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Forum Editorial

Redox Regulation of Endothelial Function

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ENDOTHELIAL CELLS play an important role in vascular homeostasis. The endothelium functions as the barrier between blood and tissue and thus serves the regulation of emigration of blood cells and transport of tissue nutrients, but also regulation of vascular tone. Therefore, endothelial dysfunction is associated with various diseases, such as diabetes, hypertension, atherosclerosis, and other chronic inflammatory diseases. Among factors that damage the endothelium, reactive oxygen species (ROS) are increasingly recognized as the major culprits responsible for compromising endothelial cell function by oxidative modification of cellular components, but also by interfering with the action of endothelial products necessary for regulation of vascular homeostasis (9). In addition, ROS influence endothelial barrier function and leukocyte adhesion and transmigration (8).

Deleterious effects of ROS on endothelial cells are mediated not only by oxidative modification of proteins, lipids, carbohydrates, and DNA, but also by interfering with signal transduction pathways (5). One consequence of ROS attack is the modulation of signaling and transcriptional events in endothelial cells. A number of transcription factors were shown to be oxidation-sensitive. These include nuclear factor-κB, AP-1, and peroxisome proliferator-activated receptors, all of which play important roles in inflammation-triggered intracellular signaling (7). In addition, ROS affect mitogen-activated protein kinase signaling pathways (7), and especially modulation of the Ras-ERK signaling by ROS has recently been shown to be important in inducing apoptosis (2). The influence of ROS on these signaling pathways has important implications in the response of endothelial cells toward injury, but also in cell growth, death, and survival (5). The mechanisms of modulation of signaling pathways by ROS have been reviewed previously (5, 7, 8).

Endothelial cells can be challenged by ROS that are produced by activated inflammatory cells, smooth muscle cells, and endothelial cells themselves. The sources for ROS are enzymes that catalyze redox reactions, such as mitochondrial respiratory chain enzymes, cell membrane-associated enzymes such as NADPH oxidase, and cytosolic enzymes involved in lipid metabolism. The mechanisms of generation of ROS, modulation of signaling pathways, and protection of the endothelium from oxidative damage are discussed in this issue of *Antioxidants & Redox Signaling.*

One of the most important enzymes in the generation of free radicals is NADPH oxidase, which consists of several subunits. This enzyme has been discovered and well studied in granulocytes, where it plays a key role in antibacterial defense. NADPH oxidase is assembled and activated in granulocytes in response to bacterial products. More recently, NADPH oxidase has been shown to play a major role also in endothelial cells. NADPH oxidase is believed to be the major source for superoxide anion ($O_2^{\cdot -}$) in endothelial cells, and its activity is regulated by various stimuli, such as oxidized low-density lipoprotein, angiotensin II, and endothelin-1, for all of which a role in atherogenesis has been suggested. Thus, activation of endothelial NADPH oxidase could play an essential role in the pathogenesis of atherosclerosis. The structure and function of this enzyme in endothelial cells are discussed in a review article by Rueckschloss *et al.* in this issue (11). In addition, recent advances in identification of new subunits of NADPH oxidase with potentially novel functions are discussed.

Both enzymatic and free radical-induced modification of lipids leads to the formation of signaling mediators that play important roles in cell activation and communication. In her review, O'Donnell describes the enzymes and mechanisms involved in the formation of eicosanoids in endothelial cells (10). Among these enzymes, various forms of cyclooxygenases play an important role in the constitutive production of prostaglandins, as well as their induced formation during inflammatory reactions and activation of endothelial cells. Furthermore, the tight regulation of arachidonate-utilizing enzymes like lipoxygenases, as well as cytochrome P enzymes such as thromboxane synthase and prostacyclin synthases, is extremely important to produce adequate amounts of respective lipid mediators. On the other hand, free radical-induced peroxidation of arachidonic acid leads to the formation of isoprostanes, prostaglandin-like substances, which can stimulate endothelial cells to induce various signaling pathways that may interfere with inflammatory reactions (4). Moreover, endothelial cell function can also be influenced by free fatty

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acids derived from triglycerides, and thus free fatty acid overload could contribute to vascular complications by altering intracellular signaling and nitric oxide (NO) production (3).

Free radical species are thought to have a major impact on the regulation of other molecules that are involved in signaling in endothelial cells. One molecule that plays an important role in vascular homeostasis is NO. However, the bioavailability of NO is compromised in settings of oxidative stress, mainly through inactivation by $O_2^{\cdot -}$. In their review, Thomas *et al.* describe NADPH oxidase and xanthine oxidase as the major enzymes responsible for production of $O_2^{\cdot -}$ in endothelial cells (12). Furthermore, under certain circumstances, $O_2^{\cdot -}$ can be produced by nitric oxide synthase (NOS) itself, a process known as "NOS uncoupling." Other substances that interfere with NO bioavailability are lipid peroxyl radicals produced by the action of lipoxygenases. In addition to chemical inactivation, oxidized low-density lipoprotein can decrease NO generation by displacement of endothelial NOS from caveoli. Another mechanism of regulation of NO production by ROS is through increased endothelial NOS expression induced by hydrogen peroxide. In addition, it has been shown that myeloperoxidase contributes to an impairment of NO bioavailability during inflammation. Finally, treatment strategies to prevent reduction of NO bioavailability and to increase the production of this important molecule are discussed. Similar to NO, carbon monoxide produced by heme oxygenase has been suggested to play an important protective role in blood vessels. In addition to its antiinflammatory activity, heme oxygenase-derived carbon monoxide affects vascular endothelial growth factor production and thus influences angiogenic activity of endothelial cells (6).

In order to be protected against overwhelming free radical production and to keep the balance between pro- and antioxidant mechanisms inside the cell, various enzymatic systems have evolved. Protective mechanisms in endothelial cells against free radical-induced damage and dysregulation of signaling pathways by ROS are mediated by selenium-dependent enzymes. In their review, Brigelius-Flohé *et al.* discuss glutathione peroxidases and thioredoxin reductases as major protective enzymes in endothelial cell physiology (1). The influence of these enzymes on signaling events in endothelial cells is mediated by a direct effect on the function of redox-sensitive transcription factors, thus influencing gene expression, but also by influencing the bioavailability of messenger molecules such as NO and eicosanoids. Thus, a potential involvement of malfunction of these enzymes in the pathogenesis of inflammatory diseases such as atherosclerosis is suggested.

Taken together, free radicals play an essential role in endothelial cell function and vascular physiology. A delicate balance between pro- and antioxidative mechanisms needs to be maintained for physiologic function of the endothelium. Hyperactivation of free radical-generating mechanisms, oxidative modification of signaling molecules, and a resulting influence on intracellular signaling pathways leads to endothelial dysfunction and development of pathophysiological features such as hypertension, atherosclerosis, and diabetes. Further research is needed in order to devise strategies for intervention, both on the level of free radical generation and on the level of modification of cellular components by ROS.

ABBREVIATIONS

NO, nitric oxide; NOS, nitric oxide synthase; $O_2^{\cdot -}$, superoxide anion; ROS, reactive oxygen species.

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