Expression of heat shock proteins in myocardium of patients with atrial fibrillation

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Abstract Atrial fibrillation (AF) is the most common sustained arrhythmia. Because heat shock proteins (Hsp) can protect cells from stress, we compared the levels of Hsp60, Hsp72, Hsc73, and Hsp27 in atrial myocardium from 17 patients with AF (8 paroxysmal and 9 persistent) and 7 controls in sinus rhythm (SR). Hsp60, Hsp72, and Hsc73 levels were not significantly different among the 3 groups. Hsp27 expression was slightly higher in paroxysmal AF than in SR and in persistent AF, and a borderline significant difference ($P = 0.064$) was seen between the paroxysmal and persistent AF subgroups. Hsp60 levels in the moderate, severe, and profound myolysis groups were significantly lower than the light myolysis group, but no differences were found in other Hsps. In summary, the data indicate that expression of Hsp27 and Hsc73 may be associated with different stages of AF and that Hsp60 also may be associated with the degree of atrial myolysis.

INTRODUCTION

Atrial fibrillation (AF) is the most common sustained arrhythmia encountered in clinical practice. Recent data suggest that hospital stays for AF are markedly greater than for any other arrhythmia (Jahangir et al 2001). AF commonly occurs with rheumatic heart disease, and particularly with mitral stenosis, as well as with other cardiac disorders, including coronary heart disease, congestive or hypertrophic cardiomyopathy, mitral valve prolapse, and mitral valve annular calcification. Following acute myocardial infarction or cardiac surgery, AF is a common but usually self-limiting problem. Continuing problems with AF most commonly are associated with rheumatic heart disease; hypertension, especially when left ventricular hypertrophy is present; and chronic coronary heart disease. AF also occurs in the absence of oth-

er preexisting conditions and is referred to as lone or primary AF.

Heat shock proteins (Hsp) act directly on protein molecules as molecular chaperones, preventing their denaturation or facilitating their refolding (Hartl and Hayer-Hartl 2002; Walter and Buchner 2002). Hsps, in association with their cochaperones, also can influence many cell processes (Gabai and Sherman 2002; Nollen and Morimoto 2002). Interestingly, Hsp also have been shown to protect cells and organs, including the heart, against harmful and cytotoxic conditions (Benjamin and McMillan 1998; Pockley 2002; Xu 2002). However little is known about the functions of Hsps in human myocardium. Hsp levels have been examined in cardiac biopsies taken before, during, and after cardiac surgery. Thus Schäfler et al (2002a; 2002b) reported that AF patients exhibited an upregulation of expression of Hsp60 and Hsp10 in chronic AF. Kirmanoglou et al (2004) also found that the mitochondrial member of the Hsp70 family, Hsp75/mortalin, showed an increased expression in chronic AF. Because Hsps have cytoprotective effects, we determined

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Table 1 Clinical characteristics of the atrial appendage donors for the study

Patient	Gender	Age (years)	Rhythm	Diagnosis	EF (%)	LA (mm)	RA (mm)	LV (mm)	RV (mm)
1	Male	67	SR	CAD	49	3.7	3.1	5.2	2.6
2	Male	63	SR	IE	65	4.2	4.1	5.4	3.8
3	Male	45	SR	CAD	56	3.2	3.3	5.0	3.2
4	Female	26	SR	CHD	55	3.5	3.6	4.8	3.6
5	Male	34	SR	CHD	47	4.0	5.4	4.4	7.1
6	Male	79	SR	CAD	57	4.1	4.1	5.4	4.4
7	Female	40	SR	MS + MR	62	3.8	3.2	5	3.4
8	Male	49	PaAF	MS + MR	64	5.4	2.4	6.2	3.0
9	Male	69	PaAF	CAD + AVR	59	3.4	3.4	4.2	3.5
10	Female	19	PaAF	MS + MR + AVR	42	5.0	3.7	6.4	3.0
11	Male	48	PaAF	MS	64	5.8	3.9	5.2	2.8
12	Male	33	PaAF	MS + MR + AVR	50	6.6	4.6	4.3	4.9
13	Male	51	PaAF	MR	70	5.4	5.0	6.0	3.6
14	Female	30	PaAF	MS + MR	57	6.5	3.2	4.6	3.4
15	Male	58	PaAF	MR + AVR	23	5.7	5.0	7.3	3.0
16	Male	32	PeAF	MS + AVR + AVS	57	5.0	4.4	5.9	3.4
17	Female	46	PeAF	MS + MR + AVR + AVS	76	4.6	4.5	5.6	4.2
18	Male	57	PeAF	MS + MR	39	4.8	3.4	4.9	3.1
19	Male	50	PeAF	IE	57	5.0	5.2	7.5	4.3
20	Male	54	PeAF	MS + MR	64	5.7	4.7	4.6	3.6
21	Female	30	PeAF	MS + MR	66	7.3	3.2	7.3	3.2
22	Male	55	PeAF	MR	57	5.3	3.8	6.6	3.8
23	Female	40	PeAF	MS + AVR	50	5.5	4.1	4.3	3.7
24	Female	24	PeAF	MS + MR	53	8.1	3.1	6.9	2.9

