

Heat Shock Proteins and Protection Against Ischemic Injury

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ABSTRACT

Heat shock proteins present a complex family of proteins exerting chaperone-like activities that are classified according to their molecular weight. We especially explored protective functions of inducible heat shock protein 70, the mitochondrial heat shock protein 60 and 10, and the small heat shock proteins HSP27 and α B-crystallin against ischemic, reoxygenation-mediated injury using transgenic animals and hearts under in vivo conditions and in isolated cardiac myocyte-derived cells using adenoviral vectors. We noted with great interest that differential protective effects are exerted by specific hsp. For example, α B-crystallin and constitutive hsp70 markedly protect microtubular structure in cardiac myocytes from ischemia-induced injury. Inducible hsp70, hsp60 and hsp10 when coexpressed, and hsp27 and α B-crystallin have an overall protective effect against ischemic injury as determined by the release of enzymes like creatine kinase and LDH. We did not note inflammatory or immune responses elicited by the expression of hsp in transgenic animals and cardiac myocytes. The specific cell types in which hsp are expressed may contribute to the protective effect of hsp versus their inflammatory and immunogenic effects when expressed in other cell types. *Infect. Dis. Obstet. Gynecol.* 7:55–57, 1999. © 1999 Wiley-Liss, Inc.

KEY WORDS

heat shock proteins; chaperones; protective effect ischemic injury

Heat shock proteins (hsp) form a diverse group of proteins that are classified according to their molecular weight.¹ Most members of the hsp family exert a chaperone or chaperonin-like function inside the cell, allowing proteins in the crowded intercellular environment to reach their proper final folding state in an accelerated fashion and avoiding folding structures that are not productive and lead to protein denaturation. This chaperone function results in a protective effect of hsp against noxious insults like heat ischemia and exposure to toxic chemicals. Several investigators have indicated that hsp can also exert an inflammatory role and serve as antigenic targets for the immune system and, in this way, can be detrimen-

tal in certain pathophysiological circumstances. In this paper, the beneficial and protective features of hsp will be highlighted. The hsp discussed in further detail are the inducible hsp70, the small hsp hsp27 in humans or hsp25 in rodents, and α B-crystallin. In addition, the mitochondrial hsp hsp60 and hsp10 that form the chaperonin complex in mitochondria are also considered.

A description of the response of the inducible hsp70 to ischemia follows. We noted some time ago that ischemia of the dog heart leads to a marked increase in the messenger RNA and protein level for the inducible hsp70.^{2,3} These findings raised the question whether increased expression of hsp70 could lead to a protective effect against isch-

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emic injury. We initially investigated this question by stably transfecting H9C2 cells, leading to an H9C2-cell line which overexpressed the human inducible hsp70. We found a significant increase in resistance to an ischemic stress in the H9C2 cells overexpressing the inducible hsp70.⁴

In subsequent studies, we wanted to determine if this protective effect could also be induced under *in vivo* conditions. We therefore generated transgenic mice in which the rat inducible hsp70 is driven by a human cytomegalovirus enhancer chicken actin promoter.⁵ In initial studies, we used an isolated perfused mouse heart model to investigate protective effects of increased expression of hsp70 against ischemic injury. Hearts were harvested from transgene-positive mice and transgene-negative litter mates and submitted to perfusion and subsequently to sero flow ischemia for 20 minutes and reflow in the Langendorf perfusion setup. Recovery of contractile function was determined, the release of creatine kinase was investigated, and, in addition, myocardial infarct size was quantitated by a tetrazolium stain that exhibits a red color when exposed to viable myocardial tissue and a pale colorless stain in ischemic myocardium. We found that in hearts obtained from transgenic mice, the infarct zone was decreased by 40%.⁵ In addition, contractile function at 30 minutes of reflow was doubled, and the efflux of creatine kinase was decreased by 50%. These findings, therefore, clearly indicate that protection against ischemic injury occurred in this isolated perfused heart model.

