EFFECTS OF NITRITE AND NITROXYL ON HUMAN VASCULAR AND PLATELET FUNCTION

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Dedicated to my parents,

my love Inara

and children

Zilya, Latipha and Temir

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Abstract

The identification of Nitric oxide (NO) as an endothelium-derived relaxing factor stimulated research into the physiology of this most important biological messenger, which maintains a healthy vascular endothelium and an anti-thrombotic intravascular environment.

Healthy endothelial cells constantly produce NO to create 'basal' vasorelaxation via the classical L-arginine/sGC/cGMP activation cascade. Under physiological conditions this NO pathway is the fundamental to maintenance of normal cardiovascular health, and conversely it is the substrate for development of many cardiovascular disease states, when the balance in this system becomes impaired.

Endothelial dysfunction, with the closely associated phenomenon of "NO resistance", can affect any NO-sensitive tissues including blood vessels and platelets, and is now believed to trigger atherogenesis and thrombogenesis.

Treatment of cardiovascular diseases associated with this phenomenon utilizing NO donors often has proved to be ineffective. Furthermore, treatment with organic nitrates is subject to development of nitrate tolerance, limiting efficacy of this class of agents. Several agents can ameliorate NO resistance over days or weeks, but there remains a problem in circumventing NO resistance in cardiac emergencies. In this thesis we demonstrate for the first time in humans partial circumvention of NO resistance with nitroxyl, a structural analogue of NO.

Additionally, another NO sibling nitrite (NO_2^-) has been attracting substantial interest in the last decade. Evidence has been accumulating that effects of nitrite are increased during hypoxia: - nitrite becomes a potent vasodilator and anti-aggregant when compared to normoxic environment. This is especially important in the situation of chronic tissue hypoxia or in acute vascular emergencies.

Key findings from the experiments in this thesis are:

1. Nitrite is a potent vasodilator compared to GTN: in general nitrite vasodilator effects are significantly potentiated in hypoxia in human saphenous veins. However, in human internal mammary arteries, nitrite-induced vasodilation is not potentiated under hypoxia. Prolonged exposure of human saphenous vein to nitrite does not cause tolerance or cross-tolerance to GTN. Nitrite effects in saphenous veins are substantially inhibited by ODQ, suggesting that they are largely mediated by soluble guanylate cyclase. Haemoglobin, myoglobin and red blood cells significantly increase hypoxic potentiation of nitrite vasodilator effects in human saphenous veins. Hypoxic potentiation of nitrite is diminished when saphenous vein intrinsic myoglobin is blocked by ferricyanide.

2. In platelets, the anti-aggregatory effects of nitrite are markedly and selectively potentiated under hypoxia. However, nitrite is subject to "NO resistance". Anti-aggregatory actions of nitrite are more potent in venous relative to arterial blood and correlate with (greater) deoxyhaemoglobin levels. Deoxyhaemoglobin is the primary nitrite reductase in blood. We have also presented evidence that continuous generation of NO from endogenous nitrite is important in homeostasis of platelet aggregability.

3. Nitroxyl is a more potent anti-aggregant than SNP. Anti-aggregatory effects of nitroxyl are partially sGC mediated. Nitroxyl partially circumvents the phenomenon of "NO resistance" in platelets. Nitroxyl is also a potent dilator of human saphenous veins. Its effects are not NO-mediated but partially sGC-mediated.

Declaration

I, Rustem Dautov, certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Rustem Dautov

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Publications, presentations and awards related to the work conducted towards this thesis

Publications related to the work conducted in this thesis:

1. **Dautov, RF**, Stafford, I, Liu, S, Cullen, H, Chirkov, YY & Horowitz, JD 2014, 'Hypoxic potentiation of nitrite effects in human vessels and platelets', *Nitric Oxide*, May 22. In press. Doi: 10.1016/j.niox.2014.05.005

2. **Dautov, RF**, Ngo, DT, Licari, G, Liu, S, Sverdlov, AL, Ritchie, RH, Kemp-Harper, BK, Horowitz, JD & Chirkov, YY 2013, 'The nitric oxide redox sibling nitroxyl partially circumvents impairment of platelet nitric oxide responsiveness', *Nitric Oxide*, Sep 4.

