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The opposing roles of NO and oxidative stress in cardiovascular disease

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ABSTRACT

Nitric oxide (NO) plays a pivotal role in the maintenance of cardiovascular homeostasis. A reduction in the bioavailability of endogenous NO, manifest as a decrease in the production and/or impaired signaling, is associated with many cardiovascular diseases including hypertension, atherosclerosis, stroke and heart failure. There is substantial evidence that reactive oxygen species (ROS), generated predominantly from NADPH oxidases (Nox), are responsible for the reduced NO bioavailability in vascular and cardiac pathologies. ROS can compromise NO function via a direct inactivation of NO, together with a reduction in NO synthesis and oxidation of its receptor, soluble guanylyl cyclase. Whilst nitrovasodilators are administered to compensate for the ROS-mediated loss in NO bioactivity, their clinical utility is limited due to the development of tolerance and resistance and systemic hypotension. Moreover, efforts to directly scavenge ROS with antioxidants has had limited clinical efficacy. This review outlines the therapeutic utility of NO-based therapeutics in cardiovascular diseases and describes the source and impact of ROS in these pathologies, with particular focus on the interaction with NO. Future therapeutic approaches in the treatment of cardiovascular diseases are highlighted with a focus on nitroxyl (HNO) donors as an alternative to traditional NO donors and the development of novel Nox inhibitors.

1. NO as a vaso- and cardio-protective molecule

The biologically active gas, nitric oxide (NO) plays a key role in maintaining cardiovascular homeostasis. NO is synthesised from L-arginine, by the activity of nitric oxide synthase (NOS) and its critical cofactor tetrahydrobiopterin (BH_4) [1]. Endothelial NOS (eNOS) is principally expressed in endothelial cells and cardiomyocytes [2] and is a key source of NO in the cardiovascular system. NO exerts many vasoprotective effects ranging from vasodilatation, anti-aggregatory and anti-inflammatory actions to inhibition of lipid oxidation and vascular smooth muscle proliferation [1]. At the level of myocardium, NO is a powerful antihypertrophic agent, exerting its actions independently of changes in loading conditions [3–5]. Moreover, NO enhances LV relaxation and improves left ventricular (LV) diastolic distensibility, favourable therapeutic properties [6,7].

Most of the actions of NO are mediated via its intracellular receptor, soluble guanylyl cyclase (sGC) and subsequent cyclic guanosine-3′,5′-monophosphate (cGMP) generation. sGC is an α/β hemoprotein and by binding NO to its heme group, sGC is stimulated to convert GTP to cGMP. This in turn activates cGMP-dependent protein kinase (cGK, also known as protein kinase G) [8], cGMP-regulated phosphodiesterases (PDEs) and cGMP-gated cation channels (CNGs) [9]. NO also exerts a range of sGC-independent effects, largely via S-nitrosylation of a range of proteins, with resultant alteration in their biological activities [10].

An impairment in endogenous NO signaling is associated with a multitude of cardiovascular diseases including hyperten-

Abbreviations: ACE, angiotensin-converting enzyme; ALDH, aldehyde dehydrogenase; ApoE$^{-/-}$, apolipoprotein E knockout; cGMP, cyclic guanosine-3′,5′-monophosphate; DEA-NO, diethylamine NONOate; eNOS, endothelial nitric oxide synthase; GTN, glyceryl trinitrate; HNO, nitroxyl; IPA-NO, isopropylamine NONOate; IPC, ischaemic preconditioning; I-R, ischaemia-reperfusion; ISDN, isosorbide dinitrate; ISMN, isosorbide mononitrate; LDLR$^{-/-}$, lipoprotein receptor-deficient; LV, left ventricular; NO, nitric oxide; Nox, NADPH oxidases; $ONOO^-$, peroxynitrite; PDE, phosphodiesterase; P-VASP, phosphorylated vasodilator-stimulated phosphoprotein; ROS, reactive oxygen species; RyR, ryanodine receptor; sGC, soluble guanylyl cyclase; SOD, superoxide dismutase; SNAP, S-nitroso-*N*-acetyl-D,L-penicillamine; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase.

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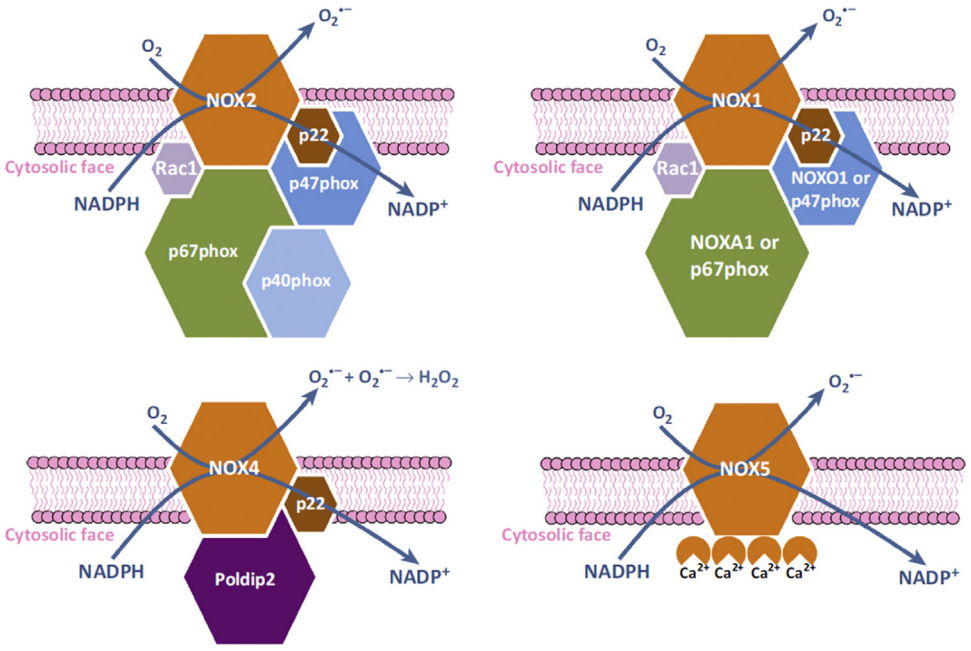

