Endothelial Dysfunction, Impaired Endogenous Fibrinolysis, and Cigarette Smoking
A Mechanism for Arterial Thrombosis and Myocardial Infarction

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Background—Effective endogenous fibrinolysis requires rapid release of tissue plasminogen activator (tPA) from the vascular endothelium. Smoking is a known risk factor for arterial thrombosis and myocardial infarction, and it causes endothelial dysfunction. We therefore examined the effects of cigarette smoking on substance P–induced tPA release in vivo in humans.

Methods and Results—Blood flow and plasma fibrinolytic factors were measured in both forearms of 12 smokers and 12 age- and sex-matched nonsmokers who received unilateral brachial artery infusions of substance P (2 to 8 pmol/min). In both smokers and nonsmokers, substance P caused dose-dependent increases in blood flow and local release of plasma tPA antigen and activity ($P<0.001$ for all) but had no effect on the local release of plasminogen activator inhibitor type 1. Compared with nonsmokers, increases in forearm blood flow ($P=0.03$) and release of tPA antigen ($P=0.04$) and activity ($P<0.001$) caused by substance P were reduced in smokers. The area under the curve for release of tPA antigen and activity decreased by 51% and 53%, respectively.

Conclusions—Cigarette smoking causes marked inhibition of substance P–induced tPA release in vivo in humans. This provides an important mechanism whereby endothelial dysfunction may increase the risk of atherothrombosis through a reduction in the acute fibrinolytic capacity. (Circulation. 1999;99:1411-1415.)

Key Words: plasminogen activators ■ endothelium ■ endothelium-derived factors ■ blood flow
forearm. Moreover, we have been able to demonstrate a reduction in tPA release after inducing experimental “endothelial dysfunction” with nitric oxide synthase inhibition. We therefore hypothesized that cigarette smoking might impair endogenous fibrinolysis by reducing the capacity of the endothelium to release tPA acutely. The aim of the study was to compare substance P–induced tPA release from the forearm vascular bed of smokers and age- and sex-matched nonsmokers.

Methods

Subjects

Twelve healthy smokers (5 to 20 cigarettes/d) and 12 age- and sex-matched nonsmokers between 25 and 55 years old participated in the study, which was undertaken with the approval of the local research ethics committee and in accordance with the Declaration of Helsinki. The written informed consent of each subject was obtained before entry into the study.

All subjects were normotensive without a history of diabetes mellitus or vascular disease. Female subjects were premenopausal and not receiving hormonal contraceptives. They were clinically well and taking no regular medications. Control subjects were lifelong nonsmokers and were not exposed to regular environmental tobacco smoke. Smokers had a history of regular daily cigarette smoking of at least 5 years’ standing and maintained their normal smoking habits in the week before attendance. None of the subjects received vasoactive or nonsteroidal anti-inflammatory drugs in the week before the study, and all abstained from alcohol for 24 hours before and from food, tobacco, and caffeine-containing drinks on the day of the study. All studies were performed in a quiet, temperature-controlled room maintained at 23.5°C to 24.5°C.

Intra-Arterial Drug Administration

The brachial artery of the nondominant arm was cannulated with a 27–standard wire gauge steel needle (Cooper’s Needle Works Ltd) under local anesthesia. The cannula was attached to a 16-gauge epidual catheter (Portex Ltd), and patency was maintained by infusion of saline (0.9%; Baxter Health Care Ltd) via an IVAC P100 syringe pump (IVAC Ltd). The total rate of intra-arterial infusions was maintained constant throughout all studies at 1 mL/min. Pharmaceutical-grade substance P (Clinalfa AG) was administered after dissolution in saline.

Measurements

Blood flow was measured in both forearms by venous occlusion plethysmography as previously described. Blood pressure was monitored in the noninfused arm at intervals throughout each study with a semiautomated noninvasive oscillometric sphygmomanometer (Takeda UA 751, Takeda Medical Inc).

Venous cannulas (17-gauge) were inserted into large subcutaneous veins of the anteceital fossae of both arms. Blood (10 mL) was withdrawn simultaneously from each arm and collected into acidified tubes and stored at 80°C before assay. Plasma PAI-1 and tPA concentrations were determined as previously described with an ELISA (Cozaika PAI-1 and Cozaika tPA, Chromogenix AB) and a photometric method (Coastel PAI-1 and Coaset tPA, Chromogenix AB). Hematocrit was determined by capillary tube centrifugation at baseline and during infusion of 8 pmol/min of substance P. Plasma lipid fractions were measured by an enzymatic colorimetric method (Boehringer Mannheim GmbH Diagnostica). LDL cholesterol was derived according to the method of Friedewald et al.

