Review

Heat shock proteins and cardiac protection

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Abstract

The heat shock proteins (hsps) are expressed in normal cells but their expression is enhanced by a number of different stresses including heat and ischaemia. They play important roles in chaperoning the folding of other proteins and in protein degradation. In the heart a number of studies have shown that prior induction of the hsps by a mild stress has a protective effect against a more severe stress. Moreover, over-expression of an individual hsp in cardiac cells in culture or in the intact heart of either transgenic animals or using virus vectors, also produces a protective effect, directly demonstrating the ability of the hsps to produce protection. These findings indicate the potential importance of developing procedures for elevating hsp expression in a safe and efficient manner in human individuals using either pharmacological or gene therapy procedures. © 2001 Elsevier Science B.V. All rights reserved.

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1. Heat shock proteins

It is now nearly 40 years since Ritossa observed that exposure of the larval salivary gland of Drosophila to elevated temperature resulted in the appearance of new puffs in the giant chromosomes of these cells [1]. It is now known that these puffs represent the transcriptional induction of specific genes which encode a group of proteins known as the heat shock proteins (for review, see Refs. [2,3]). Although originally demonstrated in Drosophila, the induction of a small number of heat shock proteins by elevated temperature is observed in all organisms studied ranging from prokaryotic bacteria such as E. coli to mammals including man. Moreover, this evolutionary conservation extends not only to the existence of the response in widely different organisms but also to the induced proteins themselves which are very similar to one another in very different organisms. Thus, the best characterised hsps, hsp90, hsp70 and hsp65 (each hsp is named according to its mass in kilodaltons), are induced in response to heat in all organisms studied from bacteria to man and are highly conserved between different species. For example, the hsp90 protein from mammals shows 60% amino acid identity with the corresponding yeast protein and 78% with the Drosophila protein [4]. The various hsps and their characteristics are listed in Table 1.

Although originally identified on the basis of their induction by elevated temperature and therefore named the heat shock proteins, these proteins are in fact induced by a wide range of stimuli which are potentially damaging to the cell. Such inducers include infections with a wide variety of different viruses [5-7], treatment with ethanol [8], sodium arsenite [9], steroid hormones [10] and heavy metals such as cadmium [11]. Similarly, and of considerable relevance from the cardiac point of view, hsps can also be induced by stimuli such as anoxia and/or ischaemia and generators of free radicals such as hydrogen peroxide (for discussion, see Ref. [12]). The effects of these stimuli on hsp synthesis in cardiac cells will be discussed in more detail below. It is clear, however, from the extensive studies which have been carried out in a variety of cell types that the hsps are induced in response...
Table 1
Major eukaryotic hsps

<table>
<thead>
<tr>
<th>Family</th>
<th>Members</th>
<th>Prokaryotic homologue</th>
<th>Functional Role</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hsp90</td>
<td>Hsp100, Hsp90, Grp94</td>
<td>C62.5 (E. coli)</td>
<td>Maintenance of proteins such as steroid receptor. Src. in an inactive form until appropriate</td>
<td>Drosophila and yeast homologues of hsp90 are known as hsp83</td>
</tr>
<tr>
<td>Hsp70</td>
<td>Grp78 (=Bip) Hsp72, Hsp73 Hsx70</td>
<td>dna K (E. coli)</td>
<td>Protein folding and unfolding: assembly of multimeric complexes</td>
<td>Hsx 70 only in primates</td>
</tr>
<tr>
<td>Hsp60</td>
<td>Hsp60</td>
<td>gro EL (E. coli) Mycobacterial 65 kD antigen</td>
<td>Protein folding and unfolding: organelle translocation</td>
<td>Major antigen of many bacteria and parasites which infect man</td>
</tr>
<tr>
<td>Hsp56</td>
<td>Hsp56</td>
<td>–</td>
<td>Protein folding, component of steroid receptor complex</td>
<td>Binds FK506 (tacrolimus) and is also known as FKBP56</td>
</tr>
<tr>
<td>Hsp32</td>
<td>Hsp32</td>
<td>–</td>
<td>Cleaves heme to yield carbon monoxide and the protective anti-oxidant molecule, biliverdin</td>
<td>Also known as heme oxygenase-1</td>
</tr>
<tr>
<td>Hsp27</td>
<td>Hsp27, Hsp26, etc.</td>
<td>Mycobacterial 18 kD antigen</td>
<td>Unclear</td>
<td>Very variable in size and number in different organisms</td>
</tr>
<tr>
<td>Ubiquitin</td>
<td>Ubiquitin</td>
<td>None</td>
<td>Protein degradation</td>
<td>Also conjugated to histone H2A in the nucleus leading to potential role in gene regulation</td>
</tr>
</tbody>
</table>