EF, ejection fraction of the left ventricle; LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle; SR, sinus rhythm; CAD, coronary artery disease; IE, infective endocarditis; CHD, congenital heart disease; MS, mitral stenosis; MR, mitral regurgitation; PaAF, paroxysmal atrial fibrillation; PeAF, persistent AF; AVR, aortic valve regurgitation; AVS, aortic valve stenosis.

the levels Hsp60, Hsp72, Hsc73, and Hsp27 in atrial myocardium from patients with paroxysmal and persistent AF and in sinus rhythm. Because myolysis is associated with the progression of AF and may contribute to the loss of atrial contractile force (Ausma et al 1997; Allesie et al 2002), we also determined the degree of the myolysis of atrial myocytes to investigate the possible association of the myolysis with the expression levels of the 4 Hsps.

MATERIALS AND METHODS

Patients

The present study was reviewed and approved by the Ethics Committee of Tongji Medical College. Specimens of right atrial appendage were obtained from 24 patients aged from 19 to 79 years undergoing heart surgery for coronary artery disease ([CAD], $n = 4$); infective endocarditis ([IE], $n = 2$); heart valve diseases ($n = 17$), including mitral regurgitation (MR), mitral stenosis (MS), aortic valve regurgitation (AVR), aortic valve stenosis (AVS); and congenital heart defects ([CHD], $n = 2$) and from patients with sinus rhythm ([SR], $n = 7$) as the control group. Cardiac examination by ultrasound was performed on all patients prior to operation and the atrial and ventricular diameter are listed in Table 1.

Definitions of AF subgroups

The AF patients were ultimately divided into 2 subgroups, paroxysmal and persistent AF. Paroxysmal AF typically

is occurring in episodes of a duration shorter than 24 hours (but longer lasting paroxysms are not unusual) with intermittent SR and is either converting spontaneously or terminated with intravenously administered antiarrhythmic drug (Gallagher and Camm 1997; Levy et al 1998). Persistent AF is continuously present until cardiac surgery, ie, at least 2 consecutive electrocardiograms of AF more than 24 hours apart, without intermittent SR and has a nonspontaneously converting character (Gallagher and Camm 1997; Levy et al 1998). Previously, persistent AF was classified as chronic AF.

Isolation of the atrial myocardium

The same cardiac anesthesiologist, perfusionist, and surgical team participated in all the interventions. All patients underwent cardiopulmonary bypass with moderate hypothermia (33–34°C), antegrade crystalloid cardioplegic arrest, and local hypothermia with ice-slash; cardioplegic solution was readministered every 20–30 minutes. Two to three millimeters of atrial tissue at the right atrial appendage site were taken as soon as the pericardium was opened. Right atrial samples from 24 patients undergoing elective cardiac surgery were excised and immediately frozen in liquid nitrogen.

