

***TISSUE RESPONSES TO NITRIC
OXIDE DONORS: STUDIES IN
PLATELETS AND IN MYOCARDIUM***

MATTHEW I. WORTHLEY

M.B.,B.S., F.R.A.C.P.

Department of Medicine

University of Adelaide

Adelaide, SA, 5005

AUSTRALIA

AND

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Department of Medicine

University of Adelaide

AUSTRALIA

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To my beautiful wife Alexandra and children

Thomas and Charlotte.

DECLARATION

This body of work contains no material which has been accepted for the award of any other degree or diploma in any university or 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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Dr. Matthew I. Worthley
Department of Medicine
University of Adelaide
Adelaide
South Australia 5000
AUSTRALIA

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THESIS RELATED PUBLICATIONS

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SPECIFIC CONTRIBUTION BY CANDIDATE TO EACH MANUSCRIPT

In this paper blood samples were collected by Dr. M Worthley, with HPLC and manuscript written by Tamila Heresztyn. The other 2 thesis-related publications were being submitted at the time of thesis completion.

THESIS RELATED ABSTRACTS

1. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD. Effects of diabetes mellitus and associated glycaemic control on platelet function in unstable angina pectoris. *Heart, Lung and Circulation* 2001; 10:A 101.
2. **Worthley MI**, Holmes AS, Chirkov YY, Zeitz CJ, Horowitz JD. Glycaemic control and platelet function in diabetic patients with threatened myocardial infarction. *Proceedings of A.P.P.S.*2001;32(2):Suppl 2 47P.
3. **Worthley MI**, Holmes AS, Chirkov YY, Zeitz CJ, Horowitz JD. Glycaemic control and platelet function in diabetic patients with threatened myocardial infarction. *Circulation* 2001;104(17):II-378.
4. **Worthley MI**, Holmes AS, Chirkov YY, Stepien JM, Zeitz CJ, Horowitz JD. Platelet responsiveness to nitric oxide in diabetics with acute coronary syndromes: Effects of correction of hyperglycaemia. *JACC* 2002;39(9):13B.
5. **Worthley MI**, Holmes AS, Chirkov YY, Zeitz CJ, Horowitz JD. Acute resolution of hyperglycaemia normalizes platelet responsiveness to nitric oxide in diabetes with acute coronary syndromes. *European Heart Journal* 2002;23:495.
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8. Willoughby SR, **Worthley MI**, Kelly EA, Horowitz JD. Acute glucose loading in normal volunteers does not affect platelet function, nitric oxide responsiveness, superoxide content or arterial stiffness. CSANZ 2003 (accepted).
9. **Worthley MI**, Willoughby SR, Kucia AM, Holmes AS, Chirkov YY, Zeitz CJ, Horowitz JD. Determinants of platelet nitric oxide response and superoxide levels in diabetic patients with threatened myocardial infarction. *European Heart Journal* 2003;24:128.
10. **Worthley MI**, Holmes AS, Willoughby SR, Kucia AM, Heresztyn T, Stewart S, Chirkov YY, Zeitz CJ, Horowitz JD. The deleterious effects of hyperglycemia on platelet function in diabetic patients with acute coronary syndrome: Mediation by superoxide production, resolution with intensive insulin administration. *Circulation* 2003;108(17):IV-311.
11. Heresztyn T, **Worthley MI**, Horowitz JD. Utility of an improved HPLC assay for the cardiovascular risk factor Asymmetric Dimethylarginine: application to Diabetic/Ischaemic patients. *Proc. Aust. Soc. Exp. Pharmacol. Toxicol* 2003;10:67.

THESIS RELATED AWARDS

1. First prize: Higher Degree (clinical research) Research Day Queen Elizabeth Hospital 2001. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD: Glycaemic control and associated platelet function in diabetic patients presenting with a threatened myocardial infarction.
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3. Second Prize: 3rd National Registrars Competition 2001. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD: Glycaemic control and associated platelet function in diabetic patients with a threatened myocardial infarction.
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1. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD :
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2. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD: Glycaemic control and associated platelet function in diabetic patients presenting with a threatened myocardial infarction. National Cardiovascular Registrars Research Forum 2001.
3. **Worthley MI**, Holmes AS, Zeitz CJ, Chirkov YY, Horowitz JD: Glycaemic control and associated platelet function in diabetic patients presenting with a threatened myocardial infarction. 4th annual SA and WA cardiovascular meeting 2001.
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LIST OF ABBREVIATIONS

