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Review

THE ROLE OF HEME OXYGENASE AND HSP70 IN TRAUMA

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Summary

The so called Heat-shock proteins (Hsp) are the product of different genes induced by a sudden and/or short-lasting temperature elevation. However, the term Hsp is generally used with great flexibility to indicate proteins induced also by other stressors besides heat shock. Many Hsp are also chaperones since they assist nascent polypeptides to fold correctly. Other canonical functions of chaperones include, in addition to protein folding, assisting protein refolding and translocation through membranes, ushering proteins damaged beyond repair to degradation, and dissolution of protein aggregates. Hsp play a key role in various human chronic diseases such as cancer, neurodegenerative diseases, cardiovascular diseases, diabetes and in acute injury (i.e. trauma). Furthermore, Hsps may be used as possible circulating biomarkers help the clinicians for diagnosis, prognosis and treatment. In the present review we focus our attention on the possible role and different clinical significance of two important heat shock proteins (i.e. heme oxygenase-1 and Hsp70) in traumatic injury.

In the mid 1950's the Nobel Prize Laureate Christian B. Anfinsen from his research on the folding of ribonuclease A (1), began to concentrate on the problem of the relationship between structure and function in enzymes. On the basis of studies on ribonuclease, he proposed that the information determining the tertiary structure of a protein resides in the chemistry of its amino acid sequence. He also elegantly showed that, after cleavage of disulfide bonds and disruption of tertiary structure, many proteins could spontaneously refold to their native forms (2). However, in the following years several studies demonstrated that this assertion is not valid for all synthesized proteins thus suggesting that some proteins may violate the Anfinsen's dogma (3). In fact, some highly specific 'steric chaperones' do convey unique structural (steric) in-

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formation onto proteins, which cannot be folded spontaneously. This finding changed dramatically the way of studying protein synthesis and their maturation following the translation process. In addition, further studies demonstrated that impairment of the chaperone machinery might lead to various pathologies, which were termed as "chaperonopathies" (4).

The response to heat shock or stress response is a highly conserved defense mechanism of the cell to various insults (i.e. trauma, infections, free radicals, etc.). Such defense mechanism is characterized by an increased expression of stress proteins conferring resistance to insults that otherwise could result lethal for the cell. Many heat shock proteins act as molecular chaperones being involved in the processes of protein folding and maturation of proteins (5). Stress proteins are typically intracellularly localized but recently their presence has also been demonstrated in the extracellular space and in various biological fluids (ie plasma, cerebrospinal fluid, human milk, etc.) (6-10). The exact mechanism of release of these proteins by the cells and its significance has not yet been elucidated completely but some authors suggest that these proteins might play an important role in the modulation of the immune system functioning as danger signals and/or as antigen carriers for cells antigen-presenting cells (APC) (11). Finally, the presence of such proteins in the extracellular space may be exploited for diagnostic and prognostic purposes (12). In the present review article, we summarize the possible significance of different heat shock proteins in the extracellular space in various animal models of trauma and in patients in order to provide intensive care physicians a valid tool for their prognostic and diagnostic accuracy and to understand important molecular and systemic insights of trauma.

Heat shock protein 32 (Hsp32 or heme oxygenase-1)

Heme oxygenase (HO)-1 is an evolutionarily conserved enzyme expressed in mammalian cells. HO-1 is the first and rate-limiting enzyme in heme catabolism,

degrading heme to equimolar quantities of carbon monoxide (CO), free iron and biliverdin; biliverdin later converted to bilirubin; free iron is directly sequestered by ferritin (13, 14). HO-1 is expressed at low levels under basal conditions and it is rapidly and vigorously induced by polyphenols (15) and a variety of stimuli such as inflammation, oxidative stress, hyperoxia, hypoxia and trauma. Such upregulation represents an intrinsic defense mechanism to maintain cellular homeostasis and enhance cell survival (16). The essential role of HO-1 in inflammation is based on the observation that both HO-1 knockout mice and human cases of HO-1 gene deficiency exhibit a significantly increased pro-inflammatory state, indicating the indispensable role of HO-1 in the regulation of inflammation (17, 18). These results were further confirmed in human studies associating gene promoter polymorphisms with different protein expression and vulnerability to many inflammatory disorders such as rheumatoid arthritis, coronary atherosclerosis, chronic obstructive pulmonary disease and graft injury after transplantation (19-22). Likewise, in experimental disease models, either HO-1 upregulation or supplementation of HO-1 bioactive products has been found to be able to dampen the inflammatory response and prevent from tissue injury (23, 24). By contrast, suppression of HO-1 expression is associated with an exaggerated inflammatory reaction and pathological alteration. Taken together, HO-1 represents an essential negative regulator in the modulation of inflammatory process.

