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Widespread Downregulation of Cardiac Mitochondrial and Sarcomeric Genes in Patients with Sepsis

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Abstract

Objective—The mechanism(s) for septic cardiomyopathy in humans is not known. To address this, we measured mRNA alterations in hearts from patients who died from systemic sepsis, in comparison to changed mRNA expression in non-failing and failing human hearts.

Design—Identification of genes with altered abundance in septic cardiomyopathy, ischemic heart disease (IHD), or dilated cardiomyopathy (DCM), in comparison to non-failing hearts.

Setting—Intensive care units (ICUs) at Barnes-Jewish Hospital, St. Louis, Missouri.

Patients—20 sepsis patients, 11 IHD, 9 DCM, 11 non-failing donors.

Interventions—None other than those performed as part of patient care.

Measurements and Main Results—mRNA expression levels for 198 mitochondrially-localized energy production components, including Krebs cycle and electron transport genes, decreased by $43 \pm 5\%$ (mean $\pm$ s.d.). mRNAs for 9 genes responsible for sarcomere contraction and excitation-contraction coupling decreased by $43 \pm 4\%$ in septic hearts. Surprisingly, the alterations in mRNA levels in septic cardiomyopathy were both distinct from and more profound than changes in mRNA levels in the hearts of patients with end stage heart failure.

Conclusions—The expression profile of mRNAs in the heart of septic patients reveals striking decreases in expression levels of mRNAs that encode proteins involved in cardiac energy production and cardiac contractility, and is distinct from that observed in patients with heart failure. Although speculative, the global nature of the decreases in mRNA expression for genes

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involved in cardiac energy production and contractility suggests that these changes may represent a short-term adaptive response of the heart in response to acute change in cardiovascular homeostasis.

Keywords for indexing

Humans; sepsis; heart; ventricular function; left; genes; mitochondrial

Introduction

Septic shock is associated with complex changes in circulatory hemodynamics that are secondary to alterations in left ventricular (LV) and right ventricular (RV) structure and function, as well as changes in peripheral arterial tone that result in absolute or relative decreases in central blood volume (1). Patients with sepsis may develop a “hypokinetic” circulatory status that is accompanied by LV dilation and decreased LV ejection fraction (EF), or a “hyperkinetic” circulatory status, which typically follows volume resuscitation, and is characterized by decreased arterial tone, tachycardia, and an increased LV EF. Given that load-dependent indices of contractility such as the EF are sensitive to peripheral arterial resistance (afterload), as well as circulating blood volume (preload), and that multiple inotropes and/or vasopressors are often used to support patients with sepsis, it has been extremely difficult to precisely define the mechanisms that contribute to sepsis-induced cardiomyopathy.

Aside from the inherent complexity of studying LV function in sepsis, one of the problems with defining mechanisms for septic cardiomyopathy is that much of the information regarding potential mechanisms has been obtained from experimental models. Perhaps not surprisingly, different animal models have yielded different and sometimes conflicting results. In order to better understand the mechanisms for human septic cardiomyopathy, in the present study we examined the levels of gene expression in hearts from patients who died from sepsis and compared this with the expression levels of genes in the hearts from patients with advanced heart failure, who were undergoing cardiac transplantation. Here, we show that the changes in gene expression in hearts from patients with septic cardiomyopathy are largely distinct from and more widespread than the changes in gene expression that occur in patients with end-stage heart failure. The alterations in septic cardiomyopathy are characterized by widespread downregulation of cardiac mitochondrial genes, as well as multiple alterations in sarcomeric genes and genes that maintain the structural integrity of the sarcolemma.

Materials and Methods

Human hearts

All studies were approved by the Washington University Human Research Protection Office. For tissue obtained by rapid autopsy from septic patients who died in the ICU, the studies were determined by the Washington University Human Research Protection Offices not to constitute human subjects research, as there was no interaction with the subject prior to death. After the patients had died, permission to collect postmortem tissues for research

purposes was obtained from the subject's next-of-kin as previously described (2). For heart tissue obtained from heart transplant recipients, heart tissue was collected from the failing heart after removal from the patient in the operating room and consent for tissue sampling and study was provided by the patient or the patient's next-of-kin prior to surgery. Hearts obtained from the Mid-America Transplant Service were from brain-dead organ donors whose next-of-kin gave consent for use of tissues for research purposes.

