The Effects of Omega-3 Fatty Acids in an Ovine Model of Anthracycline-induced Non-ischaemic Cardiomyopathy

Angelo Carbone BSc

Discipline of Medicine
Faculty of Health Sciences
The University of Adelaide, South Australia

&

Cardiovascular Research Centre
The Royal Adelaide Hospital, South Australia

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Abstract

Anthracycline drugs, such as Doxorubicin (Adriamycin) (DOX), have been widely used since the 1960s for treatment of various forms of cancer. Despite their excellent anti-tumour affects, their clinical use may be complicated by various forms of cardiotoxicity, most notably dose dependent, non-ischaemic dilated cardiomyopathy (NICM) leading to congestive heart failure (CHF). Increasingly, different strategies have been devised in recent years to mitigate the adverse cardiovascular effects of anthracycline administration. However these have had variable success and the burden of anthracycline induced NICM remains substantial.

Marine derived omega-3 polyunsaturated fatty acids (PUFA) have been shown to have cardio-protective properties in a number of clinical settings. These include anti-arrhythmic, anti-inflammatory and anti-thrombotic properties and which are predominantly mediated by the longer chain omega-3 PUFA, eicosapentaenoic (EPA) and docosahexaenoic acid (DHA).

Previously, a limited number of basic and small animal studies have evaluated the protective actions of omega-3 PUFA against anthracycline-induced cardiotoxicity, with mixed findings. Therefore the current study set out to expand on these results by investigating omega-3 PUFA supplementation in the translational setting of a large animal model of DOX-induced NICM.

Initially, a pilot study was performed to assess fatty acid bio-distribution in Merino wether sheep receiving marine fish oil (containing 300mg/mL EPA+DHA), administered by oral drenching of 23mL volumes three times
weekly for up to 20 weeks. Plasma and erythrocyte fatty acids were monitored serially and myocardial membrane concentrations were determined at study end. Systemic and myocardial uptake of long-chain omega-3 PUFA was demonstrated, with plasma, erythrocyte and myocardial concentrations increasing by two to three-fold from baseline levels (p<0.05).

For the main study, 17 age and weight-matched Merino wethers received fortnightly dosing with intracoronary DOX (1.2mg/kg for three doses) to induce cardiotoxicity. Animals were randomised to oral supplementation with fish oil (n=8) or olive oil placebo (n=9) commencing two to three weeks before DOX dosing and continued until 12 weeks after final DOX dose. Comparisons between the fish oil and placebo groups were made for left ventricular remodelling and function by cardiac magnetic resonance imaging (CMR), transthoracic echocardiography and histomorphometric analysis of myocardial fibrosis burden. Surprisingly, by comparison to placebo animals, sheep in the fish oil group showed greater decline in left ventricular ejection fraction (LVEF) (p<0.05), and greater end-diastolic and end-systolic dilatation after DOX (p<0.05). However, both groups demonstrated similar levels of left ventricular fibrosis, suggesting that the accentuation of systolic dysfunction observed in the omega-3 PUFA cohort was not mediated by excess myocardial collagen deposition.

In summary, this is the first large animal study to evaluate omega-3 PUFA supplementation in the setting of anthracycline cardiotoxicity. Despite augmenting circulating and tissue long-chain fatty acid levels, oral intake of fish-oil exacerbated cardiac remodelling induced by intracoronary DOX.
Given these new observational findings, we recommend deferring clinical investigation until further basic mechanistic studies can better define the interactions between fatty acids and cardiac biology in the presence of anthracycline exposure.
Declaration

I declare that this thesis contains no material that has been accepted for the award of any other degree or diploma in any university or tertiary institution to Angelo Carbone. To the best of my knowledge and belief, this thesis contains no material published or written by another person, except where due reference has been made in the text.

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Angelo Carbone, BSc.
July 2011
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Table of Contents

Abstract ..............................................................................................................2
Declaration ........................................................................................................5
Acknowledgements ..........................................................................................6

Introduction

1.1 Cancer and Chemotherapy

1.1.1 Cancer .....................................................................................................18
   1.1.1.1 Current Treatment Options .........................................................18

1.1.2 Anthracyclines ......................................................................................19

1.1.3 Cytotoxic Effects of Doxorubicin .......................................................19
   1.1.3.1 Generation of reactive oxygen species (ROS) .........................20
   1.1.3.2 Antioxidant adjuvants to DOX therapy ....................................22
   1.1.3.3 Mechanisms of anthracycline-induced anti-tumour activity ..............22
   1.1.3.4 Apoptosis ...................................................................................23

