Impaired cardiac autophagy in patients developing postoperative atrial fibrillation

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Objectives: Postoperative atrial fibrillation (POAF) is a common complication after on-pump heart surgery. Several histologic abnormalities, such as interstitial fibrosis and vacuolization, have been described in atrial samples from patients developing POAF. This ultrastructural remodeling has been associated with the establishment of a proarrhythmic substrate. We studied whether atrial autophagy is activated in patients who develop POAF.

Methods: A total of 170 patients in sinus rhythm who had undergone elective coronary artery bypass grafting were included. Systemic inflammatory markers were measured at baseline and 72 hours after surgery. During the procedure, samples of the right atrial appendages were obtained for evaluation of remodeling by light and electron microscopy. Protein ubiquitination and autophagy-related LC3B processing were assessed by Western blot.

Results: Of these patients, 22% developed POAF. The level of high-sensitivity C-reactive protein, fibrosis, inflammation, myxoid degeneration, and ubiquitin-aggregates in the atria did not differ between patients with and without POAF. Electron microphotographs of those with POAF showed a significant accumulation of autophagic vesicles and lipofuscin deposits. Total protein ubiquitination was similar in the patients with and without POAF, but LC3B processing was markedly reduced in those with POAF, suggesting a selective impairment in autophagic flow.

Conclusions: This study provides novel evidence that ultrastructural atrial remodeling characterized by an impaired cardiac autophagy is present in patients developing POAF after coronary artery bypass surgery. (J Thorac Cardiovasc Surg 2012;143:451-9)

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Postoperative atrial fibrillation (POAF) is a common complication after coronary artery bypass surgery (CABG), affecting approximately 30% of patients.[1] Although it is usually considered benign, POAF accounts for increased short- and long-term mortality, postoperative hemodynamic instability, increased stroke risk, and overall increased health costs.[2,3]

The underlying mechanisms for POAF remain largely unknown. Evidence has shown that inflammation and oxidative stress associated with surgical procedure serve as triggers in patients with a biologic substrate that renders them susceptible for arrhythmia development.[4,5] However, the characterization of such a substrate has proved elusive. The histologic abnormalities described in the atria of patients developing POAF include interstitial fibrosis, vacuolization, and nuclear derangement of myocytes[2]; however, the association of such findings with POAF development is not conclusive.[6] This suggests that patients with POAF have pre-existing ultrastructural atrial remodeling; however, simple histologic examination might not be sufficient to predict arrhythmia development.

One of the histologic findings most consistently reported in patients who develop POAF is cardiomyocyte vacuolization.[2] This morphologic feature has been associated with normal aging, as well as myocardial ischemia. Intriguingly, cardiomyocyte vacuolization is also the dominating morphologic feature related to autophagy.[7] Autophagy is a dynamic and highly regulated cellular process in which components of the cytoplasm are self-digested by lysosomes, playing a role in the removal and recycling of

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Abbreviations and Acronyms

AF = atrial fibrillation
CABG = coronary artery bypass grafting
IP_3R = IP_3 receptor
POAF = postoperative atrial fibrillation
RAA = right atrial appendage

long-lived or damaged protein and organelles.[8] In pathologic conditions, such as nutrient starvation and hypoxia, autophagy is upregulated and provides substrates for catabolic processes in a condition of metabolic stress. However, an excessive activation of autophagy can lead to cell death and has been associated with the early stages of pathologic cardiac remodeling.[9] The protein LC3B (also called Atg8) is a key player in the autophagic process. LC3BI (its unprocessed form) is a constitutive cytoplasmic protein. In conditions of metabolic stress, LC3BI is processed by Atg4; the resulting protein (LC3BII) becomes enriched in the autophagosomes. The LC3BII/LC3BI ratio has been used as a measurement of autophagy.[8] In the present study, we present biochemical and histologic evidence of impaired autophagy in the atria of patients who developed POAF, suggesting that ultrastructural atrial remodeling is associated with the establishment of a proarrythmic substrate. The present study provides relevant information in the search for a better understanding of the mechanisms involved in POAF development.