Septic cohort

We obtained hearts that from patients who died from systemic sepsis in the surgical/medical intensive care units (ICUs) at Barnes-Jewish Hospital (2009-2012). Most patients in this study were previously included in a recently completed parallel study on the effects of sepsis on host immunity (2). Sepsis was defined using a consensus panel definition: microbiologically-proven, clinically-proven, or suspected infection and presence of systemic inflammatory response syndrome (SIRS) (3). To limit conditions potentially impacting septic host-response, patients receiving high dose corticosteroids (>300 mg/day of hydrocortisone) or other immunosuppressive medications, patients with chronic viral infections and patients with autoimmune disease were excluded.

Non-failing and failing human hearts

Non-failing human donor hearts obtained from the Mid-America Transplant Service, St. Louis, MO were used for these studies. Failing human hearts were obtained at the time of transplantation from patients with both ischemic (IHD) and dilated (DCM) cardiomyopathy, who underwent cardiac transplantation at Barnes-Jewish Hospital, St. Louis, MO. Hearts were processed and stored, as described previously (4).

Measurement of gene expression in human cardiac tissue

Tissue samples were obtained from the left ventricular free wall between 5 - 180 minutes after time of death or transplant and either snap-frozen in liquid nitrogen or placed immediately on dry ice and then stored at -80 C. Total RNA was extracted from frozen tissue, which was thawed in QIAzol (Qiagen, Valencia, CA) and homogenized with a TissueRuptor (Qiagen). RNA was purified by using a Qiagen RNeasy kit according to the manufacturer's instructions. Processing of RNA on Affymetrix HuGene 1.0-st-v1 microarrays, data analysis, and statistics and informatics procedures are described in the Supplemental Digital Content. Microarray data files have been deposited at the NCBI GEO under accession GSE79962 (reviewer link <http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=abkvssuivpknbd&acc=GSE79962>).

Results

Clinical characteristics

Twenty patients who died of sepsis were included in the final analysis. The mean age of the patients was 70 ± 3 years (Table 1). Save for one patient with an ischemic bowel, all of the patients were supported by at least one inotrope and/or a pressor for hypotension at some time point during their septic course. Importantly, 35% of the patients were supported by two vasopressors/inotropes and 25% of the patients were supported by three or more

vasopressors/inotropes. The median length of sepsis duration and ICU duration was 3 and 9 days respectively (Table 1).

Two-dimensional echocardiographic data were available for 12 of the 20 septic patients included in this study. The mean duration of days between the 2-D echo and the time of death was 5.6 ± 2.5 days. Figure 1A summarizes the LV end-diastolic dimension, LV end-systolic dimension and LV ejection fraction for the cohort of septic patients. The overall median LV dimensions were within normal limits, and the mean LV ejection fraction was 53 ± 5.1 %. There was, however, tremendous heterogeneity in the LV size and LV ejection fraction in the patient cohort, with LV ejection fractions ranging from 20 to 70%.

