Endothelial NADPH oxidase 2: when does it matter in atherosclerosis?

Judith Haendeler*, Anna Eckers, Margarete Lukosz, Klaus Unfried, and Joachim Altschmied

IUF - Leibniz Research Institute for Environmental Medicine, Auf’m Hennekamp 50, Duesseldorf 40225, Germany

Online publish-ahead-of-print 23 February 2012

This editorial refers to ‘Endothelial-specific Nox2 overexpression increases vascular superoxide and macrophage recruitment in ApoE−/− mice’ by G. Douglas et al., pp. 20–29, this issue.

Reactive oxygen species (ROS) act as a double-edged sword in numerous cardiovascular conditions. In addition to their known detrimental effects on all cellular macromolecules, referred to as oxidative stress, lower amounts of ROS also directly modify molecules and thereby modulate their functions - this process has been termed redox signalling. Both oxidative stress and redox signalling have been implicated in the process of atherosclerosis. The sources of cellular ROS include mitochondria, NADPH oxidases (Noxs), dysfunctional nitric oxide synthases, xanthine oxidase, and other oxygenases. Among these, the Noxs are unique, since their primary function is to produce ROS.1,2 The Nox family comprises seven members, Nox1–5 and DUOX1–2, each based on a distinct core catalytic subunit.3,4 Nox1, 2, 4, and 5 are expressed in cardiovascular cells, although Nox5 is only found in humans and not in rodents.5 An involvement of Nox2 in atherosclerosis has been suspected. It was the first NADPH oxidase to be identified and is responsible for the phagocytic oxidative burst of neutrophils. It has subsequently been found to be expressed at lower levels in endothelial cells, cardiomyocytes, fibroblasts, vascular smooth muscle cells, monocytes, and macrophages, the latter two being central players in atherogenesis.6 However, findings in mice with global Nox2 deficiency on an atherosclerosis-prone ApoE−/− genetic background and fed a high-fat diet are contradictory. Kirk et al.7 found no protection against atherosclerosis when examining aortic sinus sections, whereas Judkins et al.8 demonstrated a 50% reduction in lesion area in the region between the aortic arch and iliac bifurcation. However, using global Nox2 deficiency, it is impossible to identify the cell types relevant for atherosclerosis. Thus, it is important to investigate the role of Nox2 in a cell-specific manner.

Douglas et al. describe ApoE−/− mice with endothelial-targeted overexpression of human Nox2 (Nox2-Tg ApoE−/−).9 The key findings of this study are that increased endothelial ROS production leads to enhanced vascular cell adhesion molecule-1 levels and increased monocyte/macrophage recruitment at 9 weeks of age. However, this initial increase in monocyte/macrophage recruitment did not alter plaque progression at 16 and 24 weeks of age. Furthermore, endothelial-specific overexpression of human Nox2 did not influence angiotensin II-driven atherosclerosis.7,8 At a first glance, this study suggests that endothelial-specific, Nox2-dependent ROS production is only responsible for the initiation of atherosclerosis but not involved in its progression.

These findings raise several questions. Why does the initial increased monocyte/macrophage recruitment not result in plaque progression or altered plaque composition? One could speculate that the invasion process of the monocytes is not altered since the authors observed no difference in oxidized low-density lipoprotein (oxLDL) and endothelial cell apoptosis in Nox2-Tg ApoE−/− mice and ApoE−/− animals. Thus, the initial recruitment of monocytes is increased by the Nox2-mediated ROS formation because an inflammatory response is mimicked by the overexpression of Nox2 in the endothelium. However, this does not result in changes in lipid composition and therefore not in plaque progression.

The picture might be completely different with a high-fat diet, which was not investigated by the authors. Since the high-fat diet stimulates lesion growth by elevated lipid levels, the increased monocyte recruitment in the Nox2-Tg ApoE−/− mice could, under these conditions where oxLDL levels may be higher, result in accelerated plaque progression. The authors demonstrate further that the endothelial ROS production persists for 24 weeks in the Nox2-Tg ApoE−/− mice, although they do not show whether the ROS levels are different between 9 and 24 weeks of age. However, it seems that these permanently elevated Nox2-derived ROS are not sufficient to drive plaque progression. This would indicate that during plaque progression, ROS from other sources within the endothelium may be more important, such as uncoupled endothelial nitric oxide synthase, which has been found to be elevated in atherosclerosis progression.8 Finally, it is also possible that endothelial-derived ROS from Nox2 are only responsible for the initiation of plaque formation and not relevant for further progression, although the Nox2 enzyme is up-regulated in atherosclerosis.6
Why does increased endothelial-derived ROS production not result in changes in angiotensin II-driven atherosclerosis? Unfortunately, the authors did not measure angiotensin II effects on vascular ROS formation in ApoE−/− and Nox2-Tg ApoE−/− mice. It is known from previous studies that angiotensin II increases ROS formation in vascular cells; however, it is unclear whether further elevated ROS levels would be observed in the angiotensin II-treated Nox2-Tg ApoE−/− animals compared with the non-infused mice. One could argue that the levels of ROS produced from the endothelium are already saturated and cannot be further increased. Moreover, the initial recruitment of monocytes is not shown in treated and untreated mice. Thus, one cannot completely rule out a role for endothelial-derived ROS formation in angiotensin II-driven atherosclerosis.

What are the implications of this study for atherosclerosis in humans? Activation of Nox2 in the endothelium increases the monocyte/macrophage recruitment and thereby the initiation of plaque development. Thus, reducing this initial Nox2 activation could delay plaque development. However, one has to take into account that a global inhibition of Nox2 may result in reduced infectious defence. The absence of a role for endothelial, Nox2-derived ROS in plaque progression is supported by findings from Sorescu et al. who found that Nox2 and p22phox are predominantly co-localized in macrophages of human atherosclerotic specimens and that mRNA levels for p22phox and Nox2 correlate with lesion severity. Moreover, increased p22phox has been detected in endothelial cells, smooth muscle cells, infiltrating macrophages, and adventitial fibroblasts of human atherosclerotic coronary arteries. The present study did not investigate the levels of p22phox; however, since the authors demonstrate no atherosclerosis progression in Nox2-Tg ApoE−/− mice compared with ApoE−/− mice, an increase in p22phox levels, as it is found in advanced atherosclerotic plaques in humans, is not expected.

In summary, the study by Douglas et al. importantly contributes further to our understanding of initiation of atherosclerosis and its progression. Overexpression of Nox2 in the endothelium results in increased ROS formation. This ROS formation indeed leads to activation of the endothelial cells and increased monocyte recruitment. However, as long as the lipid composition does not change, the macrophage function is not altered, and inflammatory markers are not elevated, an increase in endothelial-derived ROS formation alone will not result in atherosclerotic plaque progression.

Conflict of interest: none declared.

References