ACE	Angiotensin Converting Enzyme
ACS	Acute Coronary Syndrome
ADMA	Asymmetric Dimethylarginine
ADP	Adenosine diphosphate
AGE	Advanced Glycation End products
AI(x)	Augmentation Index
AMI	Acute Myocardial Infarction
AP	Activation Protein
ATP	Adenosine Triphosphate
BAEC	Bovine Aortic Endothelial Cells
BH4	Tetrahydrobiopterin
BSL	Blood Sugar Levels
[Ca²⁺]	Intracellular Calcium Concentration
CAD	Coronary Artery Disease
CAM	Cellular Adhesion Molecules
CAT	Cationic Arginine Transporters
CFR	Coronary Flow Reserve
CHD	Coronary Heart Disease
CRP	C-Reactive Protein
CVD	Cardiovascular Disease
DAG	Diacylglycerol
DDAH	Dimethylarginine Dimethylaminohydrolase
EDRF	Endothelium-derived relaxing factor
cAMP	cyclic adenosine 3',5' monophosphate
cGMP	cyclic guanosine 3',5' monophosphate
HRE	Hypoxia Responsive Element
HUEVC	Human Umbilical Endothelial Cells
FAD	Flavin Adenine Dinucleotide
FFR	Force Frequency Relationship
FMD	Flow Mediated Dilatation
FMN	Flavin Mononucleotide
FGF	Fibroblast Growth Factor
GAPDH	Glyceraldehyde 3-phosphate Dehydrogenase
GAS	Gamma Activating Sequence

IBMX	3-isobutyl-1-methylxanthine
IFG	Impaired Fasting Glucose
IFN	Interferon
IGT	Impaired Glucose Tolerance
IL	Interleukin
IP₃	Inositol 1,4,5- triphosphate
LDCL	Lucigenin Derived Chemiluminescence
MAPK	Mitogen-activated Protein Kinase
NADPH	Nicotinamide adenine dinucleotide phosphate
NEFA	Non-esterified fatty acids
NO[·]	Nitric Oxide
NOS	Nitric Oxide Synthase
ODQ	1 <i>H</i> -[1,2,4]oxadiazolo-[4,3-2]quinoxalin-1-one
O₂⁻	Superoxide
PDE	Phosphodiesterase
PDGF	Platelet-Derived Growth Factor
PKA	Protein Kinase A
PKC	Protein Kinase C
PKG	Protein Kinase G
PLC	Phospholipase C
PRMT	Protein arginine Methyltransferase
PRP	Platelet Rich Plasma
ROS	Reactive Oxygen Species
SDMA	Symmetric Dimethylarginine
SNAP	S-nitroso-N-acetylpenicillamine
SNP	Sodium Nitroprusside
SOD	Superoxide Dismutase
SR	Sarcoplasmic Reticulum
STAT	Signal transducers and activators of Transcription
STZ	Streptozotocin
TGF-β	Transforming Growth Factor- beta
TNF-α	Tumour Necrosis Factor-alpha
TXA₂	Thromboxane A ₂
VEGF	Vascular Endothelial Growth Factor
vWF	von Willebrand Factor

THESIS SUMMARY

The nitric oxide molecule (NO[·]) is without doubt one of the most influential on the pathophysiology of the cardiovascular system. Its effects are broad ranging in this system influencing almost all of its components, particularly the vasculature, platelets and the myocardium. The beneficial effects of this molecule not only in inhibiting the development of cardiovascular disease (CVD) but in minimizing the risk of its acute thrombotic complications makes it an obvious target for therapies designed to enhance its effects. Assessing the tissue effects of NO[·] in platelets and the myocardium has been the main focus of this thesis.

Determinants of platelet responsiveness to nitric oxide in diabetic patients with acute coronary syndromes: Effects of glycaemic control

Background

We chose to perform our initial experiments in diabetic patients admitted with an acute coronary syndrome (ACS). This cohort offered us a unique opportunity to assess NO[·] bioavailability in a group with one of the highest cardiovascular morbidity and mortality rates. We assessed this via platelet NO[·] responsiveness, which has been shown from our laboratory to be impaired in ACS but to date had not been assessed specifically in a diabetic group. As hyperglycaemia is a known independent predictor of mortality in this group we chose in our first set of experiments to primarily assess the effects of glycaemic control (acute and chronic) on platelet NO[·] responsiveness.

Study

In diabetic patients admitted with an ACS the relationship between glycaemic control and the main determinants of platelet NO[•] responsiveness, superoxide (O₂⁻) generation and guanylate cyclase activity were assessed. Secondary hypotheses assessed the effects of acute glycaemic control on other potential modulators of NO[•] responsiveness such as asymmetric dimethylarginine (ADMA), L-arginine, L-arginine/ADMA ratio, platelet activated O₂⁻ release and C-reactive protein (CRP). Other values assessed included CK rise, non-esterified fatty acid (NEFA) levels, ADP induced platelet aggregation and neutrophil count. We also assessed the determinants of both platelet SNP response and of O₂⁻ levels by a stepwise multivariate analysis; parameters assessed were age, sex, blood sugar level (BSL), statin therapy, insulin therapy, ACE-inhibitor therapy and CK elevation.

Conclusions

In diabetic patients admitted with an ACS;

- Admission BSL was inversely correlated with platelet SNP responsiveness and directly correlated with O₂⁻ generation, but not with guanylate cyclase activity.
- Admission BSL also correlated with CK rise, CRP, neutrophil count and ADP enhanced O₂⁻ generation. Admission BSL was inversely correlated with ADMA and L-arginine levels.
- On multivariate analysis, admission BSL was a significant determinant of O₂⁻ levels and an inverse determinant of SNP response; with increasing age also a significant inverse determinant of SNP response.

