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Original article

Decreased gene expression of fatty acid binding protein 3 in the atrium of patients with new onset of atrial fibrillation in cardiac perioperative phase

Yasushige Shingu (MD, PhD)[a,*], Takashi Yokota (MD, PhD)[b], Shingo Takada (PhD)[b], Haruki Niwano (MD)[a], Tomonori Ooka (MD, PhD)[c], Hiroki Katoh (MD, PhD)[d], Tsuyoshi Tachibana (MD, PhD)[c], Suguru Kubota (MD, PhD)[c], Yoshiro Matsui (MD, PhD, FJCC)[a]

[a] Department of Cardiovascular and Thoracic Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[b] Department of Cardiovascular Medicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan
[c] Department of Cardiovascular and Thoracic Surgery, Hokkaido University Hospital, Sapporo, Japan
[d] Emergency and Critical Care Center, Hokkaido University Hospital, Sapporo, Japan

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ABSTRACT

Background: Post-operative atrial fibrillation (POAF) frequently occurs after cardiac surgery. However, the mechanisms of POAF have not been fully elucidated. We aimed to examine whether pre-operative atrial gene expression related to cardiac metabolism is changed in patients with POAF.
Methods: Right atrial tissue was obtained during surgery from 38 patients who underwent cardiac surgery from 2013 to 2015. Atrial expression levels were determined by reverse transcription polymerase chain reaction for the following genes: glucose transporter type 4, peroxisome proliferator-activated receptor-α, fatty acid translocase, carnitine palmitoyltransferase 1B, and fatty acid binding protein 3 (*FABP3*). To investigate fatty acid β-oxidation and tricarboxylic acid cycle capacities in the mitochondria, β-hydroxyacyl CoA dehydrogenase and citrate synthase activity levels were spectrophotometrically determined.
Results: POAF within 7 days after surgery was observed in 18 (47%) patients. POAF patients were significantly older, had a larger left atrial diameter, and had reduced expression of *FABP3*, a fatty acids transport gene in the cytosol, compared to those in the non-POAF group. Reduced *FABP3* expression predicted POAF independent of age and atrial size. In contrast, fatty acid β-oxidation enzymatic activity was comparable between the groups.
Conclusions: *FABP3* gene expression in the atrium was reduced in patients with POAF. These findings suggest a potential link between altered fatty acid transport in the atrium and increased AF onset after cardiac surgery.

Introduction

Atrial fibrillation (AF) is the most common cardiac arrhythmia. Post-operative AF (POAF) frequently occurs after cardiac surgery and can induce thromboembolic events and heart failure, prolong the hospital stay, and increase the total medical cost, leading to a poor prognosis [1]. The incidence of POAF is as high as 20–30%, even with the use of beta-blockers, which are the only recommended prophylactic medication for POAF [2,3]. Furthermore, the mechanisms of POAF have not been fully elucidated.

It has been suggested that a reduced utilization of fatty acids in the heart contributes to the progression of left ventricular (LV) hypertrophy, LV remodeling, and chronic heart failure [4,5]. In addition, various types of arrhythmia, including atrial tachycardia, are observed at a higher rate in children with inherited fatty acid oxidation deficiencies [6]. Therefore, POAF might be attributable to impaired fatty acid metabolism in the atrium.

Fatty acid binding protein-3 [FABP3; also known as heart FABP (H-FABP)], a small cytoplasmic protein, has been isolated from a wide range of tissues, including heart and skeletal muscles. FABP3 is involved in the uptake of fatty acids and their subsequent transport toward the mitochondrial β-oxidation system [7].

* Corresponding author at: Department of Cardiovascular and Thoracic Surgery, Hokkaido University Graduate School of Medicine, Kita-14 Nishi-5, Kita-Ku, Sapporo 060-8638, Japan.
E-mail address: shingu@huhp.hokudai.ac.jp (Y. Shingu).

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The aim of the present study was to determine whether gene expression related to cardiac fatty acid metabolism, including fatty acid transport, is changed in the atrium of patients with POAF.

Materials and methods

Subjects

A prospective short-term, observational study was conducted at a single center. A total of 52 consecutive patients who underwent cardiovascular surgery between 2013 and 2015 were potentially eligible for the study. The exclusion criteria included emergent surgery, a lack of myocardial specimen availability due to severe scar formation, and a clinical history of chronic or paroxysmal AF. After applying the exclusion criteria, 38 patients remained. The university ethics committee approved the research protocol (No. 012-0141) and written informed consent was obtained from each patient. The study was registered in the UMIN Clinical Trials Registry: UMIN000012405.

Post-operative AF

POAF has been reported to occur within 2–4 days after cardiac surgery, with a peak incidence of ~70% by the end of the fourth post-operative day and in >80% the arrhythmia terminates by the end of the fifth post-operative day [8,9]. Various definitions of POAF duration (from 30 s to 30 min) have been reported [10–12]. In the present study, POAF was defined as AF lasting at least 5 min within 7 days after the surgery. The electrocardiogram was monitored for 24 h a day using a telemetry system (FUKUDA DENSHI, Tokyo, Japan). The therapeutic strategy for POAF involved anticoagulation, normalization of serum potassium, and infusion of class I antiarrhythmic agents. Cardioversion was performed when hemodynamic compromise was evident. Amiodarone was used for refractory POAF.

