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Association between Heat shock protein-60 and Development of Atrial Fibrillation: Results from the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Background—During atrial fibrillation (AF), a high rate of myocyte activation causes cellular stress and initiates the process of atrial remodeling which further promotes persistence of AF.

Although heat shock proteins (HSPs) have been shown to prevent atrial remodeling and suppress

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the occurrence of AF in cellular and animal experimental models, increased levels of HSP-60 have been observed in patients with post-operative AF, likely reflecting a response to cellular stress. To better understand the role of HSP-60 in relation to AF, we examined the association of HSP-60 levels in relation to the future development of AF in the Multi-Ethnic Study of Atherosclerosis (MESA).

Methods—MESA is a cohort study that recruited 6,814 participants aged 45–84 years and free of known cardiovascular disease at baseline (2000–2002), from six field centers. We investigated 983 participants, selected at random from the total cohort, who had HSP-60 measured and were free of AF at baseline. We tested the association of HSP-60 levels with the incidence of AF using multivariate Cox models after adjustment for demographics, clinical characteristics and biomarkers.

Results—During an average of 10.6 years of follow-up, 77 participants developed AF. We did not observe a significant association between the log-transformed HSP-60 levels and development of AF on either unadjusted or multivariate analysis (adjusted HR: 1.02 per unit difference on natural log scale, 95% CI: 0.77–1.34 ln (ng/ml)).

Conclusion—Contrary to the findings from the pre-clinical studies which demonstrated an important role of HSP-60 in the pathogenesis of AF, we did not observe a significant association between HSP-60 and occurrence of AF.

Keywords

Atrial fibrillation; heat shock protein; atrial stress response

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia and represents a challenging public health problem due to its association with increased mortality and stroke risk [1, 2]. Epidemiological risk factors for AF such as aging, valvular heart disease, hypertension and cardiomyopathy cause atrial stretch and adverse remodeling, which mediate the pathogenesis of AF [3]. Preclinical studies have demonstrated that induction of heat shock proteins (HSPs) has a cytoprotective role against various cardiac diseases [4, 5]. HSPs play an important role in protein-protein interactions such as the establishment of proper protein confirmation and prevention of unwanted aggregation that occurs during cardiac stress [5, 6]. In cardiomyocytes, HSPs bind to damaged proteins such as sarcomeric proteins and prevent further adverse structural remodeling and functional loss [6, 7]. Structural changes in the atria such as myolysis and increased oxidative stress have been observed to be features of adverse atrial remodeling associated with subsequent occurrence of AF [8, 9]. An inverse correlation between the levels of HSP-60 and mitochondrial oxygen consumption rate of myocardium was observed by Toga et al. It is plausible that an increase in HSP-60 levels during the state of increased oxidative stress may reflect its potential cardioprotective role (by prevention of denaturation of mitochondrial protein and repair of damaged proteins) [10]. Although HSPs are typically regarded as being intracellular, they have been measured in the serum of healthy individuals as well [11]. In a study by Brundel et al on HL-1 myocytes subjected to rapid pacing, pharmacological induction of HSPs was observed to

attenuate the degree of myolysis [8]. Clinical studies by Schafler et al described elevated levels of HSP-60 in the myocardial samples of patients with AF as compared to patients in sinus rhythm [12]. Cardiomyocytes have a greater number of mitochondria to generate the high amount of ATPs which are required for the normal cardiac contractility. The majority of proteins for ATP generation are synthesized in the cytoplasm and then transported to the mitochondria [13]. Once they are imported to the mitochondria in an unfolded state, HSP-60 along with HSP-10 form a chaperonin complex which helps maintain the normal configuration of these proteins [14, 15]. In AF, there is a need for increased ATP synthesis secondary to increased atrial activity. This process is also accompanied by a higher protein turnover which leads to upregulation of HSP production in the mitochondrial tissue. The presence of high HSP-60 levels therefore reflects high atrial activity and consequent ATP synthesis [16]. In addition to the high atrial activity, atrial stress (an increased oxidative and inflammatory state) also ensues during the initiation and maintenance of AF. This atrial stress leads to an increased HSP-60 mRNA and protein synthesis as an adaptive response [17]. The upregulation of HSP-60 in acute stress is followed by gradual and age-related exhaustion of HSPs, which leads to impairment of proteostasis and contributes to adverse structural remodeling and progressive nature of AF [18].

