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Original article

High recurrence of atrial fibrillation in patients with high tissue atrial natriuretic peptide and amyloid levels after concomitant maze and mitral valve surgery

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ABSTRACT

Background: Hemodynamic burden is thought to play a role in valvular atrial fibrillation (AF), but the detailed pathophysiology is unclear. We hypothesized that atrial natriuretic peptide (ANP) tissue levels and amyloid deposits in the left atrial appendage (LAA) were associated with the pre-operative hemodynamic status and post-operative rhythm outcome in patients undergoing a concomitant mitral valve and maze surgery.

Methods: We quantified the fibrosis, atrial amyloid deposits, ANP tissue levels, and multiple biomarker proteins (Western blot) in LAA tissues taken from 26 patients (53.8% male, 58.4 ± 9.7 years) who underwent concomitant maze and mitral valve surgery. The histologic and biochemical results were compared with the pre-operative pulmonary artery pressure (PAP) and post-operative rhythm outcome.

Results: The ANP tissue level was positively correlated with the atrial amyloid deposit areas ($R = 0.880$, $p < 0.001$), but not with the degree of fibrosis. The pre-operative systolic PAP negatively correlated with both the ANP tissue expression level ($R = -0.467$, $p = 0.019$) and atrial amyloid deposit area ($R = -0.589$, $p = 0.008$). The angiotensin II tissue expression level was significantly higher in tissues without ANP expression than in those with expression ($p = 0.003$). AF recurrence after the maze operation was significantly lower in patients without than in those with ANP expression (log rank $p = 0.031$, HR 3.779, 95% CI 1.163–12.277, $p = 0.027$).

Conclusions: A lower ANP atrial tissue expression and amyloid deposits were correlated with a high pre-operative hemodynamic loading, and those patients had a paradoxically lower AF recurrence after relief of the hemodynamic burden by concomitant maze and mitral valve surgery.

Introduction

The alteration of the atrial substrate in patients with chronic persistent atrial fibrillation (AF) has not been thoroughly defined. The structural basis of short- and long-term electrical remodeling has been described in different experimental models [1,2]. Although structural remodeling can result from AF, atrial substrate changes can themselves exacerbate the persistence and recurrence of AF [3], and influence worse outcome after cardiac surgery [4,5]. Atrial fibrosis has been reported in patients with AF, and is related to the activation of the renin–angiotensin system [6,7]. Previously, we reported that the existence of AF is closely related to a thickened subendocardial smooth muscle layer with extensive fibrosis in patients who undergo mitral valve surgery

Abbreviations: LAA, left atrial appendage; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ADMA, asymmetric dimethylarginine; AF, atrial fibrillation; ANP, atrial natriuretic peptide; BMI, body mass index; BSA, body surface area; CVP, central venous pressure; ECG, electrocardiography; ECL, enhanced chemiluminescence; eNOs, endocardial nitric oxide; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ICAM, intercellular adhesion molecule; LAVI, LA volume index; PAP, pulmonary artery pressure; PBS, phosphate-buffered saline; phospho-eNOs, phosphorylated eNOs; PVDF, poly vinylidene fluoride; SD, standard deviation; TBS, Tris Buffered Saline; VIF, variance inflation factors; vWF, von Willebrand factor.

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[8]. Another significant structural change associated with AF is atrial amyloid deposits [9]. Atrial amyloid deposits frequently appear in an older age group and in females, and are associated with AF and atrial thromboembolisms [10,11]. Atrial amyloid deposits affect the atrial conduction and increase the risk of AF, and are associated with an increased synthesis and secretion of the atrial natriuretic peptide (ANP) [12]. ANP is a peptide hormone synthesized and secreted predominantly by atrial cardiomyocytes [13,14]. Although atrial amyloid deposits and ANP expression play important roles in the pathophysiology of AF, their clinical applications are limited because of the difficulty with their quantification. In this regard, we hypothesized that the ANP expression in the left atrial (LA) appendage tissue was associated with the pre-operative hemodynamic burden and post-operative rhythm outcome in patients who underwent concomitant maze and mitral valve surgery. The purpose of this study was to evaluate (1) the correlations among the ANP tissue level, amyloid deposits, degree of fibrosis, and other related protein biomarkers, (2) the relationship between the ANP tissue level and pre-operative hemodynamic burden indicated by the systolic pulmonary artery pressure (PAP), and (3) the clinical outcome of the rhythm control (concomitant maze operation) in AF patients with mitral valve disease.