Pre-operative echocardiography

A Vivid Seven system (GE/Vingmed, Milwaukee, WI, USA) with an M3S (2.5–3.5 MHz) transducer, Aplio system (Toshiba Medical Systems, Tokyo, Japan) with a 2.5 MHz transducer, or a Philips system (Philips Ultrasound, Bothell, WA, USA) with a 2.5 MHz transducer was used for pre-operative echocardiography. LV end-diastolic/end-systolic dimensions and the left atrial diameter were measured from the parasternal long-axis view. LV ejection fraction was measured using the modified Simpson method. The mitral regurgitation grade was determined using the regurgitation jet area-to-left atrium ratio (1: mild, <20%; 2: moderate, 20%–40%; 3: severe, >40%). The tricuspid regurgitation grade was determined using the regurgitation jet area (1: mild, <5 cm^2; 2: moderate, 5–10 cm^2; 3: severe, >10 cm^2). Aortic valve stenosis was defined using the valve area (1: mild, >1.5 cm^2; 2: moderate, 1.0–1.5 cm^2; 3: severe, <1.0 cm^2). Aortic valve regurgitation was determined using a combination of jet width/outflow tract, pressure half time, and diastolic reverse flow at the abdominal aorta (1: mild; 2: moderate; 3: severe) [13]. The E and A waves of the transmitral flow (E/A) were measured using pulse Doppler. Deceleration time was measured as the time interval from the maximum E wave point to baseline levels.

Atrial electromechanical delay was determined using tissue Doppler as the time interval from the onset of the P-wave on the electrocardiogram to the beginning of the A' wave at the lateral and medial mitral annulus [14]. Intra-left atrial electromechanical delay was then calculated as the difference between the lateral and medial electromechanical delay [15].

Blood glucose, insulin, and free fatty acid levels

Within two days before surgery, blood was collected in the early morning after 10 h of fasting in all patients. Blood glucose, insulin, and free fatty acid levels were measured using enzymatic and colorimetric methods. Just after the blood collection, an oral glucose tolerance test (OGTT, 75 g) was performed in the non-diabetic patients (n = 32/38; one non-diabetic patient could not tolerate glucose intake). The glucose tolerance types were defined according to the guidelines of the Japan Diabetes Society (2012) using the blood glucose level at 120 min after OGTT ingestion as follows: normal, blood glucose <139 mg/dl; impaired, blood glucose 140–199 mg/dl; diabetic, blood glucose >200 mg/dl. Homeostasis Model Assessment (HOMA) R and β (parameters of insulin resistance and secretion, respectively) were calculated as follows: HOMA-R = (fasting insulin, μU/ml) × (fasting glucose, mg/dl)/405; HOMA-β = 360 × (fasting insulin, μU/ml)/[(fasting glucose, mg/dl) − 63].

Myocardium biopsy, surgical procedures, and myocardial protection

Before the establishment of cardiopulmonary bypass, right atrial myocardial tissue (10 mm × 10 mm) was excised from the insertion point of a two-staged drainage cannula. The tissue was frozen and stored at −80 °C until analysis. Aortic valve replacement was performed in 27/38 patients (71%), aortic root replacement in 5/38 (13%), total arch replacement in 6/38 (16%), and concomitant coronary artery bypass grafting in 5/38 (13%). All procedures were conducted under cardiac arrest using both antegrade (15 ml/kg) and retrograde (7.5 ml/kg) cardioplegia (15 °C) with a 1:1 mixture of blood and Myotector® (Mochida. Pharmaceutical Co., Ltd., Tokyo, Japan). Cardioplegia was introduced every 30 min thereafter. The amount of antegrade solution was reduced to 7.5 ml/kg starting at the second infusion. A terminal warm antegrade cardioplegia was conducted just before de-clamping of the aorta.

Analysis of gene expression related to cardiac energy metabolism in the atrium

Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was used to analyze gene expression related to cardiac energy metabolism in the atrium for the following genes: glucose transporter type 4 (*GLUT4*), peroxisome proliferator-activated receptor-α (*PPAR-α*), cluster of differentiation 36/fatty acid translocase (*CD36*), carnitine palmitoyltransferase 1B (*CPT1B*), and fatty acid binding protein 3 (*FABP3*). Myocardial mRNA was isolated from frozen tissue samples using the High Pure RNA Tissue Kit (Roche, Penzberg, Germany), and was then reverse transcribed into cDNA using the Transcriptor First Strand cDNA Synthesis Kit (Roche). Subsequently, RT-PCR was performed using the FastStart Essential DNA Probes Master (Roche) and Real-time Ready Assay (Roche Assay ID: 146462, *GLUT4*; 146286, *PPAR-α*; 144833, *CD36*; 126501, *CPT1B*; 118811, *FABP3*; 102054, *GAPDH*). Polymerase chain reaction amplification was then performed with a reaction volume of 20 μL using LightCycler Nano (Roche), under the conditions specified by the manufacturer. After the initial denaturation and activation of the enzyme for 10 min at 95 °C, 45 cycles of denaturation at 95 °C for 10 s, and annealing and extension at 60 °C for 30 s were performed. The results were normalized to *GAPDH* transcription levels.

Enzymatic activities related to mitochondrial fatty acid β-oxidation and tricarboxylic acid cycle in the atrium

β-Hydroxyacyl CoA dehydrogenase (β-HAD; a key enzyme in fatty acid β-oxidation) and citrate synthase [CS; a key enzyme in

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