Endothelial dysfunction in human hand veins is rapidly reversible after smoking cessation

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Division of Clinical Pharmacology, Department of Medicine, Stanford University School of Medicine, Stanford 94305-5130; and Geriatric Research, Education and Clinical Center, Veterans Affairs Palo Alto Health Care System, Palo Alto, California 94304

Moreno, J R., Heitor, Stephan Chalon, Akinori Urea, Oranee Tangphao, Ademola K. Abiose, Brian B. Hoffman, and Terrence F. Blaschke. Endothelial dysfunction in human hand veins is rapidly reversible after smoking cessation. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1040–H1045, 1998.—Cigarette smoking has been shown to impair endothelium-dependent dilation in arteries. We tested the hypothesis that cigarette smoking also impairs endothelium-dependent venodilation and evaluated changes in this response after smoking cessation in a time-course study using the dorsal hand vein technique. Dose-response curves were constructed in smokers and nonsmokers by infusing bradykinin (1–278 ng/min), an endothelium-dependent vasodilator, and nitroglycerin (0.006–1583 ng/min), an endothelium-independent vasodilator, into hand veins preconstricted with the selective α1-adrenergic agonist phenylephrine. The maximal venodilation induced by bradykinin was 89 ± 5% in controls (n = 16) and 61 ± 7% in smokers (n = 18, P = 0.02). No difference in nitroglycerin-induced venodilation was observed between the two groups. Confusion of L-arginine (0.33 mg/min) markedly improved the bradykinin-induced venodilation in smokers (52 ± 7 to 90 ± 9%, P < 0.01). After acute smoking cessation (n = 7), restoration to normal bradykinin-induced venodilation was observed within 24 h, whereas no change in the response to a maximally effective dose of nitroglycerin (1583 ng/min) was detected. In a human vein model appropriate for testing vascular functional alterations, this study demonstrates that smoking impairs endothelium-dependent venodilation in heavy smokers. Moreover, this endothelial dysfunction appears to be rapidly reversible after smoking cessation. This model may be useful in studies evaluating mechanisms of endothelial dysfunction and interventions to modify it.

endothelium; vasodilation; bradykinin

The endothelium plays a major role in modulating vascular tone by synthesizing and metabolizing vasoactive substances, including nitric oxide (NO), a powerful vasodilator (33). Over the past decade, a large number of clinical studies have assessed endothelial function in health and disease (6). Many of these studies have tested the ability of normal endothelium to release NO in response to local infusion of endothelium-dependent agonists such as acetylcholine and bradykinin in brachial arteries. With the use of this invasive pharmacological tool, endothelial dysfunction, as evidenced by an impaired availability of NO, has been identified in patients with hypertension (29), hypercholesterolemia (9), and diabetes (18). This aspect of endothelial dysfunction is increasingly regarded as a key link between cardiovascular risk factors and atherothrombotic disease (31).

More recently, several studies have investigated the effects of smoking on vascular function in nonatherosclerotic human arteries. Impaired basal NO-mediated vasodilation, demonstrated by a decrease in response to local infusion of Nω-monomethyl-L-arginine (L-NMMA), an inhibitor of NO production, has been a consistent finding in smokers (19, 22). However, studies on the effects of long-term cigarette smoking on blood flow response to intrabrachial artery infusion of muscarinic agonists (acetylcholine, methacholine) have found depressed (13, 14) or neutral effects (17, 22) when compared with controls. This heterogeneity in flow augmentation responses to endothelium-dependent agonists may relate to the differing study protocols. Importantly, differences in duration of smoking cessation (which varied from 8 to 24 h) before experimental investigations raise the possibility of a rapid recovery of the stimulated release of NO after smoking cessation. There has been considerable interest in the effects of smoking on endothelial control of vascular tone in both arteries in vivo (7, 13, 14, 17, 19, 22, 35) and vein rings in vitro (15, 16), but less information is available regarding the effects of smoking on venous endothelial function in vivo. The venous bed can be explored in humans using the dorsal hand vein method (1–3), a technique appropriate not only for testing functional alterations caused by cigarette smoke components and a normal endothelium but also for studying at regular intervals the influence of acute smoking cessation on vascular responsiveness.

The present study was designed to test the hypothesis that smoking impairs endothelium-dependent venodilation in humans and to evaluate the time course of this deficit after acute smoking cessation. Endothelium-dependent relaxation was investigated using the venodilator response to bradykinin, a more effective releaser of NO in human veins than acetylcholine (32).

