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REVIEW

Heat Shock Proteins in Vascular Disease—A Review

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Introduction. *There is growing evidence that heat shock proteins (HSPs), a family of stress-inducible proteins may be involved in the pathogenesis of atherosclerotic vascular diseases. Here, we systematically review the evidence behind this notion.*
Methods. *A detailed literature search and extensive bibliographic review of literature relating to HSPs and atherosclerotic vascular disease.*
Results. *Atherosclerotic vascular disease is classified into four main areas of presentation: carotid, coronary, aortic and peripheral vascular disease, for consideration in this review. In each of these vascular diseases, the evidence linking HSPs and atherosclerosis is outlined in a systematic manner. Current evidence suggests that components of the immune system may be involved in the pathogenesis of atherosclerosis, with HSPs acting as auto-antigens in the immune response. HSPs are detected in atherosclerotic lesions and antibodies to HSPs are increased in patients with vascular disease; the rise often correlating with the severity of atherosclerosis. The levels of anti-HSP antibodies have been shown to be independent predictors of risk and have prognostic value.*
Conclusion. *There is a strong link between heat shock protein expression and the principal manifestations of atherosclerotic vascular diseases. A better understanding of this involvement could lead to the development of new and improved treatment strategies.*

Keywords: Heat shock proteins; Vascular disease; Review.

Introduction

More than 40 years ago, Ritossa observed that exposing larval salivary glands from Drosophila to heat, induced specific genes in the giant chromosomes of these cells.[1] It is now known that these genes code for a family of proteins called heat shock proteins. Heat shock proteins (HSPs) are a group of highly conserved proteins found in the cells of all organisms, from the simplest of prokaryotes to the most complex mammals including man.[2] The term heat shock protein, however, is a misnomer. A better title would be 'stress proteins', because in addition to raised temperature, HSP synthesis is increased in response to many environmental stresses (stress-inducible) like oxidative stress, nutritional deficiency, ultraviolet radiation, chemicals, viruses and ischaemia-reperfusion injury. Although stress-inducible, low levels of HSP expression occurs in normal physiological conditions (constitutive expression) making up 5–10% of the total protein content in healthy growth conditions.[3,4]

HSPs function as molecular chaperones guiding newly formed polypeptides through folding/unfolding steps to achieve correct functional configuration.[5] They are also involved in protein transport across intracellular membranes and the repair of denatured proteins (Fig. 1). HSPs are grouped into various families according to their molecular weight: namely the 110, 90, 70, 60, 40 kDa and low molecular weight families. A selection of the more important members of the HSP family are detailed in Table 1. HSPs have been implicated in the pathogenesis of several disease processes, however, their role in atherosclerosis

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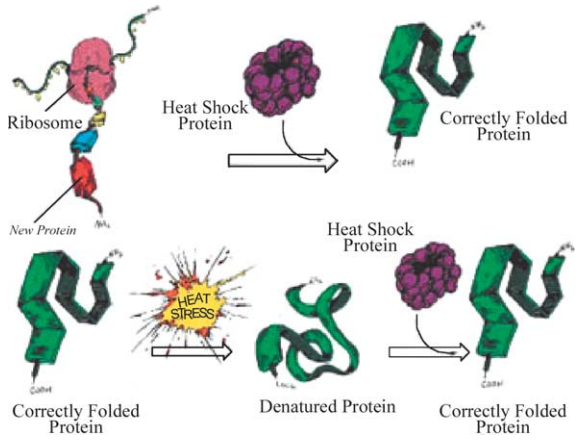

Fig. 1. Two functions of heat shock proteins. Top: as new polypeptide chains (proteins) are being produced by ribosome within the cell, heat shock proteins assist in correct folding of polypeptide chain into functional protein. Presence of heat shock protein (purple) assures that the new protein will assume its functional three-dimensional configuration. Bottom: after stress event, heat shock proteins also assist in refolding or degradation of damaged or denatured proteins.[3]

particularly is widely studied. The aim of this review is to highlight the link between HSPs and atherosclerotic vascular disease.

