Atrial protective effects of n-3 polyunsaturated fatty acids: A long-term study in ovine chronic heart failure

Dennis H. Lau, MBBS, PhD,* Peter J. Psaltis, MBBS, PhD,* Angelo Carbone, BSc,* Darren J. Kelly, PhD,† Lorraine Mackenzie, PhD,* Michael Worthington, MBBS,* Robert G. Metcalf, PhD,* Pawel Kuklik, PhD,* Adam J. Nelson, BMedSc,* Yuan Zhang, MD,† Christopher X. Wong, MBBS,* Anthony G. Brooks, PhD,* David A. Saint, PhD,* Michael J. James, PhD,* James Edwards, MBBS,* Glenn D. Young, MBBS,* Stephen G. Worthley, MBBS, PhD,* Prashanthan Sanders, MBBS, PhD*

*From the *Cardiovascular Research Centre, Departments of Cardiology, Cardiothoracic Surgery, and Rheumatology, Royal Adelaide Hospital and the Disciplines of Medicine and Physiology, University of Adelaide, Adelaide, Australia, and †Department of Medicine, St. Vincent's Hospital, University of Melbourne, Melbourne, Australia.*

BACKGROUND It has been suggested that omega-3 polyunsaturated fatty acids (n-3 PUFAs) may prevent the development of atrial fibrillation (AF).

OBJECTIVE The purpose of this study was to evaluate the impact of these agents on development of the AF substrate in heart failure (HF).

METHODS In this study, HF was induced by intracoronary doxorubicin infusions. Twenty-one sheep [7 with n-3 PUFAs treated HF (HF-PUFA), 7 with olive oil–treated HF controls (HF-CTL), 7 controls (CTL)] were studied. Open chest electrophysiologic study was performed with assessment of biatrial effective refractory period (ERP) and conduction. Cardiac function was monitored by magnetic resonance imaging. Atrial n-3 PUFAs levels were quantified using chromatography. Structural analysis was also performed.

RESULTS Atrial n-3 PUFAs levels were twofold to threefold higher in the HF-PUFA group. n-3 PUFAs prevented the development of HF-related left atrial enlargement ($P = .001$) but not left ventricular/atrial dysfunction. Atrial ERP was significantly lower in the HF-PUFA group ($P < .001$), but ERP heterogeneity was unchanged. In addition, n-3 PUFAs suppressed atrial conduction abnormalities seen in HF of prolonged P-wave duration ($P = .01$) and slowed ($P < .001$) and heterogeneous ($P < .05$) conduction. The duration of induced AF episodes in HF-PUFA was shorter ($P = .02$), although AF inducibility was unaltered (P = NS). A 20% reduction of atrial interstitial fibrosis was seen in the HF-PUFA group ($P < .05$).

CONCLUSION In this ovine HF study, chronic n-3 PUFAs use protected against adverse atrial remodeling by preventing atrial enlargement, fibrosis, and conduction abnormalities leading to shorter AF episodes despite lower ERP.

KEYWORDS Atrial fibrillation; Congestive heart failure; Omega-3 polyunsaturated fatty acid; Remodeling

ABBREVIATIONS **AF** = atrial fibrillation; **ANOVA** = analysis of variance; **CTL** = control; **ERP** = effective refractory period; **HF** = heart failure; **LA** = left atrium; **n-3 PUFAs** = omega-3 polyunsaturated fatty acids; **RA** = right atrium

Introduction

The role of omega-3 polyunsaturated fatty acids (n-3 PUFAs) in the prevention of atrial fibrillation (AF) remains unclear, with clinical research showing conflicting results to date.[1–9] This is largely due to the heterogeneity of patient populations or underlying atrial substrates, variable n-3 PUFAs formulations or dosing, and different types of AF studied. In contrast, the bulk of preclinical evidence suggests a beneficial atrial antiarrhythmic effect, although questions remain regarding its mechanisms of action.[10–13]

Specifically, n-3 PUFAs have been associated with a reduction in incident heart failure (HF), with mortality ben-

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efits and fewer HF hospitalizations.[14–17] Whether this association can be extended to HF-related AF remains unknown in the clinical setting. In this study, we evaluated the effects of long term n3-PUFAs supplementation on atrial remodeling in a recently characterized ovine model of doxorubicin-induced, nonischemic cardiomyopathy.[18]

Methods

A total of 21 Merino Cross wethers were studied. All procedures were conducted in accordance with the guidelines outlined in the "Position of the American Heart Association on Research Animal Use" adopted on November 11, 1984, by the American Heart Association. Approval for the performance of the study was provided by the Animal Ethics Committees of the Institute of Medical and Veterinary Services and the University of Adelaide, Adelaide, Australia.