Transcriptional Profiling

We performed transcriptional profiling on septic heart samples (n=20), 11 non-failing (NF) human LV heart samples, 11 human LV heart samples from patients with ischemic heart disease (IHD) and 9 LV heart samples from with dilated cardiomyopathy (DCM) (**see Supplemental Digital Content for further details**). IHD and DCM hearts were examined separately, based on the known differences in transcriptional profile between these two classes of heart failure (5). We analyzed 6,719 mRNAs from our Affymetrix microarray data that were present and above a minimum detection limit of RPKM (RPKM: reads per kilobase of exon per million mapped reads, a standard measure of RNA abundance in next-generation RNA-sequencing studies) value 2 in a prior study in human hearts (5). As shown in Figure 1B, principal component analysis on these mRNAs suggested that mRNAs from patients with septic cardiomyopathy had a distinct transcriptional profile when compared to NF, IHD and DCM hearts. Considering the greater sample size, we employed a more stringent cutoff of false discovery rate (FDR) < 0.01 when evaluating the differentially expressed mRNAs in septic hearts; FDR < 0.05 was used for IHD and DCM hearts when compared to NF hearts (Figure 1C). Unsupervised hierarchical clustering of the data from differentially expressed mRNAs in septic hearts revealed similar profiles among all samples from septic hearts, non-failing hearts, and IHD and DCM hearts, demonstrating that no individual hearts were outliers that may have skewed the overall results of the transcriptional profiling data (Figure 1D). To begin to elucidate potential gene transcription mechanisms responsible for septic cardiomyopathy, we performed two different data analyses. First, we examined changes in gene expression that were common to both septic hearts and the hearts from patients with IHD and DCM. Second, we performed an in-depth analysis of the dysregulated genes that were unique to septic cardiomyopathy, and that were not observed in patients with IHD or DCM.

Shared transcriptional profile of septic hearts and failing hearts (Figure 2)

Figure 2A shows that of the 169 dysregulated genes that were common to septic, IHD and DCM hearts, ~ 61% (n=104) were of decreased abundance and ~ 39% were of increased abundance (n= 65). We performed an enrichment analysis for Gene Ontology (GO) biological process using BiNGO (6) on the mRNAs that were dysregulated (compared to nonfailing control hearts) in the hearts of patients with sepsis, IHD and DCM hearts (**details in Supplemental Table 1 in Digital Content**) (6). As shown in Figure 2A the dysregulated genes that were common to septic and failing hearts were significantly enriched for genes

that were involved in fatty acid and lipid metabolism, consistent with the known shift from fatty acid metabolism to glycolytic metabolism reported in heart failure and septic cardiomyopathy (see details in Supplemental Table 2 in Digital Content). Several canonical markers of cardiac stress (upregulation of atrial natriuretic peptide (*NPPA*) and B-type natriuretic peptide (*NPPB*), and downregulation of SERCA2a (*ATP2A2*) were also observed in both heart failure and sepsis (Figure 2C).

Unique Transcriptional Profile of Septic Hearts (Figures 3 – 4)

To better understand the gene sets that were unique to septic hearts, we performed enrichment analysis for Gene Ontology (GO) biological process categories using BiNGO (6) on the 1185 mRNAs that were uniquely dysregulated in septic hearts. Of these 1185 unique mRNAs, 979 (83%) mRNAs could be allocated to one or more GO biological process categories. Further GO cellular component analysis of these 979 genes showed that they were enriched for mitochondrially-localized gene products, myocyte sarcomeric and structural/membrane genes (Supplemental Figure 1 in Digital Content).

Mitochondrial genes—A total of 216 dysregulated mitochondrial genes were detected in septic hearts (see Supplemental Table 3 and Supplemental Figure 2A in Digital Content for details). Overall, 198 of these genes, including Krebs (TCA) cycle and electron transport components, decreased by $43\pm 5\%$ (mean $\pm$ s.d.). Indeed, Figure 3A shows that the most significantly over-represented mitochondrial gene GO categories in the dysregulated septic heart genes were related to energy production and/or oxidative phosphorylation. However, the salient finding shown by Figure 3B is that the majority of the dysregulated mitochondrial genes were coordinately downregulated. As shown in Figure 3B, > 50% of the genes involved in complexes I through IV of the electron transport chain had decreased expression levels, whereas 43% of the genes in complex V were downregulated and 4% of the genes were upregulated. Moreover, there was even more profound decreased expression of genes related to the TCA cycle. With the exception of mitochondrial isocitrate dehydrogenase, which converts isocitrate to α -ketoglutarate (*IDH2*), all of the other 19 genes involved in the TCA cycle were decreased in expression by $43\pm 5\%$ (mean $\pm$ s.d.) (see Supplemental Table 4 and Supplemental Figure 2B in Digital Content for details). In addition, 12 of the 35 detected mitochondrial ribosomal protein genes detected showed reduced expression, potentially limiting the capability of mitochondria to translate mitochondrial mRNA transcripts (see Supplemental Table 6 and Supplemental Figure 2C in Digital Content for details).

