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Endothelial NADPH oxidases: friends or foes?

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Reactive oxygen species (ROS) can cause oxidative stress and might promote cardiovascular diseases [22]. On the other hand, increasing evidence supports beneficial effects of ROS as important signaling molecules in the cardiovascular system [5]. NADPH oxidases are main sources of ROS in the vessel wall. Substantial efforts have been made in the 1990s and mainly in the last decade to identify different NADPH oxidase (Nox) isoforms in the vessel wall [3]. The major endothelial Nox isoforms are Nox2 and Nox4 [26]. They might be friends or foes of endothelial function.

Initial studies focussed on the detection and regulation of the classical NADPH oxidase complex containing the catalytic subunit gp91phox (Nox2) in endothelial cells [10, 13, 15, 18, 29]. This complex was first discovered in granulocytes and consists of four subunits, membrane-bound subunits Nox2 and p22phox and cytosolic subunits p47phox and p67phox. Phosphorylation of cytosolic subunits leads to translocation from the cytosol to the membrane and activation of the complex. Furthermore, its activity requires small G proteins like Rac. Of critical importance is the subunit Nox2 which mediates the electron transfer from NADPH to oxygen, thus causing superoxide anion formation. However, the impact of endothelial-specific overexpression of Nox2 on vascular structure and function in vivo has not been resolved.

In the current issue of *Basic Research in Cardiology*, Murdoch et al. [27] report that endothelial-specific overexpression of Nox2 in transgenic mice enhances angiotensin II-induced endothelial dysfunction and contributes to vascular remodeling and hypertension. The mice had a twofold increase in endothelial Nox2 expression. This cell-specific increase was compensated under basal conditions with respect to vascular NADPH oxidase activity, endothelial function, and blood pressure. However, the potent vasoconstrictor angiotensin II potentiated vascular ROS formation and endothelial dysfunction in these animals. This is in agreement with previous in vitro studies showing a low expression of Nox2 in healthy endothelial cells, but an induction of Nox2 in response to pathophysiological stimuli like angiotensin II or oxidized low-density lipoprotein (oxLDL) in human endothelial cells [31, 32, 37]. Exposure to angiotensin II potentiated NADPH oxidase activity in Nox2 transgenic animals compared to wild-type controls. This was accompanied by an impaired endothelial function in response to angiotensin II. These data strongly support a crucial role of endothelial Nox2-generated superoxide anions in endothelial dysfunction. Especially interesting are the data analyzing the blood pressure in Nox2 transgenic mice in response to low and high doses of angiotensin II. Low angiotensin II dosages had no effect on hemodynamics in wild-type animals, but increased systolic, diastolic, and mean blood pressure in mice overexpressing endothelial Nox2. Higher dosages of angiotensin II raised systolic and mean blood pressures to a similar degree, like in the wild-type, but did not potentiate the response in transgenic Nox2 animals. Only the diastolic values were increased in these animals compared to wild-type. A potential explanation could be the increasing stimulation of AT_2 receptors in response to higher angiotensin II dosages. While the rise in blood pressure is mainly mediated by AT_1 receptors, AT_2 could act in counterbalancing the effects. Similar dose-dependent findings have been observed after induction of Nox2 by

