Deficiency in Heat Shock Factor 1 (HSF-1) Expression Exacerbates Sepsis-induced Inflammation and Cardiac Dysfunction

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Received: November 25, 2013; Accepted: January 10, 2014; Published: January 27, 2014

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Abstract

In the present study, we investigated whether absence of heat shock factor 1 (HSF-1) and inability to increase myocardial expression of heat shock proteins alter septic responses of inflammatory cytokines and myocardial contractility. HSF-1 knockout (*hsf*^{-/-}) mice and wild type litter mates underwent a sterile (lipopolysaccharide; LPS) or infectious (*Streptococcus pneumoniae* or *Klebsiella pneumoniae*) septic challenge. Production of cytokines, TNF, IL-1 β , IL-6 and IL-10, in the blood and from cardiomyocytes was exaggerated in the *hsf*^{-/-} mice compared to responses measured in wild type mice given an identical septic challenge. This enhanced compartmentalized myocardial inflammation was associated with significantly decreased cardiac contraction and diminished relaxation in the *hsf*^{-/-} mice. However, lacking HSF-1 expression did not affect intracellular calcium and sodium responses in cardiomyocytes isolated from septic challenged mice, suggesting that ion loading was not a major or sustaining cause of the greater myocardial contractile defects in *hsf*^{-/-} mice. In conclusion, our data indicated that HSF-1 and downstream heat shock proteins are essential components to support cardiac function in sepsis. Further studies are warranted to further define the precise mechanisms of HSF-1 mediated cardiac protection.

Keywords: HSF-1; Heat shock proteins; Sepsis; Cardiac dysfunction; Inflammation; Cytokines; Cesium handling

Introduction

Severe sepsis is a leading cause of death in intensive care units [1,2]. Despite improvements in antibiotic therapies and critical care techniques [3], approximately 215,000 Americans still die from sepsis each year [4]. Present treatment for sepsis continues to be supportive care and source control, such as using intravenous fluids and oxygen, and/or antibiotics and procedural interventions [3]. Most attempts at molecule-based treatments have failed clinically [5,6]. To date, our understanding of sepsis pathogenesis and therapeutic options are still limited. Therefore, investigation of the pathological mechanisms and exploration of new therapeutic interventions are clearly needed to advance the treatment of this devastating condition.

Cardiac dysfunction is a vital component of multi-organ failure during severe sepsis [7-9]. Increased cardiac expression of heat shock protein 70 (HSP70) has been described after ischemic injury [10], hypoxia [11] and major burn [12]. A cardioprotective role has been suggested for HSP70, which is believed to function as a molecular chaperone that inhibits apoptosis and necrosis secondary to inhibitory effects on caspases [13]. HSPs may also provide cardioprotection through their ability to interact with cytoskeletal proteins by stabilizing cytoskeletal structures and increasing resistance to stress [14,15]. HSP70 expression after injury or stress is regulated by heat shock transcription factor-1 (HSF-1) [16]. Stress promotes HSF-1 conversion from a monomeric form in the cytoplasm to a trimerized phosphorylated form, which then translocates to the nucleus to promote the transcription of heat shock proteins [17]. In the heart, HSF1 deficiency reduces cardiac expression of Hsp25, α B-crystallin and Hsp70 [16]. The role of HSF-1/HSP70 in inflammation has been emphasized by the finding that HSF-1 deficient mice exhibit chronically elevated systemic TNF levels as well as increased susceptibility to LPS challenge [18]. In this study, using HSF-1 knockout (*hsf*^{-/-}) mice as a model, we examined the role of HSF-1 in inflammation and cardiac function in response to septic challenge by lipopolysaccharide (LPS), gram-positive bacteria *Streptococcus pneumoniae* (*S. pneumoniae*), or gram-negative bacteria *Klebsiella pneumoniae* (*K. pneumoniae*).

Materials and Methods

Experimental animals and sepsis models

HSF-1 heterozygous knockout mice were obtained from Dr. Ivor Benjamin at University of Texas Southwestern Medical Center (UTSW) [19,20]. Males and females of these mice were intercrossed to produce homozygous knockout, *hsf*^{-1/-} (*Hsf1*^{tm1ljb}), mice, and gene knockout was confirmed by RT-PCR.

