Clinical and Biological Determinants of the Coronary Slow Flow Phenomenon

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Declaration

I, Victoria Kopetz, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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* Kopetz V.A, Penno M.A.S, Hoffmann P, Wilson D.P, Beltrame J.F. Potential mechanisms of the Acute Coronary Syndrome Presentation – Insight from a plasma proteomic approach**.**

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Abbreviations

2D-DIGE = Two-dimensional Difference Gel Electrophoresis

AAT = Alpha-1 Anti-trypsin

ACE = Angiotensin Converting Enzyme

ACE-1 = Angiotensin Converting Enzyme Inhibitor

ACh = Acetylcholine

ACN = Acetonitrile

ACS = Acute Coronary Syndrome

ACT = Alpha-1 Anti-chymotrypsin

ADMA = Asymmetric Dimethylarginine

ADP = Adenosine Diphosphate

AF = Angina Frequency

AIx = Augmentation Index

ATP = Adenosine Triphosphate

BH_4 = Tetrahydrobiopterin

CAD = Coronary Artery Disease

CBG = Corticosteroid Binding Globulin

CCU = Coronary Care Unit

CFR = Coronary Flow Reserve

CHAPS = [3- (3 Cholamidopropyldimethylammonio) -1- propanesulfonate]

CHD = Coronary Heart Disease

Cl^- = Chloride ion

CK = Creatine Kinase

CMD = Coronary Microvascular Dysfunction

CRP = C-reactive protein

CSFP = Coronary Slow Flow Phenomenon

CSX = Coronary Syndrome X

CT = Computed Tomography

DDAH = Dimethylarginase

DNA = Deoxyribose Nucleic Acid

DTT = Dithiothreitol

E-selectin = Endothelial selectin

ECG = Electrocardiogram

EDHF = Endothelial Derived Hyperpolarising Factor

EDTA = Ethylenediaminetetraacetic Acid

ELISA = Enzyme-linked immunosorbent assay

ET-1 = Endothelin-1

eNOS = Endothelial NOS

FA = Formic Acid

FMD = Flow-mediated Dilatation

FN = Fibronectin

GTN = Glyceryl Trinitrate

H_2O_2 = Hydrogen Peroxide

HMG-CoA = 3-hydroxy-3-methyl-glutaryl-CoA

HOCl = Hypochlorous Acid

HPLC = High Performance Liquid Chromatography

hsCRP = High-sensitivity C-Reactive Protein

ICAM-1 = Intercellular Adhesion Molecule

IEF = Isoelectric Focusing

IFN-γ = Interferon Gamma

IL-1 = Interleukin 1

IL-6 = Interleukin 6

IL-8 = Interleukin 8

IPG = Immobilised pH gradient

iNOS = Inducible NOS

IP-10 = Inducible Protein 10

IVUS = Intravascular Ultrasound

LAD = Left Anterior Descending

LRα2GP = Leucine-rich alpha-2-glycoprotein

LV = Left Ventricular

MARS = Multiple Imunnoaffinity Removal System

MCP-1 = Monocyte Chemotactic Protein-1

MDA = Malondialdehyde

MMP = Matrix Metalloproteinases

MPO = Myeloperoxidase

MRI = Magnetic Resonance Imaging

MS = Mass Spectrometry

MVA = Microvascular Angina

mRNA = Messenger RNA

NADPH = Nicotinamide adenine dinucleotide phosphate

NO = Nitric Oxide

NO_2^- = Nitrite

NOS = Nitric Oxide Synthase

NMMA = N-monomethylarginine

NMR = Nuclear Magnetic Resonance

nNOS = Neuronal NOS

O_2^- = Superoxide

OD = Optical Density

$ONOO^-$ = Peroxynitrite

oxLDL = Oxidised Low-density Lipoprotein

PA = Persistant Angina

PAF = Platelet Activating Factor

PBS = Phosphate Buffered Solution

PBS-BSA = Phosphate Buffered Solution + 0.1% Bovine Serum Albumin

PBST + Phosphate Buffered Solution + 0.001% Tween

PCI = Percutaneous Coronary Intervention

PDGF = Platelet Derived Growth Factor

PET = Positron Emission Tomography

PGH_2 = Prostaglandin

PGI_2 = Prostacyclin

PON-1 = Paraoxonase -1

PTM = Post-translational Modification

PVD = Primary Microvascular Dysfunction

ROS = Reactive Oxygen Species

RCA = Right Coronary Artery

SAQ – Seattle Angina Questionnaire

SDMA = Symmetric Dimethylarginine

SDS- PAGE = Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

SF-36 = Short-Form 36

SNC = Sublingual Nitrate Consumption

SOD = Superoxide Dismutase

SPECT = Single-photon Emission Computed Tomography

TBA = Thiobarbituric Acid

TBST = Tris-buffered saline + Tween

TIMI = Thrombolysis in Myocardial Infarction

TNF-α = Tumor Necrosis Factor Alpha

TXA_2 = Thromboxane A_2

TnT = Troponin T

U.S = United States

VSMC = Vascular Smooth Muscle Cell

VCAM-1 = Vascular Cell Adhesion Molecule

vWF = von Willebrand Factor

Abstract

Background

This thesis investigates the clinical and biological factors that contribute to the cardiovascular condition known as the Coronary Slow Flow Phenomenon (CSFP). From its initial description, little remains understood regarding the mechanisms contributing to this curious condition. The research efforts in this thesis have focused upon further characterising the CSFP and identifying an effective therapy, by investigating the mechanisms involved during different periods of presentation.