Determination of heat shock proteins in the right atria

Determination of heat shock proteins was performed as described earlier (Xiao et al 2002) with minor modifica-

Table 2 Preoperative data of the patients

Variables	Sinus rhythm group	Atrial fibrillation (AF) group		P-value
		Paroxysmal AF subgroup	Persistent AF subgroup	
No. of patients	7	8	9	—
Mean of age (years)	50.6 ± 19.4	44.0 ± 16.0	43.7 ± 12.5	0.644
Range of age (years)	26–79	19–69	24–58	—
Men/women	5/2	6/2	5/4	0.663
Mean of LA (mm)	3.79 ± 0.35	5.18 ± 0.88**	5.97 ± 1.15**	0.000
Mean of RA (mm)	3.83 ± 0.80	3.75 ± 0.83	4.18 ± 0.76	0.509
Mean of LV (mm)	5.03 ± 0.35	5.83 ± 1.10	5.64 ± 1.26	0.310
Mean of RV (mm)	4.01 ± 1.45	3.40 ± 1.49	3.58 ± 0.64	0.435
Mean of EF (%)	55.9 ± 6.4	57.1 ± 6.8	54.6 ± 16.6	0.900

LA, diameter of left atrium; RA, diameter of right atrium; LV, diameter of left ventricle; RV, diameter of right ventricle; EF, ejection fraction.

** $P < 0.01$, reference group is the SR group.

tions. Protein content was determined using the Biorad protein assay reagent (BioRad Laboratories, Hercules, CA, USA). The supernatant was taken and diluted with the same buffer to adjust the protein concentration. Samples in sodium dodecyl sulfate (SDS) buffer at a final protein concentration of 1.3 ug/uL, were boiled at 100°C for 5 minutes, and 20 uL of each sample was subjected to 10% SDS–polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred electrophoretically to nitrocellulose membranes. The transfer and protein loading were monitored by staining with Ponceau S. Membranes were saturated with blocking buffer (phosphate-buffered saline [PBS] containing 5% skim milk powder) for 1 hour at 37°C, washed with PBS-0.05% Tween 80 for 5 minutes, and incubated sequentially at 37°C for 1 hour with rabbit anti-Hsc73 (1:1 000), anti-Hsp72 antibody (1:2 000), anti-Hsp60 antibody (1:2 000), and anti-Hsp27 antibody (1:5 000) in PBS containing 5% skim milk powder. All antibodies were from StressGen Biotechnologies (Victoria, British Columbia, Canada). After washing the membranes 6 times (6 × 10 min) with 10 mL PBS-0.05% Tween 80, horseradish peroxidase-labeled goat anti-rabbit IgG in blocking buffer (1:1 000, Beijing Zhongshan Biotechnology Company, Beijing, China) was added and the membrane further incubated at 37°C for an additional 1 hour. Membranes were washed 6 times with 10 mL PBS-0.05% Tween 80. The membranes were incubated with 0.20 mL/cm² ECL-detection reagent (Amersham Ltd, Buckinghamshire, UK) for 1 minute and exposed to Hyperfilm ECL for 1 to 10 minute. Hsps were quantified using an imaging densitometer (Shimadzu CS-930, Shimadzu Corp, Tokyo, Japan) and integrated optical density (IOD) was used to represent their relative levels. The amount of Hsps was measured densitometrically and expressed as ratio of the IOD of samples to the IOD of a control sample and called arbitrary units. The control consisted of a mix of all samples and was added on each

gel. Experiments were repeated 3 times and the mean of these IOD was scored.

Microscopic analysis of myolysis

Sections from atrial appendage were stained with periodic acid–Schiff (PAS) and toluidine blue and examined by light microscopy (Ausma et al 2001). At least 3 sections per patient were examined with a minimum of 200 cells in total. The degree of myolysis was defined, according to the percent of myolytic myocytes, as light (<30%), moderate (30% to 50%), severe (50% to 70%), and profound (>70%).

Statistical analysis

Data analysis was carried out using the statistical analysis software SPSS 11.0 package. The data are presented as the mean ± standard deviation. The analysis of covariance (one-way ANOVA), Chi-square test, and Pearson correlation test were used for statistical comparisons between groups. Statistical inference was based on the different levels of significance, ie, $P < 0.05$ and $P < 0.01$.