Methods

Literature relevant to this review was identified by a systematic search in the Medline (PubMed) database from 1960 onwards. A random search of Medline using HSP as a key word revealed more than 20,000 citations. HSP, vascular disease and human were used to limit the search, which was further restricted to papers in the English language. Papers dealing with *in vivo* studies involving the role of HSPs in the pathogenesis of atherosclerotic vascular disease were accepted rather than *in vitro* studies in tissue and cell samples. The search was refined by careful review of references cited in key papers on HSP. For a clearer understanding of the complex role of HSPs in atherosclerotic vascular disease, such diseases are classified into four main areas namely—carotid, coronary, aortic and peripheral vascular disease. After an initial overview of the pathogenesis of atherosclerosis with particular reference to HSPs, the link between HSPs and atherosclerosis is explored in more detail in the four areas of vascular disease.

HSP and Atherosclerosis

In relation to atherosclerosis, HSPs from the HSP60 and HSP70 families are most widely investigated. Studies have shown that HSP60 localizes selectively in atherosclerotic lesions as opposed to non-atherosclerotic regions of the arterial wall.[6] In advanced atherosclerotic lesions, HSP70 is over expressed in several cell types, including monocytes, macrophages, dendritic cells and smooth muscle cells. In early atherosclerotic lesions, however, only dendritic cells, which are key cells in the immune response, over express HSP70.[7] Interestingly, HSPs, which are normally intracellular, have also been found in a soluble form in serum along with specific anti HSP antibodies, and some studies suggest a correlation between the levels of these antibodies and the severity of atherosclerosis.[8,9]

HSP have been investigated in association with

Table 1. Key members of the heat shock protein family in humans[3,4]

HSP member	Location	Description
Low molecular weight HSPs		
Ubiquitin	Cytoplasm/nucleus	Facilitates targeting and removal of denatured proteins
HSP10	Mitochondria	Cofactor for HSP60
HSP27	Cytoplasm/nucleus	Involved in cytoskeletal stabilisation
Aβ-crystallin	Cytoplasm	Involved in intracellular actin dynamics
HSP40		
HSP40	Cytoplasm/nucleus	Regulates activity of HSP70, binds non-native protein
HSP47	Endoplasmic reticulum	Processing of pro-collagen
HSP60		
HSP60	Mitochondria	Molecular chaperone
HSP70		
HSP72	Cytoplasm/nucleus	Highly stress inducible, protects against ischaemia
HSP73	Cytoplasm/nucleus	Constitutively expressed molecular chaperone
HSP75	Mitochondria	Induced by stress including hypoxia
HSP90		
HSP90	Cytoplasm (migrates to nucleus)	Part of the steroid receptor complex
HSP110		
HSP110	Nucleolus/cytoplasm	Thermal tolerance
HSP105	Cytoplasm	Protein refolding

many established risk factors for the development of atherosclerosis and also as independent markers of the disease. In a large population based study, high levels of soluble HSP60 correlated with LDL cholesterol.[10] Levels of anti-HSP 70 antibodies were significantly and independently elevated in 111 established hypertensive men as compared with 75 normotensive controls.[11] The same group also showed that HSP 70 antibodies may have a protective effect in hypertensive subjects by modifying the progression of atherosclerosis.[12] In British civil servants, an association was found between HSP60 and various psychosocial measures like low socio-economic status, social isolation and psychological distress.[13] In addition to traditional risk factors for atherosclerosis, prominent immunoreactivity against HSP60 was associated with atherosclerosis in male youngsters as measured by intima-media thickness in carotid and femoral arteries.[14] HSPs also have prognostic significance in predicting morbidity and mortality due to atherosclerosis. In a cohort of 79 men with documented coronary artery disease, significantly higher levels of anti-HSP 65 antibodies were found in those who went on to have cardiovascular events than those who did not.[15]

Another HSP widely studied is Hemeoxygenase-1, classified as HSP 32. Hemeoxygenase-1 is the inducible form of hemeoxygenase, an enzyme essential for heme degradation. It is induced by a variety of stressors and performs anti-atherogenic functions in the arterial wall like scavenging reactive oxygen species, reducing monocyte adhesion and chemotaxis.[16] It is unclear whether HSP are protective or destructive for the organism and the role of HSPs in atherosclerosis is most probably multifaceted.