METHODS

Subjects

Eighteen smokers (mean age ± SD: 37 ± 6 yr, range 28–53 yr, 15 men and 3 women) and 16 healthy nonsmokers (mean ± SD: 32 ± 7 yr, range 22–47 yr, 7 men and 9 women) were involved in this study. A complete physical examination,
electrocardiogram, and routine laboratory tests were performed. Subjects were excluded if they had a blood pressure in excess of 140/90 mmHg, a fasting glucose level >115 mg/dl, a serum total cholesterol concentration >200 mg/dl, or a serum creatinine level >1.0 mg/dl. Smokers who smoked an average of 30–90 cigarettes/day for 16 ± 7 yr were asked to continue smoking before the study (last cigarette 1–1.5 h before infusion of vasodilators). Each participant gave written informed consent; the protocol was approved by the Stanford Administrative Panel on Human Subjects in Medical Research (Stanford, CA).

Dorsal Hand Vein Technique

The dorsal hand vein technique, previously modified by Aellig (1), has been described in detail as used in our laboratory (28). Briefly, a 23-gauge butterfly needle was inserted into a suitable vein on the back of the hand, with the arm positioned at an upward angle of 30° to allow the complete emptying of the veins. A tripod, holding a linear variable differential transformer (LVDT; Shaevitz Engineering, Pennsauken, NJ), was mounted on the back of the hand with the central aperture of the LVDT, containing a movable metal core, at a distance of 10 mm downstream from the tip of the needle. The signal output of the LVDT, which is linearly proportional to the vertical movement of the core, gives a measure of the diameter of the vein. Readings were made under a congestive pressure of 40 mmHg by inflating a blood pressure cuff placed on the upper portion of the arm under study. Results are presented as normalized dose-response curves in which the diameter of the vein during saline infusion was defined as 100% dilatation. The vein was preconstricted to 20% of the baseline size by infusing increasing doses of phenylephrine, a selective α1-adrenergic receptor agonist (99–3,166 ng/min). The infusion rate of phenylephrine inducing 80% vasoconstriction was kept constant during the entire study, rate, and this degree of constriction was defined as 0% dilatation for the purpose of subsequent calculations. The vasodilator response expressed in this study was calculated as a percentage of the range between 0 and 100% dilatation. Drugs were infused using a Harvard infusion pump (Harvard Apparatus, South Natick, MA) at a flow rate of 0.3 ml/min. Blood pressure and heart rate were monitored in the opposite arm with a Dinamap Blood Pressure Monitor (Critikon, Tampa, FL).

Study Design

Protocol 1: vascular responsiveness. Because venorelaxation was assessed in our studies after preconstriction with phenylephrine, preliminary experiments were conducted to compare the sensitivity to the α1-adrenoceptor agonist between the two groups. Full dose-response curves to phenylephrine (99–3,166 ng/min) were generated in six smokers and eight nonsmokers.

To evaluate vascular smooth muscle (endothelium-independent) venodilation, full dose-response curves to nitroglycerin (0.006–1,583 ng/min), an NO donor, were performed in six subjects from each group. Endothelium-dependent venodilation was induced by infusing increasing doses (1–278 ng/min) of bradykinin (18 smokers, 16 nonsmokers). In separate studies, the venodilation induced by a single dose of bradykinin (278 ng/min) was determined (10 smokers, 7 nonsmokers). These additional studies were performed to exclude any potentially confounding effects of desensitization induced by cumulative doses of bradykinin (11).

We tested the effects of L-arginine, the precursor of NO, on bradykinin-induced relaxation in 10 smokers and 7 nonsmokers. A low rate of infusion of L-arginine (0.33 mg/min) was selected, based on a pilot study in healthy controls showing that this infusion rate of L-arginine alone did not induce venodilation. In these studies, the response to a single-dose infusion of bradykinin (278 ng/min) was determined. After a 35-min infusion of phenylephrine alone (to wash out the bradykinin), L-arginine was added to the infusion for 10 min, and the response to a second single dose of bradykinin (278 ng/min) was measured.

