JOURNAL OF SURGICAL RESEARCH 193 (2015) 874–887

Available online at www.sciencedirect.com

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journal homepage: www.JournalofSurgicalResearch.com

# Cardiac fibroblasts: contributory role in septic cardiac dysfunction

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ARTICLE INFO

*Article history:*
Received 19 July 2014
Received in revised form
26 August 2014
Accepted 5 September 2014
Available online 16 September 2014

*Keywords:*
Cardiac dysfunction
Cardiac fibrosis
Cardiac fibroblast
Inflammation
Polymicrobial sepsis

ABSTRACT

*Background:* Cardiac dysfunction is a frequent and severe complication of septic shock and contributes to the high mortality of sepsis. Although several mechanisms have been suspected to be responsible for sepsis-associated cardiac dysfunction, the precise cause(s) remains unclear to date.

*Materials and methods:* We tested the hypothesis that cardiac fibroblasts may play a critical role as a disease modifier involved in sepsis-associated cardiac dysfunction. Human cardiac fibroblasts (HCFs) cultured *in vitro* were exposed to lipopolysaccharide (LPS). Changes in cardiac morphology and function were assessed in mice with cecal ligation and puncture-induced sepsis.

*Results:* In LPS-stimulated HCFs, messenger RNA and protein levels of proinflammatory molecules, including tumor necrosis factor-α, interleukin-1β, interleukin-6, and monocyte chemoattractant protein-1, were strikingly upregulated. LPS also increased expression and activity of matrix metalloproteinase (MMP)-9, but not MMP-2. LPS-induced expression of α-smooth muscle actin, a classical marker for myoblast differentiation, which was abrogated when MMP-9 small interfering RNA was transfected into HCFs. High gene expression levels of proinflammatory cytokines and MMP-9 were observed in the heart tissues of cecal ligation and puncture-induced septic mice. Histology sections of the hearts from septic mice showed perivascular and interstitial cardiac fibrosis, and echocardiography demonstrated that septic mice had profound cardiac dysfunction. The broad-spectrum MMP inhibitor ONO-4817 significantly alleviated these histologic and functional changes during the acute phase.

*Conclusions:* We suggest that cardiac fibroblasts are of pathogenetic importance in inflammation and fibrosis in the heart during sepsis, leading to cardiac dysfunction that would affect the outcome of sepsis syndrome.



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http://dx.doi.org/10.1016/j.jss.2014.09.012

## 1. Introduction

Sepsis and especially its life-threatening complication, septic shock, are major causes of death in intensive care units, despite advancements in antimicrobial therapy [1]. Cardiac depression is a well-recognized manifestation of organ dysfunction in sepsis. Because of the lack of a generally accepted definition and the absence of large epidemiologic studies, its accurate frequency is uncertain. However, clinical studies report that sepsis-induced cardiac dysfunction occurs in approximately 40%–50% of patients with prolonged septic shock and is associated with increased mortality [2]. Interestingly, cardiac contractility is found to be reduced even in septic patients in the absence of changes in ventricular preload or afterload [3]. Although studies using animal models of sepsis have extensively explored the etiology of cardiac dysfunction, the underlying mechanisms linked to effective methods for preventing and/or treating sepsis-induced cardiac dysfunction have remained elusive.

It has been shown that bacterial endotoxin lipopolysaccharide (LPS) causes cardiac dysfunction by enhancing cardiac-derived inflammatory mediator expression, associated with the release of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β, and overproduction of nitric oxide (NO) [4,5]. In this regard, cardiac fibroblasts may serve critical roles as intermediate sensors and amplifiers of inflammatory signals from immune cells and myocytes in response to LPS, through production of cytokines and NO [6], because they make up 60%–70% of the total cell number in the heart. Furthermore, cardiac fibroblasts play a central role in synthesizing extracellular matrix (ECM) proteins, and their activation and transition into a myofibroblastic phenotype, which are controlled by a variety of stimuli, including cytokines, could contribute to the development of cardiac fibrosis and adverse ventricular remodeling [6]. Thus, the genesis of reduced cardiac function during sepsis may involve the abnormal responses of cardiac fibroblasts.