One of the important areas of current research is to investigate the role of HSPs in the association between infection and atherosclerosis. Based on their observations, Wick *et al.* have proposed an autoimmune hypothesis of atherogenesis. HSP60 is expressed by the endothelial cells of stressed arteries and because of sequence homology between microbial and human HSP60, the cost of immunity against micro organisms may be responsible for endothelial cell damage and early atherosclerosis.[17] This notion is explored further in relation to key areas of atherosclerotic vascular disease.

Carotid Disease

Initial work in atherosclerosis and HSPs focussed on carotid disease, possibly because of the ease of measuring atherosclerotic lesions in the carotid artery by ultrasound scanning. In a landmark paper, Xu and colleagues studied 867 randomly selected normal inhabitants of South Tyrol and showed that serum anti-HSP65 antibodies were significantly elevated in elderly subjects with carotid atherosclerosis. Further, a significant positive correlation was found between anti-HSP65 antibodies and the thickness of the atherosclerotic plaque in the carotid artery as measured by ultrasonography.[18] Anti-HSP65 antibody titre elevation was sustained in persons with severe and progressive carotid disease and was an independent predictor of mortality at 5 years.[19] Carotid atherosclerosis is an important cause of ischaemic stroke. In 180 stroke patients, anti-HSP65 and anti-HSP70 antibodies were significantly elevated 48 h after ischaemic stroke compared to controls, and multiple regression analysis showed these antibodies to be independent risk factors for stroke.[20] Not surprisingly although anti-HSP70 antibody level was a risk factor for ischaemic stroke, HSP70 itself may be a marker for neuroprotection in the early stages of ischaemic stroke.[21] Many mechanisms have been suggested for HSP70 protection from cerebral ischaemia including defence against apoptotic and necrotic cell death.[22]

Anti-HSP antibodies maybe produced in response to infection and this possibly provides a link between atherosclerosis and chronic infection. Infection with *Chlamydia pneumoniae* seems a likely suspect. Anti-*C. pneumoniae* antibodies are most closely associated with carotid atherosclerosis as opposed to antibodies to other infectious agents. This association is independent of other risk factors, consistent over time and for different stages of atherosclerosis. The antibody titre correlates with intima-media thickness and with anti-HSP65 antibodies. Anti-*C. pneumoniae* antibodies may be produced by sub-clinical infection, but interestingly evidence of chronic *C. pneumoniae* respiratory infection was more effective in predicting atherosclerotic carotid disease.[23] In addition to humoral factors, cell-mediated immunity to *C. pneumoniae* has also been associated with the pathogenesis of atherosclerosis. Established T-cell lines were propagated from activated T-lymphocytes isolated from tissue specimens of patients undergoing carotid endarterectomy. Forty-one percent of the propagated T-cell lines reacted with *C. pneumoniae* peptides and majority of these were from the HSP60 antigen.[24] In another study, *C. pneumoniae* was present in the endothelial cells, activated macrophages and smooth muscle cells in 90% of the specimens harvested at carotid endarterectomy. *Chlamydial* HSP60 was found in all specimens positive for *C. pneumoniae*-specific antigen and mainly co-localised with this antigen in the activated

macrophages, suggesting the role of *Chlamydia*-infected macrophages in the pathogenesis of carotid atherosclerosis.[25] In summary, microbial infection causes immune reactions involving HSPs via humoral and cell-mediated immune systems. Since, HSPs are preserved with similar antigenic properties across different species, antibodies and stimulated T cells may then cross-react with host endothelial cells expressing such molecules perhaps promoting atherogenesis.

