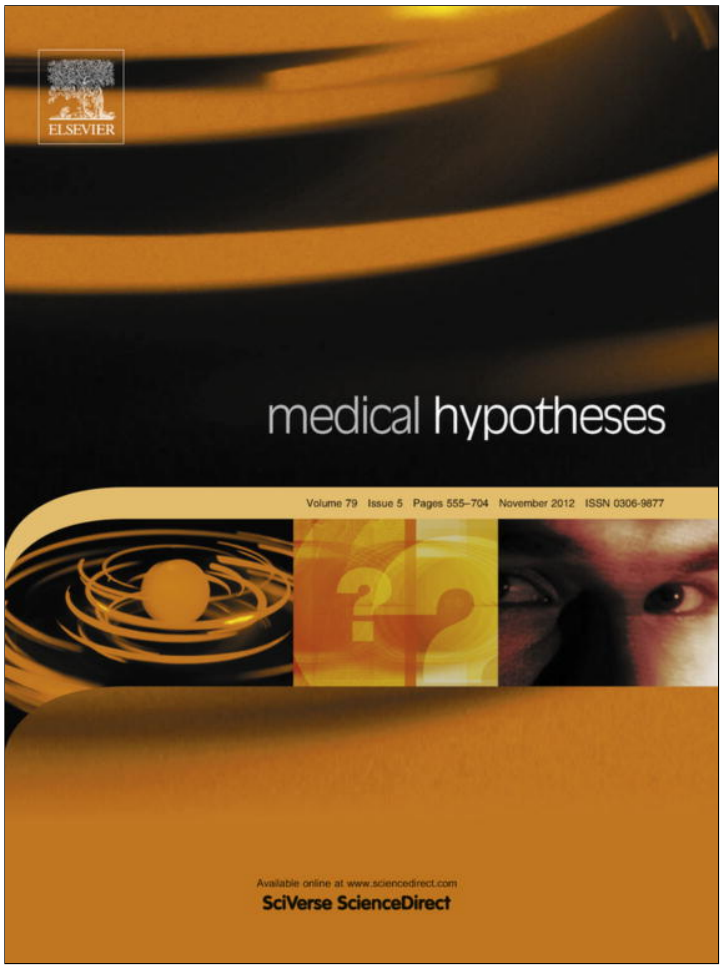

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Can we assess an acute myocardial infarction in patients with acute coronary syndrome according to diagnostic accuracy of heat shock proteins?

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ABSTRACT

Heat shock proteins (HSPs) have changed very little with evolution, suggesting that they play important role(s) in cellular survival. Specifically, HSPs protect cells from induced cell death. Their expression is triggered by heat or other stress, such as ischemia. HSPs provide protection against protein denaturation, although they slightly differ with respect to group affiliation. Release of HSPs from necrotic and ischemic cardiomyocytes into the intercellular space and plasma may correlate with the intensity of the pro-inflammatory response observed during and immediately after myocardial infarction. We hypothesized that the plasma concentration of particularly inducible forms of HSPs from different groups (HSP 90, HSP 70, HSP 60 and/or HSP 20) can be used as early specific markers for diagnosing myocardial infarction in patients with acute coronary syndrome. Our hypothesis is supported by the following data: (I) HSP expression occurs very early after acute coronary events; (II) HSP concentrations increase rapidly in the peripheral blood; (III) HSP concentrations correlate with markers of myocardial necrosis and pro-inflammatory biochemical parameters. The magnitude of the increase in plasma HSP concentrations over initial concentrations during the period of highest sensitivity and specificity of the assay could be important for early detection of myocardial infarction and distinguishing it from unstable angina. We suggest that these parameters, along with close observation of patients with chest pain, will assist providers who must differentiate between acute myocardial damage and other organ diseases.