Fig. 1. Subunit composition of NADPH oxidase isoforms (Noxs) expressed in cells relevant to cardiovascular (patho)physiology. All four Nox isoforms are comprised of a catalytic 'Nox' subunit which may reside in the nuclear, endoplasmic reticulum (ER) or plasma membrane. For Nox 1, 2 and 4, additional subunits are required for full enzyme activity. By contrast, Nox5 is a 'stand alone' protein whose activity is regulated by Ca^{2+} binding. Reactive oxygen species (ROS) generated by Noxs are likely to be released either into the extracellular space or within the intraluminal space of cellular organelles. Whilst Nox1, 2 and 5 generate superoxide ($^{\bullet}O_2^-$), Nox4 appears to generate hydrogen peroxide (H_2O_2).
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sion [11,12], atherosclerosis [13] and diabetes [14], as well as heart failure [15,16]. Indeed in patients with cardiovascular disease, impaired NO signaling is an independent predictor of poor outcomes, including mortality [17,18]. Oxidative stress is a key contributor to compromised NO function in vascular and cardiac pathologies [19,20] and there has been much interest in the development of therapeutics which limit or prevent oxidative stress [21–23].

2. Cardiovascular disease and oxidative stress

Oxidative stress can be defined as an imbalance between the production and removal of reactive oxygen species (ROS) within a given cellular compartment such that these species accumulate to levels that disrupt normal cellular function. Oxidative stress within the vascular wall and myocardium is a hallmark of conditions such as hypercholesterolemia, hypertension, diabetes, atherosclerosis and cardiac hypertrophy; studies over the past two decades have provided strong evidence that upregulation of one or more members of the NADPH oxidase (Nox) family of ROS-producing enzymes is a major cause [19,24].

The Nox family is comprised of 7 members including Nox 1–5 and Duox 1 and 2 [2,22]. Of these, only Noxes 1, 2, 4 and 5 appear to be expressed in cells that are relevant to cardiovascular (patho)physiology i.e. endothelial cells, vascular smooth muscle cells, cardiac myocytes, adventitial and cardiac fibroblasts, macrophages, neutrophils and T cells [25,22,23] (Fig. 1). Nox enzymes generate ROS by abstracting electrons from the cytosolic substrate, nicotinamide adenine dinucleotide phosphate reduced (NADPH), transferring them through their membrane-bound catalytic subunit, and depositing them on oxygen molecules bound to the enzyme complex by a conserved heme domain [24,26]. In addition to the catalytic subunits, the different Nox isoforms rely to varying extents on a range of regulatory subunits including a membrane-bound stabilizing protein, $p22^{phox}$; cytosolic 'organizer' proteins such as $p47^{phox}$, $p40^{phox}$ or Noxo1; cytosolic 'activator' proteins including $p67^{phox}$ and Noxa1; and the G-proteins Rac1 or Rac2 [22,23,25].

The different Nox isoforms are localised to varying extents on the plasma membrane, particularly within lipid raft structures such as caveolae, endosomal membranes, mitochondrial membranes, and nuclear membranes [22]. Moreover, their topology is such that the ROS they generate are likely to be released either into the extracellular space, or within the intraluminal space of cellular organelles [23,24]. As we have highlighted in recent reviews on the topic, this is likely to have major ramifications for the range of cellular processes that certain Nox members may influence [23]. Specifically, Noxes 1, 2 and 5 are thought to generate superoxide as their primary ROS product. As a charged anion, superoxide is unlikely to cross lipid bilayers and so will most likely only interact with molecules present within the same sub-cellular compartment. Thus, while molecules present within the cytosol are likely to be spared from direct attack by superoxide, those present in the extracellular space or inside caveolae, endosomes or the nucleus, might be vulnerable. One of these vulnerable molecules is NO, which must traverse the extracellular space to mediate its protective actions on target cells such as smooth muscle cells, leukocytes and platelets. This consequence of the reaction between superoxide and NO is discussed further in the following section.

3. Interaction between NO and ROS

Cardiovascular homeostasis is achieved when there is a balance between the generation of NO and ROS, whereby NO protects against ROS-induced damage to macromolecules and ROS in turn limits the effects of NO. In the setting of cardiovascular disease, the excess generation of ROS, as described above, overwhelms the antioxidant defence mechanisms and can lead to impairment of the NO/sGC/cGMP signaling system. ROS can compromise NO function