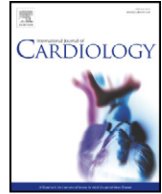
International Journal of Cardiology 220 (2016) 580–588

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard



Post-operative atrial fibrillation is associated with a pre-existing structural and electrical substrate in human right atrial myocardium

Junaid A.B. Zaman [a,d,1], Leanne Harling [a,b,c,1], Hutan Ashrafian [b,c], Ara Darzi [a], Nigel Gooderham [c], Thanos Athanasiou [a,b,*], Nicholas S. Peters [a]

[a] Myocardial Function, National Heart & Lung Institute, Hammersmith Hospital, Imperial College London, UK
[b] Department of Surgery and Cancer, St Mary's Hospital, Imperial College London, UK
[c] Department of Biomolecular Medicine, South Kensington Campus, Imperial College London, UK
[d] Cardiovascular Medicine, Stanford University, Palo Alto, USA

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form 1 June 2016
Accepted 26 June 2016
Available online 28 June 2016

Keywords:
Atrial fibrillation
Post-operative
Calcium handling
Electrograms
Dominant frequency

ABSTRACT

Background: Post-operative atrial fibrillation (POAF) is a major health economic burden. However, the precise mechanisms in POAF remain unclear. In other forms of AF, sites of high dominant frequency (DF) in sinus rhythm (SR) may harbour 'AF nests'. We studied AF inducibility in relation to substrate changes using epicardial electrograms and cardiomyocyte calcium handling in the atria of AF naïve patients.
Method: Bipolar electrograms were recorded from the lateral right atrial (RA) wall in 34 patients undergoing coronary surgery using a high-density array in sinus rhythm (NSR). RA burst pacing at 200/500/1000 ms cycle lengths (CL) was performed, recording episodes of AF > 30 s. Co-localised RA tissue was snap frozen for RNA and protein extraction.
Results: Electrograms prolonged during AF (76.64 ± 29.35 ms) vs. NSR/pacing ($p < 0.001$). Compared to NSR, electrogram amplitude was reduced during AF and during pacing at 200 ms CL ($p < 0.001$). Electrogram DF was significantly lower in AF (75.87 ± 23.63 Hz) vs. NSR (89.33 ± 25.99 Hz) ($p < 0.05$), and NSR DF higher in AF inducible patients at the site of AF initiation ($p < 0.05$). Structurally, POAF atrial myocardium demonstrated reduced sarcolipin gene ($p = 0.0080$) and protein ($p = 0.0242$) expression vs. NSR. Phospholamban gene and protein expression was unchanged. SERCA2a protein expression remained unchanged, but MYH6 ($p = 0.0297$) and SERCA2A ($p = 0.0343$) gene expression was reduced in POAF.
Conclusions: Human atrial electrograms prolong and reduce in amplitude in induced peri-operative AF vs. NSR or pacing. In those sustaining AF, high DF sites in NSR may indicate 'AF nests'. This electrical remodelling is accompanied by structural remodelling with altered expression of cardiomyocyte calcium handling detectable before POAF. These novel upstream substrate changes offer a novel mechanism and manifestation of human POAF.
© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Background

De novo post-operative atrial fibrillation (POAF) affects approximately 30–60% of patients undergoing cardiac surgery [1,2]. It is associated with increased post-operative morbidity, mortality, and significantly worse long-term outcomes [3–6]. The make-up or existence of any predisposing atrial substrate for POAF remains poorly understood.

Methods to detect upstream substrate changes have proved challenging, but it is likely these changes exist in even 'lone AF" [7]. One electrical substrate feature is putative "AF nests" [8] whereby "fibrillar' myocardium is more likely to harbour sites of AF maintenance than so called "compact" myocardium in patients with persistent AF. These sites characteristically display a broad power spectral density (PSD) of the sinus rhythm electrogram when analysed using Fast Fourier Transform (FFT) and occur in both atria. The applicability of these findings in earlier forms of AF such as POAF is unknown.

This study addresses the hypothesis that acute onset POAF is associated with antecedent changes in the make-up of the atrial epicardial electrograms and calcium handling proteins that give rise to a vulnerable substrate in which peri-operative triggers result in clinically significant AF. This is motivated by several lines of reasoning. Firstly, work from our group has discovered that changes in intra-operative paced electrogram changes are able to identify a population who subsequently go on to develop post-operative AF, related to changes in connexin expression [9]. Secondly, we also reported changes apparent in sinus

* Corresponding author at: Department of Surgery and Cancer, Imperial College London, 10th Floor, QEQM Building, St Mary's Hospital Campus, South Wharf Road, London W2 1NY, UK.
E-mail address: t.athansiou@imperial.ac.uk (T. Athanasiou).
[1] Joint First Author.

http://dx.doi.org/10.1016/j.ijcard.2016.06.249
0167-5273/© 2016 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

rhythm electrograms in an “AF naïve” patient group as potential indicators of a predisposed substrate which the operative ‘trigger’ will interact with to produce POAF [10]. However, there is a lack of upstream atrial structure/function studies to detect, prevent or treat POAF especially in subjects with no antecedent AF history.