Introduction

Heat shock proteins (HSPs) from groups 90, 70, 60, and 20 are present constitutively in the cells. These proteins have changed only slightly during evolution, indicating their high biological significance [1]. Although HSPs differ partially, depending upon group affiliation [1], they all protect cells from apoptosis. HSPs maintain the normal structures polypeptide chains, enabling transfer polypeptides across cell membranes. HSPs also influence peptide placement in major histocompatibility complex (MHC) class I molecules [1,2]. HSP 90, particularly promotes transport of protein-kinase C epsilon [3] and connexin 43 [4] from the cytoplasm to the inner mitochondrial membrane in *ex vivo* perfused ischemic rat hearts, thereby protecting the myocardium. HSP 90 also increases the activity of nitric oxide synthase, enhancing nitric oxide-induced vasodilatation [5]. Repeated stimulation with heat in rats did not lead to an increase in cardiac HSP 90 but increased the expression of constitutionally expressed HSP 70 [6]. Interestingly, levels of HSP 70 messenger RNA did not change [6]. Heat treatment of cardiomyocytes resulted in significantly increased levels of HSP 72, an inducible HSP, but did not affect levels of the constitutively expressed HSP 73 during the first week after coronary artery ligation in rats [7]. In the same model, expression of HSP 27, which can bind to cytoskeleton proteins, increased significantly during the first, second, and eighth weeks after coronary artery ligation [7]. An increase in HSP 60 during the eighth week correlated with the development of chronic heart failure [7]. These data indicate that the expression of HSPs is triggered by heat stress or other stimuli such as ischemia, when they provide additional protection against protein denaturation and cell death [1]. However, prolonged ischemia is responsible for the death of cardiomyocytes resulting in acute myocardial infarction (AMI). In contrast, necrosis of cardiomyocytes does not occur in unstable angina (UA) [8,9]. Damaged cardiomyocytes release HSPs from their cytoplasm into the intercellular space, leading to HSPs increase in peripheral blood

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[10]. The intense pro-inflammatory response observed during and immediately after myocardial infarction may be triggered by HSPs, as it was suggested recently by many investigators [1,11–14].

Soluble HSP 60-specific T lymphocytes exist in the circulatory system and in atherosclerotic plaques in humans [12]. These T cells are thought to play a role in damaging vessels, causing plaque rupture at the time of acute coronary events [13]. High plasma concentrations of HSP 60 are associated with the development and severity of coronary artery disease [15]. HSPs associated with peptide in the extracellular space may indirectly become immunogenic after binding to cognate receptors (for example, Toll-like receptor 4 and CD91) on the surfaces of antigen-presenting cells [1]. Binding could initiate innate and acquired immune responses [1], which can trigger plaque rupture. This idea is supported by the presence of abundant Toll-like receptor 4-positive macrophages in infiltrates of ruptured plaques [16]. There are positive correlations between HSP 70 levels and the expression of Toll-like receptor 4 on monocytes and with levels of pro-inflammatory cytokines and chemokines in the peripheral blood [17]. HSP 27 is a biological marker of atherothrombosis, but its plasma concentration is not associated with cardiovascular events in previously healthy women, as shown by Kardys et al. [18]. HSP 27 expression was significantly reduced in human atherosclerotic lesions, while it is increased in the area surrounding the plaque [18]. Park et al. [19] demonstrated that plasma HSP 27 expression was increased in patients with acute coronary syndrome (ACS). Similarly, HSP 70 was found in the human myocardium early after infarction and was distributed distinctly around the area of infarction [20]. Cardiomyocytes with coagulation necrosis or myocytolysis do not express HSP 70 after ischemic damage [20]. However, plasma HSP 70 concentrations are significantly higher in patients with AMI upon admission and 6 h after admission than in patients with stable angina (SA) and in normal subjects [10,20]. Compared to HSP 70 concentrations in control subjects, concentrations of circulating HSP 70 decrease rapidly during the first 7 days after acute coronary events [17] and remain higher during the first 14 days after myocardial infarction [21]. Increase in plasma concentrations of HSP 70 is correlated with increased risk of morbidity and severity of acute coronary syndrome, while the concentration of anti-HSP 70 antibody is associated with reduced risk of acute coronary syndromes [17]. Therefore, cardiomyocytes may significantly upregulate and secrete HSP 70, among other HSPs, in response to acute ischemic damage [2]. Coronary endothelial cells are the main site of induction of HSP 70 in the heart and vessels [22].

Hypothesis

We hypothesize that HSP 90, HSP 70, HSP 60, and/or HSP 20 are released from ischemia-induced necrotic cardiomyocytes and that plasma concentrations of these HSPs are early specific markers of myocardial infarction in patients with acute coronary syndrome. If this hypothesis is correct, particularly inducible forms of HSPs may serve as diagnostic aids. Stent implantation is a valuable tool for myocardial revascularization. This procedure eliminates the cause of necrosis during myocardial infarction. We therefore expect more rapid reductions in HSP levels in stented patients than in patients treated with anti-ischemic drug therapy only.

Our hypothesis is supported by the following findings: (I) HSPs occur in peripheral blood very early after acute coronary events [10,19,20]; (II) some HSP concentrations in peripheral blood increase rapidly in proportion to the severity of ACS [15]; (III) HSPs correlate with markers of myocardial necrosis and pro-inflammatory biochemical parameters, such as creatinine kinase-MB, cardiac troponin T, IL-6, IL-8, and TNF-α [10,17].

