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

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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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Abbreviations

AEC	Animal Ethics Committee	EDD	End-diastolic dimension
ALA	Alpha-linolenic acid	EDV	End-diastolic volume
ANOVA	Analysis of Variance	EPA	Eicosapentaenoic acid
ANP	Atrial naturetic peptide	EPO	Erythropoietin
ARA	Arachidonic acid	ESV	End-systolic volume
BSA	Body surface area	ETE	Eicosatrienoic acid
CAM	Cell adhesion molecule	FOV	Field of View
CH_3	Methyl group	Fr	French
CH_3CO	Acetyl group	FS	Fractional shortening
CHF	Congestive heart failure	G-CSF	Granulocyte colony stimulating factors
CI	Confidence interval	IC	Intracoronary
CK	Creatine Kinase	IV	Intravenous
CMR	Cardiac magnetic resonance imaging	LA	Left atrium
COOH	Carboxyl group	LA	Linoleic acid
COX-2	Cyclooxygenase-2	LARIF	Large Animal Research & Imaging Facility, IMVS.
DGLA	dihomo-gamma-linolenic acid	LDH	Lactate dehydrogenase
DHA	Docosahexaenoic c acid	LV	Left ventricle
DNA	Deoxyribonucleic acid	LVEF	Left ventricular ejection fraction
DNR	Duanorubicin	LVEDD	Left ventricular end-diastolic dimension
DOX	Doxorubicin	LVESD	Left ventricular end-systolic dimension
DPA	Docosapentaenoic acid	m^2	Metre squared
ECG	Electrocardiogram	NADH	Nicotinamide adenine dinucleotide hydrogenase

Abbreviations (continued)

NICM	Nonischaemic Cardiomyopathy	SD	Standard Deviation
PG	Prosaglandin	SR	Sarcoplasmic reticulum
PLA2	Phospolipase A2	TE	Echo Time
PUFA	Polyunsaturated fatty acid	TPO	Thrombopoietin
RA	Right Atrium	TR	Repetition Time
RBC	Erythrocyte (red blood cell)	TTE	Transthoracic Echocardiography
ROS	Reactive oxygen species	VA	Ventricular Arrythmia
RV	Right ventricle	V-CAM	Vascular cell adhesion molecule
SEM	Standard Error of the Mean	WCC	White Cell Count