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

Acute rupture of a coronary atheromatous plaque and subsequent coronary artery thrombosis causes the majority of sudden cardiac deaths and myocardial infarctions.1,2 Cigarette smoking not only is strongly associated with atherosclerosis3 and ischemic heart disease4 but also is a major risk factor for acute coronary thrombosis.1,5 Indeed, 75% of sudden cardiac deaths due to acute thrombosis are in cigarette smokers.1 Smoking causes endothelial dysfunction6 and is associated with increased platelet thrombus formation.5 Small areas of denudation and thrombus deposition are a common finding on the surface of atheromatous plaques7,8 and are usually subclinical. However, in the presence of an imbalance in the coagulation or fibrinolytic systems, such microthrombi may propagate, ultimately leading to arterial occlusion.

The importance of endogenous tissue plasminogen activator (tPA) release is exemplified by the high rate of spontaneous reperfusion in the infarct-related artery after acute myocardial infarction, occurring in ≈30% of patients within the first 12 hours.9-11 It would be anticipated that high plasma tPA concentrations should protect against subsequent coronary events. However, in epidemiological studies of patients with ischemic heart disease12,13 and in a healthy male population (US Physicians Study),14 higher total plasma tPA (antigen) concentrations positively predict future coronary events. This is explained by the concomitant elevation of plasminogen activator inhibitor type 1 (PAI-1), which forms a complex with tPA and thereby causes an overall reduction in free tPA “activity.”15,16 It is this free and unbound tPA that is physiologically active and leads to endogenous fibrinolysis. However, the capacity of endothelial cells to release tPA from intracellular storage pools and the rapidity with which this can be mobilized may not necessarily be reflected in the basal circulating plasma concentrations of tPA antigen or activity.17

Using the endothelium-dependent vasodilator substance P to stimulate tPA release, we recently described an in vivo model to assess the acute fibrinolytic capacity of the human
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 epirudal catheter (Portex Ltd), and patency was maintained by infusion of saline (0.9%; Baxter Health Care Ltd) via an IVAC P1000 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 antecubital fossae of both arms. Blood (10 mL) was withdrawn simultaneously from each arm and collected into acidified buffered citrate (Biopool Stabilyte, for tPA assays) and citrate withdrawn simultaneously from each arm and collected into acidified buffered citrate (Biopool Stabilyte, for PAI-1 assays) tubes and kept on ice before being stored at 80°C before assay. Plasma lipid fractions were measured by an enzymatic colorimetric method (Coatest 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.21

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>
<thead>
<tr>
<th>TABLE 1. Baseline Subject Characteristics</th>
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<tr>
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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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<tr>
<td>Mean arterial pressure, mm Hg</td>
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<td>Heart rate, bpm</td>
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<tr>
<td>Fasting plasma glucose, mmol/L</td>
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<tr>
<td>Total cholesterol, mg/dL</td>
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<td>LDL cholesterol, mg/dL</td>
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<tr>
<td>HDL cholesterol, mg/dL</td>
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<tr>
<td>Triglycerides, mg/dL</td>
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<td>Baseline hematocrit</td>
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*P=0.01 (unpaired t test, smokers vs nonsmokers).
TABLE 2. Blood Flow and Plasma tPA and PAI-1 Antigen and Activity Concentrations in Both Forearms