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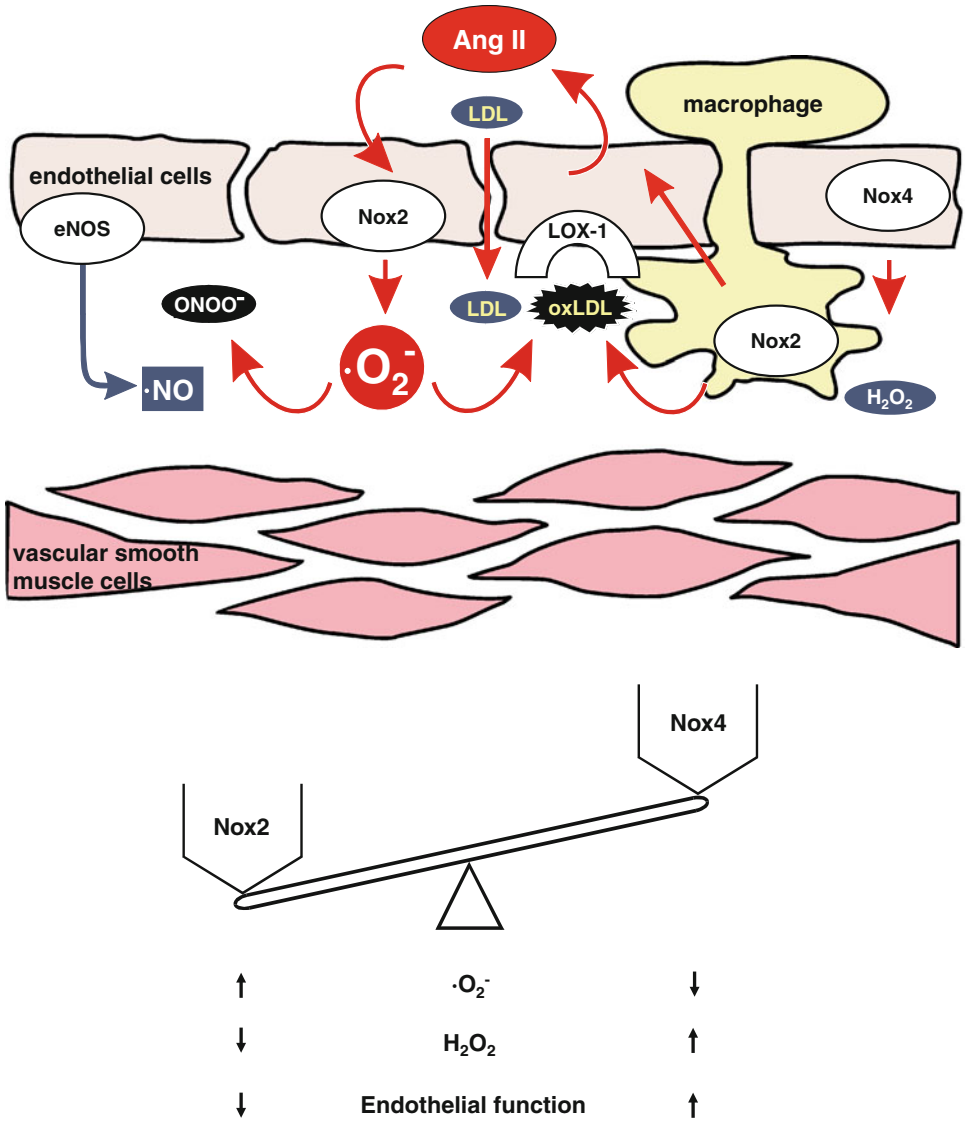

Fig. 1 Nox2 promotes a vicious cycle leading to endothelial dysfunction. The Nox2 complex releases superoxide anions ($\cdot O_2^-$). They can inactivate NO, formed by eNOS, thus leading to ONOO· formation and endothelial dysfunction. In addition, low-density lipoprotein (LDL) is oxidized to oxLDL. The oxLDL uptake is preferentially mediated by the LOX-1 receptor in endothelial cells. This vicious cycle can be potentiated by angiotensin II (Ang II). In contrast, the endothelial Nox isoform Nox4 mainly generates H_2O_2

angiotensin II in human endothelial cells [32]. Finally, Murdoch et al. show that this increased activation of ROS in response to angiotensin II affects structural aortic remodeling and activates signaling cascades like the ERK pathway.

An important player in this context could be NO. Endothelial cell in vivo are constantly exposed to hemodynamic forces like shear stress leading to increased eNOS expression and NO formation. While short-term application of shear stress activates an Nox2-containing complex, long-term application of high laminar shear stress causes downregulation of Nox2 and Nox4 [9, 11]. The flow-dependent regulation of both endothelial Nox complexes seems to involve shear stress-dependent release of NO. The crucial role of NO on endothelial function as shown in the Nox2-overexpressing mice supports this concept.

Superoxide anions can inactivate NO in a very rapid manner leading to formation of the cytotoxic peroxynitrite. Therefore, a well-controlled balance of ROS and NO is necessary to control endothelial function. Another deleterious effect of ROS is the oxidation of LDL. The oxLDL is taken up by endothelial receptors like LOX-1. Because oxLDL itself can further increase Nox2 expression and activity, this could lead to a pro-atherosclerotic vicious cycle (Fig. 1). In later stages of atherosclerosis, infiltrating monocytes/macrophages with a large number of Nox2 complexes might even further accelerate this vicious cycle.