Protocol 2: acute smoking cessation. Seven heavy smokers (20 cigarettes/day or more for at least 10yr) who had demonstrated impaired venodilation to bradykinin in the vascular responsiveness study within the previous 4 wk were enrolled in a smoking cessation study. They were admitted to the General Clinical Research Center and discontinued smoking at the time of admission. To ensure that these subjects were not smoking after admission, tobacco exposure was monitored by taking samples of expired air every 2 hr while the subjects were awake and once during the night to be analyzed for CO by a CO monitor (Bedfont Scientific).

Dorsal hand vein studies were performed shortly after admission (day 0) and then at 24-h intervals during the smoking cessation study. Additional studies were performed at the time of discharge, when the subjects (n = 6) had resumed smoking. Each hand vein study included a full dose-response curve to bradykinin (1–278 ng/min) followed, after appropriate wash out, by a single dose of nitroglycerin (1,583 ng/min) infused for 5 min in the same preconstricted vein.

Materials

Drugs were diluted in normal saline. The following drugs were used: phenylephrine hydrochloride (1% injection) and nitroglycerin (0.5% injection) (American Reagent Laboratories, Shirley, NY); bradykinin acetate salt (Sigma, St. Louis, MO), used under investigational new drug no. 32226 and L-arginine (10% injection; Kabi Pharmacia, Clayton, NC).

Data Analysis

Results are expressed as means ± SD. Depending on the protocol, two approaches were used to interpret and summarize individual dose-response curves to bradykinin. In the initial vascular responsiveness study, the maximal drug responses were not systematically determined; therefore, data from smokers and nonsmokers were fitted to a quadratic function (Sigma Plot; Jandel, San Rafael, CA). An estimation of the individual response for the infusion rate of 278 ng/min was obtained from the fitted curve, and mean values of each group were compared by an unpaired two-tailed t-test. Complete individual dose-response curves to bradykinin generated in the smoking cessation study were fitted to a four-parameter logistic equation using the ALLFIT program (21). This iterative curve-fitting program provides an estimate of the maximal response (Emax) and of the dose producing the half-maximal response (ED50). A log transformation was performed on individual ED50 values, and the geometric mean was calculated as the anti-log of the mean of log values. The same four-parameter logistic equation was used to analyze dose-response curves to phenylephrine and nitroglycerin. A Student’s paired or unpaired two-tailed t-test was used to compare the mean values for ED50 after log transformation (dose-response curves) or Emax (dose-response curves and single dose administrations). Statistical comparisons of Emax and ED50 values for bradykinin during the smoking cessation study (days 0, 1, 2, and 3 and week 2)
were made by ANOVA followed by Bonferroni correction for multiple comparisons. Statistical significance was assumed if a null hypothesis could be rejected at \( P < 0.05 \).

**RESULTS**

Drugs infusions were well tolerated. None of the pharmacological interventions resulted in significant changes in blood pressure or heart rate, indicating a lack of systemic effect of the doses used. No difference was observed between the two groups for the dose-response curves to phenylephrine. Maximal vasoconstriction achieved was 100 ± 4% in smokers and 99 ± 3% in nonsmokers; \( \log ED_{50} \) were 2.1 ± 0.4 and 2.2 ± 0.7 (geometric means: 126 and 158 ng/min), respectively.

Bradykinin caused dose-dependent venodilation in both smokers and nonsmokers. As shown in Fig. 1, maximal response to the endothelium-dependent vasodilator was significantly blunted in smokers compared with nonsmokers, either after cumulative increasing doses (61 ± 7 vs. 89 ± 5%, respectively; \( P < 0.05 \)) or on a separate occasion after a single dose (52 ± 7 vs. 99 ± 5%, respectively, \( P < 0.05 \)). Maximal endothelium-independent venodilation induced by nitroglycerin was not different between smokers and nonsmokers (Fig. 1). Similarly, no difference in \( \log ED_{50} \) to nitroglycerin was detected between the two groups. Coinfusion of L-arginine, the precursor of NO, markedly improved bradykinin-induced venodilation in smokers (52 ± 7 to 90 ± 9%, \( P < 0.01 \)), leading to similar venodilation compared with nonsmokers (Fig. 2). No significant effect was detected after coadministration of L-arginine in nonsmokers (Fig. 2).