Data Analysis and Statistics

This study’s population size, on the basis of power calculations derived from previous studies, gives 90% power of detecting an 18% difference in tPA release at a significance level of 5%. Coefficients of repeatability for plasma concentrations of tPA antigen and activity during substance P infusion at 8 pmol/min are 1.6 ng/mL and 1.4 IU/mL, respectively (data on file).

Plethysmographic data were extracted from the Chart data files, and forearm blood flows were calculated for individual venous occlusion cuff inflations by use of a template spreadsheet (Excel version 5.0; Microsoft Corp). Recordings from the first 60 seconds after wrist cuff inflation were not used because of the reflex vasoconstriction this causes. Usually, the last 5 flow recordings in each 3-minute measurement period were calculated and averaged for each arm. Estimated net release of tPA activity and antigen was defined previously as the product of the infused forearm plasma flow (based on the mean hematocrit, Hct, and the infused forearm blood flow, FBF) and the concentration difference between the infused ([tPA]inf) and noninfused ([tPA]noninf) arms: Estimated net tPA release = FBF × (1 – Hct) × ([tPA]inf - [tPA]noninf).

Data were examined, where appropriate, by 2-way ANOVA with repeated measures and 2-tailed Student’s t test using Excel version 5.0 (Microsoft). The area under the curve was calculated for the estimated net release of tPA across the study period. All results are expressed as mean ± SEM. Statistical significance was taken at the 5% level.

Results

There were no significant differences in baseline characteristics, except that smokers had a slightly lower HDL concentration (Table 1). There were no significant changes in blood pressure, heart rate, hematocrit, or blood flow in the noninfused forearm during the study (data on file; Table 2). In the noninfused arm, plasma tPA antigen concentrations were higher in smokers than nonsmokers (P = 0.02; Table 2). There were no significant differences in plasma PAI-1 antigen and activity between the groups.

<table>
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<tr>
<th>TABLE 1. Baseline Subject Characteristics</th>
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<tr>
<td>Nonsmokers</td>
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<tr>
<td>Age, y</td>
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<tr>
<td>Sex, male:female</td>
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<tr>
<td>Body mass index, kg/m²</td>
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<td>Mean arterial pressure, mm Hg</td>
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<td>Heart rate, bpm</td>
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<td>Fasting plasma glucose, mmol/L</td>
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<td>Baseline hematocrit</td>
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*P = 0.01 (unpaired t test, smokers vs nonsmokers).
have a markedly impaired capacity of the endothelium to release tPA acutely. This establishes an important mechanism whereby cigarette smoking can lead to arterial thrombosis and myocardial infarction.

The rapid mobilization of tPA from the endothelium is crucial if endogenous fibrinolysis within the arterial circulation is to be effective, with thrombus dissolution being much more effective if tPA is incorporated during, rather than after, thrombus formation.23–24 The increased risk of spontaneous thrombosis seen in smokers may therefore plausibly relate to the propagation of thrombus, which would otherwise undergo lysis and remain subclinical. Although cigarette smokers have a higher overall mortality from myocardial infarction than nonsmokers,25 the in-hospital mortality is lower.26–28 This apparent paradox can be explained by the observation that the infarct-related artery is more than twice as likely to become occluded in smokers as in nonsmokers after thrombolytic therapy.29,30 Indeed, it has been suggested31 that thrombolytic therapy should only be given to smokers and that alternative strategies such as primary angioplasty should be used in nonsmokers. These observations are consistent with the present findings because it might be anticipated that patients with impaired endothelial cell tPA release would benefit most from thrombolytic therapy, whereas those with a normal endogenous fibrinolytic capacity are more likely to have tPA-resistant thrombus, which would respond less favorably.