RESULTS

Clinical characteristics of the atrial appendages donors

Tables 1 and 2 show the clinical characteristics of the atrial appendage donors in the study. The preoperative data for the 7 patients in the SR group and the 17 patients in the AF group (8 patients in paroxysmal and 9 patients in the persistent AF subgroups) are summarized in Table 2. The mean age of sinus rhythm group and paroxysmal and persistent AF subgroups are 50.6 ± 19.4, 44.0 ± 16.0, and 43.7 ± 12.5 years, respectively, but the differences were not statistically significant ($P = 0.644$). No signifi-

Fig 1. Graph showing the relative amounts of (A) Hsp60, (B) Hsp72, (C) Hsc73, and (D) Hsp27 present in sinus rhythm (SR), paroxysmal atrial fibrillation (PaAF), and persistent atrial fibrillation (PeAF). The combined scatter plot (a) shows individual levels in all three groups, and the mean and standard deviation (SD) is shown to the right of each scatter plot. The representative results of Western blotting were shown in (b): sample 1 and 2 belonged to SR group; sample 3 and 4 belonged to PaAF group; sample 5 and 6 belonged to PeAF group.

cant differences occurred either in the sex distribution among the 3 groups.

In the preoperative data, the diameter of left atrium (LA) in paroxysmal AF subgroup and persistent AF subgroup were both statistically larger than in the sinus rhythm group with diameters of left atrium of 3.79 ± 0.35 , 5.18 ± 0.88 , and 5.97 ± 1.15 mL in sinus rhythm and paroxysmal and persistent AF subgroups, respectively ($P < 0.01$). In other preoperative measurements,

such as the ejection fraction (EF), the diameter of left ventricle (LV), the diameter of right atrium (RA), and the diameter of right ventricle (RV), there were no significant differences among the 3 groups.

Expression of heat shock proteins in 3 groups

As shown in Figure 1A, the expression levels of Hsp60 in sinus rhythm, paroxysmal AF and persistent AF were

Table 3 Correlations among age, gender, the kind of disease, left atria, left ventricle, ejection fraction, and the level of Hsps in the patients

	Pearson correlation coefficient									
	Age	Gender	Kind of disease	Left atria	Left ventricle	Ejection fraction	Hsp60	Hsp72	Hsc73	Hsp27
Age	1.00	-0.649**	0.171	-0.499*	-0.177	0.017	-0.245	-0.245	0.059	0.102
Gender	—	1.00	-0.219	0.316	0.079	0.136	0.266	0.004	-0.140	-0.223
Kind of disease	—	—	1.00	-0.607**	-0.405*	-0.147	-0.143	-0.126	-0.255	0.001
Left atria	—	—	—	1.00	0.417*	-0.013	0.247	0.120	0.122	-0.210
Left ventricle	—	—	—	—	1.00	-0.111	0.172	-0.183	-0.140	0.037
Ejection fraction	—	—	—	—	—	1.00	0.073	-0.101	-0.258	-0.156
Hsp60	—	—	—	—	—	—	1.00	0.529**	0.019	0.282
Hsp72	—	—	—	—	—	—	—	1.00	0.217	0.322
Hsc73	—	—	—	—	—	—	—	—	1.00	-0.342
Hsp27	—	—	—	—	—	—	—	—	—	1.00

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is highly significant at the 0.01 level (2-tailed).

1.21 ± 0.55 , 1.39 ± 0.59 , and 1.36 ± 0.91 arbitrary units, respectively. The amount of Hsp60 in both paroxysmal AF and persistent AF were slightly higher than that in the sinus rhythm group but the difference between groups was not statistically significant ($P = 0.882$, one-way ANOVA). In the case of the stress-inducible Hsp72, the expression levels in the 3 groups were similar (0.93 ± 0.17 , 0.96 ± 0.14 , and 0.94 ± 0.20 arbitrary units for the sinus rhythm, paroxysmal and persistent AF groups, respectively; Fig. 1B) and the difference was not statistically significant ($P = 0.925$).

The expression level of the constitutively expressed Hsc73 in the 3 groups is shown in Figure 1C. The levels of Hsc73 in sinus rhythm, paroxysmal AF, and persistent AF subgroups were 0.51 ± 0.14 , 0.67 ± 0.44 , and 0.82 ± 0.49 arbitrary units, respectively, and the difference between the 3 groups was not statistically significant ($P = 0.339$, one-way ANOVA). However, the Hsc73 levels of both paroxysmal and persistent AF subgroups appeared to be higher than that of the sinus rhythm group. In particular, the level of Hsc73 in the persistent AF subgroup was apparently 1.6 times higher than that of the sinus rhythm group ($P = 0.146$).

