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ORIGINAL CONTRIBUTION

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Heat shock protein 27 protects the heart against myocardial infarction

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Abstracts Heat shock proteins (hsp) represent a group of chaperones which protects the cells against a diversity of stresses. It has been demonstrated that hsp27 is constitutively present in cells where it plays an important role in different cytoprotective processes which ultimately inhibit cell death. We investigated the response of the isolated perfused mouse heart over expressing hsp27 to the ischaemia/reperfusion injury using infarct size as an end point. Our results show for the first time that mice over expressing hsp27 (verified by Western blotting analysis) were found to be protected from lethal ischaemia/reperfusion injury compared to their negative littermates.

Key words Heat shock protein 27 – reperfusion injury – infarct size – mouse

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Introduction

The heat shock proteins are induced by exposure to heat or other stresses and play a protective role in minimising the damaging effect of such stress [for review see 10]. A number of different hsps have been defined and are named according to their molecular weight, e.g. hsp90, hsp70, etc [11].

In the heart, initial studies in cultured cardiac cells demonstrated a clear protective effect of over-expressing hsp70 against subsequent challenge with elevated temperature or hypoxia [5]. Subsequently, the results were extended by several groups which reported that hearts from transgenic mice over-expressing hsp70 demonstrated enhanced resistance to ischaemia/reperfusion injury [see for example, 13, 14, 16, for review see 9].

Interestingly, in cultured cardiac cells over-expression of hsp27 also has a potent protective effect whereas over-expression of hsp90 produces a much more limited protection and over-expression of hsp56 is not protective [3, 6]. Moreover, in other studies of the hsp70 transgenic mice relatively poor protection was observed in neuronal cells [12], whereas using the first transgenic mice over-expressing hsp27 it has recently been demonstrated that this protein is associated with a potent protective effect in the nervous system of the intact animal [1].

In view of these previous findings, we hypothesised that over expression of hsp27 would confer protection against myocardial ischaemia-reperfusion injury. To

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investigate this, two independent lines of transgenic mice (TG +ve: +18, +64) and their negative litter mates (TG –ve: –18, –64) were studied.

Materials and methods

Transgenic mice (TG) were created using a transgene containing human hsp27cDNA with a chicken beta-actin promotor and cytomegalovirus enhancer (pCAGGS). In order to track expression of the transgene, a hemagglutinin (HA) tag was placed contiguous with the hsp27 cDNA sequence. Two independent line: 18 and 64, were established with similar expression properties as previously described [1]. These lines were maintained by breeding to wild-type F1 hybrid (C57BL10 × CBA/Ca) mice and transgenic mice were identified using PCR from a small ear biopsy.

The experimental protocol consisted of isolated Langendorff perfused hearts from the positive (TG+ve), and negative littermates (TG–ve) being subjected to 35 minutes of global normothermic ischaemia followed by 30 minutes reperfusion. Infarction (I/R%) was analysed using tetrazolium staining and quantification of total hsp27 performed by Western blot analysis as previously described [2] using the human hsp27 antibody (Upstate, UK).

Results

These results demonstrate that mice expressing hsp27 have significantly reduced infarction compared to their negative litter mates: TG line 18: TG+ve 28.25 ± 3.13 % (n = 6) vs TG–ve 39.15 ± 2.9 % (n = 11), $p < 0.05$; TG line 64: TG+ve 26.36 ± 2.09 % (n = 7), vs TG–ve 38.86 ± 4.54 % (n = 8), $p < 0.05$ (Fig. 1). Additional experiments for Western blotting analysis confirmed the presence of human hsp27 in the transgenic animal hearts (Fig. 2).

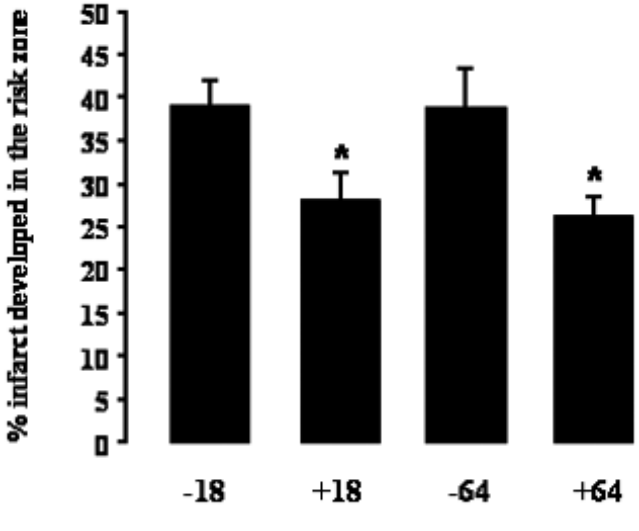

Fig. 1 Infarct developed in the risk zone in transgenic mice (+18; +64) and their negative littermates (–18; –64). *= $p < 0.05$

Discussion

In has been suggested that overexpression of hsp27 may be important in protecting the myocardium from ischaemia/reperfusion injury [7]. However our experiments demonstrate, for the first time, that over-expression of hsp27 can protect the intact heart against myocardial injury by significantly reducing infarct size. As hsp27 is known to be regulated by phosphorylation, it will be particularly interesting in the future to determine whether protection against infarction, by over-expressing hsp27, involves such phosphorylation by preparing transgenic mice expressing mutant forms of hsp27 in which the phosphorylated serines have been converted either to a non-phosphorylatable amino acid or to a phosphomimetic amino acid.

With regard to a possible mechanism by which hsp27 could protect against cell death, it has been suggested that hsp27 can interact with Akt [15] performing a chaperone function by maintaining the kinase in a biologically active conformation. This resultant increase in active AKT, by phosphorylating protein Bad or caspase 9, directs the cell to choose an antiapoptotic course.

Further evidence to support the role of hsp27 in the control of apoptosis, by regulating AKT activation, has been provided by Rane et al. [17], who showed in neutrophils that interaction between hsp27 and AKT was necessary for activation of AKT. This description of the role of hsp27 in regulating the AKT prosurvival cascade, provides support for possible combined synergistic antiapoptotic properties. For additional information on potential mechanisms relating to hsp27 see review article 4.

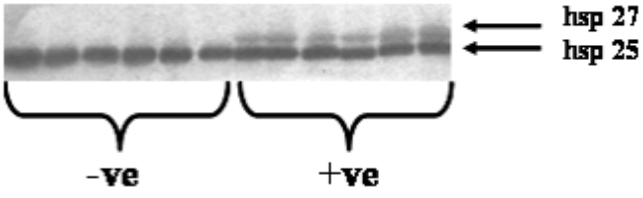

Fig. 2 Western blots comparing the expression of the human hsp27 in transgenic mice hearts (+ve littermates) and their negative controls (–ve littermates). Heart homogenates were probed with primary antibody which reacts with both human hsp27 and murine hsp25 (n = 6 per group)

Conclusion

It is well known that over-expression of hsp70 can protect the myocardium from ischaemic/reperfusion injury [9, 10]. From the results of our study it is clear that over-expression of hsp27 can also have a protective effect against infarction. It has also recently been demonstrated that, in the case of the nervous system, hsp27 can have a protective effect not only in transgenic animals but when administered to the adult animal with a viral vector [8]. Hence, future therapeutic approaches to cardiac disease may involve the over-expression of hsp27 either alone or together with hsp70 which might be achieved either by using viral vectors to deliver exogenous hsp genes or by using pharmacological compounds to induce the endogenous hsp genes [for further discussion see ref 10].

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