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Increased Levels of Plasma Thrombomodulin in Patients with Acute Myocardial Infarction Who Had Thrombolytic Therapy and Achieved Successful Reperfusion

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Summary

Background: There is a growing body of evidence from animal and in vitro studies for the existence of reperfusion injury after thrombolytic therapy for acute myocardial infarction (AMI), but the patient data are limited.

Hypothesis: We aimed to examine the plasma thrombomodulin (TM) levels as a marker of endothelial injury and to investigate the effect of successful reperfusion on these levels.

Methods: The study included 32 patients who had a first episode of acute myocardial infarction (AMI) and received intravenous streptokinase therapy.

Results: Thrombomodulin levels increased significantly at 60 min after thrombolysis compared with the levels before thrombolytic therapy (0 min) in 21 (66%) patients who had successful reperfusion (49.09 ± 10.51 vs. 25.76 ± 5.55 ng/ml, $p < 0.001$). There was no difference between the TM levels at 0 and at 60 min of thrombolysis in the remaining 11 (34%) patients who could not achieve reperfusion (27.81 ± 6.32 vs. 28.72 ± 7.28 ng/ml, $p = 0.35$).

Conclusion: There was a significant increase in TM levels at 60 min after thrombolysis in a group of patients with AMI who achieved successful reperfusion; this increase may have been caused by the activation/injury of endothelial cells. Data also suggest that the increment in TM levels may be predictive of the potential success of thrombolytic therapy.

Key words: acute myocardial infarction, thrombomodulin, reperfusion injury

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Introduction

There is a growing body of evidence from animal and in vitro studies for the existence of reperfusion injury after thrombolytic therapy for acute myocardial infarction (AMI),[1–3] but the patient data are limited. It has been suggested that thrombomodulin (TM), an endothelial cell surface protein with antithrombotic action mediated via the thrombin-thrombomodulin complex, is a marker of endothelial dysfunction or activation.[4, 5] Accordingly, we aimed to investigate the plasma TM levels as a marker of endothelial injury in patients with AMI who underwent thrombolytic therapy, and the effect of successful reperfusion on these levels.

Materials and Methods

In all, 32 consecutive patients (20 men, 12 women, mean age ± standard deviation [SD] = 62.3 ± 12.4 years), who were admitted to our clinic because of a first episode of transmural AMI within the first 12 h after symptom onset, were enrolled in this study. The diagnosis of AMI was based on the typical chest pain lasting > 30 min and unrelieved by intravenous nitroglycerin, accompanied with a new ST-segment elevation of > 0.2 mV in at least two contiguous leads on a standard 12-lead electrocardiogram (ECG) and followed by two-fold elevation of serum creatine kinase with an myocardial band (MB) > 5%. Criteria of exclusion were history of previous myocardial infarction, evidence of valvular heart disease or cardiomyopathy, complete left bundle-branch block, severe liver disease, septicemia, and contraindication to thrombolytic therapy. All patients received 1.5 million IU streptokinase intravenously (IV) in 60 min. Immediately after thrombolytic treatment, an initial IV bolus of 5000 IU heparin was injected and followed by continuous infusion for 3 to 5 days, beginning at 1000 IU/h and maintaining activated partial thromboplastin time at 1.5 to 2 times of control. Antiplatelet therapy with aspirin (150 mg/day) was started on admission in all patients. A 12-lead ECG was obtained from all patients immediately after admission and at least every 4 h for the first 24 h. Creatine kinase (CK) MB isoenzyme was measured every 4 h for the first 24 h, every 8 h for the next 24 h, and once a day until Day 7.

Time to peak release of CK-MB isoenzyme was calculated by plotting the CK-MB (IU/l) against time in hours. The ECG resolution of maximal ST-segment elevation by at least 50% within 2 h from the start of streptokinase administration, as well as the occurrence of peak plasma CK-MB level within 12 h from the onset of symptoms were considered as noninvasive markers of reperfusion. Patients in whom both of these two criteria were achieved were considered to have had successful reperfusion. In all patients, left ventriculography and selective coronary angiography were performed by the Judkin's technique within 10 days of the infarction. The clinical features analyzed included age, gender, risk factors for coronary artery disease (hypertension, cigarette smoking, serum cholesterol > 250 mg/dl, diabetes mellitus, and family history), and history of angina pectoris before AMI. The control group consisted of 24 healthy persons (15 men and 9 women, mean age ± SD = 60.5 ± 10.2 years). All patients and control subjects gave informed consent, and the study protocol was approved by the ethics committee of our institution.

Peripheral venous blood samples of the patients for measuring TM levels were drawn without venous occlusion immediately after admission and before administration of streptokinase (0 min) and at 60 min after the start of thrombolysis, using 21 G multiple drawing blood collecting needles into 3.8% 1:9 trisodium citrate-containing tubes. The blood samples were centrifuged immediately at 3000 g for 15 min and then the plasmas were stored in several aliquotes at −70°C until assayed. Blood samples were obtained between 8 and 10 A.M. from the control group in the same manner. Plasma TM (Asserachrom Thrombomodulin, enzyme immunoassay of thrombomodulin. Diagnostica Stago, Asnières, France) concentrations were measured by the solid phase "sandwich" enzyme-linked immunosorbent assay method.

Values for plasma TM levels were given as mean ± SD. The statistical significance of change in TM levels was evaluated by 1-way analysis of variance for repeated measures and Duncan's test. Probability levels < 0.05 were considered statistically significant.

