Changes in Coagulation and Fibrinolysis Markers in Acute Ischemic Stroke Treated With Recombinant Tissue Plasminogen Activator

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**Background and Purpose**—Shifts of the balance between coagulation and fibrinolysis play a crucial role in pathogenesis and treatment of cerebral ischemia. In this study, we characterized the kinetics of hemostatic abnormalities induced by acute ischemic stroke and its thrombolytic (recombinant tissue plasminogen activator [rtPA]) or anticoagulant (heparin) treatment.

**Methods**—Systemic generation of molecular markers of hemostasis (fibrin monomer, D-dimer, thrombin-antithrombin complex, and fibrinopeptide 1.2) was monitored in acute ischemic stroke, and the effects of thrombolytic and anticoagulant treatments were analyzed.

**Results**—Thrombolysis with rtPA induced a massive response of markers of coagulation activation and fibrin formation that peaked after 1 to 3 hours and persisted for up to 72 hours. In contrast, only minor hemostatic changes were induced by acute ischemic stroke itself. Administration of heparin did not significantly affect these hemostatic abnormalities.

**Conclusions**—This first characterization of the coagulation activation induced by rtPA treatment for acute ischemic stroke and the failure to abolish such hemostatic abnormalities by heparin may be of value for further refinement of the currently discussed thrombolytic therapy and the controversial adjunctive anticoagulant prophylaxis in stroke patients. *(Stroke. 1999;30:2101-2104.)*

**Key Words:** coagulation ■ stroke, ischemic ■ thrombolysis ■ tissue plasminogen activator, recombinant

Ischemic stroke is caused by blockade of the vascular supply of cerebral tissue. Two multicenter, double-blind, and randomized trials (European Cooperative Acute Stroke Study [ECASS], National Institute of Neurological Diseases and Stroke [NINDS] rtPA Stroke Study)1,2 showed the potential benefit of thrombolysis with recombinant tissue plasminogen activator (rtPA) in ischemic stroke when administered within the first 3 to 6 hours, but they also delineated the frequent failure to achieve recanalization and the increased risk of intracranial hemorrhage. Although this thrombolytic agent has been approved by the Food and Drug Administration as the only accepted therapy specifically directed at acute ischemic stroke, its use is highly controversial.3 In myocardial infarction, adjunctive anticoagulation with heparin appears to improve initial infarct-related artery patency, although thus far there is no proven survival benefit to this strategy.4 Despite considerable differences in pathophysiology, this therapeutic concept has been transferred to ischemic stroke.

Molecular markers of hemostasis allow the detection of in vivo coagulation activation.5-10 D-Dimer (DD) antigen is an indicator of the formation of factor XIIIa cross-linked fibrin. Current assays detect DD antigen irrespective of the size of the fibrin compound containing the epitope that is present in high-molecular-weight fibrin complexes as well as in small fragments generated during plasmin proteolysis of cross-linked fibrin.11 Fibrin monomer (FM) is the product of the thrombin-mediated cleavage of fibrinopeptide A from fibrinogen. Prothrombin fragment fibrinopeptide 1.2 (F1.2) is coreleased with each thrombin molecule from prothrombin by factor Xa. Thrombin–antithrombin III complex (TAT) is the product of a complex formation between active thrombin and antithrombin III.

The aim of this study was to characterize the extent and kinetics of hemostatic abnormalities in acute ischemic stroke patients treated with rtPA and to assess its possible modulation by anticoagulation with heparin.

**Subjects and Methods**

**Patients and Control Subjects**

Forty-four patients (23 women, 21 men; median age, 72 years; range, 39 to 97 years) presenting for treatment of acute ischemic stroke...
within 2 to 6 hours after onset of symptoms were studied. The etiology was artery-to-artery embolism (n = 13), cardioembolic stroke (n = 17), lacunar stroke (n = 5), and other or unknown (n = 9).

After the first blood sampling, thrombolysis with rtPA was initiated in 23 patients (10 women, 13 men; median age, 71 years; range, 40 to 97 years), 5 of whom participated in the ECASS trial (alteplase 1.1 mg/kg IV, administered within 6 hours after onset of first symptoms, followed by adjunctive high-dose heparin therapy after 24 hours).1 Eighteen patients were treated with rtPA independently from such study protocols with inclusion criteria adopted from the NINDS trial2 (alteplase 0.9 mg/kg IV, administered within 3 hours after onset of symptoms, followed by adjunctive high-dose heparin therapy after 1 to 2 hours). rtPA administered in patients who participated in the ECASS trial and in those who were studied independently from such trials was identical.