In the present study, we hypothesized that cardiac fibroblasts may emerge as a disease modifier due to the regulation of inflammatory and fibrotic remodeling in the heart that would affect the progress, severity, and outcome of sepsis. Consequently, we initially examined whether human cardiac fibroblasts (HCFs) can highly respond to LPS. We found that LPS strikingly upregulated gene expression of proinflammatory cytokines and chemokines, and matrix metalloproteinase (MMP)-9 in HCFs. Then, we examined changes in cardiac histology and function in mice with cecal ligation and puncture (CLP)-induced polymicrobial sepsis. CLP-induced sepsis is an animal model has high clinical relevance to humans because it reproduces many hallmarks of sepsis that occur in patients [7]. To our knowledge, our present study represents the first report indicating that cardiac fibroblasts emerge as a key player contributing to cardiac dysfunction in sepsis through the regulation of two pathologic processes, cardiac inflammation and fibrosis.

## 2. Methods and materials

### 2.1. HCF cultures and treatments

Primary HCFs from the adult ventricle were purchased from Cell Applications Inc (San Diego, CA). Cells were grown in fibroblast growth medium in a 5% $CO_2$ humidified incubator kept at 37°C. Cells of passage 3–6 were used when 80% confluent. Then, cells were harvested and seeded into six-well plates. LPS (List Biological Laboratories, Campbell, CA) was usually given at a dose of 1 μg/mL. This concentration is far higher than the plasma levels reported in septic patients (110–726 pg/mL) [8]. We also examined its stimulant effect at doses ranging from 0.001–1 μg/mL. ONO-4817 (Ono Pharmaceutical Co, Osaka, Japan) was used as a synthetic MMP inhibitor. ONO-4817 has a broad inhibitory spectrum for MMPs, including MMP-2, MMP-3, MMP-8, MMP-9, MMP-12, and MMP-13, but not MMP-1 and MMP-7 [9]. Cells were usually preincubated for 30 min with ONO-4817 at a concentration of 10 μM, which remained in the medium during exposure of cells to LPS. MMP-9 small interfering RNA (siRNA) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The negative control (sc-37007) consists of a 20–25 nucleotides scrambled sequence, which does not target any known messenger RNA (mRNA). Introduction of siRNAs into cells was performed in siRNA Transfection medium (Santa Cruz Biotechnology) according to the manufacturer's protocol. MMP-9 siRNA (80 pmol) or control siRNA was transfected 1 d before LPS exposure.

### 2.2. RNA extraction and quantitative real-time polymerase chain reaction

Total RNA was isolated from HCFs or cardiac tissue with an RNeasy Mini Kit (Qiagen, Tokyo, Japan). RNA was reverse-transcribed to complementary DNA, and real-time polymerase chain reaction analyses were performed as described previously [10] using Takara RNA polymerase chain reaction kit (Takara Shuzo, Ohtsu, Japan). The sequences of specific primer pairs for target genes are available on request from a website of Takara Bio Inc (http://www.takara-bio.co.jp/).

### 2.3. Enzyme immunoassay

TNF-α, IL-1β, IL-6, and monocyte chemoattractant protein-1 (MCP-1) concentrations were determined in cell culture supernatants by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) according to the manufacture's instructions. The plate was read on a microplate reader (Nippon-InterMed, Tokyo, Japan). Assays were performed in duplicate.

### 2.4. Measurement of nitrite and nitrate production

To assess NOx (nitrite and nitrate) production by HCFs, NOx content of the medium was measured with $NO_2/NO_3$ Assay Kit-C II (Colorimetric [Griess Reagent Kit]) (Dojindo,