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METHODS

Subjects

Patients in normal sinus rhythm who underwent elective CABG at Hospital Clinico de la Pontificia Universidad Católica de Chile from January 2008 to May 2010 were included. The baseline clinical characteristics, two-dimensional echocardiogram, and basic laboratory tests were obtained at admission. All patients underwent CABG using a roller pump and a membrane oxygenator with a standard cardiotomy suction setup. Hypothermia was used in all cases, cooling the patients down to a core temperature of 30°C. Myocardial protection was obtained with antegrade and retrograde crystalloid (St. Thomas cardioplegic solution) infused alternatively every 10 to 15 minutes. No aprotinin was used in any case at any time. Venous drainage was achieved with a single double-stage cannula introduced through right atrial appendage (RAA) amputation and directed toward the inferior vena cava. The resected RAA biopsies were obtained at right atrial cannulation, before the onset of cardiopulmonary bypass, immediately introduced in a portable nitrogen tank for transportation to the laboratory, and preserved in this condition for histologic examination and biochemical studies.

The patients were monitored continuously for 72 hours with a telemetry system with automated arrhythmia detection (IntelliVue MP70; Phillips Healthcare, Andover, Mass). In our previous work,[1] the peak for POAF was obtained after 11 ± 28 hours, and most POAF events occurred before day 3. In every patient with a suspected arrhythmic event, a standard 12-lead electrocardiogram was performed and reviewed by a trained cardiologist. Episodes of atrial fibrillation (AF) longer than 15 minutes were considered an episode of POAF for the purposes of the present study.

To exclude the effect of other proinflammatory factors from the analysis, the following exclusion criteria were applied: the need for urgent revascularization or pre-operative cardiogenic shock; a history of myocardial infarction within the previous 2 months; a need for associated heart valve surgery; a history of malignancy, rheumatologic disease, or any chronic inflammatory disease; chronic steroid treatment; thyroid dysfunction; and evidence of active infection. Our institutional ethics committee approved the present study, and all patients gave written informed consent.

Tissue Array

Tissue arrays were generated from paraffin stacks containing a sample of RAA. Each tissue cylinder was sectioned from the endocardium to epicardium using a precision instrument (Manual Tissue Microarrayer MTA-1; Beecher Instruments, Sun Prairie, Wis) and placed in a recipient paraffin block. Thin sections from this block (3 to 4 μm) were stained with hematoxylin-eosin and Masson trichrome; the images were reviewed by two independent pathologists who were unaware of POAF occurrence. In the case of differences between the evaluators, a third evaluator resolved the discrepancy.

Morphologic features, including atrial myocyte vacuolization, myxoid degeneration, and fibrosis and mononuclear infiltration, were assessed using a semiquantitative scale detailing the intensity of the characteristics evaluated from none to low, mild, and high.

Immunostaining

Paraffin sections of the tissue array were deparaffinized, and the endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 15 minutes. The slides were incubated in a moist chamber for 90 minutes with a primary rabbit polyclonal antibody against ubiquitin (Chemicon International, Temecula, Calif) and subsequently incubated with a bridge-antibody consisting of biotinylated anti-rabbit IgG (1:300 dilution; BioGenex, San Ramon, Calif). Between steps, the sections were washed with Tris-buffered saline. Diaminobenzidine and 0.04% hydrogen peroxide in distilled water was used as the chromogen, and the reaction was intensified by incubating in 0.1% osmium tetroxide for 3 minutes. All specimens were counterstained with hematoxylin-eosin.

Electron Microscopy

The tissue samples were fixed in 2% glutaraldehyde in 0.05 M (pH 7.3) cacodylate buffer, postfixed in 1% osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812. Ultrathin sections were stained with aqueous uranyl acetate for 25 minutes and Reynolds lead citrate for 20 minutes and examined under a Philips Tecnai 12 BT electron microscope at 80 kV. Electron microscopy images were analyzed by two independent observers who were unaware of POAF occurrence using the NIH ImageJ software (Rasband WS, ImageJ, US National Institutes of Health, Bethesda, Md, available from: http://rsb.info.nih.gov/ij/, 1997–2009).

Total Protein Extracts

Atrial tissue was weighed and homogenized on ice in RIPA homogenization buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1% sodium deoxycholate, 1 mM ethylenediaminetetraacetic acid, 0.1% sodium dodecyl sulfate, 1 mM phenylmethylsulfonyl fluoride, 1 μg/mL aprotinin, 1 μg/mL leupeptin, 1 μg/mL pepstatin, and 1 mM sodium vanadate). The resulting homogenate was then centrifuged at 6,000g for 10 minutes. The protein concentration in the supernatant was determined by Lowry assay.

Western Blots

Aliquots of the extracted proteins (50 μg/lane) were separated according to the molecular weight on a monodimensional 15% and 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis for LC3B and protein polyubiquitination. The resolved proteins were transferred to supported nitrocellulose sheets (BioRad, Hercules, Calif) at 400 mA for 105 minutes