to a variety of stressful stimuli and are perhaps more properly called therefore “stress proteins”.

The strong evolutionary conservation of the hsps or stress proteins which was noted above together with their induction by a variety of stressful stimuli has suggested that they have some critical function in the cell’s response to stress. Interestingly, however, many hsps or stress proteins are also synthesised by normal unstressed cells with their synthesis being further increased upon exposure to stress. Thus, for example, hsp90 is one of the most abundant proteins in unstressed cells constituting approximately 1% of the total protein in mammalian cells even prior to exposure to stress. This has led to the idea that the function of the hsps is one which is required in normal cells but to an even greater extent in stressed cells.

This idea is in accordance with detailed functional studies of individual hsps. Thus, as indicated in Table 1 it appears that a number of hsps have a role in ensuring the correct protein folding of other proteins within the cell (acting as so-called “molecular chaperones”) (for review, see Ref. [13]). Thus, for example, hsp90 associates with steroid receptors such as the glucocorticoid receptor and keeps them in an inactive form located in the cytoplasm prior to exposure to steroid. Upon steroid treatment, hsp90 dissociates from the receptor which is then able to move to the nucleus and activate steroid responsive genes.

Clearly, correct protein folding is of importance in normal cells but factors which aid this process will be required at higher level in stressed cells when, for example, stimuli such as elevated temperature result in an increased level of denatured or semi-denatured proteins. This idea is also in agreement with findings which indicate that hsps can also be induced by treatment of cells with amino acid analogues which again would induce the formation of abnormally folded proteins [11].

Both in normal cells and in stressed cells there will also be a need to degrade proteins which have become abnormally folded and cannot be rescued by the action of chaperone proteins. It is therefore of interest that ubiquitin, which plays a critical role in protein turnover by being linked to proteins marked for degradation, is also induced by elevated temperature and is therefore a heat shock protein (see Table 1). A further link between the hsps and protein degradation is provided by the recent observation...
that inhibition of hsp70 synthesis enhances the cell death which is induced by inhibiting the proteasome, which mediates the degradation of ubiquitinated proteins [14].

The idea of the hsps as proteins which are of importance in normal cells but which assume a greater significance in stressed cells leads logically to the idea that the induction of these proteins by a stressful stimulus is of itself important in assisting the cell to protect itself from such a stress. In turn, this leads to the idea that the prior induction of the hsps by a mild stress or by some other non-stressful procedure would be protective against subsequent more severe stress. This idea obviously has considerable medical importance and has therefore been intensively investigated.

Over the years, this work has effectively proceeded in three stages. Firstly, the demonstration that exposure to mildly stressful stimuli which can induce hsp expression can in turn protect cells against exposure to a more severe stress. Evidently, such findings implicate the hsps as being protective but do not prove this since the protective effect could be due to some other action of the mildly stressful treatment other than its ability to induce the hsps. This idea leads directly to the second stage of these investigations, namely the use of gene constructs to over-express the hsps in cultured cells and to then demonstrate a protective effect against subsequent exposure to stress. Finally, more recently, these experiments in cultured cells have been complemented by experiments over-expressing the hsps in an intact animal and again demonstrating a protective effect.

