Cigarette Smoking and Endothelial Damage

Ben-Ami Sela MD

Institute of Chemical Pathology, Sheba Medical Center, Tel Hashomer, Israel
Section of Clinical Biochemistry, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Israel

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Cardiovascular mortality and morbidity are estimated to be increased some fourfold in smokers, yet even individuals who inhale cigarette smoke passively have a 35% increased risk of heart attack [1]. The figure of a 50% increase in stroke mortality and even greater likelihood of peripheral arterial occlusive disease among smokers are equally alarming. Cigarette smoking has also been shown to be linked directly to the risk of aortic aneurysm independently of atherosclerosis [2]. Cadmium accumulation in aortas of smokers, which is known to impair the viability of aortic smooth muscle cells, is implicated in abdominal aortic aneurysm, acknowledged as a smoking-related disorder [3]. The exact mechanism by which smoking enhances atherosclerosis and thrombosis remains uncertain. It involves adverse effects of the various tobacco combustion products absorbed into the circulation. Nicotine, the addictive component of tobacco, activates the sympathoadrenal system to increase levels of catecholamines, thus accelerating heart rate and peripheral vasoconstriction. Smoking facilitates platelet release of the pro-aggregatory thromboxane A2, which is also a potent vasoconstrictor. The platelets of smokers seem to have reduced sensitivity to the anti-aggregatory effect of prostacyclin-PG12, known also to be a smooth muscle relaxant [4]. Low density lipoprotein is more actively oxidized in smokers, thus enhancing macrophage ingestion of oxidized LDL to form foam cells and promoting monocyte adhesion and migration into the subintimal space. Other than the effect of smoking on blood lipid profile, by decreasing high density lipoprotein-cholesterol and elevating triglycerides and very low density lipoprotein, tobacco use significantly elevates blood levels of coagulatory fibrinogen. Smoking reduces plasminogen and causes marked inhibition of substance P-induced tissue plasminogen activator [5]. Smoking alters rheology and shear forces at the vascular surface, which up-regulates leukocyte adhesion molecules such as selectin P. In particular, smoking increases the release from leukocytes of soluble intercellular cell adhesion molecule-1 [6], which may in part be responsible for the increased monocyte-endothelium cell adhesion [7].

Ample experimental evidence is accumulating to suggest that smoking-induced endothelial changes are early events heralding the subsequent vascular damage. In the current issue of *IMAJ*, Sela et al. [8] discuss their study on the concomitant contribution of polymorphonuclear leukocytes to both systemic oxidative stress and inflammation in smokers. Most previous reports emphasized the injury to lung tissue conferred by either inflammation or oxidative damage. Yet, the current report analyzed PMN leukocytes at the systemic level, and found that after being primed *in vivo*, these cells release superoxide anion radicals at a faster rate in smokers as compared to non-smokers. Moreover, this study demonstrates a markedly diminished antioxidant capacity of smokers' plasma as expressed by lower reduced-glutathione, while increased oxidized glutathione species in smokers are apparent. Another interesting outcome of this study is the contribution of PMN leukocytes to chronic inflammation in smokers, and the demonstration of a leukoclastic activity in sera of smokers. Noteworthy is the finding by Sela and colleagues that heavy smoking does not correlate with the degree of oxidative stress or that of inflammation, implying that other genetic or adaptive parameters are involved. Indeed, it was shown that smoke-induced oxidative stress is more pronounced in individuals whose cardio-vascular system is lacking adaptation to smoke [9].

Increased generation of oxygen free radicals by PMN leukocytes was originally proposed by Kalra et al. [10] to be responsible for enhanced risk of smoking-related diseases. Numerous studies have indicated an increased white blood cell count in smokers mainly due to neutrophilia, associated with a shortening of the mean transit time of PMN leukocytes from bone marrow to the circulation. Such immature PMN leukocytes are larger and less deformable, thus they are preferentially sequestered in pulmonary microvessels and damage pulmonary capillary endothelium by the release of oxygen radicals and hydrolytic enzymes such as elastase [11].

