NAD(P)H oxidase: where there’s smoke, there’s fire

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IN INDUSTRIALIZED NATIONS such as the United States, tobacco represents the leading cause of deaths, with a majority of these being associated with cardiovascular disease, rather than cancer (2, 20). The vasculature develops an inflammatory and thrombogenic phenotype in response to many cardiovascular risk factors, including hypertension, hypercholesterolemia, and smoking, and these alterations have become recognized as key components in the pathogenesis of cardiovascular disease (16, 21). One of the earliest responses of vascular endothelium exposed to cardiovascular risk factors is the development of oxidative stress, which is known to mediate many aspects of the endothelial dysfunction/activation. Although the enzymatic sources of these reactive oxygen species (ROS) have been extensively characterized in the settings of hypercholesterolemia and hypertension, less is known about the oxidative stress that is induced in the vasculature by cigarette smoke (CS).

CS can be divided into two phases, the tar phase and the gas phase. Both phases contain high concentrations of ROS, including nitric oxide, peroxynitrite, peroxynitrate, and free radicals of organic compounds (1, 14). The ROS in the gas phase are highly reactive, with their short half-lives potentially limiting the area over which they can exert damage. However, in the setting of cardiovascular disease, a potentially more important source of ROS is derived from the substances in CS that have the capacity to stimulate intracellular ROS production (19). For example, pulmonary artery endothelial cells exposed to CS release elevated levels of superoxide (8), and one of the thiol-reactive stable compounds found in CS, acrolein, is also capable of increasing superoxide release from endothelial cells. Substances such as acrolein (also a component of secondhand smoke) may be carried throughout the systemic circulation and act in organs remote to the lungs (the primary site of exposure) to stimulate ROS generation (5). Interestingly, the levels of serum protein-acrolein adducts are higher in patients with cardiovascular risk factors associated with oxidative stress, such as diabetes (11). If substances in CS can promote an oxidative stress in multiple organs or vessels, then it is plausible that this could, in turn, result in a proinflammatory and thrombogenic phenotype throughout the vasculature. This could lead to a systemic inflammatory environment that could feed the development of atherosclerosis and could also generate propagate local inflammation in the arteries prone to atherosclerotic lesion development. In fact, CS extract facilitates atherogenesis in hyperlipidemic rabbits through an oxidant-dependent mechanism (22). In this issue of American Journal of Physiology-Heart and Circulatory Physiology, Orosz et al. (12) hypothesize that the water-soluble constituents of CS—which would be the most likely components to reach the systemic circulation—promote vascular formation of ROS that sustains leukocyte recruitment in major arteries via the generation of inflammatory mediators.

In their study, Orosz et al. (12) examined both carotid and coronary arteries from rats exposed to CS (“in vivo” exposure model) and arteries incubated with CS extract (“in vitro” model) and found that both modes of smoke exposure generated higher levels of superoxide and hydrogen peroxide when compared with control vessels (12). Although relatively few studies have investigated which enzymes are responsible for the generation of ROS from the vasculature in response to CS, Jaimes et al. (8) demonstrated that superoxide was released from NAD(P)H oxidase, but not xanthine oxidase, endothelial nitric oxide synthase, or mitochondrial respiration, in pulmonary artery endothelial cells exposed to CS. The in vitro approach used by Orosz et al. (12) allowed for the targeting of specific pathways to determine both the enzyme(s) responsible for the elevated ROS production and their role in some of the inflammatory pathways initiated by this oxidative stress. They were able to confirm the participation of NAD(P)H oxidase in the vascular oxidative stress induced in carotid and coronary arteries by CS (12). Interestingly, staining with dihydroethidium suggested that both endothelial cells and smooth muscle cells contribute to the enhanced generation of superoxide. Furthermore, they went on to demonstrate that serum from a smoking donor could induce hydrogen peroxide production from carotid arteries, underlining the systemic nature of this response, and the role of stable substances derived from CS that can stimulate cellular ROS generation. Although they did not determine whether α,β-unsaturated aldehydes were responsible, Orosz et al. (12) concluded that nicotine was unlikely to contribute to the oxidative stress since nicotine itself failed to invoke the same response.

The pathophysiological relevance of these findings was highlighted by the observation that CS-induced impairment of endothelium-dependent vasodilation was attenuated by blocking the production of ROS, in particular, from NAD(P)H oxidase (12). This established a link between smoking-induced, NAD(P)H oxidase-mediated oxidative stress and the resultant endothelial dysfunction. To develop this further, Orosz et al. (12) found that levels of proinflammatory cytokines, which are normally found in atherogenic environments in these arteries, were increased in arteries exposed to smoke and that this was associated with activation of NF-κB, a redox-sensitive transcription factor. Interestingly, inhibition studies in the arteries incubated with CS extract suggested that these responses were mediated by NAD(P)H oxidase-derived hydrogen peroxide. Although it remains unclear whether these cytokines perpetuated the oxidative stress that was observed in these vessels, they are also capable of promoting oxidative stress and stimulating the expression or upregulation of adhesion molecules on endothelial cells. In fact, CS promotes superoxide-dependent leukocyte adhesion in both arterioles and venules within 30 min of exposure (10). In agreement with these findings in the microvasculature, Orosz et al. (12) demonstrated that monocyte adhesion to endothelial cells, a critical
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step in atherogenesis, was enhanced by exposure of the endothelial cells to CS extract (12). Furthermore, this was true for monocyte adhesion to carotid and coronary arteries, and all of these could be abrogated by the inhibition of NAD(P)H oxidase, further strengthening the evidence of a role for this enzyme in the generation of a proatherogenic environment by CS, at least in endothelial cells.

The in vitro studies by Orosz et al. (12) suggest that the activation of circulating cells by CS is not necessary for the induction of inflammation in the vessel wall; however, it remains plausible that blood cells contribute to the altered vascular phenotype in vivo. Leukocytes and platelets are activated and exhibit elevated oxidative stress in response to several cardiovascular risk factors. In contrast, there are several studies demonstrating that CS actually reduces the oxidant-producing capacity of neutrophils while elevating adhesion molecule expression (17, 18), although less is known about monocytes and lymphocytes. With the recent abundance in the literature on the interactions between the inflammatory and thrombogenic pathways induced in many disease states, it would be interesting to determine whether platelets, which are activated by CS (4, 6) and can themselves produce superoxide from NAD(P)H oxidase (3, 7, 13), participate in the vascular oxidative stress and inflammation generated during smoking.

Despite the fact that oxidative stress has been implicated in the pathogenesis of cardiovascular disease, antioxidant-based therapies have proven disappointing to date. Improvement of delivery or the type of antioxidant may prove beneficial, but it is also important to take into consideration that cardiovascular patients often present with more than one risk factor, which may require a multitargeted approach to therapy. Smoking not only synergizes with other cardiovascular risk factors to invoke exaggerated inflammatory responses but, in fact, exacerbates the seriousness of other risk factors. For example, smoking enhances the levels of LDL and increases the oxidative modification and nitration of LDL (23) and can also increase insulin resistance. These properties may render treatment of the other risk factors more difficult, and yet, the harmful vascular effects of CS can be attenuated almost immediately upon withdrawal of cigarette smoking (9, 15). The study by Orosz et al. (12) illuminates the role of NAD(P)H oxidase in smoking-induced inflammation in the walls of large arteries and provides the basis for the study of models where smoking is combined with other cardiovascular risk factors to gain better insights into the underlying mechanisms of oxidative stress that mediate cardiovascular disease in the smoking population.

GRANTS

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REFERENCES