Although, various mechanisms for the upregulation of HSP-60 during AF remain to be elucidated, it may reflect increased atrial activity and cellular stress associated with AF. To improve understanding of the role of HSP-60 in individuals without a history of AF, we studied the association of HSP-60 levels with future development of new-onset AF in the Multi-Ethnic Study of Atherosclerosis (MESA). We hypothesized that elevated levels of HSP-60 in MESA participants at baseline might be associated with subsequent development of AF.

Methods

Our study population consisted of 983 randomly selected participants of the MESA study who had HSP-60 levels measured at baseline and were free of baseline AF. MESA is a cohort study that recruited 6,814 subjects from six field centers who were 45–84 years of age and free of clinically-recognized cardiovascular disease at baseline (2000–2002). Informed consent was obtained from every participant at the time of enrollment in the study. MESA is to determine the progression of various subclinical cardiovascular diseases in participants without a history of clinically recognized cardiovascular disease at baseline. Details of the MESA study design have been published previously [19]. Race was self-reported as Caucasian, Chinese-American, African-American or Hispanic. Information on use of anti-hypertensive and lipid medications, smoking status (never, past, or current), and health insurance and education (as markers of socioeconomic status) was obtained by self-report. Systolic blood pressure was measured in the seated position after a 5-minute rest and was the average of the last 2 of the 3 measurements made with an oscillographic method [19]. Diabetes mellitus was defined as fasting blood glucose > 125 mg/dl or the use of hypoglycemic medications. Total and high-density lipoprotein cholesterol were measured from the blood samples obtained after a 12-hour fasting period. Considering the role of inflammation, atrial hemodynamics and increased atrial fibrosis, we included IL-6, NT-proBNP and FGF-23 as other biomarkers of interest. Serum levels of HSP-60 were

measured in ng/ml and the levels of IL-6, NT-proBNP and FGF-23 were measured in pg/ml [20–22].

Study participants were contacted every 9 to 12 months to ask about hospitalizations during follow-up. Medical records for these hospitalizations were obtained and AF was ascertained from the ICD-9 (International Classification of Diseases, Ninth Revision) codes assigned at hospital discharge. For participants older than 65 years of age who were enrolled in fee-for-service Medicare, claims data for inpatient and outpatient services were used to ascertain the diagnosis of AF [21]. A 12-lead EKG was obtained at baseline clinical examination 1 at the time of enrollment (July 2000). Subsequently, follow up EKGs were obtained at examination 2 (July 2002) and examination 3 (January 2004).

Categorical variables in the study are reported as numbers and percentages, and continuous variables as means $\pm$ standard deviations. We initially categorized participants according to the occurrence of AF. Categorical and continuous variables were compared between the two groups using Chi-square and ANOVA test, respectively. The Cox proportional hazards model was used to assess the adjusted association of HSP-60 with incident AF. We retained as covariates in the multivariate model those characteristics that were associated with incident AF in the univariate analysis with a p -value < 0.20 (Table 1). Because of the long-tailed distributions, natural log transformations for HSP-60, IL-6, CRP and NT-proBNP were used in all the analyses and the corresponding estimates are presented as per unit change on the natural log scale. As the initial step, we used a general additive model to assess if the untransformed values of HSP-60 had a non-linear relationship with AF. Considering the lack of evidence of non-linearity, we then used the natural log-transformed HSP-60. We also used the additive model to assess the presence of a non-linear relationship between the log-transformed values of HSP-60 and AF. All statistical analyses were performed with STATA 13 software (College Station, TX: Stata Corp LP).

Results

In our study sample of 983 participants, 77 developed AF over the follow-up period of 10.6 ± 1.8 years. Comparison between the two subgroups is shown in Table 1. Participants who developed AF were significantly older, had higher mean systolic blood pressure, and a larger proportion were male and used antihypertensive medication than in those who did not develop AF. No statistically significant difference was observed in the mean level of ln HSP-60 between those with and those without incident AF (9.62 vs. 9.60 ln (ng/ml), respectively). However, mean levels of IL-6, ln FGF-23 and of ln NT-proBNP were significantly higher in the AF than in the non-AF individuals ($p < 0.01$).

On multivariable analysis using the Cox proportional hazards model, there was no significant association of HSP-60 levels with incident AF, after adjustment only for age and gender, and after further adjustment for antihypertensive medication use, systolic BP, smoking status, HDL, diabetes mellitus, IL-6, NT-proBNP and FGF-23 (Table 2). However, as was seen in the unadjusted analysis, we observed in the multivariate analysis a significant and fairly strong association between NT-proBNP and AF (adjusted HR: 1.63, $p < 0.001$).

