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Marine n-3 fatty acids are incorporated into atrial tissue but do not correlate with postoperative atrial fibrillation in cardiac surgery

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ABSTRACT

Objectives: Postoperative new-onset atrial fibrillation (POAF) in cardiac surgery is associated with increased morbidity and mortality. Because n-3 polyunsaturated fatty acids (n-3 PUFA) have an antiarrhythmic effect, we hypothesized that a high content of marine n-3 PUFA in the atrial wall was associated with a reduced risk of POAF.
Design: Venous blood and tissue from the right atrial appendage were obtained from 50 patients undergoing elective cardiac surgery. We determined the content of marine n-3 PUFA in atrial tissue and in plasma phospholipids using gas chromatography.
Results: The mean age of the patients (results available from 49 patients) was 66.0 ± 10.4 years, and 22, 14, 10 and 3 patients underwent coronary artery bypass surgery, valve, combined or other cardiac surgery, respectively. Eighteen patients (36.7%) developed POAF. Concentrations of n-3 PUFA in the atrial wall and in plasma phospholipids did not predict the development of POAF, but there were significant correlations between marine n-3 PUFA in atrial tissue and plasma.
Conclusion: Levels of marine n-3 PUFA in the atrial wall was not associated with the risk of POAF following cardiac surgery, despite significant correlations of marine n-3 PUFA in the atrium and in plasma phospholipids.

1. Introduction

Postoperative new-onset atrial fibrillation (POAF) is the most common post-operative arrhythmia after cardiac surgery with an incidence between 20% and 60%, depending on the definition and methods used for obtaining the diagnosis [1]. POAF carries severe clinical and economic implications i.e. increased early and late morbidity and mortality [2,3] and prolonged hospital stay [1,4,5]. The occurrence rate of POAF remains high despite development of contemporary medical advances [6]. There have been several attempts at preventing and treating POAF, [7–9] and it is important to identify new (and potentially treatable) predictors of POAF in order to reduce its risk.

Fish oils, with their three major marine n-3 polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) have anti-inflammatory and anti-fibrotic effects, as well as direct electrophysiological effects in cardiac myocytes [10–12]. Therefore, attempts have been made to introduce marine n-3 PUFA for the prevention of POAF [6,13] and their effect on the risk of developing POAF has been investigated in some randomized studies with positive results [13]. However, a recent meta-analysis concluded that there was no convincing evidence to support that short-term fish oil use reduces POAF [14]. A possible effect of marine n-3 PUFA on atrial arrhythmias, including POAF, would mechanistically be likely to involve incorporation of n-3 PUFA in the atrium, although effects of circulating n-3 PUFA in plasma or cells cannot a priori be excluded. No previous study has related the content of marine n-3 PUFA in atrial tissue to the risk of POAF after cardiac surgery, and this was the primary aim of the present study. Furthermore, we investigated if there were correlations between levels of marine n-3 PUFA in the atrium and in plasma phospholipids.

2. Materials and methods

2.1. Study population and collection of clinical data

We enrolled 50 patients undergoing first-time elective cardiac surgery between December 1, 2014 and April 30, 2015, at Aalborg University Hospital, Denmark. Informed consent was obtained from each

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patient before participation in the study, which was approved by the Research Ethical Committee of the Northern Denmark Region (N-20140070). Twenty-two, 14, 10 and 3 patients underwent isolated coronary artery bypass grafting (CABG), isolated valve surgery, combinations or other cardiac surgery, respectively. All patients had sinus rhythm before operation. Main exclusion criteria were a history of any kind of preoperative atrial fibrillation or atrial flutter, use of a pacemaker, non-elective surgery, off-pump CABG, pregnancy, and lactating patients.

Clinical demographic data and perioperative data were retrieved from the Western Denmark Heart Registry [15] and electronic patient records. We registered the following pre- and perioperative variables: age, gender, body mass index (BMI), chronic obstructive pulmonary disease (COPD) defined as a need of long term use of bronchodilators or steroids for lung disease, left ventricular ejection fraction, peripheral vascular disease (defined as one or more of the following: intermittent claudication, carotid occlusion or >50% stenosis, amputation for arterial disease, previous or planned intervention on the abdominal aorta, limb arteries or carotids needing medical attention), acute myocardial infarction within 3 months of surgery, additive EuroSCORE, which is a 30-day risk model related to postoperative death, [16] hypertension needing medical treatment, presence of diabetes mellitus, pre-operative drug treatment, type of surgery, use of cardio-pulmonary bypass, aortic cross-clamp time, and the use of intra-aortic balloon pumping. New-onset POAF was defined as atrial fibrillation or atrial flutter (AFL) occurring during hospitalisation that required medical treatment in the form of potassium supplementation, cardioversion and medications in a patient who had no preoperative history of atrial fibrillation.