A large body of evidence points to the endothelium as a primary target of insult, preceding plaque formation and vascular injury. One of the best available measures of endothelial damage is the raised levels of von Willebrand factor, a procoagulatory protein produced specifically by endothelial cells [12]. Lipid peroxides

LDL = low density lipoprotein

PMN = polymorphonuclear

raised in smokers' serum are correlated with damaged endothelial cells to promote release of vWf. However, nicotine and thiocyanate, two major constituents of cigarette smoke, added *in vitro* at physiologic levels to endothelial cells from human umbilical vein did not induce vWf release, implying that deleterious effects exerted by smoking on the endothelium are multifactorial and complex [13].

Exposure to some of the toxic compounds contained in tobacco smoke, i.e., reactive oxygen species, polycyclic aromatic hydrocarbons such as benzo[α]pyrene, cadmium and nicotine, induces cytoprotective stress proteins in endothelial cells. Thus, in an adaptive response to smoke components, heat shock proteins, especially HSP70, emerge in endothelial cells, in addition to heme oxygenase-1; eventually, loss of mitochondrial membrane potential occurs, leading to cell death by apoptosis or necrosis [15]. Recently, Wang and co-workers [14] demonstrated that human umbilical venous endothelial cells *in vitro* respond dramatically to a 3 hour exposure to cigarette smoke, by a 3.6-fold increase of caspase-3 activity. This enzyme leads to an increased proportion of endothelial cells undergoing apoptotic changes detected by DNA fragmentation *in situ*. Apoptotic endothelial cells are likely to detach from the vessel wall, an event relevant to thrombogenesis. Although limited apoptosis of endothelial cells is physiologically essential to maintain their functional integrity, excessive apoptosis imposed on the endothelium by chronic smoking could promote atherogenesis [16].

The exact entry or binding of nicotine or its major metabolite, cotinine, to the endothelium cells might involve interaction with the nicotine-sensitive acetylcholine receptor, recently located on endothelial cells [17]. Indeed, by blocking *in vitro* nAChR with hexamethonium, the increase in DNA synthesis seen in endothelial cells treated with nicotine was abolished. Similarly, when nAChR was neutralized *in vivo* by hexamethonium in a nicotine-treated mouse hind-limb ischemia model, the increase in capillary formation evident by nicotine treatment was eliminated. One of the interesting effects of nicotine on the endothelium that was recently unveiled involves the increased synthesis of vascular endothelial growth factor, a specific mitogen normally expressed in arteries in low levels. In an intact carotid artery perfusion culture model, nicotine was shown to induce a significant increase in VEGF expression [17]. Previously it was demonstrated that nicotine induces the activity of various atherosclerosis-related endothelial genes including endothelial nitric oxide synthase, angiotensin-1 converting enzyme, platelet-derived growth factor, and basic fibroblast growth factor. The particular interest in VEGF stems from its contribution to both increased endothelial permeability and turnover, which are relevant to increased angiogenesis attributed to nicotine, and the fact that VEGF is highly expressed in human atherosclerotic plaques compared to its extremely low level in healthy arteries [18]. By increasing the permeability of endothelial cells, the transport of LDL and fibrin into the vessel wall is enhanced. A newly discovered aspect of VEGF effect on vascular permeability involves its regulation of endothelial tight junction assembly by reducing the expression of occludin and disrupting ZO-1 and occludin organization [19].

A striking direct effect of nicotine causing endothelial dysfunction even after smoking one cigarette was demonstrated by ultrasound assessment of flow-mediated dilation of the brachial artery [20]. Smoking one cigarette (1 mg nicotine, 12 mg tar) caused a 55% decline in FMD 10 minutes later, whereas a 35% decline was evident when a 1 mg dose of nicotine was applied by nasal spray. Such a profound effect of nicotine on endothelial function could be reversed by super-perfusion with superoxide dimutase, suggesting a causal role of oxidative stress [21]. Cigarette smoke impairment of endothelium-dependent vasodilation was measured as FMD of the brachial arteries in healthy smokers as compared to non-smokers [22]. While baseline diameters of brachial arteries were not different between smokers and non-smokers (4.4 versus 4.2 mm), FMD was significantly reduced in smokers (0.5%) versus non-smoking controls (6.1%). This study [22] settled some inconsistency with regard to nitric oxide generation in smokers vis à vis enzyme protein expression in the serum and the actual enzymatic activity of NOS. As reported also by others, the actual mass protein of nitric oxide synthase was surprisingly increased in smokers, perhaps as a feedback response to diminished nitric oxide caused by smoking. Yet, net NOS activity was largely reduced in smokers due to the presence of inhibitors, lack of substrate and co-factors, or the production of non-functional enzyme, eventually uncoupling the expression of eNOS gene and its activity. It is noteworthy that while nitric oxide is continuously produced by the arterial endothelium, its baseline production by venous endothelium is much lower. When shear stress, bradykinin or the calcium ionophore A23187 are applied, vein endothelium is induced to produce more nitric oxide. Even in this regard the mean maximum relaxation measured under isometric tension in the saphenous vein in response to bradykinin is twice as high in non-smokers (approximately 54%) than in smokers (about 27%), which further underlines tobacco effect on venous endothelium dysfunction [23].