3. Maher, AR, Arif, S, Madhani, M, Abozguia, K, Ahmed, I, Fernandez, BO, Feelisch, M, O'Sullivan, A, Christopoulos, A, Sverdlov, AL, Ngo, D, **Dautov, R**, James, PE, Horowitz, JD & Frenneaux, MP 2013, 'Impact of chronic congestive heart failure on pharmacokinetics and vasomotor effects of infused nitrite', *Br J Pharmacol*, vol. 169, no. 3, Jun, pp. 659-670.

Presentations of work related to this thesis on international conferences:

1. **Dautov, RF**, Chirkov, YY, Ngo, Sverdlov, AL, Kemp-Harper, BK, Ritchie, RH, Horowitz, JD. The Nitric Oxide Redox Sibling Nitroxyl Partially Circumvents Platelet Nitric Oxide Resistance in Patients With Ischaemic Heart Disease. Presented at AHA Scientific Sessions 2012, Los Angeles, USA. *Circulation 2012; 126: A12677.*
2. **Dautov, RF**, Liu, S, BK, Chirkov, YY, Horowitz, JD. Anti-aggregatory effects of nitrite are augmented in venous, relative to arterial blood. Presented at World Congress of Cardiology 2014, Melbourne, Australia. *Global Heart Journal 2014; 9:1S:PT366.*

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List of abbreviations

ADP	Adenosine diphosphate
ACEi	Angiotensin converting enzyme inhibitor
ADMA	Asymmetric dimethylarginine
ARB	Angiotensin receptor blocker
ATP	Adenosine triphosphate
BH_4	Tetrahydrobiopterin
CABG	Coronary artery bypass grafting
cAMP	Cyclic adenosine monophosphate
CCB	Calcium channel blocker
cGMP	Cyclic guanosine monophosphate
CO_2	Carbon dioxide
CPTIO	Carboxy-PTIO
DDAH	Dimethylarginine dimethylaminohydrolase
DeoxyHb	Deoxygenated haemoglobin
DeoxyMb	Deoxygenated myoglobin
EDRF	Endothelium-derived relaxing factor
eNOS	Endothelial nitric oxide synthase
FMD	Flow mediated dilatation
GP	glycoprotein
GTN	Glyceryl trinitrate
Hb	Haemoglobin
HNO	Nitroxyl
IHD	Ischaemic heart disease
IMA	Internal mammary artery
iNOS	Inducible nitric oxide synthase
IPA/NO	Isopropylamine NONOate
KPSS	Kreb's solution with KCl substituted for NaCl on an equimolar basis
Mb	Myoglobin

mcg	microgram
mg	milligram
N_2	Nitrogen
NADPH	Nicotinamide adenine dinucleotide phosphate hydrogen
$NaNO_2$	Sodium nitrite
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NO_2^-	Nitrite
NOS	Nitric oxide synthase
O_2	Oxygen
O_2^-	Superoxide
PDE	phosphodiestherase
PKC	Protein kinase C
PKG	Protein kinase G
PLC	Phospholipase C
pO_2	Partial pressure of oxygen
PPP	Platelet-poor plasma
PRP	Platelet-rich plasma
RBC	Red blood cells
ROS	Reactive oxygen species
SD	Standard deviation
SEM	Standard error of the mean
sGC	Soluble guanylate cyclase
SNP	Sodium nitroprusside
SV	Saphenous vein
TXNIP	Thioredoxin-interacting protein
VASP	Vasodilator-stimulated phosphoprotein
vWf	Von Willebrand factor
XOR	Xanthine oxidoreductase