<table>
<thead>
<tr>
<th></th>
<th>Nonsmokers</th>
<th></th>
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<th>Smokers</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Time, min</td>
<td>Time, min</td>
<td>Baseline</td>
<td>Time, min</td>
<td>Time, min</td>
<td>Time, min</td>
<td>Time, min</td>
</tr>
<tr>
<td>Substance P dose, pmol/min</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Absolute forearm blood flow, mL • 100 mL^{-1} • min^{-1}</td>
<td>2.8 ± 0.3</td>
<td>2.9 ± 0.4</td>
<td>2.9 ± 0.4</td>
<td>2.8 ± 0.3</td>
<td>2.8 ± 0.2</td>
<td>2.9 ± 0.3</td>
<td>2.9 ± 0.3</td>
<td>3.0 ± 0.3</td>
</tr>
<tr>
<td>Noninfused arm</td>
<td>3.7 ± 0.4</td>
<td>11.2 ± 1.1</td>
<td>13.5 ± 1.3</td>
<td>16.2 ± 1.5*</td>
<td>3.6 ± 0.3</td>
<td>9.4 ± 0.4</td>
<td>11.5 ± 0.7</td>
<td>14.2 ± 0.8*</td>
</tr>
<tr>
<td>Infused arm</td>
<td>3.3 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.4 ± 0.5</td>
<td>3.7 ± 0.5</td>
<td>4.0 ± 0.5</td>
<td>4.3 ± 0.5</td>
<td>4.4 ± 0.5</td>
<td>4.4 ± 0.6†</td>
</tr>
<tr>
<td>tPA antigen, ng/mL</td>
<td>3.2 ± 0.5</td>
<td>4.1 ± 0.6</td>
<td>4.4 ± 0.6</td>
<td>6.2 ± 0.8*</td>
<td>4.1 ± 0.5</td>
<td>4.5 ± 0.6</td>
<td>5.2 ± 0.7</td>
<td>5.9 ± 0.9*</td>
</tr>
<tr>
<td>Noninfused arm</td>
<td>0.8 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.3 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Infused arm</td>
<td>0.8 ± 0.2</td>
<td>2.1 ± 0.5</td>
<td>2.8 ± 0.5</td>
<td>4.6 ± 0.6*</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.2</td>
<td>1.7 ± 0.4</td>
<td>3.0 ± 0.5†</td>
</tr>
<tr>
<td>tPA activity, IU/mL</td>
<td>29 ± 6</td>
<td>29 ± 6</td>
<td>28 ± 7</td>
<td>28 ± 6</td>
<td>29 ± 6</td>
<td>26 ± 5</td>
<td>25 ± 5</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>Noninfused arm</td>
<td>28 ± 6</td>
<td>28 ± 7</td>
<td>27 ± 6</td>
<td>28 ± 5</td>
<td>26 ± 5</td>
<td>27 ± 6</td>
<td>26 ± 6</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>Infused arm</td>
<td>11.8 ± 1.7</td>
<td>11.8 ± 1.7</td>
<td>12.1 ± 1.6</td>
<td>11.4 ± 1.8</td>
<td>12.0 ± 2.0</td>
<td>11.0 ± 1.7</td>
<td>9.2 ± 1.4</td>
<td>10.2 ± 1.3</td>
</tr>
<tr>
<td>PAI-1 antigen, ng/mL</td>
<td>10.7 ± 1.6</td>
<td>8.8 ± 1.5</td>
<td>10.8 ± 1.7</td>
<td>9.3 ± 1.5</td>
<td>12.5 ± 1.9</td>
<td>10.6 ± 1.5</td>
<td>10.5 ± 1.2</td>
<td>8.5 ± 1.1</td>
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One-way ANOVA: *P<0.001; 2-way ANOVA (nonsmokers vs smokers): †P<0.05; ‡P=0.001.

Substance P caused dose-dependent increases in forearm blood flow in the infused arm in both smokers and nonsmokers (Table 2, Figure), but the increase in blood flow was greater in nonsmokers (P=0.03; 2-way ANOVA, nonsmokers versus smokers). Compared with the noninfused arm (2-way ANOVA), substance P caused dose-dependent increases in plasma concentrations of tPA antigen (P<0.001) and activity (P<0.001) in the infused arm of both smokers and nonsmokers (Table 2). There were no significant changes in plasma PAI-1 antigen or activity in either group. The increase in plasma tPA activity in the infused arm was greater in the nonsmokers (P=0.001; 2-way ANOVA, nonsmokers versus smokers).

Substance P increased the net release of tPA antigen (P=0.009) and activity (P<0.001) in smokers (Figure). In nonsmokers, substance P increased the net release of tPA antigen (P<0.001) and activity (P<0.001) significantly more than in smokers (P=0.04 and P<0.001, respectively; 2-way ANOVA, nonsmokers versus smokers). Compared with the nonsmokers, the area under the curve for net tPA antigen and activity release was reduced by 51% and 53%, respectively, in the smokers.

Subgroup analysis after exclusion of female subjects did not alter the magnitude or the statistical significance of the above findings. Qualitatively, the responses in female smokers and nonsmokers were similar to those observed in the male subjects.

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 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 patent in current smokers as in nonsmokers after thrombolytic therapy for acute myocardial infarction,26–30 Indeed, it has been suggested10 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 data12–14 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
occlusion, systemic desmopressin infusion, or exercise. However, because of confounding systemic effects and the nonuniformity of the stimuli applied, these responses can be variable and give only a relatively crude measure of fibrinolytic capacity. Moreover, although it has previously been shown that systemic desmopressin infusion causes less tPA release in smokers, this effect may not be directly endothelium-dependent. In contrast, we have used locally active doses of substance P to provide a more precise pharmacological stimulus to the endothelium and to cause a substantial and dose-dependent local release of tPA. This has allowed us to demonstrate a distinct and marked inhibition of stimulated endothelial tPA release in smokers. Although thrombin is more physiologically relevant to acute tPA release than substance P, we have used the latter because its vascular actions are endothelium-dependent, mediated in part through nitric oxide, and its administration intra-arterially is safe and well tolerated. Consistent with previous workers, we have also found an attenuation of 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. Although differences exist, the forearm model may provide a useful surrogate for the coronary vascular bed 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. 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, 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