Our findings in smokers are consistent with the previous observational data15–14 that increased basal plasma concentrations of tPA antigen are associated with future coronary events. The assessment of endogenous fibrinolysis has previously relied on measurement of basal plasma tPA concentrations and the acute release of tPA in response to venous

| TABLE 2. Blood Flow and Plasma tPA and PAI-1 Antigen and Activity Concentrations in Both Forearms |
|-------------------------------------------------|-----------------|---------|---------|---------|---------|---------|---------|---------|---------|
| Substance P dose, pmol/min | 0 | 2 | 4 | 8 | 0 | 2 | 4 | 8 |
| Absolute forearm blood flow, mL - 100 mL⁻¹ - min⁻¹ | 2.8±0.3 | 2.9±0.4 | 2.9±0.4 | 2.8±0.3 | 2.8±0.2 | 2.9±0.3 | 2.9±0.3 | 3.0±0.3 |
| Nonsmokers | Infused arm | 3.7±0.4 | 11.2±1.1 | 13.5±1.3 | 16.2±1.5* | 3.6±0.3 | 9.4±0.4 | 11.5±0.7 | 14.2±0.8† |
| Smokers | tPA antigen, ng/mL | 3.3±0.5 | 3.4±0.5 | 3.4±0.5 | 3.7±0.5 | 4.0±0.5 | 4.3±0.5 | 4.4±0.5 | 4.4±0.6† |
| | Infused arm | 3.2±0.5 | 4.1±0.6 | 4.4±0.6 | 6.2±0.8* | 4.1±0.5 | 4.5±0.6 | 5.2±0.7 | 5.9±0.9* |
| | tPA activity, IU/mL | 0.8±0.2 | 0.9±0.2 | 1.0±0.2 | 1.3±0.2 | 0.7±0.1 | 0.7±0.1 | 0.8±0.1 | 1.0±0.2 |
| | Infused arm | 0.8±0.2 | 2.1±0.5 | 2.8±0.5 | 4.6±0.6* | 0.7±0.1 | 1.1±0.2 | 1.7±0.4 | 3.0±0.5†‡ |
| | PAI-1 antigen, ng/mL | 29±7 | 29±6 | 28±7 | 28±6 | 29±6 | 26±5 | 25±5 | 26±5 |
| | Infused arm | 28±6 | 28±7 | 27±6 | 28±5 | 26±5 | 27±6 | 26±6 | 26±5 |
| | PAI-1 activity, AU/mL | 11.8±1.7 | 11.8±1.7 | 12.1±1.6 | 11.4±1.8 | 12.0±2.0 | 11.0±1.7 | 9.2±1.4 | 10.2±1.3 |
| | Infused arm | 10.7±1.6 | 8.8±1.5 | 10.8±1.7 | 9.3±1.5 | 12.5±1.9 | 10.6±1.5 | 10.5±1.2 | 8.5±1.1 |

One-way ANOVA: *P<0.001; 2-way ANOVA (nonsmokers vs smokers): † P<0.05; ‡ P=0.001.

Discussion

We have shown here, for the first time, that despite higher basal plasma tPA antigen concentrations, cigarette smokers have a markedly impaired capacity of the endothelium to...
the endothelium-dependent forearm blood flow responses in smokers. This inhibition of both the blood flow and tPA response may, in part, relate to an impairment of the L-arginine:nitric oxide pathway in smokers.\(^{19,39}\) Although differences exist,\(^{41}\) the forearm model may provide a useful surrogate for the coronary vascular bed\(^{42,43}\) and permits a readily accessible and reliable assessment of endothelial cell function. However, the present findings need to be confirmed in the coronary circulation.

We have studied the sustained effect of chronic smoking in a selected healthy and predominantly male population at a single time point. Although total and LDL cholesterol concentrations were similar in smokers and nonsmokers, HDL cholesterol concentrations were slightly lower in smokers. This is not unexpected, because cigarette smoking is known to be associated with a selective reduction in HDL cholesterol concentrations.\(^{44,45}\) However, the application of this model to other conditions associated with endothelial dysfunction, such as dyslipidemia, is warranted. Finally, because hormonal status influences fibrinolytic parameters,\(^ {46}\) the assessment of the acute fibrinolytic capacity in premenopausal and postmenopausal women and the modulating effect of hormonal therapy will also be of particular interest.

In conclusion, we have demonstrated a major impairment of tPA release from the vascular endothelium of smokers. Our findings suggest that the fundamental mechanism whereby cigarette smoking causes arterial thrombosis and myocardial infarction relates, at least in part, to impairment of the acute endogenous fibrinolytic capacity.

**Acknowledgments**

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**References**


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