Hsp27 levels in sinus rhythm, paroxysmal AF, and persistent AF subgroups were 0.87 ± 0.38 , 1.07 ± 0.43 , and 0.72 ± 0.29 arbitrary units, respectively (Fig 1D), and the difference between the 3 groups was not statistically significant ($P = 0.172$, one-way ANOVA). Again the Hsp27 level of the paroxysmal AF subgroup was higher than that of the sinus rhythm group and the persistent AF subgroup. The difference in Hsp27 levels between the paroxysmal AF and the persistent AF subgroups was borderline ($P = 0.064$).

Correlations of protein levels with the clinical characteristics

We next analyzed correlations among the known risk factors related to atrial fibrillation and the expression levels

of Hsps in the patients in order to find out which factors might contribute to the expression levels. The Pearson correlation coefficients are shown in Table 3. Significant correlations are evident among some variables: age was correlated with the sex and left atrium diameter; the disease type was correlated with the LA and LV diameters; and the LA and LV were correlated to each other. Although the expression levels of each individual Hsp did not show any significant correlations with the clinical characteristics, there was a significant positive correlation between the level of Hsp72 and Hsp60 ($P = 0.008$) with a Pearson correlation coefficient of 0.529.

Correlation of the four Hsps in the 3 groups

For a further understanding of the changes in Hsps and AF, we next examined the correlations between the 4 Hsps in each group. Table 4 summarizes the correlation results in the 3 groups. The numbers shown are Pearson correlation coefficients among the 4 Hsps. A $P < 0.05$ indicates a positive correlation between the levels of the 2 heat shock proteins.

In the sinus rhythm patients, there was a significant positive correlation between the level of Hsp72 and Hsp60 ($P = 0.043$) with a Pearson correlation coefficient of 0.769. A significant negative correlation also was found ($P = 0.030$) between the level of Hsp72 and Hsc73 with a Pearson correlation coefficient of -0.803 . No significant correlation was found among the other Hsps.

In the paroxysmal AF patients, there was a significant negative correlation between the level of Hsc73 and Hsp27 ($P = 0.005$) with a Pearson correlation coefficient of -0.874 . No significant correlation was found among the other Hsps.

Finally, in persistent AF patients, no significant correlation was found among the 4 Hsps.

Hsps expression levels of different degree of myolysis

Finally, because myolysis is an important structural change in AF, we examined whether or not there was any

Table 4a The correlation results among the 4 Hsps for sinus rhythm patients

	Pearson correlation coefficient			
	Hsp60	Hsp72	Hsc73	Hsp27
Hsp60	1.00	0.769*	-0.427	0.639
Hsp72		1.00	-0.803*	0.606
Hsc73			1.00	-0.434
Hsp27				1.00

* $P < 0.05$.

relation between the levels of Hsps and the myolysis state. The scoring was done as described in Brundel et al (2006a). As shown in Table 5, the number of patients in the slight, moderate, severe, and profound myolysis groups were 1, 6, 7, and 3, respectively. The expression levels of Hsp60 in moderate, severe, and profound myolysis groups were significantly lower than in the slight myolysis group. No significant differences were found in the expression levels of Hsp72, Hsp73, and Hsp27 among different degree of myolysis groups.

DISCUSSION

Atrial fibrillation is a cardiac arrhythmia that is electrocardiographically characterized by irregular and disorganized atrial activity in respect to both heart rate and rhythm (Myerburg et al 1998). It is one of the most frequently detected arrhythmias in the clinics. It is not only an independent risk factor for stroke, but AF itself also is associated with a 1.5- to 1.9-fold increase in mortality risk (Wolf et al 1991; Truelsen et al 1994; Benjamin et al 1998).