TABLE I Baseline characteristics of patients with acute myocardial infarction (n = 32)

Variable	Results
Age (years) (mean(SD)	62
Female/male	12/20
Infarct location	
Anterior	16
Inferior	12
Posterolateral	4
Infarct-related artery	
Left anterior descending	16
Left circumflex	7
Right	9
Time to streptokinase (h) (mean ± SD)	3.8 ± 1.2
Successful reperfusion (%)	21 (66)
Peak CK-MB (IU/l)	834 ± 254
Killip classification at admission	
Class I	9
Class II to IV	23
Coronary risk factors	
Hypertension	13
Smoking	16
Diabetes mellitus	5
Hypercholesterolemia	17
Family history	21
Obesity	14
Total white blood cell counts ($X10^{-9}$/l)	11.5 ± 2.3
Platelet counts ($X10^{-9}$/l)	270 ± 12
Fibrinogen (g/l)	4.8 ± 1.7

Abbreviations: SD = standard deviation, CK-MB = creatine kinase-myocardial band.

Results

Table I lists the baseline clinical characteristics of the study patients. Thrombomodulin levels in patients with AMI on admission were significantly higher than those in the control subjects (26.46 ± 5.82 vs. 18.21 ± 4.24 ng/ml, $p < 0.001$). Compared with the levels before thrombolytic therapy (0 min), thrombomodulin levels increased significantly at 60 min after thrombolysis in 21 (66%) patients who had successful reperfusion (49.09 ± 10.51 vs. 25.76 ± 5.55 ng/ml, $p < 0.001$) (Fig. 1). There was no difference between the TM levels at 0 min and at 60 min of thrombolysis in the remaining 11 (34%) patients who could not achieve reperfusion (27.81 ± 6.32 vs. 28.72 ± 7.28 ng/ml, $p = 0.35$) (Fig. 2).

Discussion

Thrombomodulin is a cell surface glycoprotein with anticoagulant properties that is mainly present on the luminal surface of endothelial cells. It has been suggested to be a marker of en-

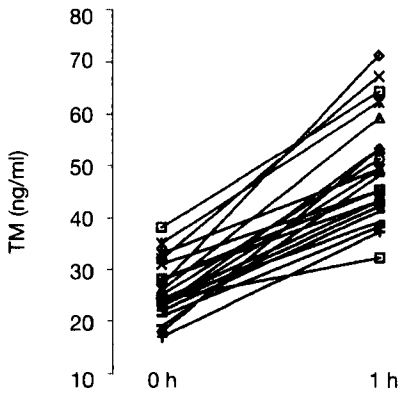

FIG. 1 Scatter plot of thrombomodulin (TM) levels before and after 1 h of thrombolytic therapy in patients who achieved successful reperfusion (n = 21).

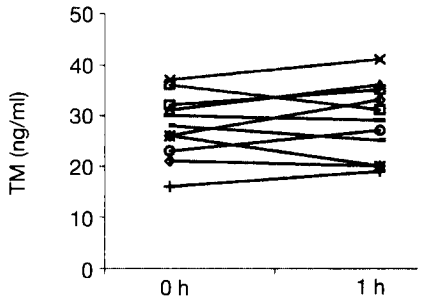

FIG. 2 Scatter plot of thrombomodulin (TM) levels before and after 1 h of thrombolytic therapy in patients who did not achieve successful reperfusion (n = 11).

dothelial cell dysfunction, and its plasma levels are increased in a number of pathological states when the endothelium is likely to have been damaged.[4–6]

The present study has shown a significant increase in TM levels at 60 min after thrombolysis in a group of patients with AMI who achieved successful reperfusion; however, such an increase could not be detected in patients with unsuccessful reperfusion. There may be two explanations for this rise in circulating TM levels associated with successful thrombolysis: First, the reperfusion activates/damages the endothelium in AMI releasing TM; thus, the elevated TM levels provide an indirect evidence for the existence of reperfusion injury in patients undergoing thrombolytic therapy. Reperfusion of ischemic myocardium generates free radicals and highly reactive chemical species toxic to endothelial cells in vitro and in animal models.[1,2,7–12] Second, reperfusion merely washes out TM molecules released before reperfusion from ischemic and infarcted endothelium. Recanalization of the occluded coronary artery may accelerate appearance of TM in plasma because of enhanced blood flow and associated increase in lymph flow permitting rapid transit of TM from necrotic endothelium into the circulation.

Although endothelial dysfunction after reperfusion has been demonstrated in animal models and in vitro experiments,[1–3] there are few data pertaining to patients. In a previous report, a significant increase was shown in von Willebrand factor VIII antigen levels which are also a marker of endothelial injury, in 10 patients with AMI who successfully reperfused after thrombolytic treatment.[13] However, because of the low number of patients who had unsuccessful reperfusion, no statistical analysis of their data was possible. In another study, Shimomura *et al.*[14] presumed that the increment in plasma soluble P-selectin levels at 4 h after initiation of thrombolysis for AMI in their study group may be caused by activation of endothelial cells and platelets after myocardial ischemia and thrombolytic therapy; but they did not investigate the role of reperfusion.

With regard to IV thrombolysis in patients with AMI, a noninvasive marker of the myocardial reperfusion in its early phase would be of great value, because patients with persistent occlusion, despite thrombolytic treatment, might benefit from further interventional procedures such as rescue angioplasty to restore blood flow. Preliminary data of the present study suggest that measurement of TM levels immediately after 1 h of thrombolytic therapy may be useful for the early evaluation of the reperfusion status in AMI. Further investigations are needed for a detailed examination of the serial changes in plasma TM levels in these particular patients.

Conclusion

There is a significant increase in TM levels at 60 min after thrombolysis in a group of patients with AMI who achieved successful reperfusion; this increase may be caused by the activation/injury of endothelial cells. The data also suggest that an increment in TM levels after thrombolysis may be predictive of the potential success of thrombolytic therapy.

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