Male *Hsf*^{-1/-} mice and wild type litter mates (C57BL/6, *Hsf*^{1+/+}), 8-10 weeks of age and body weight 20-22g for wild type or 15-18g for *Hsf*^{-1/-}, were subjected to three distinct septic

challenges: (1) lipopolysaccharide (LPS), (2) gram-positive bacteria *Streptococcus pneumoniae*, type 3, and (3) gram-negative bacteria *Klebsiella pneumoniae*. Among these models, LPS and its control vehicle were given via intraperitoneal (IP) administration at the dose of 4 mg/kg (in 20 μ L saline as vehicle). The mice were subjected to bacterial infection via intratracheal (IT) delivery of bacterial resuspension at 1×10^5 CFU per mouse (in 0.3 mL sterile PBS as vehicle). The IT inoculation procedure was performed according to our previously established protocol [21-23], and blood samples were collected 24 hours after inoculation to confirm the presence of bacteremia. All mice received IP resuscitation (lactated Ringer's solution, 2 mL), and were studied 24 hrs after septic challenge.

In the present study, animal protocol and pathogen safety plans were approved by Institutional Animal Care and Use Committee (IACUC) and the Department of Environmental Health and Safety (EH&S) at the University of Texas Southwestern Medical Center (UTSW). All work described was performed according to the current guidelines for caring animals and handling biohazard agents.

Isolation and culture of primary cardiomyocytes

Isolation of cardiomyocytes from adult mice was performed as previously described [24-26]. The mice were briefly given an intraperitoneal injection of heparin, 500 units/mouse, 20-30 minutes before they were sacrificed by decapitation. Hearts were harvested and placed in a petri dish containing heart medium at room temperature [in mM: 113 NaCl, 4.7 KCl, 0.6 KH_2PO_4 , 0.6 Na_2HPO_4 , 1.2 MgSO_4 , 12 NaHCO_3 , 10 KHCO_3 , 20 D-glucose, 10 HEPES buffer solution, 30 taurine, 2 carnitine, 2 creatine, and plus 0.5x MEM amino acids (Invitrogen, Carlsbad, CA)], which was bubbled constantly with 95% O_2 -5% CO_2 . Hearts were cannulated via the aorta and perfused with heart medium at a rate of 1 mL/min for 5 minutes in a nonrecirculating mode. Enzymatic digestion was initiated by perfusing the heart with digestion solution [prepared by adding 50 mg of collagenase II (Worthington Biochemical Corporation, Lakewood, NJ) and 50 mg bovine serum albumin (BSA), Fraction V (Invitrogen, Carlsbad, CA) to 34.5 mL of heart medium, plus 1x trypsin (Invitrogen, Carlsbad, CA), 15 μ M CaCl_2 , and 40 mM 2,3-butanedione monoxime (BDM)]. Enzymatic digestion was accomplished by recirculating this solution through the heart at a flow rate of 1 mL/min for 20 minutes. All solutions perfusing the heart were maintained at a constant 37°C. At the end of the enzymatic digestion, the ventricles were removed and mechanically disassociated in 6 mL of enzymatic digestion solution containing a 6-mL aliquot of 2x BDM/BSA solution [3 mg BSA, Fraction V to 150 mL of BDM stock, 40 mM]. After mechanical disassociation with fine forceps, the tissue homogenate was filtered through a mesh filter into a conical tube. Cells adherent to the filter were collected by washing with an additional 10 mL aliquot of 1x BDM/BSA solution [prepared by combining 100 mL of BDM stock, 40 mM; 100 mL of heart medium; and 2 g of BSA, Fraction V]. Cells were then allowed to pellet in the conical tube for 10 minutes. The supernatant liquid was removed, and the pellet was re-suspended in 10 mL of 1x BDM/BSA. The cells were washed and pelleted further in BDM/BSA buffer with increasing

concentrations of calcium (100; 200; 500; and 1,000 μ M). After the final pelleting step, the supernatant liquid was removed, and the pellet was re-suspended in MEM [contains 1x MEM (Sigma-Aldrich, St. Louis, MO); 11.9 mM NaHCO_3 ; 10 mM HEPES; and 1X penicillin/streptomycin (Invitrogen, Carlsbad, CA)]. At the time of MEM preparation, the medium was bubbled with 95% O_2 -5% CO_2 for 15 minutes, and the pH was adjusted to 7.1 with 1 M NaOH. The solution was then sterilized by filtration and stored at 4°C until use. At the final concentration of calcium, the cell viability was measured, and only cell suspensions with greater than 85% viability were used for subsequent studies.