The specific objectives include:

1) Identifying the possible mechanisms of the acute coronary syndrome (ACS) presentation in CSFP patients by comparing plasma protein profiles from samples obtained from initial presentation and during a quiescent phase of the disorder;
2) Investigating the role of the endothelium during the chronic phase of the disorder. Specifically, this includes looking at mechanisms of endothelial dysfunction, inflammation and oxidative stress and comparisons with a healthy control group;
3) Evaluating the efficacy of a dual endothelin-1 (ET-1) receptor blocker (Bosentan) in ameliorating angina symptoms in CSFP patients. This project also involves monitoring improvements in health-related quality of life, clinical profiles, endothelial function, inflammation and oxidative stress following Bosentan treatment.

Methods

This thesis employed a number of methods to comprehensively assess the pathophysiological mechanisms contributing to CSFP aetiology. In order to identify possible protein biomarker candidates, a state-of-the-art proteomic approach was used to obtain plasma protein profiles. A paired-longitudinal study design was employed by which blood samples were obtained from CSFP patients during the ACS and compared to a quiescent phase. During the chronic phase of the condition, a cross-sectional study was conducted to assess endothelial function, inflammation and oxidative stress parameters compared with a healthy control group that had no history of chest pain or coronary disease. The clinical trial employed a randomised, double-blind, placebo-controlled, cross-over design that involved evaluating changes in chest pain, clinical characteristics, endothelial function, inflammation and oxidative stress parameters following treatment with bosentan therapy

Summary of major findings

The above studies yielded the following findings:

1) Proteomic investigations identified specific inflammatory and oxidative stress protein markers that were elevated during the ACS presentation compared to the chronic phase (Chapter 2).
2) There was no evidence of impairments in endothelial vasomotor function or increases in inflammatory and oxidative stress parameters during the chronic phase of the condition compared to control subjects (Chapter 3).
3) Bosentan therapy did not significantly improve angina symptoms, clinical profiles, endothelial function, inflammation and oxidative stress

parameters compared to placebo. Despite not reaching statistical significance, reductions in angina frequency and severity in addition to improvements in quality of life parameters were identified (Chapter 4).

Conclusion

This thesis provides a new platform for future investigations into the CSFP. Pathophysiological differences identified between the acute and chronic presentations have initiated the need to further conduct research on the specific mechanisms that contribute to both the ACS presentation and persistent symptoms. Additionally, investigating the role of ET-1 receptor blockade in CSFP patients has identified a number of inherent problems associated with clinical trial design in CSFP patients.

Statement of Authorship

Potential mechanisms of the Acute Coronary Syndrome Presentation – Insight from a plasma proteomic approach.

International Journal of Cardiology 2012;156:84-91

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Other Abstracts and Presentations

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Poster Presentations

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Plasma Proteomic Investigations into the Human Coronary Vasculature: Exploring mechanisms of the acute coronary syndrome presentation. Presented at the 4th Asian-Oceanic Human Proteome Organisation Conference, June 22nd-26th, Cairns, Australia.

Preface

Coronary heart disease (CHD) is a disorder characterised by dysfunction in the large and/or small coronary vessels. Impairments in coronary vascular function result in reduced flow of oxygen and nutrient-rich blood to the myocardium thereby producing myocardial ischaemia. This in turn may manifest as chest pain (referred to as angina) or in severe cases may result in myocardial infarction or even death. Indeed, CHD is the leading cause of death globally, responsible for an estimated 17.3 million deaths worldwide in 2008 (1) and more than 2200 deaths every day in the United States of America (U.S.A) alone (2). Accordingly, it is imperative that we increase our knowledge and understanding of the condition that is responsible for considerable global morbidity and mortality.

This thesis will provide a comprehensive summary of cardiovascular pathology, with a particular focus on coronary microvascular disorders, namely the coronary slow flow phenomenon (CSFP). The introductory chapter will provide the necessary background relating to CHD and will begin by describing contemporary facets of CHD with a discussion on the contribution of both large (coronary artery disease) and small vessel dysfunction (coronary microvascular disease) in determining clinical outcomes. This will then be followed by an extended discussion of the clinical conditions associated with coronary microvascular dysfunction (CMD), with particular reference to coronary syndrome X (CSX) and the CSFP. Thereafter, the thesis objectives and proposed investigations will be outlined.