Third, in addition to these changes in connexin expression and quantity, there is growing evidence to suggest that structural and electrical remodelling occur at a transcriptional level when considering the mechanism behind abnormal atrial cardiomyocyte electrophysiology. In particular, alterations to intracellular calcium handling proteins may act as a key component of POAF pathogenesis, as with other forms of AF [11]. Reduced SERCA2A and/or increased ryanodine receptor (RYR3) gene expression may directly increase cytosolic Ca^{2+} through increased reuptake [12–14] and sarcoplasmic reticulum (SarcR) Ca^{2+} release respectively [15]. Conversely, compensatory mechanisms to increase SarcR Ca^{2+} re-uptake and restore Ca^{2+} homeostasis have also been associated with AF, including downregulation of sarcolipin (SLN), which itself negatively regulates SERCA2A [16]. Indirectly, dysregulation of RhoC signalling may promote AF pathogenesis through both perturbation of ROCK signalling pathways, and through MAPK and PI3K signalling which in turn may alter SarcR Ca^{2+} uptake [17]. Similarly, increased expression of the pro-oxidant monoamine oxidase B may produce intracellular Ca^{2+} overload through generation of H_2O_2, mitochondrial damage and increased mitochondrial Ca^{2+} release [18].

We set out to study this hypothesis in a group of patients with no previous AF history, forming one of the furthest upstream atrial structure function studies carried out in the literature to date.

2. Methods

2.1. Patient selection and recruitment

This study complies with the Declaration of Helsinki, and the study was approved by the local and regional research ethics committee (Ref: 09/H0711/23). Informed consent was obtained from all subjects. Between November 2010 and September 2011, thirty-four patients undergoing non-emergent, on-pump coronary artery bypass grafting (CABG) at Imperial College Healthcare NHS Trust, were prospectively selected to participate in this study. Emergency cases, those requiring adjunctive procedures (e.g. valve repair or replacement), patients with a prior history of any cardiac arrhythmia, thyroid disease, those taking anti-arrhythmic agents, or undergoing surgery with mini-cardiopulmonary bypass (CPB) systems were excluded.

All patients underwent continuous Holter (Novocor Vista 5 lead system, 2 channel recording) monitoring from the time of admission to the time of surgery (12–24 h). Atrial fibrillation was defined according to Heart Rhythm Society Guidelines [19]. Post-operative atrial fibrillation (POAF) was defined as new onset AF following CABG surgery in patients with pre-operative Holter recordings demonstrating sinus rhythm and no prior history of the arrhythmia. All episodes of AF, atrial flutter, or tachycardia of at least 30 s duration were documented. Only AF episodes > 30 s were categorised as AF positive (group 2) [19]. Patients were grouped retrospectively (following Holter analysis after discharge) according to the absence (Group 1) or presence (Group 2) of POAF.

2.2. Electrophysiological study

The intra-operative electrophysiological protocol was designed to minimise delay in surgery or deleterious effects to the patient should AF be induced. Immediately prior to establishing cardio-pulmonary bypass, a high-density Inquiry AFocus II catheter (Irvine Biomedical, St Jude Medical, Minnesota, USA) was placed by the surgeon on the lateral right atrial wall. Stability of contact was ensured by consistent paced capture with high signal to noise ratio on bipolar electrograms. Pacing was always performed at twice the pacing threshold.

Baseline bipolar atrial electrograms were recorded during sinus rhythm and pacing at 500 ms cycle length pacing from an outer pair of electrodes. A steady-state recording of at least 10 consecutive beats was obtained. Electrograms were digitised and stored using BARD (Boston Scientific, USA) at standard sampling rates of 1 kHz and bandpass filtered between 30.0 Hz and 300 Hz.

Atrial fibrillation (AF) was then induced by burst pacing from the same lateral electrode pair to ensure consistent pacing direction and planar wavefronts. Pacing was performed at 200 ms cycle length for 5 s (four bursts) and then 10 s (two bursts) until AF occurs. AF was considered significant if sustained for greater than 30 s [20,21].