Coronary Disease

Ischaemic heart disease is the most common cause of death in the Western world and extensive studies on HSPs and coronary atherosclerotic disease have been performed. The relationship between HSPs and most aspects of ischaemic heart disease has been studied including presence and severity of coronary artery atherosclerosis, coronary syndromes, myocardial infarction (MI), ischaemia-reperfusion injury and cardiac protection. In patients with coronary artery disease (CAD), antibodies to both HSP65 and HSP60 have been shown to be significantly associated with both presence and severity of the disease. Higher titres were found with increasing number of diseased vessels and also greater extent of disease, as measured by coronary atherosclerosis scores.[26,27] Hsp65 antibody, although elevated in patients with CAD, significantly fell in patients following acute MI. It is hypothesized, based on animal models of MI that HSPs are released in the circulation from the infarcted heart tissue and these bind to circulating anti-HSP antibodies to form antigen–antibody complexes, which are subsequently removed from the circulation by the reticuloendothelial system.[28] In addition, following percutaneous transluminal coronary angioplasty, anti-HSP65 antibody titres dropped in patients who remained disease-free, but remained elevated in those who developed restenosis. A fall in HSP antibody titre maybe associated with a favourable outcome, and may serve as a useful prognostic marker for coronary angioplasty.[29] Levels of HSP60 and HSP65 are associated with more severe forms of CAD, but some other HSPs may have a protective effect. High levels of human HSP70 were shown to be associated with low CAD risk, suggesting a more complex role for these proteins in coronary atherosclerosis.[30]

In coronary atherosclerotic disease circumstantial evidence links infection, immunity and the atherosclerotic process with HSPs, particularly infection with *C. pneumoniae*. A strong association between antibodies to the organism and coronary artery disease has long been established[31,32] and *C. pneumoniae* has also been detected in atheromatous plaques,[33,34] but how *C. pneumoniae* might induce or promote atherosclerosis was not clear until recently. In surgical specimens from human atherosclerotic arteries, *Chlamydial* HSP60 and human HSP60 were found to co-localize within atherosclerotic plaque macrophages and both were shown to potently stimulate the production of tumour necrosis factor (TNF) and MMP-9 by the macrophages.[6] *Chlamydial* lipopolysaccharide has been demonstrated to induce mononuclear phagocyte foam cell formation and *Chlamydial* HSP60 was shown to induce low-density lipoprotein (LDL) oxidation.[35] These steps are highly relevant to atherogenesis and plaque complications. Further evidence of this coronary atherosclerosis/*Chlamydial* infection/HSPs association is suggested in other clinical studies. High levels of anti-human HSP60 antibodies and *C. pneumoniae* antibodies were found to be independent risk factors for coronary atherosclerosis, and their simultaneous presence substantially increased the risk of disease development.[36,37] Using multivariate analysis to account for other risk factors high levels of anti-*Chlamydial* HSP60 antibodies were again shown to be independently associated with coronary artery disease and could identify the subset of patients with *Chlamydial* infection and significant CAD.[38] A persistent elevation in the immune response involving *C. pneumoniae* and HSP60, when present together, better predicted coronary events than transient or individual elevations in these antibodies.[39] This evidence has paved the way for research trials to evaluate the role of immunomodulation, antibiotics and vaccination against *C. pneumoniae* as interventional measures in CAD.[40,41] Although initial clinical studies showed promising results, these new anti-atherogenic measures have not yet had any long term impact on atherosclerosis. Possibly, this is because of wrong choice or short duration of antibiotics or the fact that the disease aetiology is multi-factorial and not simply dependent on one infectious agent.[42,43] Long term trials are required to investigate further this interesting area of research.