Because the basal expression of Nox2 is low in endothelial cells, its role in endothelial dysfunction has been questioned. The Nox4 complex is much higher expressed in endothelial cells [1, 12]. However, the role of Nox4 in the endothelium is not yet resolved. Increasing evidence suggest that the Nox4 complex under normal conditions mainly forms hydrogen peroxide (H_2O_2) instead of superoxide anions [33, 34]. Hydrogen peroxide acts in a dose-dependent manner as a double-edged sword on the blood pressure and endothelial function. Under physiological conditions low doses of H_2O_2 have been described to lower blood pressure. Under certain conditions, H_2O_2 is even discussed as EDHF. Murdoch et al. did not observe changes in endothelial Nox4 expression in the mice overexpressing Nox2. However, the concept of a vasoprotective role of Nox4 is also supported by recent data from the group of Ajay Shah in the novel model of mice with transgenic endothelial overexpression of Nox4 [30]. In these mice, the endothelial function is improved due to increased H_2O_2 production and H_2O_2-induced hyperpolarization. This does not involve altered nitric oxide bioactivity. Nevertheless, endothelial-specific Nox4 mice have a lower blood pressure than wild-type animals [30]. Therefore, the majority of data currently support the model that Nox4 is rather friend, Nox2 rather foe of endothelial function. This view might be wrong under certain pathophysiological conditions. A variety of in vitro studies have shown that high dosages of H_2O_2 are deleterious to endothelial cells and induce apoptosis [6]. Therefore, a strong Nox4 expression and activity or a putative "uncoupling" of Nox4 switching its activity back from H_2O_2 to superoxide anion formation might tip the balance in the other direction.

Several additional insights came from transgenic models overexpressing different Nox isoforms in specific cell types in the past years. Nox1 overexpression in vascular smooth muscle cells potentiated angiotensin II-induced hypertension and vascular hypertrophy [7]. Overexpression of p22phox in vascular smooth muscle cells was counterbalanced by increased NO formation and ecSOD expression [23]. Bendell et al. [4] could show a crucial role of endothelial Nox2 in vascular oxidative stress and hemodynamics. Their data strongly support the recent findings of Murdoch et al. [27]. However, angiotensin II-dependent chronic hypertension in mice overexpressing human renin was not affected by the Nox2 complex [36]. Overexpression of a constitutive active human mutant of Rac1 in vascular smooth muscle cells caused increased superoxide anion and peroxynitrite formation and hypertension [14]. For Nox4, first data from knockout animals do not support a basal effect of Nox4 on blood pressure, but suggest additional roles of Nox4 in heart failure, cardiac angiogenesis, and stroke [20, 21, 38]. In addition, with the help of knockout models, crucial roles for Nox2 and Nox1 have been shown in renovascular hypertension and angiotensin II-induced hypertension [19, 24]. These data suggest a role of Nox2 in the development of hypertension.

Inhibition of NADPH oxidase expression and activity might have even important clinical implications. AT_1 receptor blockers and statins have been shown to reduce Nox2 expression in vessels of patients with coronary artery disease [31, 32]. Both medications can improve endothelial and cardiac function [8, 16, 25, 28, 35]. This could involve decreased NADPH oxidase activity and improved redox state in the vessel wall [2]. In contrast, most clinical trials with synthetic vitamins have failed to mediate protective effects in cardiovascular diseases and stroke [17]. Potential reasons might include the fact that the vitamin concentration in some trails was too low, or that a single vitamin in comparison to a combination of vitamins C and E was not efficient. Furthermore, the typical Western-type diet is supplemented with a variety of antioxidants. Finally, the vitamin concentration might not reach a protective concentration in the target organ after oral uptake. Therefore, a major aim of the vascular redox field is the development of novel antioxidants or isoform-specific Nox inhibitors. They should directly target individual Nox isoforms to reach local antioxidative protection in the affected target tissue.

In conclusion, increasing evidence supports a role of Nox4 as friend and Nox2 as foe of endothelial function. In this context, the study of Murdoch et al. is an important proof of concept of the critical role of the deleterious effects of the Nox2 complex in endothelial dysfunction and hypertension. Nox2 is back to front.

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Conflict of interest The author declares that he has no conflict of interest.

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