As controls, 21 stroke patients (9 women, 13 men; median age, 74 years; range, 39 to 89 years) who were treated with heparin at a low dose (3 × 5000 IU/d SC; n = 10) or a high dose (5000 IU, IV bolus, followed by 1000 IU/h infusion; partial thromboplastin time, 65 to 90 seconds; n = 11) were studied. In addition, 50 age- and risk factor–matched (hypertension in 67%, hypercholesterinemia in 53%, smoking in 49%, and diabetes mellitus in 21%) subjects (19 women, 31 men) aged from 40 to 79 years (median, 68 years) and 75 subjects (45 women, 30 men) aged from 19 to 69 years (median, 29 years) without vascular risk factors were included as additional control groups.

Blood Sampling and Serial Quantification of Markers of Hemostasis

Blood samples were collected before treatments and at hours 1, 3, and 5 and at days 1, 3, and 5 after initiation of the therapy. Blood sampling, plasma preparation, and storage were performed according to DIN 58 905 part I, published by the Deutsches Institute for Normung (DIN) (Berlin, Germany, 1994). Blood was drawn with syringes containing 1/10 volume of 3.13 citrate solution. Plasma was obtained by centrifugation at 2000g for 10 minutes at 8°C. Aliquots of the plasma samples were transferred to polystyrene sample tubes, snap-frozen on liquid nitrogen, and stored at −70°C for analysis; samples were thawed in a water bath at 37°C and analyzed within 30 minutes after thawing.

Concentrations of the DD antigen were measured by an immunoturbidimetric assay (Tina-quant). Concentrations of FM, F1.2, and TAT were determined by enzyme-linked immunosorbent assays (Enzymun-Test FM and Enzygnost F1.2 and TAT, respectively). In 4 exemplary patients, changes of activity of tPA in peripheral blood were also serially determined with the use of a commercially available enzyme immunoassay (Immuno Ltd). Blood was collected to determine rPA with the use of a special tube (Stabilyte) from Biopool.

Concentrations were expressed as mean ± SE. As a derivative parameter of the net change of concentrations of DD, FM, F1.2, and TAT, ΔMAX values (maximal concentrations minus basal concentrations) were calculated. For statistical analyses, the Wilcoxon matched rank test and the Mann-Whitney test were used, both with a Bonferroni correction at a level of α < 0.05.

Results

Fibrinolysis and Coagulation Induced by rtPA Thrombolysis for Acute Ischemic Stroke

Markers of coagulation activation and fibrin formation concomitantly responded to rtPA treatment with a similar massive and sustained increase in concentrations. Concentrations of these molecules increased within 1 hour after initiation of rtPA thrombolysis, peaked between hours 1 and 3, and declined toward baseline within 1 to 3 days (Figures 1 and 2). No significant correlations between ΔMAXs of the coagulation markers were observed. FM did not correlate with other hemostatic indicators. However, ΔMAXTAT significantly correlated with ΔMAXF1.2 (r = 0.56, P < 0.05).

Fibrinolysis and Coagulation Induced by Vascular Risk Factors and Acute Ischemic Stroke

Compared with 75 control subjects without vascular risk factors, 50 otherwise healthy subjects with such risk factors exhibited significantly increased values of FM (1.05 ± 0.13 versus 4.19 ± 0.40 µg/mL; P < 0.0001) and DD (136.42 ± 10.02 versus 10.40 ± 5.30 ng/mL; P < 0.0001). Compared

Figure 1. Kinetics of liberation of FM and DD in acute ischemic stroke treated with rtPA (n = 23) and low-dose (n = 10, broken line) or high-dose (n = 11, dotted line) heparin only. Con indicates risk factor–matched control subjects (n = 50). *Significant after Bonferroni correction at a level of α < 0.05.

Figure 2. Kinetics of liberation of F1.2 and TAT and of the appearance of tPA in acute ischemic stroke treated with rtPA (tPA was quantified only in 4 exemplary patients). Con indicates risk factor–matched control subjects (n = 50). *Significant after Bonferroni correction at a level of α < 0.05.
with these age- and risk factor–matched control subjects, patients with acute ischemic stroke showed significantly elevated levels of DD before heparin treatment and after treatment at hours 1, 3, and 5 and at day 1 ($P<0.05$ after Bonferroni correction; Figure 1). FM levels were also significantly increased at hours 1, 3, and 5 after initiation of heparin treatment ($P<0.005$; Figure 1).