In addition to chemical and enzymatic changes induced in the endothelium by smoking, the actual original evidence on endothelial injury was obtained from morphologic changes in umbilical arteries of smoking women. These included irregular appearance of the endothelium with formation of blebs or microvillous-like projections [24]. Such morphologic alterations were associated with reduced endothelial production of prostacyclin as well as enhanced adhesion of platelets. Of the numerous biomarkers for oxidative damage to the endothelium, the F_2-isoprostanes, products of free radical-catalyzed lipid peroxidation of arachidonic acid, were found increased in smokers [25]. F_2-isoprostanes are known to activate the thromboxane A2 receptor and are efficient vasoconstrictors, and their elevated levels in smokers both in their free and esterified forms are significantly reduced following 2 weeks of abstinence [26]. Using a system of isolated porcine aortic rings

vWf = von Willebrand factor
nAChR = nicotine-sensitive acetylcholine receptor
VEGF = vascular endothelial growth factor

FMD = flow-mediated dilation
NOS = nitric oxide synthase

exposed to cigarette smoke, the addition of ifetroban reversed almost completely the effects of cigarette smoke exposure on vascular function, while indomethacin was only partially effective. Ifetroban is a blocker of the thromboxane A2/prostaglandin endoperoxidase H_2 receptor, while indomethacine is a cyclo-oxygenase inhibitor. Thus, it is suggestive that in addition to cyclooxygenase-derived vasoconstrictive eicosanoids, molecules such as F_2-isoprostanes, which are generated after cigarette smoke exposure, are important mediators of endothelial dysfunction [26].

Smoking-induced damage to the endothelium relates only to the "superficial" facet of the blood vessel wall, which is adversely affected by tobacco use as a whole. Numerous vascular effects triggered by chronic smoking have been documented. To mention some – intima-media thickening of the carotid arteries, reduced distensibility in medium-sized and large elastic arteries causing systemic artery stiffening, and progressive vasculitis in medium and small limb vessels in thromboangiitis obliterans, almost exclusively a disease of the heavy smokers. These are just some of the solid reasons for smokers to kick their habit.