As far as concern the role of HO-1 in trauma, previous studies evaluated the changes of HO activity after spinal cord injury (SCI), and the effect of pharmacological treatments with hemin (an inducer) and (Sn)-protoporphyrin (an inhibitor) of HO, upon motor recovery after SCI. Animals were submitted to SCI by trauma and sacrificed at different time points (2, 4, 8, 12 and 24 h) after injury to evaluate HO activity. The authors showed that HO activity increased following SCI, at all times evaluated, as compared to sham group values. Interestingly, animals treated with hemin 2 and

8 h after SCI, showed a better motor recovery and higher spared cord tissue, as compared to control group values thus suggesting that activation of HO is a beneficial mechanism when attained during the acute phase after spinal cord injury (SCI) (25, 26). These results were further confirmed by Okubo S et al showing that HO-1 protein is upregulated in brain parenchyma following traumatic brain injury (27). Such upregulation could be related to increased oxidative stress following brain injury which is involved in the progression of intracerebral haemorrhage (ICH)-induced secondary brain injury. The pathway involving Kelch-like ECH-associated protein 1 (Keap1) and nuclear factor erythroid 2-related factor 2 (Nrf2) is currently recognised as the major endogenous regulatory system against oxidative injury and play a key role in HO-1 expression regulation. To this regard, Shang H showed that after blood infusion, Keap1 showed decreased expression starting at 8 h, whereas Nrf2 began to show a significant increase at 2 h with a peak at 24 h (28). The hypothesis that HO-1 upregulation could be mediated by increase of oxidative stress is further corroborated by previous results showing that N-acetylcysteine (NAC), an antioxidant, prevents HO-1 upregulation following TBI (29).

The role of HO-1 was also evaluated in other TBI models such as penetrating ballistic-like brain injury (PBBi) (30). The authors showed that following a 10% frontal PBBi, HO-1 mRNA and protein was increased at all time points studied, reaching maximum expression levels at 24-48 h post-injury. Furthermore, the authors showed an increase in the heme concentration and the development of brain edema coincided with the upregulation of HO-1 mRNA and protein during the 7-day post-injury period. Interestingly, selective brain cooling (SBC) significantly decreased PBBi-induced heme concentration, attenuated HO-1 upregulation, and concomitantly reduced brain water content. These results suggest that the neuroprotective effects of SBC may be partially mediated by reducing the heme accumulation, which reduced injury-mediated upregulation of HO-1,

and in turn ameliorated edema formation. Collectively, these results suggest a potential value of HO-1 as a diagnostic and/or therapeutic biomarker in hemorrhagic brain injury.

The above mentioned results obtained in animal model were also confirmed in patients with traumatic brain injury (8). In particular, the authors measured HO-1 concentration in cerebral spinal fluid samples from 48 infants and children following TBI. The authors showed that HO-1 was seen in TBI versus control patients. Furthermore, increased HO-1 concentration was associated with increased injury severity and unfavorable neurological outcome.

The importance of HO-1 in trauma was also demonstrated in an animal model of trauma hemorrhage (31-33). The authors showed that trauma hemorrhage increased hepatic oxidative stress and inflammation and this effect could be reversed by various HO-1 inducers such as resveratrol, estrogens or heme arginate.

Heat shock protein 70 (Hsp70)

The HSP70 family was discovered in the '60s, from an accidental discovery made in *Drosophila* (fruit fly). A sample of this model was incubated at a temperature higher than normal and microscopic observation of chromosomes was observed a high transcriptional activity of some unknown protein. This transcriptional activation triggered by the heat was called "Heat Shock Response" and the proteins expressed in this response were called "Heat Shock Proteins" (HSPs) (34).

All HSP70 have three main functional domains: a) an ATPase domain at N-terminal, able to bind and hydrolyze ATP. This domain has a regulatory function in the exchange between ATP and ADP induces a conformational change in the other two regulatory domains; b) a substrate-binding domain, which contains a groove that has affinity for neutral and hydrophobic amino acid residues; c) a C-terminal domain, with alpha-helix structure that works as a "lid" for the binding of the substrate. When a protein HSP70 is linked to ATP, the "lid" is open and the peptides bind and are released quickly. When proteins HSP70 are linked to ADP

the "lid" is closed, and the peptides are firmly bound to the binding site of the substrate.

Hsp70 are involved in the pathophysiology of various human disease such as neurodegenerative disorders, cardiovascular diseases, inflammatory chronic diseases, cancer and trauma (35). To this regard, previous studies showed in animal model of TBI showed that Hsp70 is mainly found in injured regions and subcortical white matter (36). In particular, the major cellular sources of Hsp70, were neurons in perilesion and macrophages/microglia in lesion areas and astrocytes in subcortical white matter. Similarly, DeGracia DJ et al (37) showed that HSP-70 immunofluorescence revealed a steady increase from 4 to 48 hours following TBI, culminating in a ubiquitous expression with the capillary bed 48 hours post-TBI. These results were also confirmed in different organs and different animal models. In particular, previous studies showed that in dental pulp trauma there is a statistically significant difference in Hsp70 staining between traumatized and non-traumatized teeth only in the group observed 24 h after the trauma thus suggesting that the expression of Hsps form part of the early pulpal response to trauma (6).

Conclusions

Both HO-1 and Hsp70 protein are over-expressed in different organs following a traumatic event. Such upregulations is likely to occur as a response to the increased oxidative stress and inflammatory response at the site of injury. Furthermore both proteins were detected in the plasma of patients following different traumatic injury, however the presence of these proteins in the extracellular space may have different possible significance. In fact, as far as concern HO-1, it was demonstrated a significant correlation between plasma levels and various clinical data (i.e. site of injury, age, etc) whereas non significant correlation was found for Hsp70. Therefore, it is possible to conclude that both proteins play a major role in the pathophysiology of tissue injury but only HO-1 may be used a pos-

sible circulating biomarker to evaluate traumatic injury.

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