To culture, cardiomyocytes were plated at a density of 10^6 cells/plate on 100 mm petri dishes and incubated with MEM medium at 37°C incubator. Cells were harvested 12-18 hours after culture.

Measurement of cytokines

In blood serum and in the conditional medium of primary cardiomyocyte cultures, levels of TNF, IL-1 β , IL-6 and IL-10 were measured using immunoassay kits (Biosource, Camarillo, CA). Blood was collected from sacrificed animals using Vacutainer rapid serum tubes (RST) (BD Diagnostics, Franklin Lakes, NJ) followed by immediate centrifugation at 3,000g for 15 min at 4 °C, for preparing serum. The serum preparations were then allocated and stored at -80°C until analyzed. Cardiomyocyte medium was collected when isolated myocytes were cultured at 1×10^5 /mL per well on 12-well plates (Corning, Corning, NY) for 18 hours.

Measurement of intracellular calcium and sodium levels

Levels of intracellular calcium and sodium were measured in primary cardiomyocytes harvested from all experimental groups. Cells were loaded with either fluorescent calcium probe Fura-2 AM (Sigma-Aldrich, St. Louis, MO) for 45 min or sodium-binding benzofuran isophthalate (SBFI) (Sigma-Aldrich, St. Louis, MO) for 60 min at room temperature in the dark. Myocytes were then suspended in 1.88 mM calcium-containing Tyrodes solution and washed to remove extracellular dye; myocytes were placed in a perfusion chamber on the stage of a Nikon inverted microscope which was interfaced with Grooney™ optics for epi-illumination, a triocular head, phase optics, and a 10X phase-contrast objective and mechanical stage. The excitation illumination source (300 W compact Xenon arc illuminator) was equipped with a power supply. In addition, this InCyt Im2™ Fluorescence Imaging System (Intracellular Imaging, Cincinnati, OH) included an imaging workstation and computer. The computer-controlled filter changer allowed alternation between the 340 and 380 nm excitation wavelengths. Images were captured by a monochrome charge coupled device camera equipped with a TV relay lens. InCyt Im1™ and InCyt Im2™ Image software allowed measurement of the intracellular calcium and sodium concentrations from the ratio of the fluorescent signals generated at the two excitation wavelengths; auto fluorescence of myocytes that had not been loaded with fluorescent dye was measured with each experiment and subtracted. The calibration procedure included measuring

the fluorescence ratio with buffers having different calcium or sodium concentrations. At each wavelength, the fluorescence emissions were collected for 3 msec intervals, and the time between data collections was 12 msec. Because quiescent or non-contracting myocytes were used in these studies, the calcium and sodium concentrations measured reflect those in diastole.

Analysis of cardiac function

As previously described, cardiac contraction and relaxation were examined using Langendorff heart perfusions [27]. Mice were anticoagulated with sodium heparin (200 U; Elkins-Sinn, Inc., Cherry Hill, NJ) and killed by decapitation. The heart was removed rapidly and placed in a petri dish containing ice-cold (4°C) Krebs-Henseleit bicarbonate-buffered solution (in mM: NaCl 118, KCl 4.7, NaHCO₃ 21, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, and glucose 11). All solutions were prepared each day with demineralized, deionized water and bubbled with 95% O₂-5% CO₂ (pH 7.4; pO₂ 550 mm Hg; pCO₂ 38 mm Hg). A cannula placed in the ascending aorta was connected via glass tubing to a buffer-filled reservoir for perfusion of the coronary circulation at a constant flow rate. Hearts were suspended in a temperature-controlled chamber maintained at 38±0.5°C, and a constant-flow pump (Holter, Model 911; Critikon, Inc., Tampa, FL) was used to maintain perfusion of the coronary artery by retrograde perfusion of the aortic stump cannula as previously described [28,29]. Coronary perfusion pressure was measured, and effluent was collected to confirm the coronary flow rate. Left ventricular pressure (LVP) was measured with a Statham pressure transducer (Model P23ID; Gould Instruments Inc., Oxnard, CA) attached to the cannula placed in the left ventricle, and the rate of LVP rise (+dP/dt) and fall (-dP/dt) were obtained using an electronic differentiator (Model 7P20C; Grass Instruments, Inc., Quincy, MA) and recorded (Model 7DWL8P, Grass Instruments). The LVP and ±dP/dt responses to increase in perfusate calcium were also examined. Data input from the Grass recorder was transferred to a computer and a Grass Poly VIEW Data Acquisition System was used to convert data into digital form.