**Effects of Heparin Treatment on Fibrinolysis and Coagulation in Acute Stroke Patients**

Importantly, concentrations of hemostatic markers did not significantly differ between patients treated with high- or low-dose heparin (Figure 1). Their massive response to rtPA treatment was not prevented by heparin (Figures 1 and 2). Finally, comparison between 5 patients treated with a study protocol (ECASS),$^1$ with heparin administration 24 hours after rtPA, and 18 patients treated according to the NINDS protocol,$^2$ with heparin 1 to 2 hours after rtPA infusion, revealed no significant reduction of MAX values of markers of coagulation (FM, 22.44±14.24 versus 44.10±8.16 μg/mL, $P=NS$; F1.2, 1.47±0.36 versus 1.81±0.20 pmol/L, $P=NS$; TAT, 18.53±15.27 versus 20.24±3.79 μg/L, $P=NS$).

The increase in DD antigen may be caused by either proteolysis of cross-linked fibrin or other fibrin derivatives or due to de novo formation of cross-linked fibrin by the action of thrombin. The pronounced response of the markers of procoagulant activity—FM, TAT, and F1.2—in stroke patients treated with rtPA indicates that thrombolytic therapy of ischemic stroke, apart from its desired fibrinolytic effects, strongly activates the coagulation cascade, resulting in thrombin formation. No correlation between DD and any of the coagulation markers was observed, indicating their different regulation. However, F1.2 significantly correlated with TAT, consistent with their common relationship to the process of thrombin formation.

In the course of fibrin formation, considerable amounts of thrombin and factor Xa are absorbed at the clot, where they are protected from effects of inhibitors.$^6$ The systemic procoagulant activation in stroke patients treated with rtPA may be explained by exposure of clot-bound thrombin and factor Xa as the clot undergoes lysis. Platelet activation by rtPA shown in vitro$^7$ may be an alternative explanation.

In ischemic stroke patients, these abnormalities induced by rtPA could contribute to the pathogenesis of severe complications, such as the failure to achieve initial reperfusion, early reoclusion after successful thrombolysis, or recurrent stroke. These results are consistent with observations of procoagulant tendencies (eg, increase in fibrinopeptide A) induced by thrombolysis for myocardial infarction.$^{13–15}$

Interestingly, control subjects with vascular risk factors exhibited a significant activation of hemostasis compared with control subjects without such risk factors. Compared with risk factor–matched subjects, patients with acute ischemic stroke exhibited a significant increase in hemostatic indicators. This may be induced by the chronic and acute endothelial activation, respectively, shown in these conditions.$^{16}$ However, hemostatic changes due to the presence of risk factors or secondary to cerebral ischemia were modest compared with those observed in response to rtPA treatment.

We could not separately study the effects of rtPA in stroke patients because we did not modify treatment protocols for the purposes of this study. However, this study showed that heparin has no major effects on systemic hemostasis in acute ischemic stroke because low- or high-dose heparin treatment in stroke patients did not result in visible hemostatic differences, although subpopulations were heterogeneous and consisted of a relatively small number of patients. Since we did not study the effects of rtPA treatment in the absence of any heparin anticoagulation, it cannot be excluded that this condition would be associated with an even more pronounced coagulation.

Heparin did not abolish the pronounced procoagulant response to rtPA, and comparative analysis in patients receiving heparin 1 or 24 hours after rtPA also revealed a marked procoagulant activation in patients with early high-dose heparin.

Such failure of intravenous heparin to suppress thrombin activity was also observed after coronary thrombolysis.$^{17}$ Recently, it has been suggested that heparin binding sites on thrombin are masked when this enzyme is bound to fibrin or its soluble fragments. A new generation of inhibitors of thrombin, which inactivate free and fibrin-bound thrombin equally well,$^{18}$ is thought to provide a better anticoagulant prophylaxis after rtPA thrombolysis than the currently used heparin.

In conclusion, whereas the presence of vascular risk factors and ischemic stroke itself had only minor effects on hemostasis, rtPA thrombolysis induced a massive and sustained increase of activation markers of coagulation. This kinetic study on coagulation in ischemic stroke treated with rtPA revealed important similarities with hemostatic abnormalities induced by thrombolysis for myocardial infarction, supporting a transfer of therapeutic concepts, ie, adjunctive anticoagulation from myocardial infarction to ischemic stroke, despite differences in pathophysiology of these diseases. There is, however, evidence that currently investigated novel anticoagulants may be more effective as suppressors of rtPA-induced coagulation than currently used heparin.

**References**


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