A number of studies have shown that prior induction of Hsps by a mild stress has a protective effect against a more severe stress in the heart. Pretreatment with heat to induce all the Hsps results in subsequent reduction in infarct size after ischemia in rodent models (Donnelly et al 1992; Currie et al 1993; Marber et al 1994). Transgenic overexpression of human Hsp70 (Plumier et al 1995) or rat Hsp70 (Marber et al 1995) provides significant resistance to mouse hearts from ischemic injury. Although studies have shown that overexpression of various Hsps in cultured primary cardiac cells can provide protection from simulated ischemia, Hsps need to be tested individ-

Table 4c The correlation results among the 4 Hsps for persistent atrial fibrillation patients

	Pearson correlation coefficient			
	Hsp60	Hsp72	Hsc73	Hsp27
Hsp60	1.00	0.553	0.093	0.215
Hsp72		1.00	0.300	0.637
Hsc73			1.00	0.243
Hsp27				1.00

Table 4a–c summarizes the correlation results for the 3 different groups. The numbers shown are Pearson correlation coefficients between the levels of the different heat shock proteins. A $P < 0.05$ indicates a positive correlation between the levels of the 2 heat shock proteins.

ually for their protective effect in specific stressful situations (Cumming et al 1996). Kirmanoglou et al (2004) and Schäfler et al (2002a; 2002b) previously measured the levels of the 2 mitochondrial chaperones mortalin and Hsp60, respectively, in AF. The present data show that the levels of Hsp27 in the paroxysmal AF subgroup appeared to be higher (but did not reach significance, $P = 0.064$) compared to the sinus rhythm group and the persistent AF subgroup. The expression level of Hsc73 in both paroxysmal AF and persistent AF subgroups was not significantly higher than that in the sinus rhythm group. While this manuscript was finalized, another group (Brundel et al 2006a) also measured levels of Hsp40, Hsc70 (Hsc73), Hsp70 (Hsp72), Hsp27, and Hsp90 in a group of paroxysmal and chronic lone AF patients. They did not find changes in amounts of Hsc70, Hsp40, and Hsp90 between AF patients and SR controls, but there was a significant increase of Hsp27 and a borderline increase of Hsp70 (Hsp72) in patients with paroxysmal AF compared to chronic AF. The expression level of Hsp27 (but not Hsp70) also was correlated inversely with myolysis in AF patients.

Our results on Hsp27 in the paroxysmal form of AF are consistent with the data of Brundel et al (2006a). Hsp 27 is a member of the small Hsp family. Its intracellular concentration increases several-fold after heat and other metabolic stresses and its expression has been associated closely with the acquisition of thermotolerance and with the protection of the cytoskeletal actin network (Lavoie et al 1993). Hsp27 was phosphorylated in response to various stimuli, including cellular stressors such as heat shock, oxidants, and cytokines, as well as growth factors and protein kinase C (PKC) activators (Kato et al 1994; Knauf et al 1994; Cuenda et al 1995). In amphibian heart, stimulation of p38-mitogen activated protein kinase (MAPK) by oxidative stress and the consequent phosphorylation of Hsp27 may be important in cardioprotection (Gaitanaki et al 2003). Angiotensin II and heat shock treatment also can induce high-level expression of Hsp27 and Hsp70 (Chen et al 2004). Interestingly, Brundel et al (2006b) just reported that induction and phosphorylation

Table 4b The correlation results among the 4 Hsps for paroxysmal atrial fibrillation patients

	Pearson correlation coefficient			
	Hsp60	Hsp72	Hsc73	Hsp27
Hsp60	1.00	0.233	-0.098	0.220
Hsp72		1.00	0.465	-0.243
Hsc73			1.00	-0.874**
Hsp27				1.00

** $P < 0.01$.

Table 5 The Hsps expression levels in different degree of myolysis

Variables	Degree of myolysis				P-value
	Slight (<30%)	Moderate (30–50%)	Severe (50–70%)	Profound (>70%)	
No. of patients	2	7	11	4	
Hsp72	1.070 ± 0.058	0.966 ± 0.129	0.897 ± 0.209	0.982 ± 0.112	0.533
Hsp73	0.380 ± 0.028	0.831 ± 0.654	0.600 ± 0.193	0.788 ± 0.361	0.445
Hsp27	1.152 ± 0.455	0.775 ± 0.409	0.910 ± 0.398	0.861 ± 0.311	0.678
Hsp60	2.564 ± 0.871	1.531 ± 0.542*	1.015 ± 0.564**	1.214 ± 0.535*	0.014

* $P < 0.05$; ** $P < 0.01$; reference group is the slight myolysis group.

of Hsp27 protected against atrial-tachycardia remodeling in a dog model.