In this review, I will discuss these three stages of work on the protective effect of heat shock proteins focusing on studies involving cardiac cells in culture or in the intact heart.

2. Protective effect of stimuli which induce hsp synthesis

During the 1980s, a very large number of studies demonstrated that, in cells in culture, stimuli which induced hsp synthesis such as a mild stress resulted in protection against exposure to a subsequent more severe stress. Moreover, it was also demonstrated that the levels of the hsps induced by such mildly stressful procedures correlated with the level of protection which was observed against the subsequent stress (for reviews, see Refs. [2,3]).

In general, however, these studies were carried out in fibroblast cells and other non-cardiac cell types rather than in cardiac cells. It was some time, however, before these studies attracted the attention of those in the cardiac field and their obvious clinical implications were realised. However, once such studies were initiated they moved rapidly to the intact heart either perfused ex vivo or within the intact animal in vivo.

Thus, Currie [15] demonstrated that exposure of isolated rat hearts to ischaemia or elevated perfusion temperature on a Langendorff perfusion apparatus resulted in the induction of hsp70. Similarly, Dillmann et al. [16] demonstrated that hsp70 was induced in the intact dog heart in vivo by occlusion of the left anterior descending coronary artery resulting in myocardial ischaemia. These initial studies were supplemented by others which demonstrated that, as in other cell types, hsps could be induced in the heart by exposure to a wide variety of stimuli (for review, see Ref. [17]). Such stimuli include, for example, pressure or volume overload [18], exposure to heavy metals [19], drugs such as vasopressin or angiotensin [20] or isoproterenol [21].

Evidently, studies demonstrating the induction of the hsps in the heart lead to the question of whether such hsp induction can protect the heart against subsequent exposure to a more severe stress. This was first demonstrated by Currie et al. [22] who exposed rats to elevated temperature and then removed their hearts and exposed them to ischaemia on a Langendorff perfusion apparatus. They demonstrated that the hearts from rats which had been exposed to an elevated temperature showed improved recovery of contractile function following subsequent ischaemia and reperfusion compared to control hearts. Furthermore, the reperfusion damage, as measured by creatine kinase release, was significantly reduced in the heat shock hearts. These findings therefore demonstrated for the first time that a stimulus which induced hsp induction in the intact heart was able to produce a protective effect against subsequent exposure to ischaemia/reperfusion. These results have subsequently been extended both by examining other parameters of heart function and by using other species such as the rabbit [23,24].

These studies demonstrating a protective effect in the heart on a Langendorff perfusion apparatus following prior exposure to elevated temperature in vivo, lead naturally to the question of whether a similar protective effect would be observed in hearts exposed to myocardial ischaemia within the intact animal following a prior exposure to heat shock.

Donnelly et al. [25] demonstrated that this was indeed the case with an effective reduction of infarct size being observed when rat hearts were exposed to 35 min of left coronary artery occlusion in the intact animal following exposure to heat shock. Moreover, this protective effect is not confined to the use of heat shock itself to induce the hsps. Thus, Marber et al. [26] were able to demonstrate that four brief periods (5 min each) of cardiac ischaemia were able to induce hsp synthesis and were also able to reduce infarct size when the hearts were subsequently exposed to 30 min of ischaemia in the intact animal.

Hence, stimuli which result in hsp induction in the intact heart in vivo can produce a protective effect against subsequent exposure of the heart to ischaemia/reperfusion either on a perfusion apparatus or within the intact animal. In addition, a number of studies have demonstrated that the protective effect correlates with the amount of heat shock protein which is induced. Thus, for example, Marber et al.
[27] showed a correlation between the amount of hsp70 produced by heat stress of papillary muscle and the muscle’s ability to recover function following a period of hypoxia. Similarly, Hutter et al. [28] demonstrated a similar correlation between the amount of hsp70 and the ability to limit infarct size following exposure of the heart to ischaemia and subsequent reperfusion. Evidently, however, the stimuli which induce the hsps may also induce other proteins (see, for example, Refs. [20,23,29]). This has led to much discussion as to the protective role of the hsps in the protective effect of particular inducing stimuli. In particular, much discussion has taken place as to whether the phenomenon of preconditioning (in which a series of short periods of ischaemia protect the heart against a subsequent more prolonged ischaemic period) mediates its protective effect via induction of hsps (for discussion, see Refs. [24,30]).

