OSTEOPROTEGERIN:

A PATHOLOGICAL ROLE IN HUMAN ABDOMINAL AORTIC ANEURYSM

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Corey Stephen Moran

October 2006

Declaration on Ethics

The research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *National Statement on Ethics Conduct in Research Involving Human* (1999), the *Joint NHMRC/AVCC Statement and Guidelines on Research Practice* (1997), the *James Cook University Policy on Experimentation Ethics. Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). The proposed research methodology received clearance from the James Cook University Experimentation Ethics Review Committee (approval numbers H1464 and A964)

Corey Moran	October 16, 2006
	(Date)

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Communications & Awards

1. CONFERENCES

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2. PAPERS

Moran CS, McCann M, Karan M, Norman P, Ketheesan N, Golledge J. Association of osteoprotegerin with human abdominal aortic aneurysm progression. *Circulation* 2005;111:3119-3125

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3. AWARDS

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Abstract

Rupture of Abdominal Aortic Aneurysm (AAA) is the end-stage, catastrophic failure of the aneurysmal aortic wall and is associated with a mortality rate of up to 95 percent. Presently, surgery is the only treatment option available but carries with it a mortality rate of up to five percent and is usually reserved for repair of aneurysms showing high probability of rupture. What is required for the treatment of AAA, and essentially the basis of research in this area, is to understand the pathology of the disease well enough so that non-surgical intervention aimed at inhibiting small aneurysm progression can be developed.

The lack of non-invasive medical treatment for the disease, especially at the initial stages of development, stems from an incomplete understanding of its pathogenesis. Despite extensive laboratory and clinical research, the precise mechanisms leading to aneurysm formation remain unclear. The hallmark features of an aneurysmal aortic wall are degradation and fragmentation of the medial extracellular matrix (ECM), and significant reduction in smooth muscle cell (SMC) density, believed to be associated with the marked cellular inflammatory response also observed in the aneurysmal tissue.

A newly identified member of the tumour necrosis factor receptor superfamily known as osteoprotegerin (OPG) is constitutively expressed within the human artery wall and, under pathological conditions, is upregulated and associated with vascular disease. Elaboration on the involvement OPG of in AAA will determine its potential as a pharmacological target for the treatment of aneurysmal disease.

The focus of this study was to understand whether OPG might be important in the development of AAA. Two hypotheses were proposed:

1. Expression of OPG is upregulated in the aneurysmal aorta
2. Osteoprotegerin actively promotes aneurysm phenotype within the aortic wall

The specific aims of the study were to:

a) Assess relationship between aortic concentration of OPG and the presence of aneurysm

b) Define possible mechanism(s) by which OPG may be functionally active in the promotion of aneurysm development

c) Modulate aortic expression of OPG and assess the effect on aneurysm development

Serum OPG was correlated with aneurysm growth rate in 146 men with small AAA followed by ultrasound for 3 years (R=0.20; P=0.04), and a demonstrated predictor of aneurysm expansion on multiple-regression analysis (P=0.02; coefficient 1.33, SE 0.51) in a model consisting of patient age, diabetic status, smoking history, initial aortic diameter, serum cholesterol, and C-reactive protein. Western analysis showed 3-fold, 8-fold, and 12-fold greater OPG concentrations in human AAA biopsies compared to age and gender-matched atherosclerotic narrowed aorta (AOD; 1.4±0.1 ng/mg tissue vs 0.5±0.1 ng/mg tissue; P=0.002), post-mortem non-diseased abdominal aorta (PAA; 1.4±0.1 ng/mg tissue vs 0.2±0.1 ng/mg tissue; P<0.001), and non-diseased thoracic aorta (TA; 1.4±0.1 ng/mg tissue vs 0.1±0.06 ng/mg tissue; P<0.001), respectively. Resident vascular smooth muscle cells (VSMC) and infiltrating macrophages were identified as primary sources for OPG within the aneurysmal aortic media. The association between aortic expression of OPG and the presence of AAA was confirmed in an animal model of experimental aneurysm formation, in which levels of OPG protein were 4-fold greater in aneurysmal aortic tissue compared to non-aneurysmal tissue. Furthermore, aortic tissue levels of OPG in this model correlated strongly with vessel diameter.