Hsc73 is a constitutive protein, which helps to fold newly synthesized proteins and to facilitate transportation of proteins across membranes. Hearts in AF are in a state of paroxysmal or persistent stress. Factors that have been shown to increase Hsps in the heart include tumor necrosis factor (TNF)- α , stretch, and decreased shortening. The continual stress stimuli might blunt or block the stress response and interfere with normal protein synthesis. One of the most intriguing properties of AF is its tendency to become more persistent over time. Consequently, as many as one-third of patients with paroxysmal AF will develop persistent AF (Godtfredsen 1975). The mechanism(s) by which AF is sustained first was proposed by Gordon K. Moe in his multiple wavelet hypothesis to describe the cause and maintenance of AF (Moe and Abildskov 1959). Hsc73 generally is thought to change minimally with stress, but our study shows some increase in Hsc73 expression. Whether or not myolysis increased expression of Hsp27 and Hsc73 plays any role in the pathology or maintenance of AF warrants further investigation with larger groups.

Schäfler et al (2002a; 2002b) have reported a 2.5-times increase of Hsp60 expression in chronic AF patients compared to SR patients. However, in the present study, the amount of Hsp60 in both the paroxysmal and persistent AF subgroups were not significantly higher than that in the SR group. A possible explanation for the discrepancy may be ethnic or cultural differences. Again a larger study will be needed to assess the reasons for differences between both studies.

Hsp72, expressed at low levels in normal tissue, can be induced rapidly in response to stress. For example, brief ischemia increased Hsp72 levels in rabbit hearts (Knowlton et al 1991a). Mechanical stimuli, such as stretch and decreased shortening known to be important trigger mechanisms in AF (Godtfredsen 1999), caused a rapid increase in Hsp72 in the isolated, perfused rabbit heart (Knowlton et al 1991b). In our study, no difference was found in the levels of Hsp72 between the SR and AF hearts. This supports the notion of a time-dependent character of Hsp72 expression. Studies have shown that chronic stimulation of cardiac myocytes with TNF- α will

lead to an initial increase in Hsp72 levels. However, the myocytes become desensitized within 24 hours and the levels of Hsp72 return to baseline values (Nakano et al 1996). This may be true for AF hearts as well.

The present study found some significant correlations among the four Hsps, and the correlations were different in each group of patients. Some studies have shown that at least two Hsps are needed to play an important role in protecting cardiac cells in culture from the effects of damaging stimuli. Thus overexpressing Hsp60 or Hsp10 individually in cultured cardiac cells had no protective effect; when both proteins were overexpressed simultaneously, a clear protective effect was observed, suggesting that overexpression of more than 1 Hsp may be required to produce an optimal therapeutic effect (Lau et al 1997). However, we did not find any significant correlation between risk factors of AF and the expression levels of Hsps.

It has been suggested that the number of myocytes affected by myolysis increased with the duration of AF, and the extent of myolysis within individual myocytes also increased during AF (Ausma et al 2001). Recent data suggest that the elevated Hsp27 expression in paroxysmal AF might contribute to protecting myocytes from myolysis and limit the progression to persistent AF (Brundel et al 2006a). Our data show that the expression level of Hsp60, not Hsp27, seems associated with different degree of myolysis of the atrial myocytes. The level of Hsp60 was not measured in the studies of Brundel (2006a; 2006b). The underlying mechanisms of protection and the roles of these 2 Hsps certainly warrant further investigation.

In summary, our findings indicate that the expression of Hsp27 and Hsc73 may be associated with different stage of AF. The expression of Hsp60 might be associated with the degree of atrial myolysis. It remains to be determined why the Hsps have differential expression levels during the different stages of AF and whether the Hsps play any role in the pathogenesis of AF and in myolysis. A study on a larger population of AF patients is warranted due to the following factors: the present study only included 8 patients with paroxysmal AF and 9 patients with persistent AF; the patients had various underlying heart diseases that might blur the relation sought between AF stress and Hsps expression; and a

recent report was published showing the positive effect of a treatment inducing Hsps in vivo in a dog model (Brundel et al 2006b).

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