References

1. Glantz SA, Parmley WW. Even a little second hand smoke is dangerous. *JAMA* 2001;286:462–3.
2. Lee AJ, Fowkes FGR, Carson MN, et al. Smoking, atherosclerosis and the risk of abdominal aortic aneurysm. *Eur Heart J* 1997;18:671–6.
3. Abu-Hayyeh S, Sian M, Jones KG, et al. Cadmium accumulation in aortas of smokers. *Arterioscler Thromb Vasc Biol* 2001;21:863–7.
4. Rangemark C, Wennmalm A. Smoke-derived nitric oxide and vascular prostacyclin are unable to counteract the platelet effect of increased thromboxane formation in healthy female smokers. *Clin Physiol* 1996;16:301–15.
5. Newby DE, Wright RA, Labinjoh C, et al. Endothelial dysfunction-impaired endogenous fibrinolysis, and cigarette smoking: a mechanism for arterial thrombosis and myocardial infarction. *Circulation* 1999;99:1411–15.
6. Mazzone A, Cusa C, Mazzucchelli I, et al. Cigarette smoking and hypertension influence of nitric oxide release and plasma levels of adhesion molecule. *Clin Chem Lab Med* 2001;39:822–6.
7. Adams MR, Jessup W, Celermajer DS. Cigarette smoking is associated with increased human monocyte adhesion to endothelial cells: reversibility with oral L-arginine but not vitamin C. *J Am Coll Cardiol* 1997;29:491–7.
8. Sela S, Shurtz-Swirski R, Awad J, et al. The involvement of peripheral polymorphonuclear leukocytes in the oxidative stress and inflammation in cigarette smokers. *IMAJ* 2002;4:1015–19.
9. Glantz SA, Parmley WW. Passive smoking and heart disease: mechanisms and risk. *JAMA* 1996;273:1047–53.
10. Kalra J, Chaudhary AK, Prasad K. Increased production of oxygen free radicals in cigarette smokers. *Int J Exp Pathol* 1991;72:1–7.
11. Terashima T, Klut ME, English D, et al. Cigarette smoking causes sequestration of polymorphonuclear leukocytes released from the bone marrow in lung microvessels. *Am J Respir Cell Mol Biol* 1999;20:171–7.
12. Blann AD, Kirkpatrick U, Devine C, et al. The influence of acute smoking on leucocytes, platelets and the endothelium. *Atherosclerosis* 1998;141: 133–9.
13. Blann AD, McCollum CN. Adverse influence of cigarette smoking on the endothelium. *Thromb Haemost* 1995;70:707–11.
14. Wang J, Wilcken DEL, Wang XL. Cigarette smoke activates caspase-3 to induce apoptosis of human umbilical venous endothelial cells. *Mol Gen Metabol* 2001;72:82–8.
15. Vayssier-Taussat M, Camilli T, Aron Y, et al. Effects of tobacco smoke and benzo[α]pyrene on human endothelial cell and monocyte stress responses. *Am J Physiol Heart Circ Physiol* 2001;280:H1293–300.
16. Haunstetter A, Izumo S. Apoptosis: basic mechanisms and implications for cardiovascular disease. *Circ Res* 1998;82:1111–29.
17. Conklin BS, Zhao W, Zhong D-S, et al. Nicotine and cotinine up-regulate vascular endothelial growth factor expression in endothelial cells. *Am J Pathol* 2002;160:413–18.
18. Celletti FC, Waugh JM, Ambile PG, et al. Vascular endothelial growth factor enhances atherosclerotic plaques progression. *Nature Med* 2001;7:425–9.
19. Wang W, Dentler WL, Borchardt RT. VEGF increases BMEC monolayer permeability by affecting occluding expression and tight junction assembly. *Am J Physiol Heart Circ Physiol* 2001;280:H434–40.
20. Neunteufl T, Heler S, Kostner K, et al. Contribution of nicotine to acute endothelial dysfunction in long-term smokers. *J Am Coll Cardiol* 2002;39: 251–6.
21. Mayhan WG, Sharp GM. Superoxide dismutase restores endothelium-dependent arteriolar dilatation during acute infusion of nicotine. *J Appl Physiol* 1998;85:1292–8.
22. Baruna RS, Ambrose JA, Eales-Reynolds L-J, et al. Dysfunctional endothelial nitric oxide biosynthesis in healthy smokers with impaired endothelium-dependent vasodilation. *Circulation* 200;104:1905–10.
23. Higman DJ, Greenhalgh RM, Powell JT. Smoking impairs endothelium-dependent relaxation in saphenous vein. *Br J Surg* 1993;80:1242–5.
24. Pittilo RM. Cigarette smoking, endothelial injury and cardiovascular disease. *Int J Exp Pathol* 2000;81:219–30.
25. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers: smoking as a cause of oxidative damage. *N Engl J Med* 1995;332:1198–203.
26. Raij L, DeMaster EG, Jaimes EA. Cigarette smoke induced endothelium dysfunction: role of superoxide anion. *J Hypertens* 2001;19:891–7.

Correspondence: Dr. B-A. Sela, Institute of Chemical Pathology, Sheba Medical Center, Tel Hashomer 52621, Israel.
Phone: (972-3) 530-2553
email: benamis@post.tau.ac.il

Capsule

Human stem cell research approved

The Israel Bioethics Committee has endorsed legislation that would allow both human embryonic stem cell research and the derivation of embryonic cells from unwanted embryos created during fertility treatments, and subsequent research into therapeutic cloning.

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