1.2 Doxorubicin-induced Cardiomyopathy

1.2.1 Mechanisms of Anthracycline-induced Cardiomyopathy .................25
   1.2.1.1 Apoptosis and oxidative stress ................................................25
   1.2.1.2 Down-regulation of cardiac specific muscle proteins ....................26
   1.2.1.3 Release of vasoactive substances .............................................26

1.2.2 Cardiac Monitoring of Patients Receiving Anthracyclines ...28
1.3 Treatment Strategies to Reduce Anthracycline-induced NICM

1.3.1.1 Dosing regime………………………………………………29
1.3.1.2 Anthracycline analogues………………………………29
1.3.1.3 Liposomal preparations………………………………30

1.3.2 Cardioprotective Adjuncts

1.3.2.1 Dexrazoxane…………………………………………30
1.3.2.2 Hematopoetic cytokines………………………………30
1.3.2.3 Antioxidants…………………………………………31
1.3.2.4 Improved prognosis with current generation heart failure medications…………………………………31

1.4 Omega-3 Polyunsaturated Fatty Acids

1.4.1. Fatty acid synthesis………………………………………33
1.4.2 Dietary Sources of Polyunsaturates……………………35
1.4.3 Cardioprotective Effects of Omega-3 PUFA……………37

1.4.3.1 Absorption of dietary omega-3 PUFA………………38
1.4.3.2 Anti-inflammatory effects……………………………39
1.4.3.3 Anti-arrhythmic effects………………………………40
1.4.3.4 Anti-thrombotic effects………………………………40
1.4.3.5 Other cardiovascular benefits………………………41

1.5 Effect of Omega-3 PUFA on Anthracycline-induced Cardiomyopathy - Current Perspectives………………41
1.6 Ovine Model of DOX-induced Cardiomyopathy ................. 44

1.7 Effects of Omega-3 PUFA in an Ovine Model of DOX-induced Cardiomyopathy ........................................... 45

1.7.1 Thesis Studies Proposal ........................................... 45

1.7.1. Study Hypotheses ............................................. 46

Materials and Methods

Animal Ethics Approval ............................................. 48

Use of Animals and Study Management .................................. 48

2.1 Omega-3 PUFA Dosing Study

2.1.1 Drenching Protocol ............................................. 49

2.1.2 Collection of samples for fatty acid level assessment ............... 49

2.1.2.1 Myocardial sample preparation ..................................... 50

2.1.2.2 Blood sample preparation ......................................... 50

2.1.2.3 Separation of phospholipids, preparation of fatty acid methyl esters (FAMEs) and identification by gas chromatograph ..................... 50

2.2 Ovine Model of DOX-induced Cardiomyopathy

2.2.1 General Anaesthesia ............................................. 51

2.2.2 Pericardial Windows ............................................. 52
2.2.3 Cardiac Magnetic Resonance Imaging ....................... 52
   2.2.3.1 Measurement of Left Ventricular Ejection Fraction ...... 53
2.2.4 Transthoracic Echocardiogram ................................ 54
2.2.5 Blood Samples .................................................. 54
2.2.6 DOX-Infusion Protocol
   2.2.6.1 Establishment of dosage ..................................... 54
   2.2.6.2 Group allocation ............................................... 55
   2.2.6.3 Catheterisation and DOX-infusion ......................... 55
2.2.7 Retrieval .................................................................. 56
2.2.8 Histopathology Protocol ........................................... 57
2.2.9 Histological Assessment of Percent Area Fibrosis .......... 57
2.2.10 Sample size calculation .......................................... 58
2.2.11 Statistical analysis ............................................... 58

Results

3.1 Omega-3 PUFA Dosing Study

3.1.1 Omega-3 PUFA Levels
   3.1.1.1 Omega-3 PUFA Baseline levels ............................. 64
   3.1.1.2 Omega-3 PUFA Drenching study - erythrocyte membrane bound levels ................................. 66
   3.1.1.3 Omega-3 PUFA Drenching study-Myocardial levels ... 66
   3.1.1.4 Myocardial arachidonic acid levels ...................... 68

3.2 Ovine DOX-infusion Study

3.2.1 Clinical Results
   3.2.1.1 Mortality rate .................................................... 69
3.2.1.2 Electrocardiographic changes……………………………70