Acute Resolution of hyperglycaemia normalizes platelet responsiveness to nitric oxide in diabetics with acute coronary syndromes

Background

We then explored whether improving glycaemic control had any effect on platelet NO[•] responsiveness. This was performed by randomizing diabetics with ACS to either intravenous infusions or subcutaneous injections of insulin. This experiment was designed to determine possible mechanisms to explain the results of the DIGAMI study, which showed that tight glycaemic control decreased mortality in diabetics admitted with an acute myocardial infarction (AMI).

Study

Sixty diabetic patients admitted with an ACS were randomized to aggressive IV insulin therapy or standard subcutaneous insulin therapy over 12 hours. The primary objective of this study, was to assess the acute effects of tight glycaemic control on platelet NO[•] responsiveness, O₂⁻ generation and guanylate cyclase activity. To explore all possible mechanisms of any observed effect δ ADMA, L- arginine and L- arginine/ADMA ratio, CRP, non esterified fatty acids (NEFA) and platelet aggregation were assessed.

Conclusions

In diabetic patients admitted with an ACS;

- Aggressive glycaemic control resulted in significantly enhanced platelet responsiveness to SNP, related to a reduction in O₂⁻ generation. No effect on guanylate cyclase activity was seen.

- A significant reduction in ADMA and L-arginine levels were observed with intravenous compared to subcutaneous insulin therapy.

Effects of an oral glucose load on platelet responsiveness to nitric oxide

Background

Elevated BSLs are associated with adverse cardiovascular outcomes in ACS in both diabetic and non-diabetic subjects. Hyperglycaemia is no longer considered an ‘innocent bystander’ however, having detrimental mechanistic implications on a number of protective biological systems. Indeed, harmful effects of sudden glucose loads such as post-prandial hyperglycaemia on the cardiovascular system are becoming apparent. While our previous experiments had focused on the impact of hyperglycaemia and its subsequent correction, on NO[•] bioavailability, our follow up experiments assessed the effect of an oral glucose load in normal subjects and patients with known cardiovascular disease. In light of the previous findings, we assessed the effects of an acute glucose load on platelet NO[•] responsiveness and vascular reactivity as assessed by applanation tonometry.

Study

8 healthy and 10 ‘high-risk’ cardiovascular subjects were enrolled into this study. A 75 gm glucose load was administered and baseline and 2 hour data were assessed. Platelet SNP responsiveness and O₂⁻ generation along with augmentation index (AIx) and ADP-induced platelet aggregation were assessed in all subjects. In the ‘high-risk’ cohort cGMP generation and insulin levels were also evaluated.

Conclusions

In the healthy volunteers;

- No significant change occurred in any of the variables assessed

In the 'high-risk' cohort

- 80% of patients had undiagnosed impaired glucose handling
- A significant increase in soluble guanylate cyclase activity was associated with an oral glucose load possibly related to an increase in insulin levels

Lack of inotropic effect of nitric oxide in rat papillary muscle.

Background

Our final set of experiments moved away from platelet studies to the assessment of myocardial contractility. Differing methodologies and animal models have resulted in variable results in the assessment of the inotropic effects of NO[•]. While the consensus is that NO[•] donors have a positive inotropic effect in low doses and are negatively inotropic at high doses, this has been difficult to show in the papillary muscle of most animal models. We addressed this issue in the papillary muscle of the Sprague-Dawley rat.

Study

The experimental protocol involved the assessment of NO[•] effects on contractility on the left ventricular muscle of the Sprague-Dawley rat. These studies initially involved assessing the most stable preparation, comparing a 10bpm with a 35bpm

protocol. The effect of the NO[•] donor SNP was then assessed not only on the isolated LV papillary muscle but also in the presence of β -adrenoceptor stimulation and an ischaemic/reperfusion model (15mins of anoxia/ 30 mins of reperfusion).

Conclusions

In the left ventricular papillary muscle of the Sprague-Dawley rat

- A stimulation frequency of 10bpm resulted in a more stable preparation over a 60 minute protocol, compared to 35bpm.
- The isolated rat papillary muscle had no measurable response to exogenous NO[•] donors. The inotropic effects of β -adrenoceptor stimulation and ischaemia-reperfusion were NO[•]-independent in this model.

While the field of NO[•] research is rapidly expanding, we forget that it is only just over 20 years since we were first aware of its existence. From Furchgott and Zawadzki's (Furchgott and Zawadzki, 1980) initial observation of an endothelium-derived relaxing factor (EDRF) to the subsequent studies by Palmer et al. (Palmer et al., 1987) and Ignarro et al. (Ignarro et al., 1987) showing that EDRF was indeed NO[•], many questions remain unanswered in relation to the 1992 'molecule of the year'. This thesis contributes to our understanding of NO[•] and its associated bioavailability in a number of tissues, particularly in relation to a high-risk cohort, the hyperglycaemic diabetic with an ACS.