2.3. Electrogram analysis

Intra-operative bipolar electrograms were analysed for the following parameters in an automated software package written in Labview based on previous studies [8,22,23]: duration, peak-to-peak amplitude, dominant frequency (DF) and activation time. Electrogram duration was calculated as time from first deviation from baseline to return [24]. Activation time was defined as the time from first to last activation of the AFocus. Conduction velocity (CV) was calculated from the manually annotated activation times using Matlab [25]. This enabled visualisation of propagation across the AFocus II as an isochronal map and estimated CV both using planar and circular conditions. An overall direction of propagation was also calculated and maps visually assessed for atrial conduction patterns. We present mean electrophysiological data from at least 6 consecutive beats. All electrophysiological analyses were conducted blinded to the patient POAF status.

2.4. Laboratory methods

2.4.1. Tissue sampling

Atrial tissue biopsies were taken (without the use of electrocautery) from the free wall of the right atrium prior to cannulation and institution of cardiopulmonary bypass. This corresponded to the site of the electrogram recordings at a consistent site at the lateral border of the AFocus catheter. All biopsies were taken prior to pacing protocols to avoid confounding effects of AF induction on calcium handling proteins. Due to ethical considerations of taking left atrial biopsies in patients undergoing routine coronary surgery, with no need for left atrial access, only right atrial studies were performed.

2.4.2. RNA extraction

Whole RNA was extracted from atrial tissue using TRIzol® Reagent as described previously [26]. RNA quality and concentrations were assessed using the Nanodrop 1000 spectrophotometer and Agilent 2100 Bioanalyser. All RNA integrity numbers (RIN) were >7.5.

2.4.3. RT-qPCR

mRNA levels were quantified using specific TaqMan™ qPCR gene expression assays for the following gene targets: SLN, PLN, Triadin (TRDN), SERCA2A, MYH6 (Applied Biosystems). For each study probe an identical PCR reaction was also carried out using ribosomal U6 as a control gene.

2.4.4. Reverse transcription

Reverse transcription was performed utilising a high-capacity cDNA universal RT kit in accordance with the manufacturer protocol (TaqMan, Applied Biosystems, Life Technologies, Paisley, UK). A total of 10 μl RT master mix was added to 5 μl of sample containing 1 μg total RNA. On completion of the RT reaction, resultant cDNA was either used immediately or stored at −80 °C.

2.4.5. qPCR reaction

qPCR reactions were performed using TaqMan gene expression assays according to manufacturer protocol. A total reaction volume of 10 μl was used for qPCR and an identical control PCR reaction (U6) carried out for each sample reaction. All reactions were carried out in three technical replicates using the Applied Biosystems 7500 fast Real-Time PCR System [27]. Amplification plots were examined for adequate amplification and a delta R_n threshold value of 0.2 (within the exponential phase of amplification) was set to ensure comparability across plates. C_t values were recorded for both samples and controls and compared using the $\Delta\Delta C_t$ method.

2.4.6. Western blotting

Protein was extracted from RA tissue samples (25–50 mg) using RIPA buffer with added protease inhibitor cocktail (*Sigma*). Prior to commencing western blotting, protein concentrations were determined by BCA assay. Proteins were separated by SDS–polyacrylamide gel electrophoresis (10-well 10% NuPAGE® Bis-Tris Precast Gels (Life Technologies) with MES running buffer) and blotted onto a 0.45 μm Protran® membrane. Successful protein transfer was confirmed with Ponceau S stain. Membranes were blocked for 1 h at room temperature in 5% milk blocking buffer and then incubated with primary antibody overnight at 4 °C (Rabbit polyclonal Anti-Sarcolipin (#ABT13, Millipore UK), Mouse monoclonal Anti-Phospholamban 2D12 (#ab2865, Abcam), and Mouse monoclonal Anti-SERCA2A ATPase (2A7-A1; #ab2861, Abcam). Membranes were then washed in PBS-T (PBS with 0.01% Tween) and incubated for 1 h in blocking buffer containing secondary antibody. After washing, protein bands were visualised using enhanced chemiluminescence (Thermo Scientific, Rockford, IL). Quantitation of protein concentrations was preformed using Kodak Image Station 2000M analysis software. Normalisation of protein loading was performed against β-Actin (primary mouse β-Actin (Abcam)).

2.5. Statistical analysis

Inter-group comparisons were performed using student t-test if two groups, or one-way ANOVA if multiple groups. Statistical significance is reported if $p < 0.05$. All calculations were performed using Prism 6.0 (GraphPad, La Jolla, USA).

3. Results

34 patients undergoing non-emergent, on-pump CABG were recruited. 21 did not develop POAF and were classified into Group 1, 13 patients developed POAF and were classified into Group 2. The mean time to onset of AF was 2.5 days.

Download English Version:

https://daneshyari.com/en/article/5963589

Download Persian Version:

https://daneshyari.com/article/5963589

Daneshyari.com