Non-mitochondrial genes—To determine the functional significance of the non-mitochondrial genes that were dysregulated in septic hearts, we excluded the mitochondrial genes from the list of 1185 unique septic heart genes. Figure 4A shows that there were 991 non-mitochondrial genes (465 downregulated genes and 535 upregulated genes) that were uniquely dysregulated in septic hearts (see Supplemental Table 5 in Digital Content for details). These 991 genes were then further analyzed using a KEGG (Kyoto Encyclopedia of Genes and Genomes) functional pathway analysis. As shown in Figure 4B three KEGG pathways were significantly dysregulated in the septic hearts ($FDR < 0.05$), including genes involved in ribosomal function, as well as genes identified in hypertrophic cardiomyopathy

and dilated cardiomyopathy. By combining the overlapping gene lists in the hypertrophic cardiomyopathy and dilated cardiomyopathy KEGG pathways, we were able to identify four different groups of genes with distinct function in the heart, which we refer to as functional gene modules. The gene modules included genes involved in sarcomere contraction, maintenance of the sarcolemma and intercellular junctions (i.e., structural integrity), excitation contraction coupling and the extracellular matrix/collagen (see Supplemental Table 6 in Digital Content for details). The important finding shown by Figure 4C is that the majority of dysregulated genes in the septic hearts were involved in sarcomere contraction and excitation contraction, analogous to what we observed with the analysis of mitochondrial TCA and electron transport genes (Figure 3B). In contrast ~20% of the genes involved in structural integrity of the myocyte and/or the extracellular matrix and collagen had increased levels of expression. Thus, septic cardiomyopathy is accompanied by a profound dysregulation of genes involved in ATP production from upstream metabolites, sarcomere contraction and excitation-contraction coupling, any or all of which could adversely affect contractility.

Discussion

The myocardial dysfunction that occurs during sepsis, commonly referred to as septic cardiomyopathy, involves abnormalities of both systolic and diastolic LV function (7). A number of mechanisms have been proposed, including the presence of circulating depressant factors such as endotoxin, nitric oxide, and complement activation, pro-inflammatory cytokines and extracellular histones, as well as myocyte cell death and abnormalities of cardiac energetics (8-13). Not surprisingly, many of these mechanisms have been defined in animal models, and may therefore not reflect the relevant pathophysiological mechanisms observed in patients with sepsis. Here we show for the first time that, although the cardiomyopathy in patients with sepsis shares some characteristics with advanced heart failure (Figures 1 and 2), the majority of the alterations in mRNA expression levels that occur in septic cardiomyopathy (referred to herein as 'transcriptional reprogramming'), are largely distinct from and more profound than those that are observed in the failing human heart.

The dysregulation of cardiac mitochondrial genes included downregulation of genes involved in almost every step of the tricarboxylic acid (TCA) cycle (Supplemental Figure 2B), as well as the majority of genes coding for members of the electron transport chain (Figure 3). These findings are consistent with the observation that there is increased myocardial use of glucose and decreased use of fatty acids in septic hearts from both patients and experimental animals, and are in line with the notion that myocardial metabolism shifts from aerobic to anaerobic glycolysis in septic hearts (8, 14). These findings are also consistent with the observed global downregulation of oxygen consumption that has been observed in sepsis (i.e. cytopathic hypoxia) (15). This acquired defect in oxidative phosphorylation prevents cells from using molecular oxygen for ATP production, albeit potentially at the expense of LV contractile function (16). While a short-term rodent model of sepsis (48 h) revealed similar transcriptional dysregulation in a polymicrobial cecal ligation model (17), these studies are the first to demonstrate that human septic hearts undergo transcriptional reprogramming of this nature. In support of our findings, direct

enzyme assays of mitochondrial electron transport complexes I and IV indicated depressed mitochondrial function in skeletal muscle of septic patients. Interestingly, this occurs despite increased expression of mitochondrial biogenesis genes, which may represent a counter-regulatory response in this tissue (18).