Statistical analysis

All data were expressed as mean ± SEM of at least 3 independent experiments using 4-8 animals/group. Student's t-tests were used to assess the difference between the sham and the various sepsis groups. Because two outcome measures (wild type and knockout) were tested against three hypothesized predictors (sepsis by LPS, *S. pneumoniae* or *K. pneumoniae*), a Bonferroni's-adjusted probability value less than 0.0083 was considered statistically significant.

Results

In the present study, wild type and *hsf*^{-/-} mice were given sham or septic challenge by LPS, gram-positive bacteria *Streptococcus pneumoniae* (*S. pneumoniae*), or gram-negative bacteria *Klebsiella pneumoniae* (*K. pneumoniae*). Animals in all groups survived 24 hours of septic challenge.

Cytokine production

To evaluate the effect of HSF-1 deficiency on systemic inflammation, cytokine levels were measured in the blood serum collected 24 hours after septic challenge (or sham challenge). As summarized in Figure 1, in response to sham septic challenge, absence of HSF-1 did not cause significant changes in the basal levels of TNF, IL-1β, IL-6, and IL-10 in circulation. Both LPS and bacterial infection, either by *S. pneumoniae* or *K. pneumoniae*, induced a significant rise of cytokines in the wild type mice. Further, *hsf*^{-/-} mice presented amplified cytokine responses in peripheral blood compared with their wild type counter parts, (*p*<0.05).

We then estimated whether HSF-1 deficiency alters inflammation in myocardium. Levels of cytokines secreted from primary cardiomyocytes isolated from wild type and *hsf*^{-/-} mice receiving sham or septic challenge were compared. The absence of HSF-1 did not alter the production of IL-1, IL-6, IL-10 and TNF in cardiomyocytes from sham subjects (Figure 2). However, a robust secretion of cytokines was detected in all cardiomyocytes isolated from mice given LPS challenge or bacterial infection. Similar to what we observed in circulating cytokine responses (Figure 1), LPS, *S. pneumoniae* or *K. pneumoniae* triggered significantly higher levels of cytokine productions in myocytes from *hsf*^{-/-} animals, compared with the cells isolated from the wild type mice.

In previous studies, we confirmed that fewer than 2% of the total cells in a cardiomyocyte preparation were non-cardiomyocytes using this technique; therefore, a majority of the inflammatory cytokines measured in the cardiomyocyte medium was indeed cardiomyocyte derived [30]. The impact of HSF-1 deficiency on cytokine levels in wild type versus *hsf*^{-/-} mice suggests that HSF-1 plays a significant role in control of both systemic and myocardial inflammation during sepsis.

Intracellular calcium and sodium levels in cardiomyocytes

Our previous studies have suggested that, in cardiomyocytes, changes in calcium and sodium flux affect contractility as well as the inflammatory responses [31-33]. In this report, we measured calcium and sodium levels in the cardiomyocytes isolated 24 hrs after septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae*. In these cells, intracellular calcium (Figure 3A) and sodium (Figure 3B) levels were similar in all mice in the absence of septic challenge. Septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae* promoted calcium and sodium loading in myocytes, however, we found no significant differences detected between wild type and *hsf*^{-/-} mice. With regard to intracellular sodium (Figure 3B), the difference between LPS-challenged wild type and knockout mice was subtle and repeatable, however not statistically significant.

Cardiac function analysis

We next explored the functional consequence of HSF-1 deficiency in sepsis-induced cardiac dysfunction. According to previously established protocol, we applied isolated hearts from

Figure 1: HSF-1 deficiency elevates circulating cytokines in response to septic challenges: Wild type (WT) and HSF-1 knockout mice were subjected to sham or septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae*. Levels of TNF, IL-1 β , IL-6 and IL-10 were measured in the blood serum collected 24 hours later. All values are means $\pm$ SE. Statistical significances are labeled with * for a difference between sham and sepsis subjects and † for a difference between HSF-1 KO and wild type, each challenged with LPS, *S. pneumoniae* or *K. pneumoniae* (n=6-8 per group).

