Macrophage Infiltration Into the Endothelium of Atrial Tissue in Atrial Fibrillation

Yuko Sonoda; Yasushi Teshima, MD, PhD; Ichitaro Abe, MD; Yuki Ebata, MD; Takahiro Oniki, MD; Shintaro Kira, MD; Hidekazu Kondo, MD, PhD; Shotaro Saito, MD, PhD; Kunio Yufu, MD, PhD; Shinji Miyamoto, MD, PhD; Tatsuo Shimada, PhD; Naohiko Takahashi, MD, PhD

Figure 1. (A) Masson trichrome staining. Atrial endocardium was thick (red arrow), and there was a large area of subendocardial and interstitial fibrosis accompanied by atrophy and disarrangement of cardiomyocytes (yellow arrows). (B) Transmission electron microscopy (TEM) showing numerous collagen fibers in the stroma of the atrial muscle tissue accompanied by the appearance of a myofibroblast (red arrow). The cardiomyocytes were atrophied (yellow arrows). (C) The number of mitochondria was increased. In some areas, most of the mitochondria were small, condensed, and abnormally shaped, and the structure of myofibrils was deformed and discontinuous (Upper). In other areas, most of the mitochondria were swollen and the structure of cristae was destroyed (Lower). (D) Periodic acid-Schiff staining showing glycogen deposited in a large area of atrial tissue (Upper, dark purple area). (Lower) TEM showing accumulation of a large amount of glycogen granules (yellow arrows) and collapse of the mitochondrial cristae structure (red arrow).
Atrial fibrillation (AF) is a common but critical arrhythmia because of the high risk of fatal cerebral thrombosis. In a model of renal dysfunction, monocytes adhere and infiltrate the endothelium of atrial tissue, which may contribute to AF induction. To explore the pathogenesis of AF, we investigated the microstructural morphology of the left auricle in a representative case of AF.

A 76-year-old man was diagnosed with rheumatic valvular disease at the age of 49 years, and mitral stenosis/regurgitation and aortic stenosis were observed. The patient also had a history of hypertension, hyperuricemia, and dyslipidemia, but no history of diabetes mellitus (HbA1c, 5.7%). The patient was prescribed diuretics, β-blockers, and calcium channel blockers, which controlled his blood pressure to approximately 120/70 mmHg. Paroxysmal AF emerged 9 months prior to the hospitalization for cardiac surgery. On preoperative echocardiogram the left atrial dimension had enlarged to 54.8 mm and there was no asynergy in the left ventricular wall motion, with a 62.8% ejection fraction. The peak and mean aortic stenosis pressure gradient was 50.5 and 27.5 mmHg, respectively. The mitral valve area calculated by pressure half-time was 1.04 cm². Mild aortic and mitral regurgitation were detected. Plasma brain natriuretic peptide was 139.2 pg/mL. The patient underwent aortic and mitral valve replacement using biological valves (Magna ease, 21 and Magna Mitral ease, 29 mm) and the maze procedure. The left appendage was surgically removed and immediately frozen in liquid nitrogen for use in the present study.

On histological assay using Masson trichrome staining, the endocardium was found to be thick and have a large area of subendocardial and interstitial fibrosis, which was accompanied by atrophy and disarrangement of cardiomyocytes (Figure 1A). On transmission electron microscopy (TEM; JEM-1200EXII; JEOL, Tokyo, Japan), numerous collagen fibers in the stroma of the atrial muscle tissue were observed, accompanied by myofibroblast. These collagen fibers mechanically dissociated the connection of atrial cardiomyocytes, and the cardiomyocytes were atrophied (Figure 1B). The number of mitochondria was increased (Figure 1C). Around the areas where inflammatory cells were collected, the mitochondria were small and condensed and the structure of the myofibrils was deformed and discontinuous (Figure 1C Upper). In other areas of the same tissue, mitochondria were swollen and the structure of cristae was destroyed (Figure 1C Lower). On periodic acid-Schiff (PAS) staining, glycogen was deposited in a large area of the atrial tissue (Figure 1D Upper). TEM also showed the accumulation of a large amount of...
glycogen granules and that the structure of the mitochondrial cristae had collapsed (Figure 1D, Lower). Figure 2A,B shows scanning electron microscopy (SEM; Hitachi S-4800; Hitachi High-Technologies Corporation, Tokyo, Japan) of the atrial endothelium. Numerous pores of various sizes were observed in the junction between atrial endothelial cells (Figure 2A). Figure 2B shows a macrophage infiltrating into a pore. Figure 2C,D shows TEM of atrial tissue; a monocyte is observed just above the atrial endothelium (Figure 2C), and a macrophage in the subendothelium is observed incorporating the wreckage of a myocyte (Figure 2D). SEM and TEM were analyzed by an anatomist skilled in electron microscopy.

The morphological abnormalities in the atrial tissue of patients with AF have been previously reported, such as the loss of myofibrils, which are replaced by a large number of glycogen granules. The large number of glycogen molecules may impede electrical conduction, and these heterogeneously distributed islands of impaired conduction may create a re-entry circuit into the atrium. Malformation of mitochondria was also observed in the atrial tissue of patients with AF. In a previous report, sarcomeres were depleted and replaced by a large number of abnormally shaped small mitochondria. Similar to these observations, we noted disruption of sarcomeres along with the accumulation of glycogen and the presence of abnormally shaped mitochondria in the present study. The decrease in myofibrils and sarcomeres due to the increased number of glycogen granules and abnormal mitochondria may induce cardiac dysfunction by reducing ATP production.

Inflammation may critically contribute to the development of AF. Rheumatic valvular disease may not contribute to the inflammation and structural changes observed (Figure S1), but Shenthar et al recently showed that inflammation and interstitial fibrosis in the atrium are commonly observed in rheumatic mitral stenosis irrespective of the rhythm (sinus rhythm or AF). Hence, we cannot exclude the possibility that rheumatic disease per se may be responsible for the inflammation and atrial interstitial fibrosis. The tissue of a patient without AF showed a smaller area of interstitial fibrosis (Figure S2A). Furthermore, the atrial muscle fibers in these patients maintained structural integrity (Figure S2B). This indicates an association between AF and the structural changes observed in the present study. We cannot, however, exclude the possible influences of comorbidities, such as valvular disease, hypertension, and dyslipidemia, which may facilitate atrial structural changes. In conclusion, we have demonstrated that ultrastructural alterations in the atrium may develop into AF substrate.

Disclosures
The authors declare no conflict of interest.

References

Supplementary Files

Supplementary File 1

Figure S1. Atrial tissue in a patient without a history of rheumatic heart disease.

Figure S2. Morphological analysis of atrial tissue in a patient without atrial fibrillation (AF).

Please find supplementary file(s): http://dx.doi.org/10.1253/circj.CJ-16-1072