABLE 1: Summary of location and function of important HSPs

<table>
<thead>
<tr>
<th>HSP</th>
<th>Intracellular Location</th>
<th>Proposed Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp27</td>
<td>cytosol &amp; nucleus</td>
<td>antiapoptotic; microfilament stabilization</td>
</tr>
<tr>
<td>Hsp60</td>
<td>mitochondria</td>
<td>involved in protein folding after post-translational import to the mitochondria; prevents aggregation of denatured proteins; proapoptotic</td>
</tr>
<tr>
<td>Hsp70</td>
<td>cytosol, ER, &amp; nucleus</td>
<td>molecular chaperone, thermotolerance, antiapoptotic</td>
</tr>
<tr>
<td>Hsp90</td>
<td>cytosol, ER, &amp; nucleus</td>
<td>maintains steroid receptors &amp; transcription factors, antiapoptotic</td>
</tr>
<tr>
<td>Hsp104</td>
<td>cytosol</td>
<td>protein folding</td>
</tr>
</tbody>
</table>

* ER = endoplasmic reticulum.
Heat shock proteins

Other studies using an acute traumatic SCI model in rats have provided evidence that HSPs probably play a role in SCI. Using DNA microarray technology, Song et al. demonstrated that Hsp70 is among the most frequently produced proteins after SCI. In a similar model using gene expression, Tachibana and coworkers found that Hsp27 showed a > 2-fold increase 24 hours after SCI.

The role of HSPs is complex: they can be proinflammatory and yet they may also have significant antiinflammatory and cytoprotective actions. Studies have shown that HSPs have a multifaceted modulatory role in inflammation. These proteins have been shown to promote inflammation initially and then exert an antiinflammatory effect, thereby limiting chronic sequelae. In fact an elevation in autologous extracellular Hsp70 has been shown to protect against a wide range of chronic inflammatory diseases including arthritis, atherosclerosis, and Type 1 diabetes. It may be that extracellular HSPs in the cerebrospinal fluid inhibit the development of chronic inflammatory disease after acute injury to the central nervous system.

Unlike microglial and ependymal cells, motor neurons express very low levels of HSPs in the setting of acute SCI. Heat shock proteins, however, have been shown to be directly cytoprotective of motor neurons. Robinson et al. have demonstrated the dependence of motor neurons on circulating extracellular HSPs. Considering the size of motor neurons and the metabolic demands placed on them, the authors hypothesized that motor neurons are only capable of synthesizing amounts of HSP necessary for maintenance of cell function and survival and did not appear to increase production in response to the greater demands of stressful stimuli. The extracellular Hsp70 derived from other cell types may compensate for this deficit. It is conceivable, therefore, that in situations of environmental stress, the amount of extracellular Hsp70 available to motor neurons becomes limited as utilization of these proteins is increased in the surrounding cells. This situation would then place the motor neurons in a position of insufficient access to Hsp70, rendering them more susceptible to damage and death than if Hsp70 were more abundant. Such results suggest a critical role for extracellular Hsp70 in promoting motor neuron survival.

Rajdev et al., Yenari et al., and Kelly et al. have also shown that Hsp70 serves a cytoprotective function and confers ischemic tolerance in the brain when overexpressed by a transgene or viral vector. Similarly, Cizkova and associates have demonstrated that sublethal cellular stress mediated by hyperthermia or mild transient ischemia can also elevate Hsp70 levels and confer ischemic tolerance in the spinal cord. In other animal studies, the spinal cord was noted to have improved tolerance of ischemic effects after ischemic preconditioning. Some authors have noted that ischemic preconditioning may have a beneficial effect through the increased synthesis of HSPs. Perdrizet and colleagues found that preoperative stress conditioning in the form of whole body hyperthermia or tin(II) chloride administration and recovery can prevent paralysis in a rabbit model of aortic surgery. This ischemic spinal cord protection is associated with an increased level of stress proteins within the spinal cord (such as Hsp70) and in other organs. These changes in function and stress protein synthesis were associated with preservation of the histological morphology of the spinal cord. In another study, Matsumoto and coworkers elucidated the temporal efficacy of ischemic pretreatment in the spinal cord: the protective effect was apparent 4 days after pretreatment. In their study the amount of Hsp70 in the cytoplasm did not explain the temporal profile of ischemic tolerance and they hypothesized that nuclear Hsp70 might play a role in the acquisition of ischemic tolerance.

Asea et al. have reported on the expanded role of Hsp70 and have ascribed novel functions to the Hsp70 protein depending on its localization. Surface-bound Hsp70 specifically activate natural killer cells while Hsp70 released into the extracellular milieu specifically binds to TLR2 and TLR4 on antigen-presenting cells and exerts immunoregulatory effects including upregulation of adhesion molecules and costimulatory molecule expression as well ascytokine and chemokine release, a process known as the “chaperone” activity of Hsp70. The chaperone activities of HSPs have been shown to play an important role in host protection against pathogenic infections.

The authors of numerous studies have suggested that the beneficial effects of various drugs in SCI are due to modulation of HSPs. Shin et al. demonstrated that cyclosporin A reduces neurological injury in a rabbit model of 25-minute spinal cord ischemia. They concluded that this neuroprotective effect against ischemia was related to overexpression of neural nitric oxide synthetase and Hsp70. The neuroprotective effect was more evident on postoperative Day 7 than on Day 2, indicating the role of HSPs in modulating secondary injury. Park and associates demonstrated improved outcome after SCI in rats pretreated with peroxisome proliferator–activated receptor inhibitors. They also hypothesized that this neuroprotective action was partially mediated by the upregulation of HSPs. Guzman-Lenis et al. examined on the neuroprotective effects of an experimental drug called FK506 (tacrolimus) and concluded that the drug’s beneficial effects were due to Hsp70 downregulation. Lee and colleagues postulated that the neuroprotective action of bupivacaine and hypothermia in SCI was due to HSP downregulation. Sharma and colleagues reported that the experimental drug H-290/51 induced neuroprotection by preventing the upregulation of HSPs that occurs after focal SCI. Their results suggested an important contribution of the oxidative stress response in HSP induction, microvascular permeability disturbances, edema formation, and cell injury after SCI. Waza et al. reported similar results indicating a neuroprotective effect with inhibition of HSPs. They demonstrated that inhibition of Hsp90 by 17-AAG, a potent Hsp90 inhibitor, ameliorated motor impairment, and attenuated motor neuron degeneration in a mouse model of spinal and bulbar muscular atrophy.

Based on these studies, both up- and downregulation of HSPs appear to have neuroprotective effects. Such contradictory findings probably reflect the complex role of HSPs in modulating secondary injury after SCI. Similar to other inflammatory mediators, HSPs probably have

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both beneficial and deleterious effects in SCI. Given the involvement of HSPs in SCI, investigators have even used HSP levels as a measure of SCI.39

Conclusions
At present our understanding of the role of HSPs in SCI is limited. There is a preponderance of evidence in animal studies suggesting that HSPs are extremely important in modulating inflammation and are aptly referred to as the guardians of proteins due to their ability to stabilize proteins. Heat shock proteins are mainly synthesized by microglial, ependymal, and endothelial cells after SCI and seem protective of motor neurons. Certain experimental drugs that have shown to be neuroprotective to the spinal cord appear to function by modulating HSPs. Further experiments elucidating the exact role of HSPs in SCI may lead to clinically useful improvements in outcomes.

References


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Heat shock proteins


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