Discussion

Our study was aimed at investigating the association between HSP-60 and subsequent occurrence of AF during the follow-up period in the MESA study. We did not find a significant association between HSP-60 and subsequent development of AF. Studies in the preclinical setting have shown that the HSPs have a cardioprotective role against cellular stress. Sensing of cellular stress leads to activation of heat shock transcription factor-1 (HSF-1) which upregulates the expression of HSPs [5]. HSP-60 plays an important role in cytoprotection by preventing denaturation and enhancing proper assembly of cellular proteins [23]. In animal models, pretreatment with the HSP inducer, geranylgeranylacetone (GGA) was shown to attenuate the adverse changes in electrical properties and prevent initiation of AF [24]. On the other hand, Schaffler et al reported a 2.5 fold increase in the levels of HSP-60 expression in the myocardial samples from patients with chronic AF as compared to patients in sinus rhythm. Despite its potentially protective role, up-regulation of HSP-60 may be reflective of cellular stress which may predispose to the development of AF, or it may be up-regulated as a direct or indirect consequence of AF [12].

We did not find a significant difference in baseline levels of HSP-60 in participants who developed AF over an average of 10.6 years of follow-up compared with those who did not develop AF. There could be several explanations for the findings of our study. In animal models, atrial remodeling and AF were induced relatively acutely so it is possible that the increased levels of HSP-60 might reflect its role as an acute response to cellular stress. Clinical studies such as ours are inherently dependent on the participants enrolled. Considering that the participants in MESA did not have known cardiovascular disease at baseline and developed AF during the follow up period of several years, it is conceivable that the baseline levels of HSP-60 may have increased in closer proximity to the occurrence of AF. Acknowledging that the mechanism of initiation and perpetuation of AF is multifactorial, it is quite plausible that other pathways (such as inflammation and atrial dilatation) may be more relevant to clinical AF [25, 26]. It is also possible that a protective role of HSP-60 may be roughly counterbalanced by its serving as a marker for cellular stress, with no net association between baseline HSP-60 and incidence of AF. Although we adjusted for various risk factors of AF (age, gender, hypertension), it is not possible to exclude residual confounding factors in an observational study such as ours. Our findings of elevated baseline levels of NT-proBNP in the study participants who developed AF are consistent with previous publications from MESA and the Cardiovascular Health Study (CHS) [21, 22].

Experimental studies focused on pre-induction of HSPs using either hyperthermia [8] or GGA [26] do support its role as an interesting target to mitigate the adverse changes in the atrial substrate and subsequently suppress the initiation of AF [27]. These findings seem to be the basis of the paradigm that prior induction of HSPs by a mild stress has a protective effect against more severe stress in the cardiomyocytes. These studies investigating the role of pre-induction of HSPs using GGA have also observed that usually its higher doses are required for a discernible cardioprotective effect of HSPs. Various derivatives of GGA with improved pharmaco-chemical and HSP-inducing properties have been synthesized. Future results from the HALT & REVERSE consortium program aimed at investigating the role of

pre-induction of HSPs using these derivatives of GGA to prevent the progression of adverse electropathological changes in patients with AF may yield further insights into the role of HSPs [18, 28].

Despite preclinical and clinical studies suggesting a potential role of HSP-60 in the occurrence of AF, our study based on a large cohort of participants free of cardiovascular disease at baseline, did not find a significant association between baseline HSP-60 and development of AF.

Study Limitations

We would like to acknowledge certain limitations of our study. Considering that the presence of AF was ascertained based on the 12-lead EKGs and ICD-9 codes assigned in hospital records, we did not have the data on types of AF. The use of these two methods to ascertain the diagnosis of AF is less sensitive than the data obtained from interrogation of devices and loop recorders which were not used in MESA to assess the occurrence of AF. We also did not have the data on progression of AF, so we could not perform the analysis to assess the levels of HSP-60 among the different types of AF and according to its progression during the follow up period of MESA.

Conclusions

Although pre-clinical studies have suggested the role of HSP-60 in atrial stress and pathogenesis of AF, our study based on data from participants in MESA did not find a significant association between the HSP-60 levels and baseline and subsequent development of AF. It is plausible that the protective role of HSP-60 might have been counterbalanced by its role as a marker of cellular stress and its level may have diminished over the course of the study. These could be potential explanations for lack of a significant association between baseline HSP-60 and subsequent occurrence of AF in MESA. Further clinical studies will help understand the role of HSP-60 in initiation and progression of AF. Use of GGA derivatives, which is being investigated in the HALT and REVERSE program may also provide further insights in its role as a therapeutic target.

