Role of atrial endothelial cells in the development of atrial fibrosis and fibrillation in response to pressure overload

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A B S T R A C T

Background: Monocyte chemoattractant protein-1 (MCP-1)-mediated inflammatory mechanisms have been shown to play a crucial role in atrial fibrosis induced by pressure overload. In the present study, we investigated whether left atrial endothelial cells would quickly respond structurally and functionally to pressure overload to trigger atrial fibrosis and fibrillation.

Methods and results: Six-week-old male Sprague–Dawley rats underwent suprarenal abdominal aortic constriction (AAC) or a sham operation. By day 3 after surgery, macrophages were observed to infiltrate into the endocardium. The expression of MCP-1 and E-selectin in atrial endothelium and the expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and ED1 in left atrial tissue were enhanced. Atrial endothelial cells were irregularly hypertrophied with the disarrangement of lines of cells by scanning electron microscopy. Various-sized gap formations appeared along the border in atrial endothelial cells, and several macrophages were located just in the endothelial gap. Along with the development of heterogeneous interstitial fibrosis, interatrial conduction time was prolonged and the inducibility of atrial fibrillation by programmed extrastimuli was increased in the AAC rats compared to the sham-operated rats.

Conclusions: Atrial endothelium responds rapidly to pressure overload by expressing adhesion molecules and MCP-1, which induce macrophage infiltration into the atrial tissues. These processes could be an initial step in the development of atrial remodeling for atrial fibrillation.

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1. Introduction

Leukocyte adhesion to the endothelium is a first step in atherosclerosis progression. Leukocyte migration into the intima is mediated via monocyte chemoattractant protein-1 (MCP-1), a key chemokine that recruits monocytes to atherosclerotic lesions [1,2]. Endothelial function is impaired by oxidized low-density lipoprotein and the turbulent blood flow in arterial branching areas, which increases endothelial cell expression of adhesion molecules, such as E-selectin, intercellular adhesion molecule (ICAM-1), and vascular cell adhesion molecule (VCAM-1) [3]. Hence, endothelium works as a key moderator in the progression of atherosclerosis.

Increasing evidence indicates that left atrial (LA) inflammation and interstitial fibrosis contribute to the pathogenesis of atrial fibrillation (AF) [4,5]. We previously reported that LA fibrosis and AF vulnerability were increased 28 days after suprarenal abdominal aortic constriction (AAC) in rats [6]. Similar to the progression of atherosclerosis, MCP-1, ICAM-1, VCAM-1, and E-selectin expressions were significantly increased and macrophages were recruited across the atrial endocardium in AF patients [7–9]. These results may indicate the possible pathologic similarity between AF and atherosclerosis, and dysfunction of the endothelium may be a critical facilitator in both pathogenesis. Chronic exposure to pressure overload overwhelms the defense mechanisms of endothelial cells and compromises its functional integrity. Endothelial dysfunction exhibits proinflammatory, pro-oxidant, proliferative, and proadhesion features, which will facilitate AF substrate formation [10]. Several studies have suggested that inflammation exerts its remodeling effects through reactive oxygen species (ROS) [11]. ROS can activate matrix metalloproteinases, resulting in an imbalance between accumulation and breakdown of extracellular matrix, enhancing LA fibrosis [12]. In fact, inflammatory cells have been demonstrated to infiltrate atrial tissue [13]. In the present study, we investigated the early morphological changes in the atrial endothelium in response to pressure overload.

Conflict of interest: none.

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overload and whether the changes in endothelium may induce inflammation and fibrosis in the LA which may lead to AF.

2. Materials and methods

All experimental procedures were conducted in accordance with the guidelines of the Physiological Society of Oita University for the care and use of laboratory animals, which follow the guidelines established by the US National Institutes of Health.

2.1. AAC procedure

Six-week-old male Sprague–Dawley rats were used for AAC procedure. AAC was performed as previously described [6]. Three days after the operation, the experiments were performed according to the procedures described below.

2.2. Physiological analyses

To measure the hemodynamic parameters, the right carotid artery was cannulated using a 22-gauge vascular indwelling needle (Terumo Corporation, Tokyo, Japan). The tip of the external cannula tube was subsequently inserted into the apex of the left ventricle (LV). LV systolic blood pressure and heart rate were analyzed using Chart v5.3 software (AD Instruments, Bella Vista, Australia).