Although such discussions are of interest with regard to the mechanisms of particular protective effects, it is certainly clear from the experiments described above that a number of stimuli which induce hsps do produce a protective effect in the heart. Rather than attempting to analyse whether the hsps are responsible for this protective effect in each individual case, it is perhaps better to investigate whether the over-expression of an individual hsp in the heart in the absence of any other effect, can produce protection. In this way it is possible to demonstrate that induction of a single hsp can be protective even though this does not mean that this effect is responsible for the protective effect in every circumstance where an hsp is induced by a particular stimulus. Studies demonstrating such a protective effect of individual hsps are discussed in the next section.

3. Protective effect of individual hsps in cardiac cells in vitro

Initial studies on the protective effect of the hsps in cardiac cells focused on hsp70 and utilised the H9c2 cell line which was derived initially from the rat heart. In 1994, two groups reported the results of experiments in which stable transfection was used to produce clonal cell lines derived from H9c2 which constitutively over-expressed hsp70 [31,32]. These cells were shown to be protected against subsequent exposure to thermal or ischaemic stress compared to control cells which did not over-express hsp70. These studies were subsequently extended by Cumming et al. [33] who demonstrated that similar protective effects against heat stress or simulated ischaemia could be observed when hsp70 was over-expressed by transfection of primary rat cardiac myocyte cultures, demonstrating that this protective effect could be observed both in primary cardiac cells and in cell lines derived from them. A similar protective effect was also observed when hsp70 was over-expressed by transfection in coronary endothelial cells [34], indicating that hsp70 can protect these cells as well as cardiac myocytes. This is of particular interest since it has been shown that when the heart is exposed to elevated temperature in vivo, hsp70 induction occurs primarily in endothelial cells rather than in cardiac myocytes [35,36].

These findings thus conclusively demonstrate that over-expression of hsp70 in cardiac myocytes and endothelial cells can indeed protect them against heat or ischaemia in vitro. It should be noted, however, that thermal or ischaemic stress of the type used in the experiments described in the previous section does not merely induce hsp70 but also induces a range of different hsps (see, for example, Refs. [20,29,37]) and it is therefore of considerable importance to determine the protective effect of these other hsps.

Interestingly, when transfection methods were used to over-express hsp90, hsp60, or hsp56 either in the H9c2 cell line [38] or in cultured primary cardiac cells [33,39], hsp90 over-expression was able to protect the cells against subsequent thermal stress but not against subsequent simulated ischaemia, whereas hsp60 or hsp56 had no protective effect. Since hsp70 over-expression protected against both thermal or simulated ischaemic stress in these experiments, these studies indicate that different hsps can have different protective effects and need to be tested individually for their protective effect in any specific situation.

Such an approach has been facilitated by the development of viral vectors for achieving high efficiency gene delivery to cardiac and other cell types, allowing studies to be undertaken with greater ease than the earlier transfection studies which were also of much lower efficiency in terms of gene delivery. Thus, our laboratory has prepared herpes simplex virus vectors over-expressing individual hsps and used these to determine their protective effect in primary cardiac cells. In these experiments [40] we confirmed our earlier results that hsp70 over-expression can protect cardiac cells against simulated ischaemia or thermal stress, whereas over-expression of hsp56 has no such protective effect. Moreover, we were able to extend these studies by showing, firstly, that hsp70 can protect against the induction of apoptosis (programmed cell death) in cardiac cells by exposure to ceramide, whereas hsp56 has no protective effect and, secondly, to demonstrate that over-expression of hsp27 (which we had not previously tested) similarly protects cardiac cells against subsequent exposure to thermal or ischaemic stress or to ceramide (Fig. 1).

