

HEART

Editorial

Stress proteins: a future role in cardioprotection?

Myocardial infarction remains a major challenge despite effective interventions which allow rapid reperfusion of jeopardised myocardium. This is in part because benefits diminish as treatment is delayed. The longer the duration of ischaemia, the more complete the necrosis and the less the salvage on reperfusion. Hence any intervention that could reduce the rate of necrosis before thrombolysis would preserve left ventricular function and reduce mortality. The rate of myocardial necrosis is determined by the collateral blood supply to the ischaemic zone and the inherent resistance of the myocardium to ischaemia.¹ After occlusion of an infarct related artery distal pre-formed collaterals are likely to be operating maximally. The need is therefore to understand the processes that determine the inherent myocardial resistance to infarction.

It is now known that the resistance of the myocardium to ischaemia can be enhanced both by classic preconditioning (short periods of ischaemia with intermittent reperfusion) and by the upregulation of cytoprotective proteins. Despite its first description over 10 years ago classic preconditioning (the subject of an editorial in the *British Heart Journal* in 1995²) has not led to the development of specific cardioprotective pharmacological agents, partly because of temporal limitations, tachyphylaxis, and the lack of a definitive end effector. Hence, an exploration of the endogenous cytoprotective proteins, particularly the heat shock proteins (hsps), may ultimately prove more fruitful.

Heat stress proteins

The stress response is a universally conserved cellular defence programme consisting of the upregulation of stress proteins. Any agent or treatment which induces the stress response will reduce the injury caused by exposure to a subsequent related or in some cases unrelated stressor. There are five major groups of hsps based on molecular size—70, 90, 50–60, 20–30, and 100–110 kDa.³ Much of the work on hsps within the myocardium has focused on hsp70, as it is the most abundant and inducible stress protein.

hsp70 as a molecular chaperone

All members of the hsp70 family bind to ATP through a highly conserved amino-terminal nucleotide binding domain, as well as binding to both unfolded proteins and short polypeptides *in vitro*. When an hsp70 family member is released, a process requiring ATP, the target protein starts folding and/or assembly.

The function of hsp70 gives us some clues as to the mechanisms by which these proteins may result in myocardial protection. During ischaemia the cellular

internal milieu changes profoundly with the intracellular accumulation of protons and sodium ions. These changes are compounded by the free radical stress and the marked increase in intracellular calcium associated with reperfusion. Under these circumstances the tertiary structure of proteins changes sufficiently to alter their function. In the presence of an excess of hsp70 these adverse conformational changes may be prevented or reassembly of denatured proteins may be promoted.

hsp70 and myocardial protection

It is now eight years since the association between heat stress proteins and myocardial protection was first described by Currie *et al.*⁴ They showed that 24 hours after raising the temperature of rats to 42°C for at least 15 minutes both cardiac hsp70i and catalase activity were increased, while at this time point hearts became resistant to ischaemia/reperfusion injury. In heat stressed hearts (compared with control hearts) post-ischaemic contractile recovery was enhanced and creatine kinase (CK) efflux was reduced. These findings were subsequently confirmed by other groups.⁵ Of greater pathophysiological relevance was the observation that ischaemia itself could result in hsp70i (the inducible form of hsp70) induction and cardioprotection,⁶ but increases also occurred in a 60kDa stress protein and in another myocardial antioxidant enzyme, superoxide dismutase.^{6,7} Thus these studies still fell short of proving a causal relation between hsp70 induction and cardioprotection.

Compelling evidence that upregulation of hsp70 in myocardial cells affords significant protection comes from recent genetic modification studies in which transfected myocyte lines overexpressing hsp70, but not hsp60 or 90, had enhanced resistance to hypoxic stress,⁸ and in which hearts from transgenic mice overexpressing the inducible hsp70 gene had enhanced resistance to ischaemic injury.⁹ In transgene positive compared with transgene negative hearts, the zone of infarction was reduced by 40%, contractile function at 30 minutes of reflow was doubled, and efflux of CK was halved.⁹ In addition overexpression of hsp70 did not alter the macroscopic phenotype of the mouse, contractility of the heart, or antioxidant protein content of the myocardium.⁹ This study provides direct evidence that an increase in myocardial hsp70 increases the inherent resistance of the myocardium to infarction.

Regulation of the stress response

Many of the heat shock proteins are in fact expressed constitutively in normal or "unstressed" cells where they play a fundamental part in several important biological processes. A diverse array of metabolic insults induce a

stress response—many of these agents share the common property of being “protein chaotropes,” they adversely affect the proper conformation and therefore function of proteins.¹⁰ The heat shock factor (HSF 1) present as an inactive monomer in the normal unstressed cell, rapidly trimerises in response to metabolic stress enabling it to bind to the heat shock element and activate transcription of the genes encoding stress proteins. The activation of the heat shock genes within myocardium is known to occur in response to brief periods of cardiac ischaemia *in vivo*.⁶ In these instances it is thought that HSF activation is a consequence of intracellular ATP depletion.¹¹

During ischaemia, substrate deprivation and metabolite accumulation results in a significant breakdown of adenosine triphosphate (ATP), causing the accumulation of interstitial adenosine that gives rise to the sensation of angina.¹² In addition, animal studies indicate that the cellular ATP content remains low for many hours after a brief period of ischaemia—hence periods of ischaemia as short as five minutes are sufficient to trigger hsp gene activation in animal studies. The obvious question is, does protection follow episodes of angina in patients?

Myocardial adaptation

It is interesting to speculate whether myocardial adaptation, perhaps by hsp70i induction, follows an episode of sublethal ischaemia or angina in humans. A history of angina for at least seven days before an acute myocardial infarction seems to predict a less complicated in-hospital course and reduced mortality.¹³ This observation is complicated by differences that may exist between symptomatic and non-symptomatic patients, particularly in terms of collateral vessel formation and concomitant medication. However, a recent analysis of a large and well documented thrombolysis trial database controlled for these variables and found that the protective benefits of a 48 hour history of angina before infarction reduced mortality independently of any of the standard predictors of outcome.¹⁴ The magnitude of the advantage associated with preinfarction angina is substantial. For example the absolute risk of death in anterior infarction without antecedent angina can be increased as much as twofold,¹⁵ with the enzyme derived myocardial damage similarly increased by a factor of 1.5–2.^{14,15} In these studies the temporal relation between angina and infarction is more consistent with protection by the upregulation of cytoprotective proteins than with classic preconditioning.

Therapeutic implications

Thus there is compelling evidence from animal studies that stress proteins, in particular hsp70, can increase the resistance of the myocardium to infarction. In addition, our understanding of the mechanisms regulating stress

protein expression is increasing.

These factors are providing an impetus to manipulate the regulation of the genes encoding hsp70 to confer a clinical advantage. For example, agents could be developed which may be able to bypass the usual stress response and directly upregulate the 70 kDa stress proteins without concomitant cell damage, thus providing a pharmacological route to cytoprotection.¹⁶ The critical question is whether these protective mechanisms are already operative in patients with acute myocardial infarction, and, if so, are they amenable to further manipulation?

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