Evaluation of hypothesis

To test this hypothesis, the diagnostic accuracy of different HSPs for AMI in ACS patients must be investigated. Because plasma concentrations of HSPs change rapidly after ischemia-induced damage [10,17,20], HSP concentrations need to be assessed upon admission for chest pain and at 3, 6, 9, 12 and 24 h after admission. Of particular interest are the patients having chest pain for <2 h at the time of admission. HSPs concentration data from these patients may help to shorten the time required for an accurate diagnosis and achieve well-timed myocardial revascularisation therapy by primary percutaneous intervention with stent implantation [23,24]. In the proposed studies, AMI should be distinguished from UA using ELISA assays for detection of cardiac troponin I (cTnI) and T (cTnT) [8,9,25], heart fatty acid binding protein [26,27] or glycogen phosphorylase isoenzyme BB [28] on the same blood sample. It could emphasize the significance of HSPs in distinguishing UA and AMI, particularly during very early ACS. It may be worthwhile to compare post-treatment HSP levels between patients receiving primary percutaneous coronary intervention and those treated with medicaments only. Comparison of diagnostic accuracies of HSPs and cardiac troponins could be performed at particular time points. Determination of sensitivity, specificity, and positive and negative predictive values and identification of the critical period(s) in which concentrations of HSPs increase most rapidly could collectively contribute to early detection of myocardial infarction. Finally, the prognostic value of elevated HSP levels for future cardiovascular events and death in AMI patients could be analysed.

Consequences of the hypothesis and discussion

The routine introduction of cardiac troponin measurements in the laboratory radically improved the diagnosis of AMI, because only necrotic cardiomyocytes release these proteins into the blood [9]. However, repeated measurements of cTnI concentrations are required during the period of the assay's highest specificity and sensitivity, which ranges from approximately 6 h to 9 h after admission [25,29]. In the present era of primary percutaneous coronary intervention and stent revascularisation, the ability to distinguish ACS patients from a very large proportion of patients with chest pain requires early biomarkers of myocardial cell injury. An early marker with the ability to distinguish AIM from UA on the basis of cellular necrosis would be even more valuable. Circulating HSPs in patients with coronary artery disease may reflect changes in the release of HSPs from cardiomyocytes, especially in the presence of substantial ischemic tissue damage [10,17,20]. This highlights the importance of assessing HSP levels in peripheral blood for early diagnosis of AMI. Since HSPs are widely distributed in the tissues [1], they may not specifically indicate myocardial tissue damage. We are of the opinion that at least some HSPs, particularly the inducible forms, could be useful for early and effective diagnosis of ACS. Rapid increase in blood concentrations of HSPs after acute coronary events could be of significance. The use of humoral biomarkers has been criticized. For example, troponins [30], H-FABP [26], and GPBB [28] are widely distributed and exist in multiple isoforms. Specificity can be improved by developing and/or using more specific ELISA assays. Furthermore, the diagnostic accuracy of HSPs may be compromised because HSP levels can be elevated in healthy individuals and in many patients with stable angina [17]. These factors may thus weaken the value of HSPs for differentiation between healthy subjects, SA patients, and patients with AMI and UA. However, the concentrations of HSP 70 were markedly higher in patients with ACS and SA than in controls, and were higher in patients with ACS than in those with SA [17]. Further investigation is required to determine whether HSP levels

are useful for distinguishing between AMI and UA early after admission in patients with chest pain for <2 h, which is the time period during which an emergency physician should plan an objective cardiac ischemia evaluation and appropriate therapy. We hope that the rapid dynamic changes in HSP blood concentrations, as measured during the period of highest sensitivity and specificity of each assay, will resolve this question. Furthermore, the understanding of the conditions under which HSP levels are elevated in the plasma will be helpful for differential diagnoses of acute coronary events. Elevation of circulating HSP 70 and/or HSP 60 was observed in peripheral and renal vascular diseases [31] and chronic heart failure [32]. The magnitude of HSP concentration change, relative to initial concentrations and clinical symptoms, appears to be important to differentiate between acute myocardial damage and other organ diseases.

In conclusion, HSP levels may improve the early detection of ACS and could be markers for detrimental effects at the acute stages of AMI. HSPs may also serve as indicators of disease states or pathological processes of AMI. We hope that our proposed model and current opinion will encourage new investigations on biomarkers of early ACS.

Conflict of interest statement

We, as the authors of this manuscript disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) our work in respect of employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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