Figure 2: HSF-1 deficiency elevates cytokine production in primary cardiomyocytes from septic challenged mice: Wild type (WT) and HSF-1 knockout mice were subjected to sham or septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae*, and cardiomyocytes were isolated from animals sacrificed 24 hours later. Levels of TNF, IL-1 β , IL-6 and IL-10 were measured in the conditional medium of cardiomyocytes cultured 18 hours after isolation. All values are means $\pm$ SE. Statistical significances are labeled with * for a difference between sham and septic subjects and † for a difference between HSF-1 KO and wild type, each challenged with LPS, *S. pneumoniae* or *K. pneumoniae* (n=6-8 per group).

Figure 3: HSF-1 deficiency does not affect intracellular calcium and sodium responses in cardiomyocytes from septic challenged mice: Wild type (WT) and HSF-1 knockout mice were subjected to sham or septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae*. Cardiomyocytes were isolated from animals sacrificed 24 hours later, and intracellular levels of calcium and sodium were compared. All values are means $\pm$ SE. Statistical significances are labeled with * for a difference between sham and sepsis subjects (n=6-8 per group).

wild type and *hsf*^{-/-} mice to the Langendorff perfusion to assess cardiac contraction and relaxation [27]. The cardiac stabilization data were collected in hearts perfused at a constant preload, constant heart rate and constant coronary flow rate (Table 1). As summarized in Figure 4, +dP/dt max, and -dP/dt max were similar in all sham animals, regardless of the presence or absence of HSF-1. As expected, giving septic challenge by LPS, *S. pneumoniae* or *K. pneumoniae* severely impaired heart contraction and relaxation in wild type mice. Significantly, this sepsis-induced deficiency in left ventricular performance was further exaggerated in the *hsf*^{-/-} mice, suggesting that HSF-1 plays a supportive role in maintaining cardiac function during sepsis.

Discussion

In this study, we observed that septic challenge by LPS, gram-positive bacteria *S. pneumoniae*, or gram-negative bacteria *K. pneumoniae* dramatically elevated systemic and myocardial inflammatory responses, indicated by the rise in production of cytokines TNF, IL-1 β , IL-6 and IL-10 (Figure 1 and 2). In these animals, all types of septic challenge also produced significant deficits in cardiac contractility, shown by the lower LVP and $\pm$ dP/dt max values measured during stabilization of the hearts at a constant preload, constant heart rate, and constant coronary flow rate (Table 1). The sepsis-related LV systolic and diastolic dysfunction was further confirmed by the impaired LVP responses to increase in coronary flow rate (Figure 4A) and increase in perfusate calcium (Figure 4B). Importantly, compared with the wild type mice, *hsf*^{-/-} mice exhibited significantly greater increase in cytokine production (Figure 1 and 2) and impairments in LV contraction and relaxation (Figure 4), suggesting that HSF is an important regulatory factor in sepsis-induced inflammation and cardiac dysfunction.

Our data suggest HSF-1 as a regulator of inflammation in the heart during sepsis, since deficiency in HSF-1 expression resulted in further amplified cytokine production in sepsis (Figure 1 and 2); this observation is consistent with previous reports in the literature. Several studies have demonstrated that HSF-1 suppresses certain cytokines through its direct regulation of gene expressions. For example, HSF-1 binds to the 5' promoter of TNF gene to repress TNF transcription [34]. HSF-1 also blocks the

transcription of IL-1 β gene by interacting with the nuclear factor of IL-6 [35]. Alternatively, HSF-1 may indirectly affect cytokine expression through inactivation of NF- κ B, the nuclear factor that regulates the transcription of cytokines such as TNF- α , IL-1 β , and IL-6. HSF-1 has also been shown to increase degradation of I κ B, providing one mechanism by which NF- κ B pathway was activated in the HSF-1 deficient models [36]. In septic responses, HSF-1 was suggested to suppress IL-1 β expression in LPS-challenged human monocytes [37]. Deficiency in HSF-1 expression in adult mice produced an exaggerated TNF response in the blood and increased mortality rate in response to endotoxin [19]. In this study, we applied multiple septic challenges to *hsf*^{-/-} mice and confirmed that HSF-1 functions as an inhibitory modulator in cardiac inflammation during sepsis.

