Cigarettes and the wages of sn-2: oxidized species of PAF in smoking hamsters.

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J Clin Invest. 1997;99(10):2300-2301. https://doi.org/10.1172/JCI119407.

Editorial

Cigarette smoking is associated with an excess of roughly 400,000 deaths annually from cardiovascular disease in the United States alone. There is a clear relationship between the degree and duration of exposure to cigarettes and the incidence of cardiovascular events. Furthermore, a decline in risk follows cessation of smoking, although residual effects may persist for a decade (1). However, the relative importance of the mechanisms which mediate this cardiovascular hazard is poorly understood. Thus, cigarette smoke will damage endothelial cells in culture and it activates platelets and leukocytes, which may, in turn release growth factors and proinflammatory cytokines. Smoke also enhances the ability of such cells to adhere to endothelium. Cigarette smoking causes endothelial dysfunction and an increase in antibodies directed against oxidized LDL in patients with hypercholesterolemia (2). Passive smoking may also result in endothelial dysfunction (3). Several lines of evidence point to excessive free radical generation in smokers and endogenous levels of vitamin C are low, presumably reflecting consumption of antioxidant defenses. Interestingly, administration of vitamin C reverses endothelial dysfunction in smokers (4).

In this issue of the *Journal*, Lehr et al. (5) report on a novel class of mediators which may modulate some of the adverse effects of smoking on the vasculature. Platelet-activating factor (PAF) is a bioactive lipid synthesized by many cells, including leukocytes and endothelial cells, in addition to platelets. It appears to mediate its effects via a single G-protein–coupled receptor (GPCR) and its effects may be regulated by a family of inactivating PAF acetylhydrolases (6, 7). Specific antagonists of this receptor are available; however, they have yet to establish clinical efficacy. McIntyre and colleagues have previously reported the partial characterization of PAF-related oxidized species which appear to mediate biological effects via the PAF receptor (6). Although these compounds remain to be structurally characterized, both phospholipase A_2 and PAF acetylhydrolase will degrade their biological activity, implying the importance of substituents at the sn-2 position. The present study takes advantage of a well characterized model, the smoking hamster. Blood was obtained after the hamsters smoked a single cigarette. Fractions eluting with the chromatographic retention time of such PAF-like lipids enhance leukocyte adherence to a gelatinized surface in vitro. This bioactivity is blocked by a PAF receptor antagonist. The same fractions also stimulate platelet–leukocyte interactions in vitro and the consequent release of proinflammatory cytokines. Again, these effects were blocked by a PAF antagonist. Potentially, interactions between platelets, leukocytes, and endothelial cells might also result in formation of transcellular products of arachidonic acid, which might further augment inflammatory and thrombogenic consequences of cigarette smoking.

How relevant are these observations to the cardiovascular consequences of smoking in humans? Lehr and colleagues note a similar suppressive effect of the PAF antagonist on adherence of leukocytes stimulated in vitro by 10^{-7} M PAF and in the samples obtained after smoking in two hamsters (5). From this result they suggest that circulating concentrations of PAF-like lipids correspond to 10^{-7} M PAF/ml of plasma. However, quantitative bioassay is an inexact science. For example, such an approach overestimated the circulating concentrations of prostacyclin by several orders of magnitude. Nonetheless, the authors do augment their ex vivo observations with in vivo experiments. Using a dorsal skinfold chamber in the hamster, they record that smoking results in leukocyte attachment to the microvasculature. Consistent with their observations in vitro, administration of a PAF antagonist reduces this response to smoking in vivo. Dosing with the antioxidant vitamin C (10 g/kg) similarly impairs smoking-induced leukocyte–endothelial interactions in vivo. This implies that the PAF-like oxidized lipids, rather than PAF itself, are being blocked by the antagonist. Application of the ex vivo bioassay of these lipids suggests that vitamin C reduces their formation in the smoking hamsters.

While it is difficult to relate effects on cytokine generation and leukocyte interactions with platelets and vascular cells to clinical events, such as myocardial infarction and stroke, such factors are thought to be of relevance to atherogenesis and thrombosis. Similarly, issues of species, model dependence and concentration–effect relationships constrain the extension of these observations to the human. Certainly, the message is not that smokers can relax if they take vitamin C.

Nonetheless, the observations of Lehr et al. are intriguing (5). They suggest that smoking-related free radical production might generate a family of PAF derivatives which exhibit biological activity of potential relevance to the cardiovascular sequelae of this dangerous habit. Perhaps these compounds may be of particular importance in Japan, where smoking is prevalent and up to 4% of the population lack the enzyme PAF acetylhydrolase (8). Interestingly, the current observations complement the recognition that free radicals may also catalyze the formation of a series of eicosanoid isomers (the isoeicosanoids) some of which also exhibit biological activity. Several of these compounds have already been structurally characterized and vitamin C reduces their augmented formation in apparently healthy humans who smoke cigarettes. Indeed, isoeicosanoids may also act as incidental ligands, in this case at eicosanoid GPCRs. It is interesting to note that PAF receptor antagonism in the present studies reduced adhesive interactions, partially, but not completely, both in vitro and in vivo. Perhaps the oxidized derivatives of PAF and arachidonic acid are but some of the bioactive lipids which mediate these cardiovascular responses to smoking.

Free radicals may modify directly both DNA and cellular proteins. Recently, the possibility that they might function as intracellular signals and regulate gene expression has attracted attention (9, 10). The present studies add to increasing evidence that lipid peroxidation may also be of functional impor-

J. Clin. Invest.

0021-9738/97/05/2300/02 $2.00
Volume 99, Number 10, May 1997, 2300–2301

tance in diseases characterized by excessive generation of reactive oxygen species.

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