Study protocol

All animals (n = 21) were acclimatized for ≥1 week prior to study commencement. Figure 1 details the timeline of the HF model with the sequence of investigative assessments and n-3 PUFAs supplementation. Animals were sequentially allocated to the following groups: control (CTL, n = 7), HF with n-3 PUFAs supplementation (HF-PUFA, n = 7), and HF controls treated with placebo of olive oil (HF-CTL, n = 7). HF was induced by repeated intracoronary doxorubicin infusions (described below with the fatty acids supplementation protocol). CTL animals were not sham operated or catheterized and did not receive any supplementation. All animals were fasted for 24 hours prior to general anesthesia for all investigational (transthoracic echocardiogram, cardiac magnetic resonance imaging) and interventional (pericardial window, cardiac catheterization, electrophysiologic studies) procedures. Intravenous sodium thiopentone (15–20 mg/kg) was used for induction before endotracheal intubation, and isoflurane (2%–4%) in 100% oxygen was used for maintenance. Invasive blood pressure, heart rate, pulse oximetry, end-tidal CO_2, and temperature were continuously monitored. Postoperative care (pericardial window) included intramuscular administration of xylazine hydrochloride and penicillin for 3 days.

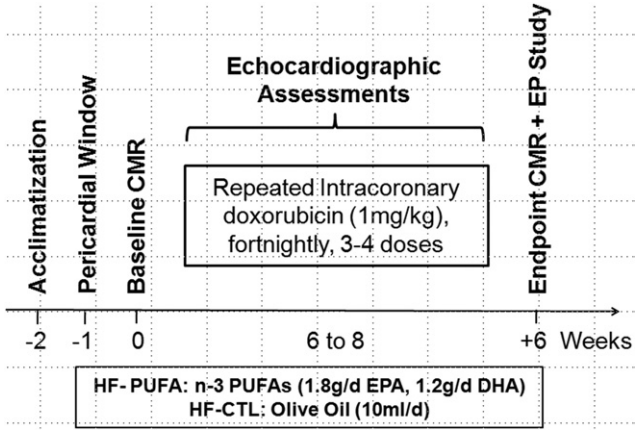

Figure 1 Model timeline showing the time sequence of interventions, supplementations, and investigations from acclimatization to endpoint electrophysiologic (EP) study. CMR = cardiac magnetic resonance imaging; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; HF-CTL = heart failure control treated with placebo of olive oil; HF-PUFA = heart failure with n-3 polyunsaturated fat supplementation; n-3 PUFAs = omega-3 fatty acid.

Doxorubicin nonischemic cardiomyopathy model

The establishment and characterization of this model has been described elsewhere.[18] In brief, animals underwent creation of a small 3-cm pericardial window to prevent inflammatory pericardial effusion at baseline prior to cardiac imaging and doxorubicin dosing. Doxorubicin (1 mg/kg) was infused over 30 minutes via catheterization of the left-sided coronary arteries (Amplatz AL1 catheter, Cordis Corp., Miami, FL, USA) under fluoroscopic guidance at fortnightly intervals. A total of three to four doses were required to achieve at least moderate left ventricular systolic dysfunction. Previous validation study showed no evidence of myocardial infarction with this HF model using delayed enhanced cardiac magnetic resonance imaging scans and histologic examination.[18]

n-3 PUFAs supplementation protocol

n-3 PUFA streated animals received 1.8 g of eicosapentaenoic acid and 1.2 g of docosahexaenoic acid per day. HF-CTL animals received 10 mL per day (equivalent volume) of olive oil, a common placebo used in n-3 PUFAs trials. Supplementation began 1 week prior and continued for 6 weeks following the last doxorubicin dosing (total 13–15 weeks). Proportions of each fatty acid present in atrial phospholipids were determined by gas chromatography as previously described.[19]

Cardiac functional assessments

Cardiac magnetic resonance imaging was used to assess both left atrial and left ventricular volumes and ejection fraction at baseline and prior to the euthanasia studies (Siemens Sonata 1.5 Tesla & Leonardo Workstation, Siemens AG, Munich, Germany) with slice thickness of 6 mm through the atria and 10 mm through the ventricles without any interslice gap. Transthoracic echocardiography was used to guide doxorubicin dosing to achieve at least moderate cardiomyopathy (Acuson XP-128, 4 M-Hz probe, Siemens Medical Systems, Malvern, PA, USA).

Electrophysiologic study

Open chest electrophysiologic studies were performed via the bilateral thoracotomy approach whereby physiologic arterial blood gases and body temperature were maintained throughout. A custom-designed 128-electrode epicardial plaque with 5-mm interelectrode distance was then applied to the right atrium (RA), left atrium (LA) and traversing the Bachmann bundle before being attached to a computerized recording system (LabSystem Pro, Bard Electrophysiology, Lowell, MA, USA).[20] Surface ECG and overlapping bipolar electrograms were continuously monitored and stored for offline analysis.