These findings suggest that hsp27 may be as protective as hsp70 in cardiac cells. Similar results were also obtained by Martin et al. [41,42] who used an adenovirus vector to over-express hsp27 or the related protein αB-crystallin in cardiac cells. They were able to demonstrate that both these proteins were able to protect cardiac myocytes from the effect of simulated ischaemia and that decreasing the
strating that a similar protective effect of over-expressing an individual hsp can be observed also in the intact animal in vivo. These studies are discussed in the next section.

4. Protective effect of hsp70 in vivo

Although, as discussed above, studies on the protective effect of hsp-inducing stimuli in the heart and on the protective effect of over-expressing individual hsps in cardiac cells in vitro, essentially recapitulated similar studies which had been carried out in other cell types, the heart was in fact the first organ for which a protective effect of over-expressing an individual hsp was demonstrated within the intact animal. Thus, in 1995–1996 several groups reported the generation of transgenic mice which over-expressed hsp70 in both the heart and other organs [44–46]. In all cases, these groups were able to demonstrate that such over-expression of hsp70 was able to protect the heart against the damaging effects of ischaemia using a variety of assays such as infarct size, creatine kinase release, recovery of high energy phosphate stores and correction of metabolic acidosis. Moreover, in a subsequent study, it was demonstrated that such a protective effect could also be observed against myocardial dysfunction caused by a brief ischaemia which was insufficient to induce an infarct [47].

These studies thus establish for the first time that the over-expression of a single hsp in vivo in the intact animal is sufficient to produce cardiac protection. Hence, although it remains to be determined whether specific treatments such as preconditioning produce their protective effect wholly or partly via their ability to induce hsps, it is absolutely clear that the over-expression of a single hsp does produce a protective effect in the heart in vivo. Interestingly, it has also been demonstrated that knock out mice lacking hsp32 (heme oxygenase-1) show enhanced infarct formation following exposure to hypoxia, indicating an important protective role for this hsp [48]. Unfortunately, transgenic animals over-expressing hsps other than hsp70 in the heart or viable knock out mice lacking hsps other than hsp32 have not yet been reported. It will clearly be of considerable importance to determine whether over-expression of other hsps has a protective effect in vivo. This is particularly true of hsp27 which, as indicated above, has a potent protective effect in cardiac cells in vitro.

5. Protective mechanisms

The clear protective effect of hsps in the heart is paralleled in a variety of other critical tissues and cell types such as the brain (for reviews, see Refs. [49,50]). This has led to a variety of investigations in different cell types aimed at elucidating the mechanism(s) mediating
these protective effects. As well as simply indicating that hsps function by chaperoning the correct folding of other proteins (for review, see Ref. [51]), these studies have also identified specific biological processes which are targeted by hsps (Fig. 2).

A major focus of attention has been the process of programmed cell death/apoptosis. One major pathway of apoptosis involves the release of cytochrome c from mitochondria. In turn, cytochrome c binds to a protein known as Apaf-1 and triggers its oligomerisation. This complex then attracts the inactive unprocessed pro-form of the proteolytic enzyme caspase-9 which is then cleaved to its active form thereby initiating the apoptotic process. Most interestingly, different hsps have been shown to inhibit this process at various points (Fig. 3). Thus, hsp27 has been shown to bind to cytochrome c and prevent it binding to Apaf-1 [52,53]. Conversely, hsp90 binds to Apaf-1 and prevents it binding to cytochrome c [54], whilst hsp70 prevents oligomerised Apaf-1 from recruiting pro-caspase-9 [55,56].

As well as this effect on caspase-mediated apoptosis, it appears that hsp70 can also inhibit apoptosis in a caspase independent manner [57]. This is likely to involve the ability of hsp70 to inhibit the c-Jun N-terminal kinase (Jnk kinase) which plays a key role in inducing apoptotic cell death in response to specific stimuli [58,59]. These mechanistic studies in non-cardiac cells on the inhibiting effect of hsps on pro-apoptotic mechanisms evidently parallel the direct demonstration that over-expression of hsps can inhibit apoptosis in cardiac cells (see above) [40].

