Proteolysis of Tissue Factor Pathway Inhibitor-1 by Thrombolysis in Acute Myocardial Infarction

Ilka Ott, MD; Valerie Malcouver; Albert Schömig, MD; Franz-Josef Neumann, MD

Background—In acute myocardial infarction (AMI), surface-bound tissue factor pathway inhibitor-1 (TFPI-1) inhibits an increased monocyte procoagulant activity. In addition, TFPI-1 is released from microvascular endothelial cells after treatment with heparin and thereby contributes to its antithrombotic properties.

Methods and Results—We examined 19 patients in a randomized study comparing intravenous fibrinolysis with alteplase (n=9) and revascularization by stent placement with additional abciximab treatment (n=10). We obtained blood samples for analysis of monocytic TFPI-1 surface expression by flow cytometry and plasma TFPI-1 concentrations by immunoassay before and after therapy. We found a significant decrease in surface TFPI-1 on circulating monocytes 24 hours after thrombolysis (P=0.006) that was not observed after stenting. Systemic plasma TFPI-1 concentrations increased immediately after stenting by 71±14% (P=0.008), whereas after thrombolysis, a decrease in TFPI-1 plasma concentrations of 21±11% was observed (P=0.075). In vitro experiments confirmed that plasmin decreased TFPI-1 surface expression dose-dependently.

Conclusions—Activation of the fibrinolytic system by alteplase in AMI decreases surface-associated TFPI-1 on circulating monocytes and plasma TFPI-1. Reduced TFPI-1 may contribute to thrombotic complications after fibrinolysis in AMI. (Circulation. 2002;105:279-281.)

Key Words: myocardial infarction ■ thrombolysis ■ leukocytes

The benefits of thrombolytic therapy for acute myocardial infarction (AMI) are limited by reocclusion of the infarct-related artery. Reocclusion occurs in up to 30% of patients after successful thrombolysis, but in only up to 6% after balloon angioplasty and stenting.1 Tissue factor (TF)-mediated activation of the coagulation cascade plays a key role in intravascular thrombus formation and is inhibited by tissue factor pathway inhibitor-1 (TFPI-1).2,3 Heparin stimulates TFPI-1 release in endothelial cells, and thus TFPI-1 may contribute to the anticoagulant activity of heparin.4,5 Soluble TFPI-1 directly inhibits factor Xa (FXa),6 whereas surface-bound TFPI-1 on endothelial cells inhibits TF-factor VIIa (FVIIa) complex in conjunction with FXa by translocation into caveolae.7,8 We have shown a similar inhibition of TF activity by TFPI-1 on TF-expressing circulating monocytes in AMI.9 Because TFPI-1 is susceptible to proteolysis by the serine protease plasmin in a recombinant system,10 a decreased anticoagulant activity after degradation of TFPI-1 during thrombolysis in AMI may contribute to rethrombosis at sites of TF expression in the ruptured atherosclerotic plaque. We therefore investigated TFPI-1 surface expression on circulating monocytes and TFPI-1 plasma levels after thrombolysis and stenting in AMI.

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Methods

Patient Selection
We included 19 patients from the Stent Versus Thrombolysis for Occluded Coronary Arteries in Patients With Acute Myocardial Infarction Study (STOPAMI I).11 In this study, fibrinolysis with alteplase was compared with stenting plus abciximab. Patients randomized to stenting were a subgroup of a previous study. The study was approved by the institutional ethics committee for human subjects. Informed consent was obtained from all patients.

All patients received 500 mg of aspirin and 5000 U of heparin intravenously in the emergency room. After randomization, fibrinolysis was performed using alteplase (n=9) and stenting was performed using abciximab (n=10) as described.11 Postinterventional therapy consisted of intravenous heparin for 48 hours and aspirin 100 mg twice daily indefinitely in the patients treated with fibrinolysis. After stenting, patients received ticlopidine 250 mg twice daily for 4 weeks and aspirin 100 mg twice daily indefinitely. Serial peripheral venous blood samples were obtained before and immediately after therapy, 24 and 48 hours after stenting. All blood samples were put on ice and processed immediately, as indicated below.

Flow Cytometry
For flow cytometry, Cyfix (donated by Dr A. Ruf, Klinikum Karlsruhe, Karlsruhe, Germany) fixed blood samples were stained for TF and TFPI-1 on circulating monocytes as described.9

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From Medizinische Klinik und Deutsches Herzzentrum der Technischen Universität München, Germany.

Correspondence to Dr. I. Ott, Deutsches Herzzentrum, Lazarettstr. 36, 80636 München, Germany. E-mail ott@dhm.mhn.de

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Baseline Characteristics of Study Patients

<table>
<thead>
<tr>
<th></th>
<th>Direct Stenting</th>
<th>Thrombolysis</th>
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<tbody>
<tr>
<td>Sex (M/F)</td>
<td>6/4</td>
<td>8/1</td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>58 (49–85)</td>
<td>60 (40–75)</td>
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<tr>
<td>Active smokers</td>
<td>3 (30)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>5 (50)</td>
<td>6 (67)</td>
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<tr>
<td>Systemic hypertension</td>
<td>7 (70)</td>
<td>6 (67)</td>
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<tr>
<td>Diabetes mellitus</td>
<td>2 (20)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Single-vessel disease</td>
<td>5 (50)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Double-vessel disease</td>
<td>3 (30)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>Triple-vessel disease</td>
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<td>3 (33)</td>
</tr>
<tr>
<td>Target vessel</td>
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<td></td>
</tr>
<tr>
<td>LAD</td>
<td>4 (40)</td>
<td>3 (33)</td>
</tr>
<tr>
<td>LCx</td>
<td>3 (30)</td>
<td>2 (22)</td>
</tr>
<tr>
<td>RCA</td>
<td>6 (60)</td>
<td>4 (44)</td>
</tr>
<tr>
<td>Peak CK, U/L (range)</td>
<td>898 (250–3742)</td>
<td>883 (187–4530)</td>
</tr>
</tbody>
</table>

Values are n (%) unless otherwise indicated.