A very similar pattern of responsiveness to bradykinin was observed in the seven subjects participating in the smoking cessation study (Table 1). Figure 3 shows the bradykinin dose-response studies in a representative subject. There was a statistically significant and rapid restoration to normal bradykinin-induced vasodilation within 24 h of smoking cessation in the subjects known to have depressed responses to bradykinin before study entry (Table 1). After smoking was resumed for 2 wk, the responsiveness to bradykinin was again impaired in all six volunteers studied at that time point. Responsiveness to a single dose of nitroglycerin did not change at the various time points in the smoking cessation study. \( E_{\text{max}} \) values for nitroglycerin were 108 ± 19, 107 ± 13, 106 ± 7, 101 ± 17, and 103 ± 5 on days 0, 1, 2, and 3 (smoking cessation) and week 2 (smoking), respectively.

**DISCUSSION**

The present study demonstrates that venodilation induced by bradykinin, an endothelium-dependent vasodilator, is impaired in heavy smokers, whereas the response to nitroglycerin, an endothelium-independent vasodilator, is not affected. These data provide in vivo evidence for smoking-induced endothelial dysfunction in veins in the absence of other major risk factors for coronary artery disease. Moreover, this selective endo-

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**Table 1: \( E_{\text{max}} \) response to bradykinin**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Day</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Week 2</th>
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<tr>
<td>1</td>
<td>26</td>
<td>94</td>
<td>99</td>
<td>65</td>
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<td>45</td>
<td>96</td>
<td>102</td>
<td>54</td>
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<tr>
<td>Mean ± SD</td>
<td>48 ± 13</td>
<td>93 ± 16*</td>
<td>95 ± 10*</td>
<td>85 ± 18*</td>
<td>58 ± 14*</td>
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</table>

Maximal responses to bradykinin (% of maximum) obtained from complete dose-response curves (1–278 ng/min) in 7 smokers enrolled in a smoking cessation study. This response was also assessed after resuming smoking (week 2: 12–18 days after the end of the smoking cessation phase). \( E_{\text{max}} \), estimate of maximal response. *Significantly different from day 0 value.
creased sensitivity to the in veins preconstricted with phenylephrine, an in-...Vascular Responsiveness Studies

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function in health and disease (4, 11, 32). In contrast to

choose to investigate vascular responsiveness in hand

studies demonstrating smoking-induced endothelial dysfunction in human veins (15, 16), we

choose to investigate vascular responsiveness in hand veins of chronic smokers. Previous clinical investigations have shown that the noninvasive dorsal hand vein technique can be used to explore endothelial function in health and disease (4, 11, 32). In contrast to the continuous production of NO by arterial endothe-

lum, the basal release of NO from venous endothelium is very low (32). However, its release from venous endothelium can be increased in response to bradyki-

n in and to a lesser extent to acetylcholine (11, 32).

Vascular Responsiveness Studies

Because the response to bradykinin was investigated in veins preconstricted with phenylephrine, an increased sensitivity to the $\alpha_1$-adrenoceptor agonist in smokers could potentially contribute to the attenuated

response to bradykinin. However, there was no differ-

ence in $E_{\text{max}}$ or $ED_{50}$ for phenylephrine between the two
groups. Another potential mechanism for decreased bradykinin response would be receptor desensitization occurring in smokers but not in controls during the period (30 min) required to generate the dose-response curve to the peptide. Arguing against this hypothesis is the observation that the response to a single infusion of bradykinin (278 ng/min for 5 min), assessed on a separate occasion, was also significantly impaired in smokers.

In human veins, bradykinin promotes venodilation mainly via activation of NO synthase through endothelial receptors (23); however, bradykinin also acts to a lesser extent through prostaglandins (4, 11, 33). Our finding that the venodilatory response to bradykinin is impaired and that produced by direct stimulation of the vascular smooth muscle by NO directly released from nitroglycerin is intact supports that the deficit is at the level of NO and/or prostacyclin production in the endo-

thelium. These results are consistent with in vitro studies on saphenous veins from heavy smokers (15) and extend previous in vivo observations obtained in brachial (13, 14) or coronary (34) arteries to hand veins. Although a possible correlation between venous and arterial endothelial dysfunction in smokers remains to be demonstrated, these findings suggest that smoking is associated with widespread endothelial dysfunction involving vasodilation.

A reduction in basal and stimulated prostacyclin production has been described in cultured human endothelial cells incubated with cigarette smoke ex-

tract (30) and in long-term smokers after smoking (26). In studies in saphenous vein rings preincubated with indomethacin, the impaired venodilation to bradykinin in vessels from smokers has been shown to be primarily dependent on NO (15). In our studies, infusion of L-arginine, the substrate for NO synthase, induced a complete restoration of bradykinin-mediated venodila-

tion in smokers; these results suggest that the im-
paired endothelium relaxation of hand veins in these subjects is likely attributable to a disturbance of the L-arginine/NO pathway.