As well as interfering with apoptotic pathways, hsps also appear to have other protective effects. Thus, for example, it has been demonstrated that over-expression of hsp27 can protect the integrity of the microtubules and the actin cytoskeleton in cardiac myocytes and endothelial cells exposed to ischaemia [60,61].

Moreover, hsp90 has been shown to bind to endothelial nitric oxide synthase (eNOS) and stimulate its activity [62] and over-expression of hsp70 enhances nitric oxide production in response to cytokine stimulation [63]. These observations are of particular interest in view of observations indicating that unstable angina activates both hsp72 and eNOS in the human heart [64] whilst nitric oxide can protect cultured cells from TNF-α-induced cell death by inducing hsp70 [65]. Hence multiple links exist between the hsp and nitric oxide systems.
The hsps therefore protect cells via multiple mechanisms which target key cellular components and regulatory processes. This leads naturally to the question of whether such protective effects can be put to therapeutic use.

6. Therapeutic potential of hsps in the heart

The experiments described in earlier sections clearly suggest that procedures which elevate hsp levels in the heart may be of significant benefit, for example, during reperfusion following a period of ischaemia, during cardiac bypass or to preserve donor heart function prior to transplantation. Indeed, in view of the use of cold storage during transportation prior to transplantation, it is of particular interest that reduced as well as elevated temperature induces hsp expression in the heart [66]. Moreover, a mild heat treatment prior to hypothermic storage has been shown to enhance subsequent recovery of the heart [67].

Such temperature-based manipulations of the hsps may thus have a role to play in cardiac transplantation procedures. Similarly, it has been shown that a protective effect across the whole heart can be obtained by using a thermal probe to produce local heating of the heart [68], suggesting that a similar procedure could be used therapeutically.

The induction of the hsps by stressful stimuli such as elevated temperature (for review, see Ref. [69]) or ischaemia [70] is mediated by the heat shock transcription factor (HSF-1) (Fig. 2). Thus following exposure to elevated temperature, the cytoplasmic HSF-1 monomer forms a trimer and moves to the nucleus where it binds to its target sites (known as heat shock elements) in the regulatory regions of the hsp genes and, following HSF-1 phosphorylation, it induces hsp gene expression.

Interestingly, the induction of the hsps by stressful stimuli diminishes with age in a variety of tissues including the heart and this has been shown to be due to impaired activation of HSF-1 by stress in the aged heart [71]. Moreover, this effect is associated with a reduced protective effect of mild heat shock or ischaemia against a subsequent severe ischaemic stress in aged hearts [71,72]. As many of the situations where the protective effect of hsps would be valuable involve elderly individuals, this suggest that other procedures not involving stressful stimuli or HSF-1 may be required for the therapeutic induction of the hsps. Such procedures can be divided into pharmacological and gene therapy procedures.

6.1. Pharmacological methods

Although the hsps were identified on the basis of their induction by stressful procedures, they are also induced naturally by specific non-stressful procedures. For example, the cytokines interleukin-6 [73] and interleukin-10 [74] can induce hsp gene expression in a non-stressful manner. It has been shown that these inducers do not act via HSF-1 but activate hsp expression via other transcription factors such as NF-IL6 and STAT3 (for review, see Ref. [75]) (Fig. 2). Based on the use of these inducers in non-cardiac cells, we demonstrated that the interleukin-6-like cytokine cardiotoxin-1 (CT-1) was able to induce hsp synthesis in cultured cardiac cells and that such treatment protects them against subsequent exposure to severe thermal or ischaemic stress [76]. Similar induction of the hsps in cultured cardiac cells and protection against subsequent severe stress is also observed with the tyrosine kinase inhibitor herbinycin A [77,78].