2.3. Echocardiography

To evaluate cardiac dimensions and ejection fraction, transthoracic echocardiography was performed using an SSD-6500 Prosound II ultrasound imaging device with a UST-5545 10-MHz linear transducer (Hitachi Aloka Medical, Tokyo, Japan).

2.4. Assay for serum levels of high-sensitivity C-reactive protein (hs-CRP) and MCP-1

The concentrations of hs-CRP and MCP-1 in the serum were measured with a Rat High-Sensitivity CRP enzyme-linked immunosorbent assay (ELISA) kit (Kamiya Biochemical Company, Seattle, WA, USA) and a Rat MCP-1 ELISA kit (Thermo Fisher Scientific, Rockford, IL, USA), respectively, according to the manufacturer's protocol. Information about the ELISA kits is summarized in Table 1.

2.5. Scanning electron microscopy (SEM)

The LA samples for SEM were prepared as previously described [14]. The samples were observed using a Hitachi S–4800 scanning electron microscope (Hitachi High-Technologies Corporation, Tokyo, Japan).

2.6. Western blot analysis

LA tissues were isolated and immediately frozen in liquid nitrogen. Immunoblots were performed as previously described [15]. Primary antibodies were purchased as follows: MCP-1 (MyBioSource, San Diego, CA, USA), E-selectin (Abcam, Cambridge, UK), ICAM-1 (Abcam), VCAM-1 (Abcam), ED1 (Abcam), α-smooth muscle actin (SMA) (Sigma-Aldrich, St. Louis, MO, USA), and GAPDH (Sigma-Aldrich). The amount of protein on the immunoblots was quantified using Image J software (ImageJ; http://rsbweb.nih.gov/ij/), and the expression levels of each protein were normalized to GAPDH expression. Information about the antibodies is summarized in Table 1.

2.7. Immunohistochemistry

Samples of isolated LA were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm sections. Some sections were incubated overnight at 4°C with a primary anti-E-selectin antibody (Abcam) diluted 1:400 in an antibody diluent, a primary antibody against MCP-1 (MyBioSource) diluted 1:25, or a primary antibody against ED1 (Abcam) diluted 1:100 in the antibody diluent. The tissues were rinsed three times in phosphate-buffered saline with agitation during each step and then incubated with EnVision + System HRP-Labeled Polymer (cat. # K4003; Dako, Glostrup, Denmark). Binding sites were visualized using the DAB substrate kit (cat. # SK4100; VECTOR Laboratories, Burlingame, CA, USA), and the sections were counterstained with hematoxylin. The micrographs were digitized using Photoshop 7.0, and the total ED1-positive area was defined as the ratio of the ED1-positive area to the reference tissue area, as measured using Image J software.

2.8. Histological studies

Isolated samples of the LA were fixed in 4% paraformaldehyde, embedded in paraffin, and cut into 5-μm sections. Masson trichrome staining was used to evaluate interstitial fibrosis. The micrographs were digitized using Photoshop 7.0 (Adobe, San Jose, CA, USA), and areas of fibrosis were analyzed using Image J software. The total fibrotic area was defined as the ratio of the area of fibrosis to the reference tissue area. We visually checked the areas after the imaging software automatically defined the areas, and corrected if necessary.

2.9. Electrophysiological studies

The hearts were isolated and subjected to retrograde perfusion using a Langendorff apparatus with a Krebs–Henseleit buffer equilibrated with a 95% O2/5% CO2 gas mixture at 36.5°C and a constant pressure of 75 mmHg. Two sets of silver bipolar electrodes coated with Teflon (except for their tips) were placed on the appendages of the right atrium (RA) and the LA. The distance between the electrodes was set at 10 mm. The effective refractory periods (ERPs) of the atria were measured by the S2 extrastimulus method using eight regularly paced beats with four cycle lengths. The interatrial conduction time (IACT) was measured by the S2 extrastimulus method using eight regularly paced beats. The intervals of S1 to S2 and S2 to S3 were the same and decremented from 100 ms to ERP of the RA. Following extrastimuli, we evaluated the appearance of AF, defined as rapid, irregular atrial excitations continuing over six beats.

2.10. Statistical analysis

The AF inducibility was analyzed using the chi-square test, and other data were analyzed by a one-way analysis of variance followed by the Bonferroni–Dunn test. Data are expressed as the mean ± S.E.M. A P value <.05 was considered statistically significant.

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**Table 1**

<table>
<thead>
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