NADPH oxidases in vascular biology and disease

C.P. Judkins,[1] H. Diep,[1] B.R.S. Broughton,[1] A.E. Mast,[1] G.J. Dusting,[2] C.G. Sobey[1] and G.R. Drummond ,[1]
[1]Department of Pharmacology, Monash University, Clayton, VIC 3800, Australia and [2]Bernard O'Brien Institute of Microsurgery, Fitzroy, VIC 3065, Australia. (Introduced by David Allen)

Until recently, reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), were thought to be mere by-products of cellular metabolism. However, it is emerging that ROS play key roles in a variety of normal cell signaling processes. Within blood vessels, ROS have been implicated in transcriptional and post-transcriptional regulation of gene expression, as well as activation and inactivation of kinases and phosphatases, respectively. ROS have even been touted as endothelium-derived relaxing factors. However, during vascular pathophysiological states such as hypertension, diabetes and hypercholesterolemia, the production of ROS in the blood vessel wall is markedly elevated such that cellular antioxidant defense mechanisms are overwhelmed and a state of 'oxidative stress' arises. Oxidative stress is thought to be an early trigger for many of the cellular processes involved in initiation of atherosclerotic lesions in the blood vessel wall including inactivation of the vasoprotective molecule, nitric oxide (NO), oxidation of lipoproteins, increased expression of adhesion molecules, and activation of pro-inflammatory signaling pathways. Hence, identification of the sources of excessive ROS production in blood vessels may lead to novel therapies to prevent atherosclerosis. There are a number of potential sources of ROS within vascular cells including endothelial nitric oxide synthase (eNOS), xanthine dehydrogenase/oxidase and components of the mitochondrial respiratory transport chain. However, for all of these enzymes, ROS production probably only occurs as a by-product of their normal catalytic function (e.g. mitochondrial respiration), or from a dysfunctional variant of the enzyme (e.g. in the cases of eNOS and xanthine dehydrogenase). Indeed, the only enzymes whose primary function appears to be the production of ROS are the NADPH oxidases, of which at least two isoforms (Nox2 and 4) are relevant to vascular (patho)physiology.

We have previously shown that, during normal physiology, blood vessels from mice express a single isoform of NADPH oxidase - Nox4 - which produces ROS, primarily in the form of H_2O_2, in a controlled manner for use in cell signaling processes (Ellmark *et al.*, 2005). However, in hypercholesterolemic apolipoprotein E-deficient (ApoE-/-) mice, the NADPH oxidase isoform expression profile in the vascular wall is substantially altered (measured by Western blotting) such that Nox4 levels in the aorta are diminished by ~80%, and Nox2 expression is elevated by up to 300% ($p < 0.05$; n=4). Immunofluorescence staining of aortic sections revealed that endothelial cells, adventitial fibroblasts and macrophages residing in the neointima were the major cellular sources of elevated Nox2 expression in ApoE-/- mice. Nox2/NADPH oxidases are likely to generate superoxide as opposed to H_2O_2. Indeed, L012-chemiluminescence studies revealed that blood vessels from ApoE-/- mice produce markedly more superoxide than those from wild-type controls ($p < 0.05$; n≥7). A likely implication of excessive superoxide production, particularly in endothelial cells, is the chemical inactivation of NO. This would not only be expected to reduce the bioavailability of this important vasodilatory and anti-inflammatory molecule, but would also give rise to the powerful oxidizing species, peroxynitrite ($ONOO^-$), thereby triggering/accelerating atherosclerosis by inducing a pro-oxidant and pro-inflammatory state in the blood vessel wall. We have recently shown that genetic deletion of Nox2 in ApoE-/- mice (*via* the generation of a novel strain of Nox2-/-/ApoE-/- double knockout mice) restores aortic superoxide production back to wild-type control levels ($p < 0.05$ *vs* ApoE-/-; n=4). Moreover, deletion of Nox2 markedly delayed the progression of atherosclerosis such that the percentage of the descending aorta covered by lesions in Nox2-/-/ApoE-/- double knockouts versus ApoE-/- mice was approximately 36% ($p < 0.05$; n≥3) and 48% at 12 and 19 weeks of age, respectively. Interestingly, by 25 weeks of age, differences in lesion size between the two strains were no longer apparent suggesting that the importance of Nox2/NADPH oxidase and oxidative stress to lesion development may diminish in the advanced stages of the disease.

In conclusion, we have provided evidence that elevated Nox2/NADPH oxidase activity is not merely a symptom of atherosclerosis but a major contributing factor to lesion development, at least in the early and intermediate stages of the disease. These findings highlight Nox2/NADPH oxidase as a possible therapeutic target for vascular disease.

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