A major heat shock protein (HSP) regulated by HSF-1 is HSP70. HSP70 has been shown to provide cardioprotection via down-regulation of myocardial calcium overload in injury models such as trauma-hemorrhagic shock and ischemia [38,39]. The possible mechanism underlying this HSP70 function may involve enhanced expression of calcium transporters such as the ryanodine receptor, sarcoplasmic/endoplasmic reticulum Ca²⁺-ATPase (SERCA), and Na⁺/Ca²⁺ exchanger (NCX) [38], as well as suppressed activation of p38-MAPKs and/or Raf-1/ERK pathways [40-42]. In the present study, we observed that intracellular calcium levels measured in both wild type shams and HSF-1 deficient shams were similar (Figure 3). An infectious insult by LPS, gram-positive or gram-negative bacteria elevated calcium loading inside cardiomyocytes. However, this alteration in calcium homeostasis was similar in all groups given a septic challenge, regardless of the presence or absence of HSF-1. These data suggest that intracellular calcium loading was not a major or sustaining cause of the greater myocardial contractile defects measure in HSF-1 deficient mice compared to contractile defects measured in wild type septic mice.

Currently, studies have tightly linked mitochondrial damage with the clinical outcomes of sepsis [43,44]. Mitochondrial dysfunction produces overproduction of potentially harmful molecules, such as mitochondrial reactive oxygen species (mtROS) [45,46] and mitochondrial DNA (mtDNA) fragments

Figure 4: HSF-1 deficiency magnifies sepsis-induced cardiac dysfunction: Wild type (WT) and HSF-1 knockout mice were subjected to sham or septic challenge. 24 hours later, hearts were removed and applied to Langendorff perfusion model for the examination of heart contractility. Data shown are the summary of left ventricular pressure (LVP) responses to incremental increase in coronary flow rate (A) or perfusate calcium (B). All values are means $\pm$ SE. Statistical significances are labeled with * for a difference between sham and sepsis subjects and + for a difference between HSF-1 KO and wild type, each challenged with LPS, *S. pneumoniae* or *K. pneumoniae*.

[47,48]. These molecules have been observed to escape from impaired mitochondria and function as danger-associated molecular patterns (DAMPs) to simulate inflammatory [49-52], apoptotic [53] and autophagic [54,55] responses. A number of studies showed that HSF-1 and related heat shock proteins play significant roles in the regulation of mitochondria function. It was previously reported that deficiency in HSF-1 expression impaired mitochondrial redox homeostasis and decreased antioxidant defense in the heart [16] and in kidney [56]. Up-regulation of HSP70, a major HSF-1-governed heart shock protein, enhanced mitochondrial superoxide dismutase activity, protected mitochondrial function, and in parallel improved cardiac function after ischemia-reperfusion injury [57,58]. We have also

previously demonstrated cardiac mitochondrial damage in the sepsis model [21]. Therefore, we suspect that a deficiency of HSF-1 expression further impairs myocardial mitochondrial structure and function, which at least in part leads to the intensified cardiac dysfunction in septic *hsf1*^{-/-} mice. However, this hypothesis needs to be further tested in our future studies.

In summary, data obtained in a mouse sepsis model suggest that HSF-1 and related heat shock protein members are essential cardiac protective factors. TNF, IL-1 β , and IL-6 secretion by cardiac myocytes after LPS, *S. pneumoniae*, or *K. pneumoniae* challenge was exaggerated in the HSF-1 deficient mice compared to responses measured in wild type counter parts. This enhanced systemic and myocardial inflammation