The mechanisms of smoking-associated endothelial dysfunction are not fully established, but cigarettes contain a number of substances that may theoretically contribute to the impairment of the functional integrity of the endothelium. Recent experimental and clinical observations point to an increased rate of NO degrada-

tion in chronic smokers. Experimental observations on porcine coronary arteries have shown that extracts of cigarette smoke induce a contraction mediated through the degradation of basally released NO by superoxide anions (25). Such findings are in agreement with cli-
nical studies showing that basal NO activity is decreased in coronary (20) and brachial (19, 22) arteries of chronic cigarette smokers. Impaired endothelium-dependent relaxation of saphenous vein rings from smokers has been causally related to a reduced activity of endothelial NO synthase, the enzyme responsible for NO synthesis (16). The ability of acute administration

Fig. 3. Bradykinin dose-response curves (1–278 ng/min) in precon-

stricted veins in a representative subject before and during smoking cessation. Dorsal hand vein studies were carried out shortly after admission (day 0) and then daily for 3 days (days 1, 2, and 3). An additional study was repeated 2 wk after discharge, when the subject had resumed smoking; estimate of maximal response at that time was 49% (data not shown).
of L-arginine, the substrate for NO synthesis, to restore endothelial function in our model would conventionally suggest that NO production in veins of chronic smokers is substrate limited. However, arginine has been also demonstrated to generate NO by a reaction with hydrogen peroxide and thereby to decrease oxidative stress (27). Thus the beneficial effects of L-arginine on bradykinin-mediated venodilatation do not exclude that superoxide-mediated degradation of NO contributes to smoking-induced endothelial dysfunction in human hand veins. Further studies are needed to determine whether the agents and the involved mechanisms in smoking-induced endothelial dysfunction in veins are the same as those in arteries.

From a clinical point of view, it is important to note that the pharmacology and physiology of the hand veins mimic those of saphenous veins (2), vessels widely used for surgical bypass of critical atherosclerotic lesions in the coronary arteries. Smoking-induced endothelial dysfunction, which has been demonstrated in saphenous veins (15, 16), increases the risk of bypass graft failure (24). Therefore, our in vivo model of smoking-induced endothelial dysfunction could be appropriate for evaluating therapeutic approaches designed to improve endothelial function and potentially to decrease the incidence of graft occlusion in smokers.

Smoking Cessation Study

We found that impaired venodilatation to bradykinin in smokers shows a rapid return (24 h or potentially less) to normal endothelial function after acute smoking cessation. Additionally, resumption of smoking was associated with a loss of responsiveness to bradykinin. Available data from forearm blood flow studies are different with our findings in veins. Indeed, although impaired basal NO-dependent vasodilation (explored by L-NMMA infusion) has been a consistent finding in the forearm arterial resistance vasculature of chronic smokers (19, 22), stimulated NO-dependent vasodilatation (explored by infusion of cholinergic agonists) has been found to be either impaired (13, 14) or preserved (22) after 12 h of smoking cessation. Importantly, in another study conducted after 24 h of smoking cessation (17), the authors were unable to detect an impaired endothelium-dependent vasodilation. Similar conflicting results on the long-term effects of smoking on epicardial and coronary blood flow responses are found in the literature. Zelher et al. (35) noted that the flow-dependent dilation response was reduced in smokers who had not smoked for at least 4 h before examination. In contrast, Vita et al. (34) and Egashira et al. (12) demonstrated no association between smoking and coronary vasomotor response to acetylcholine, and another study (10) noted no change in hyperemic blood flow in smokers compared with nonsmokers. The possibility exists that these differences between studies may relate to the time of the last cigarette smoked before vascular investigations. In view of the rapid reversibility of the effects of smoking seen in our study, great care needs to be taken in the timing of these types of experiments to reliably interpret results.

In summary, the present study demonstrates that bradykinin-induced relaxation of veins is impaired in chronic smokers and that coadministration of L-arginine is able to restore this deficit, suggesting a disturbance of the L-arginine/NO pathway in these subjects. Furthermore, 24 h of smoking cessation is associated with a complete recovery of agonist-induced endothelial venodilation. Additional studies are needed to assess whether the same temporal pattern is observed in arteries.

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