EFFECTS OF NITRITE AND NITROXYL ON HUMAN VASCULAR AND PLATELET FUNCTION

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A thesis submitted to the University of Adelaide as the requirement for the degree of Doctor of Philosophy
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. **Nitrooxyl** 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
May 2014
Publications, presentations and awards related to the work conducted towards this thesis

Publications related to the work conducted in this thesis:


Presentations of work related to this thesis on international conferences:


**Scholarship related to this thesis**

Australian Postgraduate Award, University of Adelaide, awarded in years 2011-2013
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Special thanks go to the University of Adelaide for providing me with a scholarship and comfortable research environment during my work towards this thesis.

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Last, but mostly important, I dedicate this thesis to my wonderful wife Inara! What a beautiful amazing creature she is! She helped me to go smoothly through my past 3 years helping to maintain a healthy gastric mucosa through some rough times. Her delicious borsch (rich in nitrates) at home could potentially be the secret. I saw her energy and enjoyment when she did her Honours degree and I know she wants to do a PhD next – Inara, I will fully support you in this great endeavor! A few weeks ago she gave me a true gift for persistence with my PhD - our son Temir! What a joy! One day he will read this page and write similar words on his own work to his children. I also dedicate this work to my daughters Zilya and Latipha and I hope this will encourage them towards great academic education!
# List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>ACEi</td>
<td>Angiotensin converting enzyme inhibitor</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin receptor blocker</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>BH₄</td>
<td>Tetrahydrobiopterin</td>
</tr>
<tr>
<td>CABG</td>
<td>Coronary artery bypass grafting</td>
</tr>
<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CCB</td>
<td>Calcium channel blocker</td>
</tr>
<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>CPTIO</td>
<td>Carboxy-PTIO</td>
</tr>
<tr>
<td>DDAH</td>
<td>Dimethylarginine dimethylaminohydrolase</td>
</tr>
<tr>
<td>DeoxyHb</td>
<td>Deoxygenated haemoglobin</td>
</tr>
<tr>
<td>DeoxyMb</td>
<td>Deoxygenated myoglobin</td>
</tr>
<tr>
<td>EDRF</td>
<td>Endothelium-derived relaxing factor</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilatation</td>
</tr>
<tr>
<td>GP</td>
<td>Glycoprotein</td>
</tr>
<tr>
<td>GTN</td>
<td>Glyceryl trinitrate</td>
</tr>
<tr>
<td>Hb</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HNO</td>
<td>Nitroxyl</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic heart disease</td>
</tr>
<tr>
<td>IMA</td>
<td>Internal mammary artery</td>
</tr>
<tr>
<td>iNOS</td>
<td>Inducible nitric oxide synthase</td>
</tr>
<tr>
<td>IPA/NO</td>
<td>Isopropylamine NONOate</td>
</tr>
<tr>
<td>KPSS</td>
<td>Kreb’s solution with KCl substituted for NaCl on an equimolar basis</td>
</tr>
<tr>
<td>Mb</td>
<td>Myoglobin</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>mcg</td>
<td>microgram</td>
</tr>
<tr>
<td>mg</td>
<td>milligram</td>
</tr>
<tr>
<td>N₂</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NADPH</td>
<td>Nicotinamide adenine dinucleotide phosphate hydrogen</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>Sodium nitrite</td>
</tr>
<tr>
<td>nNOS</td>
<td>Neuronal nitric oxide synthase</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>NO₂⁻</td>
<td>Nitrite</td>
</tr>
<tr>
<td>NOS</td>
<td>Nitric oxide synthase</td>
</tr>
<tr>
<td>O₂</td>
<td>Oxygen</td>
</tr>
<tr>
<td>O₂⁻</td>
<td>Superoxide</td>
</tr>
<tr>
<td>PDE</td>
<td>Phosphodiesterase</td>
</tr>
<tr>
<td>PKC</td>
<td>Protein kinase C</td>
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<tr>
<td>PKG</td>
<td>Protein kinase G</td>
</tr>
<tr>
<td>PLC</td>
<td>Phospholipase C</td>
</tr>
<tr>
<td>pO₂</td>
<td>Partial pressure of oxygen</td>
</tr>
<tr>
<td>PPP</td>
<td>Platelet-poor plasma</td>
</tr>
<tr>
<td>PRP</td>
<td>Platelet-rich plasma</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive oxygen species</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>sGC</td>
<td>Soluble guanylate cyclase</td>
</tr>
<tr>
<td>SNP</td>
<td>Sodium nitroprusside</td>
</tr>
<tr>
<td>SV</td>
<td>Saphenous vein</td>
</tr>
<tr>
<td>TXNIP</td>
<td>Thioredoxin-interacting protein</td>
</tr>
<tr>
<td>VASP</td>
<td>Vasodilator-stimulated phosphoprotein</td>
</tr>
<tr>
<td>vWf</td>
<td>Von Willebrand factor</td>
</tr>
<tr>
<td>XOR</td>
<td>Xanthine oxidoreductase</td>
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