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Original Article

Increased myocardial prevalence of C-reactive protein in human coronary heart disease: direct effects on microvessel density and endothelial cell survival

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Abstract

Background: Elevated plasma C-reactive protein (CRP) is a biomarker of cardiovascular diseases (CVDs), but its potential roles as a participant of the disease process are not well defined. Although early endothelial cell injury and dysfunction are recognized events in CVD, the initiating events are not well established. Here we investigated the local myocardial CRP levels and cardiac microvessel densities in control and CVD tissue samples. Using in vitro methodologies, we investigated the direct effects of CRP on human endothelial cells. **Methods:** Cardiac specimens were collected at autopsy within 4 h of death and were classified as normal controls or documented evidence of CVD. The regional prevalence of CRP and the cardiac microvessels (<40 μm) were investigated using immunohistochemistry. For in vitro experiments, human umbilical vein endothelial cells were incubated with CRP. Intracellular oxidant levels were assessed using 2′,7′-dichlorofluorescein diacetate fluorescence microscopy, and cell survival was concurrently determined. Effects of chemical antioxidants on endothelial cell survival were also tested. **Results:** Myocardial CRP levels were elevated in CVD specimens. This was associated with reduced cardiac microvessels, and this rarefaction was inversely correlated to adjacent myocardial CRP prevalence. CRP caused concentration-dependent increases in oxidant production and cell apoptosis. **Conclusions:** These findings provide evidence supporting myocardial CRP as a locally produced inflammatory marker and as a potential participant in endothelial toxicity and microvascular rarefaction. © 2012 Elsevier Inc. All rights reserved.

Keywords: C-reactive protein; Microvessel; Oxidants; Cardiovascular; Microvessel rarefaction

1. Introduction

Many recent studies have examined the association between circulating C-reactive protein (CRP) concentrations and increased cardiovascular disease risk. CRP is a classical plasma protein marker that is elevated during acute phase of inflammation, infection, and tissue injury [1,2]. Although CRP is mainly produced by hepatocytes, there is some recent evidence suggesting that CRP may possibly be produced by macrophages [3], smooth muscle cells [2], or adipose tissue [4]. Plasma levels of CRP have emerged as a strong independent risk factor for predicting future cardiovascular events. Several investigations have demonstrated that baseline CRP levels are associated with future coronary events in the general population without known preexisting coronary artery disease [5–8].

Recent studies have shown that CRP causes endothelial cell dysfunction and reduced expression and activation of

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endothelial nitric oxide synthase (NOS3). CRP plays a critical role in the innate immunity pathways, and presence of CRP induces important phenotypic changes in the vascular endothelium, including apoptosis [9]. NOS3 is a critical regulator of microvascular structure and function. Microvasculature in the heart and other organs is continuously and dynamically regulated by a combination of endogenous pro- versus antiangiogenic forces [10–14]. Local inflammation and oxidant status may play a key role in coronary microvessel abundance. Myocardial levels of CRP have shown to be higher in many cardiovascular diseases. However, the role of locally produced myocardial CRP in cardiac microvascular regulation and cardiovascular disease progression is not completely understood yet.

The mechanistic relevance of various concentrations of CRP on vascular endothelial cells is not completely defined. Moreover, in spite of a multitude of evidence of CRP participating in all stages of atherosclerosis, it is currently unknown whether the elevation of CRP in plasma is the cause and/or consequence of atherosclerosis [15,16]. Great interest has been triggered in understanding underlying mechanisms. However, the direct interaction of CRP and endothelial cells has not yet been specifically investigated. For this reason, here we tested the hypothesis that clinically relevant concentrations of CRP can induce oxidant production in endothelial cells. Moreover, local production of CRP by various cells may contribute significantly in the dysfunction associated with increased CRP levels, especially in the cardiac and vascular tissues. Here we tested the hypothesis that myocytes produce CRP locally and that CRP causes direct endothelial cell toxicity. Additionally, we tested the effectiveness of antioxidants in prevention of CRP-induced endothelial cell death.

2. Materials and methods

2.1. Materials

Human umbilical vein endothelial cell (HUVEC) preparations were used in all the experimental studies. ECs were obtained from BioWhittaker. For all the experiments, cells within passages 4–7 were used. Human recombinant CRP (rhCRP) was purchased from Chemicon. ECs were cultured in endothelial growth medium-2 (EGM-2). All the other chemicals were purchased from Sigma.

2.2. Human cardiac tissue sample collection

Human cardiac tissues [left ventricular (LV) anterior myocardium], full thickness, were collected at autopsy (The Ohio State University pathology/National Cancer Institute AIDS tissue depository). Deidentified patient information was obtained from autopsy reports. Mean age for all the individuals were ~34–38 years (Table 1). Patients with preexisting cardiac risk were omitted from all the investigations (diabetes, smoking, therapy involving cardiotoxic medications). Cardiac LV anterior free wall was collected at autopsy within 4 h of death. These were classified into two groups: normal controls with no evidence of cardiac dysfunction and those with documented evidence of coronary heart disease (from autopsy report). Seven to twelve subjects for each group were used in these studies. These studies were approved by the institutional review board.

2.3. Immunohistochemistry and image analysis

Formalin-fixed and paraffin-embedded tissues were used for histological analyses. Five-micromolar cardiac cross-sections were prepared for immunohistochemistry by standard procedures. Tissues were incubated for 1 h with appropriate primary antibodies. Staining was visualized with diaminobenzidine, methyl green counterstain. Staining controls were used to demonstrate the specificity of the antibodies used (primary antibody substituted for non-immune rabbit IgG). Cardiac tissue images were captured using an Insight high-resolution digital camera mounted on an Olympus microscope. Immunoreactivity was quantified by optical density measurement using an automated digital imaging subroutine (Image Pro software, Media Cybernetics, Silver Spring, MD, USA) using automated macros. In all cases, the variability was less than 2% for intrasample and <10% for intersample measurements.

Table 1
Population characteristics of patients

		Control (n=12)	CVD (n=7)
Age (years)	Mean ± S.D.	38 ± 6	34 ± 6
	Median	39	36
	Range	25–44	26–41
Evidence of cardiac involvement Patient (%)	Myocarditis	0%	29%
	Pericarditis	0%	29%
	Pericardial effusion	0%	14%
	Atherosclerosis	0%	86%
	Hypertrophy	0%	29%
	Myocardial infarction	0%	14%

LV tissues were obtained at autopsy from normal controls and patients with documented evidence of CVD. The details of all the tissues obtained are shown in this table.

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