Aortic Disease

HSPs are implicated in atherosclerotic disease involving the abdominal aorta and its visceral branches. Using immunohistochemical techniques in human aortic specimens from autopsy, HSP70 was shown to be present weakly throughout the media of apparently normal looking specimens, and was highly

concentrated in the centre of thickened atheromatous plaques around sites of necrosis and lipid accumulation. The intensity of HSP70 staining correlated with the thickness of the atherosclerotic plaque. Also the increased HSP distribution was prominent in macrophages as opposed to smooth muscle cells or other plaque cells.[44] Another study investigating HSP70 in the human aorta studied its distribution by immunohistochemistry and video image analysis software, and quantified the level of HSP70 by Western Blotting. This study showed a homogeneous staining pattern of HSP70 in 'normal-appearing' regions, but a heterogeneous pattern in areas of atherosclerosis. The image analysis indicated a significant positive correlation between the severity of atherosclerosis and altered pattern of HSP70 staining, but Western blotting showed no difference in total content with plaque progression. It is suggested that the heterogeneous pattern of HSP distribution in atherosclerotic lesions may be due to leakage of HSP70 from damaged cells into the plaque.[45] Stress-induced synthesis of HSPs normally protects cells from death, but insufficient accumulation of HSP70 in aortic smooth muscle cells, near areas of necrosis, leads to death of such cells, which further promotes plaque rupture and thromboembolic complications.[46]

Although most modern investigators question the relationship between aortic aneurysmal disease and atherosclerosis, immune responses have been studied in abdominal aortic aneurysmal disease. Serum level of anti-HSP70 antibodies was significantly higher in patients with abdominal aortic aneurysms (AAA) than controls indicating a role for humoral immune response involving HSPs.[47] Cell-mediated immunity is also implicated as HSP60 expression was found in intimal endothelial cells and mononuclear infiltrate (T-lymphocytes and macrophages) of the aorta at sites of branching and other large size arteries, but not in smaller vessels. This distribution of immune cells suggests that the stress of high velocity and haemodynamic sheer forces may be responsible for the recruitment of HSP-specific T cells.[48] Infection may be responsible for initiating the immune responses, and once again *C. pneumoniae* has been implicated, as it was detected in the vessel wall of AAA specimens using different techniques including immunohistochemistry, *in situ* hybridisation and polymerase chain reaction. To satisfy the infection hypothesis, all components of *C. pneumoniae* should be isolated in the atherosclerotic plaque. Whilst *Chlamydial* lipopolysaccharide and membrane protein antigens were detected in abundance, *Chlamydial* DNA or HSPs were not, and this was suggested to be due to the rapid degradation of the HSPs and DNA with persistence of other antigens.[49]

Peripheral Vascular Disease

Peripheral vascular disease (PVD) usually refers to atherosclerotic chronic lower limb ischaemia. Patients with PVD have three times higher mortality than age and sex matched controls mainly because of co-morbidity due to atherosclerotic disease in other vascular beds.[50] A number of studies have analysed the role of HSPs in the pathogenesis of atherosclerotic PVD and particularly the beneficial influence of exercise. Levels of circulating HSP70 were significantly elevated in 20 patients with PVD compared to controls. Levels of anti-HSP70 antibodies were also elevated but this did not reach statistical significance. In the same study, anti-HSP60 antibody levels were significantly elevated in patients with PVD and the level demonstrated positive correlation with the disease severity.[51] Similarly, in another study, levels of anti-HSP70 antibodies were found to be significantly elevated in patients with PVD as compared with controls and these levels were higher in patients with critical ischaemia than in claudicants, again suggesting that anti-HSP antibody levels bear some correlation with disease severity.[47] Diabetes mellitus is one of the more important risk factors for the development of PVD and anti-HSP70 antibody subclasses have been measured in 67 diabetic patients. IgG and IgM class of anti-HSP70 antibodies were not different as compared to controls, but IgA class anti-HSP70 antibodies titres were significantly higher in type I and II diabetics than in non-diabetic controls. It has been suggested that IgA-containing immune complexes may be implicated in the vascular complications of patients with diabetes mellitus and that HSP70 may have a role as an auto antigen in the pathogenesis of these diseases.[52]