3.2.2 Left Ventricular Ejection Fraction and Volume Changes as
Assessed by CMR……………………………………………………71

3.2.3 Fractional Shortening as assessed by TTE……………………75

3.2.4 Blood Results

3.2.4.1 Troponin-T post DOX infusion……………………………77

3.2.4.2 Haemoglobin, WCC, platelets…………………………….77

3.2.5 Histopathological Assessment

3.2.5.1 Macroscopic Appearances………………………………….80

3.2.5.2 Histopathological Findings………………………………..82

3.2.5.3 Ventricular fibrosis burden ………………………………..82

Discussion

4.1 Study Objectives……………………………………………………86

4.2 Uptake of Omega-3 PUFA in Merino Sheep……………………86

4.2.1 Elevated Omega-3 PUFA levels at baseline…………………..87

4.2.2 Implementation of Olive Oil placebo drenching for main study……88

4.3 Cardiac effect of Omega-3 PUFA in Ovine model of
DOX-induced NICM…………………………………………………88

4.3.1 Possible mechanisms for adverse effects of Omega-3 PUFA on
DOX-induced NICM…………………………………………………89

4.4 Attrition Rate…………………………………………………………90
4.5 Study Limitations

4.5.1 Anthracycline Administration and Dosage…………………………91

4.5.2 Absence of Neoplasia………………………………………………92

4.5.3 General Anaesthesia………………………………………………92

4.5.4 Follow up period…………………………………………………..92

4.5.5 Non reporting of some omega-3 PUFA levels and

histopathology samples……………………………………………93

Summary and Future Directions……………………………………94

References……………………………………………………………..95

Appendix…………………………………………………………….113
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEC</td>
<td>Animal Ethics Committee</td>
</tr>
<tr>
<td>ALA</td>
<td>Alpha-linolenic acid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ANP</td>
<td>Atrial natriuretic peptide</td>
</tr>
<tr>
<td>ARA</td>
<td>Arachidonic acid</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
</tr>
<tr>
<td>CAM</td>
<td>Cell adhesion molecule</td>
</tr>
<tr>
<td>CH₃</td>
<td>Methyl group</td>
</tr>
<tr>
<td>CH₃CO</td>
<td>Acetyl group</td>
</tr>
<tr>
<td>CHF</td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CK</td>
<td>Creatine Kinase</td>
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<tr>
<td>CMR</td>
<td>Cardiac magnetic resonance imaging</td>
</tr>
<tr>
<td>COOH</td>
<td>Carboxyl group</td>
</tr>
<tr>
<td>COX-2</td>
<td>Cyclooxygenase-2</td>
</tr>
<tr>
<td>DGLA</td>
<td>Dihomo-gamma-linolenic acid</td>
</tr>
<tr>
<td>DHA</td>
<td>Docosahexaenoic acid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>DNR</td>
<td>Duanorubicin</td>
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<tr>
<td>DOX</td>
<td>Doxorubicin</td>
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<td>DPA</td>
<td>Docosapentaenoic acid</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EDV</td>
<td>End-diastolic volume</td>
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<tr>
<td>EPO</td>
<td>Erythropoietin</td>
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<tr>
<td>ESV</td>
<td>End-systolic volume</td>
</tr>
<tr>
<td>ETE</td>
<td>Eicosatrienoic acid</td>
</tr>
<tr>
<td>FOV</td>
<td>Field of View</td>
</tr>
<tr>
<td>Fr</td>
<td>French</td>
</tr>
<tr>
<td>FS</td>
<td>Fractional shortening</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte colony stimulating factors</td>
</tr>
<tr>
<td>IC</td>
<td>Intracoronary</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>LA</td>
<td>Left atrium</td>
</tr>
<tr>
<td>LA</td>
<td>Linoleic acid</td>
</tr>
<tr>
<td>LARIF</td>
<td>Large Animal Research &amp; Imaging Facility, IMVS.</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate dehydrogenase</td>
</tr>
<tr>
<td>LV</td>
<td>Left ventricle</td>
</tr>
<tr>
<td>LVEF</td>
<td>Left ventricular ejection fraction</td>
</tr>
<tr>
<td>LVEDD</td>
<td>Left ventricular end-diastolic dimension</td>
</tr>
<tr>
<td>LVESD</td>
<td>Left ventricular end-systolic dimension</td>
</tr>
<tr>
<td>m²</td>
<td>Metre squared</td>
</tr>
<tr>
<td>NADH</td>
<td>Nicotinamide adenine dinucleotide hydrogenase</td>
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<td>Abbreviation (continued)</td>
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<tr>
<td>NICM</td>
<td>Nonischaemic Cardiomyopathy</td>
</tr>
<tr>
<td>PG</td>
<td>Prosaglandin</td>
</tr>
<tr>
<td>PLA2</td>
<td>Phospolipase A2</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>RA</td>
<td>Right Atrium</td>
</tr>
<tr>
<td>RBC</td>
<td>Erythrocyte (red blood cell)</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>RV</td>
<td>Right ventricle</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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