Healthy human aortic VSMC incubated with recombinant human OPG (0-20 ng rhOPG/10^5 cells/ml/24h) developed an aneurysmal phenotype defined by dose-dependent impaired cell proliferation (P<0.001), increased apoptosis (P<0.01), decreased interleukin (IL)-6 expression (P<0.001), and increased matrix metalloproteinase (MMP)-9 activity (P=0.01). Gene expression in OPG-treated

VSMC reflected these results exhibiting downregulation of genes associated with cell growth and survival, and upregulation of genes that negatively regulate cell growth and promote cell death.

Incubation of human monocytic cells with OPG (0-20 ng rhOPG/10^5 cells/ml/24h) resulted in up to a 2-fold dose-dependent increase in IL-6 production in lipopolysaccharide (LPS)-activated cells (P=0.005). In addition, OPG (1 ng/10^5 cells/ml/24h) acted to induce a 2-fold increase in MMP-9 expression (P<0.001), with a 1.5-fold increase in MMP-2 production (P=0.01) in resting human monocytic cells.

Treatment of human AAA tissue in culture with the angiotensin II receptor blocker, Irbesartan, and the peroxisome proliferator-activated receptor gamma (PPARγ) ligands, Pioglitazone and Rosiglitazone, inhibited OPG production by up to 50%, as well as reducing inflammatory cytokine, and proteolytic enzyme production. The effects produced by thiazolidinedione treatment on aneurysm tissue *ex vivo* were reproduced *in vivo*. Both aortic expression of OPG and MMP activity within aortic tissue from a mouse model of experimental aneurysm formation were down-regulated significantly with Pioglitazone medication.

This study demonstrates for the first time the association of OPG with AAA and identifies a possible key role for the protein in the promotion of an aneurysmal phenotype within the normal aortic wall. The ability of existing medication to limit this action potentially opens a therapeutic pathway through which to limit aneurysm expansion in humans by targeting arterial expression of OPG.

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Abbreviations

AAA	Abdominal Aortic Aneurysm
AAMø	Aortic Aneurysm-derived Macrophage(s)
AASMC	Aneurysm Aortic Smooth Muscle Cell(s)
ACE	Angiotensin Converting Enzyme
AOD	Aortic Occlusive Disease
ApoE($^{-/-}$)	Apolipoprotein E Gene (homozygous deletion)
AT_1R	Angiotensin II Receptor Type 1
DMEM	Dulbecco's Modified Eagles Media
DNA	Deoxyribonucleic Acid
ECM	Extracellular Matrix
ELISA	Enzyme-linked Immunoassay
FACS	Fluorescence-activated Cell Scanning
FBS	Foetal Bovine Serum
IFN	Interferon
IL	Interleukin
IRA	Infra-renal Aorta
LPS	Lipopolysaccharide
MMP	Matrix Metalloproteinase(s)
Mø	Macrophage(s)
NASMC	Normal Aortic Smooth Muscle Cell(s)
OPG	Osteoprotegerin
PAA	Post-mortem non-diseased Abdominal Aorta
PBM	Peripheral Blood Monocyte(s)
PPAR	Peroxisome Proliferator-activated Receptor
RANK	Receptor Activator of NFκB
RANKL (Rkl)	RANK Ligand
rhOPG	recombinant human Osteoprotegerin
RNA	Ribonucleic Acid
SEM	Standard Error of the Mean
SMC	Smooth Muscle Cell(s)
SRA	Supra-renal Aorta
TA	Thoracic Aorta
TNF	Tumour Necrosis Factor
VSMC	Vascular Smooth Muscle Cell(s)