A second major and important observation is that we observed decreased expression levels of genes encoding major proteins of the cardiac sarcomere and the excitation-contraction coupling processes in septic cardiomyopathic hearts. To our knowledge prior experimental studies have not reported widespread downregulation of sarcomeric genes, although proteasomal degradation of sarcomere components has been observed experimentally (19). Cell adhesion/communication may also be compromised in septic hearts as well, insofar as we observed downregulation of several important desmosomal genes (**see Supplemental Table 6 ‘Integrin-cytoskeleton’ module in Digital Content**). Viewed together, the widespread changes in mitochondrial genes, genes involved in excitation-contraction coupling and sarcomere contraction would have been expected to result in substantial myocardial depression. Although the mean LV ejection fraction was 53 ± 5.1 % in the entire septic cohort, the range of LV ejection fractions was quite broad, with 3 patients having an LV ejection fractions $< 35\%$ (Figure 1A). The relatively preserved LV ejection fraction observed in the septic patients likely reflects the effects of volume expansion and/or the use one or more inotropic agents and/or pressors, which were employed in the vast majority of patients for hemodynamic support. Another possible explanation is that the changes in gene expression do not necessarily reflect changes in the level of protein expression, which may have longer half-life. Further, we cannot exclude the possibility the differences in the dose of the inotropic agents and/or pressors may have contributed to the observed variability in the LV ejection fraction. Lastly, it is possible that some of the differences in LV ejection fraction may have been secondary to a difference in septic hearts vs septic cardiomyopathic hearts.

Conclusions

The major new finding of this study is that human septic cardiomyopathy is accompanied by widespread downregulation of cardiac mitochondrial genes, as well as downregulation of genes that govern cardiac myocyte contractility. Remarkably, many of the transcriptional changes in gene expression in septic cardiomyopathy were distinct from and more profound than those observed in end-stage heart failure (Figures 2-4). While the reason for these differences are not known, they may be related to previously described differences in the transcriptional profile in the heart in the setting of acute vs chronic cardiac injury (20, 21), as well as to fundamental differences in the pathobiology of heart failure vs systemic sepsis (22). Although speculative, the observation that genes related to cardiac energetics and cardiac contractility were globally downregulated in the septic heart suggests that the heart responds to sepsis in a coordinated “programmatic” fashion. This is analogous to the transcriptional reprogramming that occurs in myocardial hibernation, wherein there is a global downregulation of mitochondrial oxidative metabolism in response to decreased myocardial blood flow, with resultant tissue ischemia. Whether these changes are adaptive by allowing cardiac myocytes to downregulate oxygen consumption and thereby reduce ATP utilization and maintain cell viability, analogous to hibernating myocardium (16), or whether they are maladaptive because they lead to myocardial depression and hemodynamic collapse

cannot be addressed by the present study. This statement notwithstanding, given that previous studies from our group have shown that there is a dearth of necrotic, apoptotic, or autophagic cell death in hearts of patients dying of sepsis (23), as well as the observation from prior studies that LV function normalizes in those patients who survive sepsis, the findings of this study raise the intriguing possibility that many of these transcriptional changes in mitochondrial and sarcomeric genes may be adaptive in nature.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distinct transcriptional profile of septic cardiomyopathy

A, Box and whisker plots for cardiac structure and function for patients with sepsis. *Left*, LV end-systolic dimension; *middle*, LV end-diastolic dimension; *right*, LV ejection fraction (%). All of the patients whose data contributed to this figure (n=12) were supported by inotropes and/or vasopressors. **B**, Principal component analysis plot using all 6,719 mRNAs above a minimum detection limit as defined in Methods. The x-axis represents the first principal component, or PC (encompassing the most variance) while the y-axis represents the second principal component (PC). **C**, Venn analysis of mRNA transcripts altered in human heart failure. mRNAs from hearts failing secondary to ischemic heart disease (IHD) or to dilated cardiomyopathy (DCM), vs nonfailing hearts (NF), were selected at false discovery rate (FDR) <0.05; mRNAs from septic hearts vs NF were selected at FDR <0.01 (see main text). **D**, Standardized heatmap (mean 0, standard deviation 1) of differentially expressed mRNAs from C, using unsupervised hierarchical clustering with Euclidean distance and average linkage on both hearts and mRNAs.

