Thrombosis Research (2007) **120**, 447–450

THROMBOSIS RESEARCH

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BRIEF COMMUNICATION

Changes in blood viscosity with the recombinant tissue plasminogen activator alteplase ☆

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Received 25 December 2005; received in revised form 16 September 2006; accepted 18 September 2006
Available online 24 October 2006

KEYWORDS
Blood viscosity;
Recombinant tissue plasminogen activator;
Rheology

Abstract We measured whole blood viscosity to investigate the time course of the fibrinolytic activity of the recombinant tissue plasminogen activator alteplase. Changes in blood viscosity over time were determined using an oscillation-type viscometer at a shear rate of 400 to 500 per second. Blood viscosity initially increased with alteplase as in untreated blood, but then decreased, reflecting the fibrinolytic activity of generated plasmin. Blood viscosity subsequently stabilized at a level below the initial value owing to the dissolution of both fibrin and fibrinogen by alteplase. To our knowledge, the present study is the first to examine the time course of changes in BV during fibrin formation and degradation. The results indicated that the fibrinolyic agent alteplase might provide the additional benefit of increasing blood flow by lowering blood viscosity.

Introduction

Recombinant tissue plasminogen activators (rt-PAs) have higher affinity for bound fibrin than for circulating fibrinogen, and cause the local activation of plasminogen to plasmin and subsequent fibrin degeneration [1]. Randomized trials of patients with early acute myocardial infarction have demonstrated clear benefits of intravenous thrombolytic therapy for survival and cardiac function [1–7]. Other trials have also suggested that administering intravenous rt-PA within 3 h of the onset of ischemic stroke can improve clinical outcomes for 12 months of follow up [8,9]. Whole blood viscosity (BV), which is the intrinsic resistance of blood to flow in vessels, is determined by hematocrit, red cell deformation, red cell aggregation, and plasma viscosity [10]. To clarify the effects of rt-PA upon human blood fluidity *in vitro*, we examined changes in venous BV after

☆ The main findings of this study were presented at the 11th International Conference of Biorheology/5th International Conference of Clinical Hemorheology, Antalya, Turkey, October 2002.

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0049-3848/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.thromres.2006.09.011

collection over time, which reflects the coagulation process. We also examined the dose-dependent effects of alteplase on fibrin clots in humans.

Materials and methods

Samples

Blood samples were slowly withdrawn from the cubital veins of 20 healthy male volunteers between 9 and 11 a.m. using 21-gauge needles and examined without anticoagulants. The volunteers were of normal weight, aged between 20 and 23 y and did not smoke cigarettes. All provided written informed consent to participation in the study.

Measurement of BV and hematocrit

Powdered alteplase (24,000,000 IU; Mitsubishi Pharma, Osaka, Japan), was diluted with 24, 48, 120, or 240 ml of saline and then 30 μl of each concentration was gently mixed with 3 ml of blood by inversion. The final concentrations of alteplase were adjusted to 1000, 2000, 5000, and 10,000 IU/ml. Control blood samples contained normal saline instead of alteplase. The mixture was gently poured into a bottle incubated at 37 °C and then BV was determined using an oscillation viscometer (Viscomate VM-1G, Yamaichi Electronics, Osaka, Japan). The viscometer includes a titanium probe that periodically vibrates around an axis at 500 Hz. When the probe is immersed in blood, the resistance (viscosity) reduces the amplitude of the vibration. A piezoelectric accelerating sensor detects changes in amplitude as changes in angular acceleration. Therefore, BV can be measured at shear rates of 400 to 500 per second. This system can be used to examine changes in BV over time and to estimate coagulatory or fibrinolytic activity [11]. Because BV is almost constant at a high shear rate and anticoagulants were not included, BV was determined immediately and accurately. The viscometer was regularly adjusted with water and standard JS 2.5, JS 20, JS 50, JS 200 and JS 1000 solutions for accurate comparisons. Five samples containing each concentration of alteplase and four control samples were analyzed. Hematocrit was determined immediately after centrifugation of capillary tubes at 12,000 rpm for 5 min (Hematocrit Roter KH-120, Kubota, Tokyo, Japan).

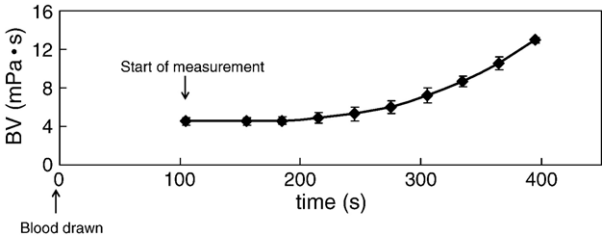

Figure 1 Time course of BV in control blood samples. Symbols represent mean ± standard deviation (n=4).

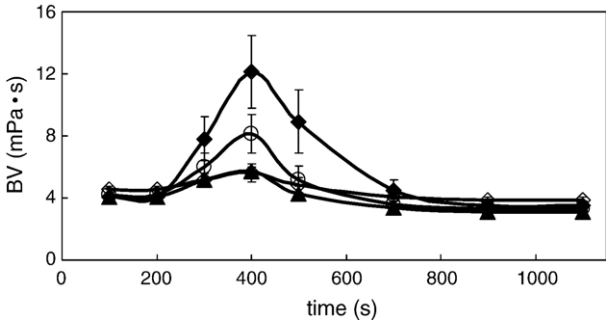

Figure 2 Time course of BV with all tested concentrations of rt-PA. Closed diamonds, 1000 IU/ml of rt-PA; open circles, 2000 IU/ml of rt-PA; closed triangles, 5000 IU/ml of rt-PA; open diamonds, 10,000 IU/ml of rt-PA.

Measurement of fibrinolytic parameters

The concentrations of fibrinogen, fibrin monomer, and d-dimer were measured in blood samples before and after mixing with 30 μl alteplase (final concentration, 1000 IU/ml) as described above. Control blood samples contained normal saline and 2.0 IU/ml of heparin sodium (Aventis Pharma Japan, Tokyo, Japan). Blood samples containing drugs or saline were mixed by inversion in glass tubes and incubated at 37 °C. Ten minutes after the blood samples were collected, 0.3 ml of sodium citrate (0.11 M) was added and fibrinolytic parameters were examined. However, blood samples containing normal saline were examined at 8 min after collection as they tended to coagulate sooner. Levels of fibrinogen were determined using the thrombin-time method; D-dimer was measured using an enhanced microlatex immunoassay (Diagnostica Stago STA Liatest D-Di); fibrin monomer was measured using a latex immunoassay with the specific antibody, F405.

Statistical analysis

Paired BV data from the experiment with rt-PA were obtained before and after adding alteplase to one blood sample. We used the Wilcoxon test to compare BV values before and after alteplase-induced fibrinolysis. We applied the Mann–Whitney test to compare the values of fibrinolytic parameters before and after adding alteplase to unpaired samples.