Material and methods

Study population

The study protocol adhered to the Declaration of Helsinki and was approved by the Institutional Review Board of Yonsei University Health System. All patients provided written informed consent. The study enrolled 26 patients who underwent mitral valve surgery (20 mitral valve replacements and 6 repairs) and a maze operation between December 2009 and January 2015 at Severance Hospital. All patients were diagnosed with persistent AF by documented electrocardiographic (ECG) monitoring for more than 7 days. Fourteen patients had mitral regurgitation and 12 had mitral stenosis; patients with acute mitral valve disease were excluded. Full wall-thickness specimens were excised from the LA appendage. All myocardial specimens were fixed in 70% alcohol and 4% formalin solution immediately after excision. The exclusion criteria were as follows: (1) specimens with a thickness of less than that of the full wall; (2) the presence of associated aortic valve disease; (3) congenital cardiac disease; or (4) prescriptions of anti-arrhythmic drugs (Class Ic or III) at the time of the operation.

Measurements of the intracardiac hemodynamics and maze operation

For continuous cardiac output monitoring, a thermodilutional pulmonary artery catheter (Swan-Ganz, COmbo, Baxter Healthcare Co., Irvine, CA, USA) was inserted via the right internal jugular vein under local anesthesia. After anesthesia was induced and the vital signs were stabilized, the central venous pressure (CVP; from right atrium) and PAP (systolic, diastolic period) were measured. The cardiac output and cardiac index were measured by using the thermodilution method. We conducted a modified Cox maze III utilizing a cryoprobe (Cryo Maze Cardioblate Cryo Flex surgical ablation probe, Medtronic Inc., MN, USA) to produce the Cox maze III lesion except the right atrial auricle resection and lesion formation from the auricle to the tricuspid valve. The lesion set of right atrium included intercaval, cavo-tricuspid and free wall lesion, and those of LA included an inferior extension of the left atriotomy to left lower pulmonary veins, inferior/superior line of pulmonary veins for the pulmonary vein isolation, endocardial LA isthmus, and epicardial coronary sinus line. The resection of LA inferior wall was performed in patients with large LA anterior–posterior diameter (≥60 mm) [15].

Tissue preparation and immunohistochemistry

Multiple 5-μm-thick serial sections were used. Immunohistochemical staining was performed using an avidin-biotin peroxidase system (Dako, Carpinteria, CA, USA) (Fig. 1A). Paraffin-embedded tissue sections were deparaffinized, and then washed with phosphate-buffered saline (PBS). A hydrogen peroxidase block (0.3% H_2O_2 in PBS) was placed on the sections for 10 min, and the slides were washed in PBS. The slides were then incubated with primary antibodies for 90 min at room temperature (approx. 25 °C). Antibodies against ANP (5 μg/ml, rabbit antihuman polyclonal; Abcam, Cambridge, UK) were used to detect amyloid deposits. After incubation, the slides were washed in PBS, and the appropriate secondary antibody (Dako) was placed on the sections for 30 min. The sections were again washed in PBS. A substrate-chromogen solution was applied for 5 min for DAB staining. The sections were then rinsed under running tap water for 5 min. Hematoxylin was added for 1 min before being rinsed under running tap water. After the specimens were dehydrated in alcohol, they were mounted and examined with light microscopy.

Quantification of fibrosis and amyloid deposits

The presence of amyloid deposits was confirmed by the appearance of green birefringence from the Congo red staining under polarized light. Sirius red staining was used to determine the presence and degree of fibrosis. Virtual microscopic images were used to quantify the degree of fibrosis and amyloid deposition, and a single investigator who was blinded to the clinical information performed the measurements. Fibrosis was quantified as the percent (%) area of the entire tissue section using the Image Pro Plus 6.0 (Media Cybernetics, Silver Spring, MD, USA). The amyloid deposits were also quantified as the percent (%) area of the entire tissue area. The amyloid deposition area was also calculated for both the endocardial and myocardial layers, and the percent area for each layer was calculated using the Image Pro.

Western blotting analysis of atrial tissue

Proteins from the LA appendage tissue were separated by 10% SDS–PAGE and then transferred to poly vinylidene fluoride (PVDF) membranes (GE Healthcare, Amersham, UK). After blocking for 1 h at room temperature with 10% skim milk in Tris Buffered Saline (TBS; Bio-world, Korea) with Tween 20 (TBS-T 0.1%, Tween 20), the membranes were incubated overnight at 4 °C with monoclonal antibodies against von Willebrand factor (vWF), a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), endocardial nitric oxide (eNOs), phosphorylated eNOs (phospho-eNOs), asymmetric dimethylarginine (ADMA), intercellular adhesion molecule (ICAM), angiotensin II, and the ANP in an antibody dilution buffer (5% bovine serum albumin in TBS with Tween 20). The membranes were washed three times with TBS-T and incubated with alkaline phosphatase-conjugated anti-IgG (1:5000 dilution in 10% skim milk in TBS with Tween 20) for 1 h at room temperature. The membranes were then washed five times with TBS-T and detected by an enhanced chemiluminescence (ECL) solution (Promega, Madison, WI, USA, 1:1 ratio of substrate solution:enhancer solution). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The tissue proteins, including ANP, were quantified by Western blotting. The relative levels of the protein expression were obtained by comparing their expression intensities to that of the reference band (GAPDH). Those tissues identified to express ANP

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