Figure 2. Shared mRNA dysregulation between cardiomyopathies

A, Plot of the degree of fold-change vs NF hearts (x-axis) against p-value for each comparison (y-axis), for the 169 mRNAs altered in IHD and/or DCM hearts together with septic hearts. A thumbnail of the Venn diagram from Figure 1C is shown at right denoting the mRNAs under consideration. **B**, Gene Ontology category over-representation for genes that were dysregulated in septic hearts and/or IHD and DCM; FDR < 0.05 using BiNGO (6). **C**, Column graphs of selected heart failure marker mRNAs (ANP / *NPPA*, BNP / *NPPB*, SERCA2a / *ATP2A2*); mean $\pm$ standard error of Affymetrix microarray abundance measurements are shown. * denotes FDR < 0.01 (for septic vs NF) or < 0.05 (for IHD/DCM vs NF).

Figure 3. Dysregulation of mRNAs encoding mitochondrial components in septic hearts
A, Gene Ontology category over-representation for genes that were uniquely dysregulated in septic hearts; FDR < 0.05 using BiNGO (6). A thumbnail of the Venn diagram from Figure 1C is shown at right denoting the mRNAs under consideration. **B**, Schematic representation of the mitochondrial oxidative phosphorylation / electron transport chain. **C**, percentage of mRNAs for each of the mitochondrial electron transport chain complexes I-V with observed dysregulation in septic hearts (FDR < 0.01 vs nonfailing).

Figure 4. Dysregulation of mRNAs encoding nonmitochondrial proteins in septic hearts
A, Plot of the degree of fold-change vs NF hearts (x-axis) against p-value for each comparison (y-axis), for the remaining 991 mRNAs altered in septic hearts. **B**, KEGG pathway enrichment and FDR for uniquely dysregulated mRNAs in septic hearts using DAVID (24). A thumbnail of the Venn diagram from Figure 1C is shown at right denoting the mRNAs under consideration. **C**, percentages of mRNAs belonging to previously described matrix-collagen, EC coupling, integrin-cytoskeleton and sarcomere gene modules (25) (derived from KEGG pathway gene lists for hypertrophic and dilated cardiomyopathy) ; lists of individual mRNAs are in Supplemental Digital Content - Table 6.

Table 1
Clinical characteristics of sepsis patients

Age (years)	Number
<i>Mean ± SEM (range)</i>	70 ± 3 (42-93)
Sex	
<i>Male</i>	10
<i>Female</i>	10
Sites of infection *	
<i>GI</i>	18
<i>Pulmonary</i>	8
<i>Urinary tract</i>	2
<i>Necrotizing fasciitis</i>	2
Hospital duration (days)	
<i>Days in hospital median (range)</i>	9 (1-195)
<i>Days in ICU median</i>	9
<i>Days of sepsis median</i>	3
Co-morbidities	
<i>Hypertension</i>	14
<i>Type II diabetes mellitus</i>	5
<i>Coronary artery disease</i>	4
<i>Hyperlipidemia</i>	3
<i>Hepatic cirrhosis</i>	2
<i>Morbid obesity</i>	2
<i>Chronic obstructive pulmonary disease</i>	4
<i>Congestive heart failure</i>	2
<i>Chronic kidney disease</i>	2
Blood, tissue, and/or urine cultures	
Gram-positive	2
Gram-negative	7
Fungal	1
Pressors	
<i>Circulatory inotropes</i>	
Dobutamine	2
Epinephrine	5
<i>Vasopressors</i>	
Norepinephrine	16
Vasopressin	10
Phenylephrine	1

* Some patients had more than one site of infection.