CK indicates creatine kinase; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; and RCA, right coronary artery.

TFPI-1 and D-Dimer Immunoassay

Plasma concentrations of TFPI-1 and D-Dimer were determined by sandwich-type immunoassay (IMMUBIND TFPI ELISA, American Diagnostica; Enzygnost D-Dimer micro, Dade Behring Marburg GmBH). The interassay variabilities in the lower assay range were 6.7% and 15.4%.

Treatment of ECV304 Cells or Human Umbilical Vein Endothelial Cells With Plasmin

The human cell line ECV304 (CR-1998, American Tissue Culture Collection) and human umbilical vein endothelial cells (HUVECs) (CellSystems, St. Katharinen, Germany) were harvested with cell dissociation buffer (Sigma) and incubated with plasmin (American Diagnostica) for 2 hours at 37°C.

Statistical Analysis

Differences between ≥2 matched samples were tested by Friedman’s test followed by Wilcoxon’s matched-pairs signed-ranks test, and differences between the groups were tested by the Mann-Whitney-Wilcoxon rank sum test or by Fisher’s exact test as appropriate (normal distribution shown by Kolmogorov Smirnov test). A value of P<0.05 in the 2-tailed test was regarded as significant.

Results

Clinical and Angiographic Data

The time from onset of symptoms to start of therapy ranged from 0.5 to 8 hours. None of the patients suffered reinfarction or died during the hospital stay. The treatment groups did not differ significantly in age, sex, risk factor profile, medication, or infarct size and location (Table). Stenting was successful in all patients, restoring TIMI grade 3 flow. Residual stenosis was <15%.

TFPI-1 and TF Surface Expression and Plasma Levels After AMI

We found a significant decrease in surface TFPI-1 on circulating monocytes after thrombolysis, whereas after stenting in AMI, no significant changes were observed (Figure 1). TF expression on monocytes remained unchanged in both groups (data not shown). Compared with the levels before therapy, plasma TFPI-1 levels decreased immediately after treatment with alteplase from 152±13 to 110±12 ng/mL (P=0.075), whereas after stenting, TFPI-1 plasma concentrations increased from 179±14 to 248±12 ng/mL (P=0.008). D-Dimer plasma concentrations were significantly increased immediately and 24 hours after thrombolysis compared with stenting plus abciximab (0 hours: 345±79 versus 17±10 µg/l, P=0.004; 24 hours: 414±99 versus 50±31 µg/l, P=0.008).

Effect of Plasmin on Cellular TFPI-1 Surface Expression In Vitro

Plasmin dose-dependently induced a decrease in surface TFPI-1 on ECV304 cells and HUVECs (Figure 2).

Discussion

Major findings of our study are (1) monocytic surface bound TFPI-1 is reduced after thrombolysis in AMI, (2) plasmin reduces surface-bound TFPI-1 on cell surfaces in vitro, and (3) the heparin-induced increase in plasma TFPI-1 found after stenting is eliminated after thrombolysis.
The fact that monocyctic TFPI-1 decreases after thrombolysis, whereas after stenting no changes in monocyctic TFPI-1 were found, shows that plasmin degrades TFPI-1 during thrombolysis in AMI. TFPI-1 is released from endothelial cells by heparin, whereas surface TFPI-1 expression on endothelial cells and monocytes remains unchanged. Elevated TFPI-1 plasma levels before reperfusion occurred because of heparin administration in the emergency room and did not differ in both treatment groups. Immediately after stenting, TFPI-1 plasma levels increased, and then gradually decreased. Although heparin was administered during thrombolysis and stenting, TFPI-1 plasma levels after thrombolysis were decreased. Previously, we showed that heparin and abciximab administration during stenting in AMI and stimulation with heparin in vitro did not affect surface TFPI-1 on monocytes. Therefore, the observed reduction in surface-associated TFPI-1 on circulating monocytes after thrombolysis is independent of heparin but caused by thrombolysis. The increase in circulating fibrin degradation product D-Dimer after thrombolysis confirmed the activation of plasmin in vivo.

This finding was confirmed by in vitro experiments showing that isolated plasmin degrades surface bound TFPI-1. Our results extend previous observations that purified plasmin degrades surface bound TFPI-1. Therefore, the observed reduction in surface TFPI-1 on monocytes after thrombolysis, an increased procoagulant activity at these sites of TF expression. This mechanism may contribute to early reocclusion after thrombolysis in AMI. The fact that excessive activation of the fibrinolytic system decreases the anticoagulant activity of TFPI-1 by proteolysis may protect from bleeding by reinforcing TF mediated coagulation. In addition to therapeutic applications of fibrinolytic agents, this new mechanism may play a role in hyperfibrinolytic states such as disseminated intravascular coagulation.

Medication between the study groups differed in respect to GPIIb/IIIa antagonists and ticlopidine. Furthermore, the interventional procedure itself may alter TFPI-1 expression. Because monocytic surface TFPI-1 is not affected by these treatments, changes can be considered as a result of fibrinolysis.

Recent clinical studies have shown that the addition of abciximab to therapy with tissue plasminogen activator increased the rate of successful flow restoration. Using a combination strategies of inhibitors of platelet aggregation and activators of fibrinolysis may therefore improve coronary flow and clinical outcome in AMI. The decrease in the anticoagulant activity of TFPI-1 after thrombolysis shown in this study suggests a further benefit of additional inhibition of TF activity by antibodies or proteolytically inactive FVIIa.

Acknowledgments
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References
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