2.2. Surgical procedures

Standard anesthetic, surgical and perfusion techniques were used. No prophylactic treatment in order to reduce the risk of POAF were given except that β-blockers and statins were not discontinued before surgery. All patients underwent surgery with the use of extracorporeal circulation (ECC) with one or two venous cannulas through the right atrial appendix and the arterial cannula in the ascending aorta. Normothermic perfusion was used in all patients although the temperature was allowed to drift towards 36 °C. Perioperative blood transfusions of any kind were given in accordance with a transfusion algorithm, and postoperative autotransfusion was not used. Temporary epicardial pace-wires (2 on the right atrial wall and 2 on the right ventricle) were used postoperatively in case of bradycardia. All patients were monitored postoperatively with continuously ECG for 2–3 days. Thereafter, conventional ECGs were obtained if any clinical suspicion of POAF developed, and all patients had an ECG taken prior to discharge. Patients were routinely discharged on postoperative day 6–8 if no postoperative complications developed.

All information regarding patient demographics, per- and postoperative data were registered on paper case report forms, and thereafter all information were transferred in an anonymous form to a research database for analyses.

2.3. Blood and tissue samples

From each patient, a fasting blood sample (10 ml) was obtained from the central venous catheter in the morning of surgery, collected into disodium EDTA tubes. After centrifugation (2500 rpm, 15 min), plasma was stored at −80 °C in multiple aliquots until fatty acid analyses were carried out.

A right atrial tissue sample (max 1 cm^3) from the atrial appendage was obtained from all patients by cutting the top of the atrial appendix during cannulation in order to connect the patient to the cardiopulmonary bypass circuit. Tissue samples were immediately transported to the research laboratory in a dry tissue container and cleaned of adipose tissue and clotted blood. Tissue, 20–30 mg, was mixed with 1.0 ml methanol (MeOH) containing butylated hydroxytoluene (BHT) (100 μg/ml) and 2.0 ml chloroform ($CHCL_3$). The suspension was sonicated for 2 min to rupture the cells, the vial was filled with nitrogen, and the sample stored at −20 °C until further extraction and analysis of fatty acids.

2.4. Extraction of total lipids from plasma

Extraction of total lipids was performed by a modified version of a method described previously [17]. For the analysis, 500 μl of plasma was mixed with 5 ml of chloroform-methanol 2:1 containing 50 μg/ml BHT as antioxidants, after which the tubes were mixed for 15 min. After adding 750 μl of 0.9% sodium chloride, the tubes were mixed for 2.5 min and centrifuged at 3220 g for 10 min at 10 °C for phase separation. The upper aqueous phase was discharged, the protein disk gently penetrated, and the lower organic phase containing total lipids was collected. A second extraction was performed to collect the remaining organic phase. The combined organic phase of the 2 extractions was dried under nitrogen for 45 min at 40 °C, dissolved in 1 ml chloroform, and briefly mixed.

2.5. Extraction of total lipids from atrial tissue

Extraction of total lipids was performed by a modified version of the method described by Folch et al. [17,18]. The sample, dissolved in chloroform and methanol, was thawed and 750 μl NaCL added, mixed, centrifuged and the organic phase collected. The extraction was repeated with 1.0 ml MeOH containing BHT, 2 ml $CHCl_3$ and 750 μl NaCl, mixed, and centrifuged. The organic phase was collected and dried under nitrogen for 45 min at 30 °C, dissolved in 1 ml chloroform, and briefly mixed.

2.6. Separation of the phospholipid fatty acid fraction from total lipids, plasma and atrial tissue

The separation was performed by a modified version of the method described by Burdge et al. [19]. The total lipid fraction dissolved in 1 ml chloroform was transferred to a Bond Elut NH_2 column 200 mg (Agilent Technologies, USA) preconditioned with 4 ml of hexane, and afterwards washed with 4 ml of chloroform. The phospholipid fatty acid fraction was eluted with 2 ml chloroform-methanol 3:2, followed by 2 ml of methanol, after which the tubes were dried under nitrogen for 1 h at 40 °C.

2.7. Methylation of fatty acids, plasma and atrial tissue

The tubes were incubated at 50 °C for 5 min before the fatty acids were dissolved in 250 μl of warm heptane (50 °C), briefly mixed, and 12.5 μl of 2 M potassium hydroxide dissolved in methanol was added. Another brief mix was undertaken, and the tubes were methylated for 2 min at 50 °C, mixed twice for 1 min and stored at room temperature for 10 min, after which they were centrifuged at 3220 g for 10 min at 10 °C. The supernatant was then transferred into vials suitable for gas chromatography.

2.8. Identification of fatty acids, plasma and atrial tissue

The fatty acid composition was analyzed by gas chromatography and expressed as percentage of the total fatty acid content using a Varian 3900 GC with a CP-8400 auto sampler (Varian, Middleburg, the Netherlands) equipped with a flame ionization detector. Split injection mode, a CP-sil 88, 60 m × 0.25 mm ID capillary column (Agilent Technologies, USA), temperature programming from 90 to 210 °C and constant flow were used. Helium was used as the carrier gas. Commercially available standards (Nu-chek-Prep Inc., Minnesota, USA) were used to identify the individual fatty acids. Results are