	Wild Type		HSF-1 KO		Wild Type		HSF-1 KO		Wild Type		HSF-1 KO	
	Sham	LPS	Sham	LPS	Vehicle	Strep	Vehicle	Strep	Vehicle	Kleb	Vehicle	Kleb
LVP (mmHg)	97.5 ± 2.3	62.8 ± 2.0*	98 ± 3.1	61.1 ± 4.6*	97.5 ± 2.3	66.3 ± 2.9*	98 ± 3.1	52.6 ± 1.8*†	97.5 ± 2.3	67.3 ± 2.4	98 ± 3.1	53.0 ± 1.0*†
+dP/dt (mmHg/sec)	2308 ± 60	1606 ± 83*	2348 ± 211	1466 ± 167*	2308 ± 60	1729 ± 58*	2348 ± 211	1337 ± 52*†	2308 ± 60	1638 ± 90	2348 ± 211	1364 ± 62*†
-dP/dt (mmHg/sec)	1940 ± 189	1229 ± 65*	2018 ± 69	1228 ± 156*	1940 ± 189	1367 ± 104*	2018 ± 69	1092 ± 66*†	1940 ± 189	1416 ± 95	2018 ± 69	1104 ± 80*†
dP40	1965 ± 59	1406 ± 88*	1955 ± 47	1200 ± 148*	1965 ± 59	1517 ± 58*	1955 ± 47	1150 ± 44*†	1965 ± 59	1383 ± 95	1955 ± 47	1157 ± 49*†
TPP (msec)	76 ± 2.6	74.4 ± 1.6	79.7 ± 2.4	74 ± 3.7	76 ± 2.6	71.8 ± 2.9	79.7 ± 2.4	73 ± 3.9	76 ± 2.6	77.2 ± 3.1	79.7 ± 2.4	73.9 ± 1.6
RT90 (msec)	79.9 ± 1.3	77.1 ± 1.9	78.6 ± 2.0	78.2 ± 2.8	79.9 ± 1.3	82.3 ± 3.1	78.6 ± 2	74.9 ± 2.3	79.9 ± 1.3	79.3 ± 2.3	78.6 ± 2	77.0 ± 2.6
TM +dP/dt (msec)	50.1 ± 1.2	49.8 ± 0.3	53.5 ± 1.7	48 ± 1.3	50.1 ± 1.2	47.1 ± 1.4	53.5 ± 1.7	47.3 ± 1.9	50.1 ± 1.2	50.7 ± 1.5	53.5 ± 1.7	49.7 ± 0.3
TM -dP/dt (msec)	51.3 ± 0.9	49.8 ± 0.5	52.6 ± 1.4	51.1 ± 1.3	51.3 ± 0.9	53.4 ± 1.1	52.6 ± 1.4	51.1 ± 1.8	51.3 ± 0.9	51.7 ± 1.7	52.6 ± 1.4	50.9 ± 1.7
CPP (mmHg)	87.9 ± 8.4	71.3 ± 8.1	82 ± 3.9	75.3 ± 9.0	87.9 ± 8.4	92.8 ± 5.3	82 ± 3.9	73.3 ± 8.5	87.9 ± 8.4	67 ± 9.4	82 ± 3.9	76.9 ± 9.5
CVR (mmHg/sec)	58.6 ± 5.6	47.4 ± 5.4	54.7 ± 2.3	50.2 ± 6.0	58.6 ± 5.6	61.9 ± 3.5	54.7 ± 2.3	48.9 ± 5.6	58.6 ± 5.6	44.7 ± 6.2	54.7 ± 2.3	51.2 ± 6.3
HR (beats/min)	333 ± 12	330 ± 10	325 ± 17	318 ± 13	333 ± 12	360 ± 10	325 ± 17	355 ± 13	333 ± 12	323 ± 9	325 ± 17	337 ± 10

Table 1: HSF-1 deficiency magnifies sepsis-induced cardiac dysfunction.

LVP, left ventricular pressure; +dP/dt, the rate of LVP rise; -dP/dt, the rate of LVP fall; dP40, dP/dt value at a developed pressure of 40 mmHg/sec; TPP, time to peak pressure; RT90, time to 90% relaxation; TM +dP/dt, time to max +dP/dt; TM -dP/dt, time to max -dP/dt;

CPP, coronary perfusion pressure; CVR, coronary vascular resistance; HR, heart rate. All values are means ± SE. Statistical significances are labeled with * for a difference between sham and sepsis subjects and † for a difference between HSF-1 KO and wild type, each challenged with LPS, *Streptococcus pneumoniae* (Strep) or *Klebsiella pneumoniae* (Kleb). ($p < 0.05$, $n = 4-6$ per group).

was associated with significantly greater deficiency in cardiac contraction and relaxation. However, we also acknowledge that a few limitations in the current report should be addressed in future investigations. First, we chose to study TNF, IL-1, IL-1 β , IL-6 and IL-10 as inflammation markers. Whether HSF-1 has any regulatory effect on the generation of other cytokines and DAMP molecules such as high-mobility group box 1 (HMGB1) [59] and hyaluronan fragments [60] will help us to further reveal the mechanism with regards to HSF-1-mediated cardiac protection during sepsis. Second, we did not examine whether lacking HSF-1 affects sepsis survival in our animal models, due to the tremendous effort to obtain relatively large quantity of animals that are needed to establish statistical significance. However, our future research effort will be extended to examine the role of HSF-1 in other organ functions in the mouse sepsis model. We believe these investigations will assist to validate whether HSF-1 and related heat shock proteins are potential therapeutic targets to encounter multiple organ dysfunction syndrome (MODS) in sepsis.

Acknowledgement

This work was supported by NIH Grant R01 GM057054-08 and 5 P50 GM 216181-41.

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