These protective effects in cardiac cells in culture have also been extended to the intact heart. For example, Vigh et al. [79] showed that bimocloclomol, a novel hydroxylamine derivative, was able to induce hsp synthesis in the intact perfused heart ex vivo and to produce a protective effect against a subsequent ischaemia. Similarly, Meng et al. [80] demonstrated that norepinephrine treatment of an intact rat resulted in hsp induction in the heart and protection against ischaemia when the heart was subsequently perfused ex vivo, whilst Dana et al. [81] showed that the pre-conditioning effect of activating adenosine A receptors was accompanied by phosphorylation of hsp27 which is believed to enhance its protective effect. This latter observation is of particular interest in view of the observation that over-expression of hsp70 in the intact heart results in the activation of the adenosine synthesising enzyme ecto-5’-nucleotidase [82], further supporting a relationship between the protective pathways involving hsps and adenosine.

Several compounds thus exist which can induce hsps in the heart and produce a protective effect, although it should be noted that in no case has this protective effect been directly shown to be due to the ability to induce hsps. Before the protective effect of any of these compounds could be exploited clinically, it is evidently necessary to investigate whether their protective effect in the heart can be achieved without any significant side-effects. For example, CT-1 was originally identified on the basis of its ability to induce cardiac hypertrophy [83], whilst herbinycin as a tyrosine kinase inhibitor is likely to have significant effects on cellular growth and division. Nonetheless, the identification of compounds able to induce the hsps without inducing a full stress response is highly promising and suggests that pharmacological induction of hsp synthesis may be a viable therapy in the not too distant future.

6.2. Gene therapy procedures

Clearly, any potential side-effects of compounds which can induce hsps could be avoided if hsp genes could be efficiently delivered to the heart. Since transgenic procedures are evidently not applicable in humans, this will require the development of procedures able to safely and
efficiently deliver hsp genes to the heart of individual patients.

Encouragingly, it has been shown that the hsp70 gene within a plasmid vector can be delivered to the heart via intra-coronary infusion of liposome particles containing it. The elevated expression of hsp70 produced by this means confers effective protection against subsequent ischaemia-reperfusion [84] or endotoxin-induced cardiac damage [85]. These experiments are of considerable importance since they demonstrate that hsp70 over-expression can have a protective effect not only in a transgenic animal but also in a situation directly relevant to the human case where hsp over-expression is produced in the adult heart by introduction of the gene construct.

Nonetheless, the problem of hsp gene delivery to the heart remains the same as for all gene therapy procedures, namely the need to safely and efficiently deliver the gene to the appropriate tissue without damaging side-effects. The development of viral vectors able to safely and efficiently deliver hsp genes to cardiac cells [40,41] gives hope that the hsps could be delivered to the heart with safe and efficient viral vectors as these are progressively developed in the future. Similarly, the effective use of liposomes to deliver hsp genes in animals [84,85] suggests that this would be an equally valid approach if highly efficient liposome preparations suitable for use in man were to be developed.

7. Conclusion

There is now overwhelming experimental evidence that over-expression of individual hsps either in cultured cardiac cells in vitro or in the intact heart has a protective effect. Similarly, such a protective effect of the hsps is likely to underlie the protection observed in earlier experiments in the heart exposed to stimuli which can induce hsps. Although other mechanisms may be involved in the protective effect in some of these circumstances, this should not divert us from the clear conclusion that hsp over-expression can be protective even in the absence of other protective procedures. Moreover, it has been demonstrated that hsp expression is elevated in the human heart of patients with unstable angina [64] or dilated cardiomyopathy [86,87]. Hence these protective mechanisms operate, although not entirely effectively, in human heart disease.

Evidently, therefore, boosting these protective mechanisms is likely to be of benefit in patients suffering repeated ischaemic episodes or at reperfusion following ischaemia as well as in hearts being transported prior to transplant. The challenge now is to identify pharmacological and/or gene therapy procedures which can efficiently elevate hsp expression in the intact heart in vivo without producing damaging side-effects which would be unacceptable in human patients and without using the stressful procedures which originally led to the identification of the hsps and which gave them their name.

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