In subsequent studies performed in collaboration with Dr. Wolfe at the University of California San Francisco, we determined infarct size in a mouse *in vivo* model.⁶ The left coronary artery was occluded for 30 minutes, followed by 120 minutes of reperfusion. Infarcts were set in anesthetized mice overexpressing the inducible hsp70 transgene and in control mice. Infarct size was then determined after sacrifice of the animals by staining with tetrazolium, and we found that the infarct size was decreased by 50% in hsp70-positive transgenic mice. Expression of the hsp70 transgene in a large number of cardiac myocytes in cell culture can also be achieved by using adenoviral vectors. The hsp70 transgene was cloned into the E1 region of the viral vector, and infection of myocytes resulted in a significant increase in inducible hsp70 levels in

marked protective effects against simulated ischemia.⁷

In subsequent studies brief episodes of myocardial ischemia were used, leading to so-called myocardial stunning. Myocardial stunning occurs under conditions in which brief periods of ischemia do not lead to permanent myocyte necrosis but result in a significant decrease in myocardial function after the ischemia is relieved.⁸ For this purpose, we developed an *in vivo* mouse model in which regional epicardial strains could be measured as an indicator of ischemia-induced myocardial malfunction. In addition, isolated perfused hearts were used to determine the contractile recovery after 10 minutes of global ischemia. In both of these models, the *in vivo* mouse model and the isolated heart, we found that expression of hsp70 protected against postischemic myocardial dysfunction induced by brief ischemic episodes or myocardial stunning.

Related to the protective effect of hsp60 and hsp10, we performed studies in collaboration with Dr. Mestril. For these studies, the hsp60 and hsp10 were cloned either by themselves or in combination into a human adenovirus five vector. Isolated neonatal myocytes were infected with the virus and then submitted to simulated ischemia. We obtained the very interesting result that only the co-expression of hsp60 and hsp10 could mediate a protective effect against simulated ischemia. In contrast, if hsp60 by itself or hsp10 by itself were expressed, no protective function could be elicited.

The last set of results relate to the protective effect of small hsps, hsp27 and alpha-B-crystallin. The small hsps arose most likely from a primordial gene and have a similar structure containing three exons.⁹ These small hsps form large multimeric units, and proteins that are not in their final folding stage bind to the outside of this large oligomeric complex. To determine if increased expression of the small hsps could result in a protective effect, we cloned alpha-B-crystallin and, separately, hsp27 into an adenoviral vector. The cardiac myocyte submitted to simulated ischemia that were infected with the adenoviruses expressing specific hsps were then subsequently examined for resultant damage. We observed that both hsp27 and alpha-B-crystallin led to a significant protective effect measured by release of creatine kinase or LDH in

isolated myocytes submitted to simulated ischemia.¹⁰ To investigate the detailed mechanism of protection, we investigated the integrity of specific skeletal structures in cardiac myocytes infected with different hsp and submitted to simulated ischemia. Especially when microtubular integrity was determined by indirect immunofluorescence using confocal microscopy and image analysis, we noted a significantly better protection of microtubular integrity in myocytes overexpressing alpha-B-crystallin. In contrast, when hsp27 was overexpressed, no protection of microtubular structure occurred. We also noted that the constitutive hsp70 provided for increased microtubular integrity, in contrast to the inducible hsp70, which had no protective effect on microtubular structures.¹¹

Related to the general protective effect of hsp27, we noted with great interest that the phosphorylation status of hsp27 does not play a significant role for this function. For example, when the three phosphorylatable serines 15, 78, and 82 were mutated to alanine, the same protective effect was mediated by the phosphorylation mutant versus the wild type hsp27.

In summary members of the hsp family, the inducible hsp70, constitutive hsp70, hsp60 and hsp10, and the small hsp hsp27 and alpha-B-crystallin have very significant protective effects against ischemic injury under in vivo conditions and in isolated cardiac myocytes. The detailed target for the protective effect is different for the different hsp. For example, related to cytoskeletal structures, the integrity of microtubules is well preserved by alpha-B-crystallin but not by hsp27. In addition, the inducible hsp70 protects microtubule structure but not constitutive hsp27. It will therefore be of great interest to determine the detailed mechanism by which specific hsp exert their protective function. It will also be of interest to determine whether increased expression of hsp in transgenic animals is tolerated without evidence of an inflammatory or immune response. In the transgenic animals we used, no such effect was observed; however, we have targeted hsp largely to cardiac myocytes that are not a competent im-

mune-presenting cell, and, in this way, we may not have noted a down-side of increased hsp expression.

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