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Table 1

Comparison of baseline characteristics of the MESA participants according to the occurrence of subsequent atrial fibrillation

Baseline variable		No AF (N = 906)	AF (N = 77)	P value
Age (years)		59 ± 9.5	67.7 ± 7.6	< 0.001
Gender	Female	530 (58.5%)	30 (39.0%)	0.001
	Male	376 (41.5%)	47 (61.0%)	
Race/Ethnicity	Caucasian	410 (45.3%)	41 (53.3%)	0.26
	Chinese-American	94 (10.4%)	20 (26%)	
	African-American	190 (21.0%)	16 (20.8%)	
	Hispanic	212 (23.4%)	17 (22.1%)	
BMI (Kg/m ²)		28.5 ± 5.7	27.7 ± 5.0	0.24
Smoking status	Never	460 (50.9%)	31 (40.2%)	0.19
	Former smoker	308 (34.1%)	31 (40.3)	
	Current smoker	136 (15.0%)	15 (19.5%)	
Education status	< High school	148 (16.4%)	15 (19.5%)	0.48
	High school	756 (83.6%)	62 (80.5%)	
Health insurance		819 (90.6%)	71 (92.2%)	0.64
Systolic BP (mm Hg)		123.3 ± 21.0	129.6 ± 20.7	0.012
Diastolic BP (mm Hg)		71.4 ± 10.3	70.9 ± 10.5	0.64
Total cholesterol (mg/dl)		195.4 ± 34.7	190.8 ± 39.2	0.27
HDL (mg/dl)		51.4 ± 14.4	48.5 ± 14.5	0.093
Lipid medications	Yes	115 (12.7%)	12 (15.6%)	0.47
Hypertension medications	Yes	295 (32.6%)	34 (44.2%)	0.039
Diabetes mellitus	Yes	98 (10.8%)	12 (15.6%)	0.20
Ln HSP-60 (ng/ml)		9.60 ± 0.86	9.62 ± 0.76	0.87
Ln IL-6 (pg/ml)		0.12 ± 0.39	0.39 ± 0.62	0.006
Ln CRP (ng/ml)		0.68 ± 1.22	0.76 ± 1.27	0.59
Ln NT-proBNP (pg/ml)		3.73 ± 1.15	4.62 ± 1.30	< 0.001
FGF-23 (pg/ml)		38.7 ± 14.2	44.2 ± 18.4	0.003

This table reflects univariate comparison between the two subgroups: “No AF” versus “AF”. BMI: Body mass index (Kg/m²), Systolic and diastolic blood pressure are expressed in mm HG, presence of health insurance is compared between the two subgroups, Log-transformed values of HSP-60, IL-6, CRP and NT-proBNP are used (Ln). Serum levels of FGF-23 are reflected in pg/ml, HDL: High density lipoprotein, IL-6: Interleukin 6, CRP: C-reactive protein, FGF-23: Fibroblast growth factor 23.

P values from ANOVA test for continuous variables and Chi-square for categorical variables.

Table 2

Multivariate Cox proportional hazards model for AF with covariates related to AF ($p < 0.20$) based on the analysis in Table 1

Variable	AF (N = 750 with 64 AF cases) HR with 95 % CI	P value
Age (per year)	1.08 [1.05, 1.11]	0.08
Gender		
Female	ref	
Male	2.31 [1.28, 4.18]	0.006
Hypertension medications	1.32 [0.79, 2.19]	0.29
Systolic BP (per SD =5.6 mmHg)	0.90 [0.68, 1.18]	0.43
Smoking Status		
Never	ref	
Former	1.10 [0.62, 1.96]	0.75
Current	1.85 [0.89, 3.83]	0.10
HDL (per unit change (mg/dl))	0.91 [0.67, 1.24]	0.55
Ln IL-6 (per unit change Ln (pg/ml))	1.41 [0.95, 2.09]	0.09
Ln (NT-proBNP) (per unit change Ln (pg/ml))	1.63 [1.27, 2.09]	<0.001
FGF-23 (per SD=14.6 pg/ml)	1.05 [0.86, 1.30]	0.62
Ln HSP-60 (per unit change Ln (ng/ml))	1.02 [0.77, 1.34]	0.90

In this multivariate cox proportional hazard model the association of HSP-60 and AF was tested along with covariates selected on the basis of p value < 0.20 on univariate analysis in table 1.