Transluminal angioplasty is a common treatment modality for patients with PVD, but the mechanical stretch injury associated with balloon angioplasty can lead to lipid accumulation, monocyte and platelet adhesion, smooth muscle cell proliferation and new plaque formation.[53] Interestingly, the mechanical stress of balloon angioplasty was shown to induce HSP70 in the smooth muscle cells in of blood vessels harvested from patients undergoing above-knee amputations. On the basis of these results it has been suggested that the induction of HSP70 expression may be an important component of the response to injury by blood vessels that is often described as the first step in atherogenesis.[54] HSP70 and HSP70 mRNA was

measured in calf muscle biopsies from patients in different stages of PVD, using gel electrophoresis and reverse transcriptase polymerase chain reaction techniques, respectively. Both HSP70 and HSP70 mRNA were significantly elevated in calf muscles from patients with Fontaine stage II, III and IV PVD as compared to controls. The highest levels were found in stage III disease, whereas levels were lower in stage IV disease, which is characterised by tissue loss. This was hypothesized to be due to lack of exercise in patients with stage IV disease. Exercise in patients with PVD may cause an ischaemia-reperfusion type injury. Since stage IV patients frequently have severely limited mobility and are unable to exercise, there is an absence of reperfusion injury and a reduction in HSP70 levels.[55] Ischaemia reperfusion injury (IRI) has been implicated in the pathogenesis of many diseases in the heart, lung and intestines. In PVD ischaemia occurs during walking to the point of claudication and reperfusion occurs when the patient stops and rests. This IRI has been suggested to cause inflammation and atherosclerotic progression, perhaps accounting for the higher mortality seen in these patients compared to age and sex matched controls.[56] Reperfusion of ischaemic tissues initiates a complex series of reactions that paradoxically injures tissues. Leucocyte-endothelial interaction is a pivotal step in this IRI. Thermotolerance, possibly mediated through the induction of HSP72, attenuates ischaemia-reperfusion induced leucocyte-endothelial interaction, the key process in IRI.[57]

In healthy individuals, a bout of acute exercise induces HSPs. Serum HSP70 levels were elevated during and after a 60 min treadmill exercise in normal humans.[58] Although exercise increases the body temperature, exercise-induced hyperthermia is not the sole factor inducing HSP70 production; this is likely to be a combination of mechanical, metabolic and chemical stresses. It is inferred that this induction of HSPs may play a role in the adaptation to exercise and training.[59] The role of HSPs in exercise in patients with PVD merits further investigation.

Conclusion

There is growing evidence that atherosclerosis may be an inflammatory and possibly an immune disorder. Due to a high degree of sequence homology between human and microbial HSPs, anti-microbial HSP antibodies produced in response to infection cross-react with HSPs on endothelial cells stressed by classical risk factors for atherogenesis. This 'antigenic mimicry' causes endothelial damage and early atherosclerotic lesions.[60,61] In each of the four key areas of vascular disease—carotid, coronary, aortic and peripheral vascular disease—studies have shown a substantial role of HSPs in the pathogenesis of these diseases. In many cases they are independent risk factors for the disease. Levels of HSPs or their specific antibodies have a positive correlation with disease severity and in some cases have prognostic value. Although for the individual cell, HSPs are helpful for cell survival as they perform important functions, it is not clear that for the organism as a whole, HSPs are beneficial or simply a double-edged sword playing a role in the pathogenesis of fatal diseases. A more comprehensive understanding of the role of HSPs in atherosclerosis is likely to lead to new approaches for the prevention and treatment for all forms of cardiovascular disease.

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