CONCISE REPORT

Blood Cells Participate in the Fibrinolytic Response to Tissue-Type Plasminogen Activator

By M. Wahbi Hammouda and Leonard A. Moroz

Exercise and venous occlusion stimulate fibrinolysis. In addition to increased concentrations of tissue-type plasminogen activator (tPA) and increased plasmin-mediated fibrinolysis in plasma, these stimuli produce additional cellular-phase activity in blood that is of the same magnitude as the plasma response. To determine whether tPA alone, rather than other consequences of these stimuli, is responsible for the cellular response, the in vitro effects of tPA on whole blood, plasma, and calculated cellular-phase (whole blood minus plasma) activities were determined by solid-phase radiofibrinogen assay on venous blood from eight normal subjects (seven men, three women). At tPA concentrations encompassing physiological and therapeutic levels (5 to 100 ng/mL; 0.7 to 14 IU/mL), increments in whole blood were consistently in excess of those in compartment plasma and represented increased cellular-phase activity equivalent in magnitude to the well-known increase in plasma activity. Fibrinolytic activity produced by 10 to 20 ng tPA/mL (1.4 to 2.8 IU/mL) was consistently detected in whole blood and plasma by 60 minutes, with higher concentrations (100 ng or 14 IU tPA/mL) detectable after a five-minute assay in all subjects. Thus, tPA alone, without invoking fibrinolytic factors extraneous to blood or other effects of exercise or venous occlusion, accounts for both cellular and plasma responses to these stimuli. The considerable cellular response, the mechanism of which remains to be elucidated, may constitute a determinant of individual therapeutic responsiveness to tPA.

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THE GENERATION of the fibrinolytic enzyme plasmin from its plasma proenzyme plasminogen by tissue-type plasminogen activator (tPA) released from vascular endothelium is a major mechanism for physiological or pathological stimulation of fibrinolytic activity in plasma and the basis for the increasing therapeutic use of tPA for thrombolysis. Two well-studied stimuli of its release in vivo are venous occlusion and exercise. In both, the participation of tPA has been documented functionally and immunohistochemically.

In addition to the plasminogen-plasmin system and other nonplasmin fibrinolytic enzymes in the plasma phase of blood, there is accumulating evidence for a quantitatively important cellular-phase component to blood fibrinolytic activity both in normal and in physiologically, pathologically, and pharmacologically altered fibrinolysis. When methods capable of simultaneously monitoring whole blood and plasma fibrinolytic activities are used, this cellular contribution is demonstrable in the blood of normal individuals; in the fibrinolytic responses to such stimuli as venous occlusion, venous thromboembolism, exercise, and surgical trauma, urokinase, and salicylates; and in decreased cellular activity in systemic lupus erythematosus. The blood neutrophil, with its abundant fibrinolytic enzymes, lysosomal elastase, and cathepsin G, is a major candidate for a participating cell. It has been directly implicated in the responses to surgical trauma, urokinase, and salicylates. However, a role for other blood cells, particularly the monocyte, which has demonstrated fibrinolytic potential, cannot be excluded.

In studies of the fibrinolytic responses to exercise and venous occlusion, we found the expected increases, attributable to tPA, in plasma fibrinolytic activity. With both stimuli, an additional response was measurable in blood that was of the same magnitude as the response in plasma and that could only be explained by the participation of blood cells. This cellular-phase increment did not correlate with blood counts of individual cells, thereby suggesting a functional change in one or more cell types. Such a response might be due to direct or indirect effects of tPA on plasminogen, other plasma proteins and blood cells, or might reflect parallel but unrelated metabolic effects of venous occlusion or exercise on cellular fibrinolysis. To clarify these alternatives, we have examined the effects of tPA on fibrinolytic activities of whole blood and plasma in vitro.

MATERIALS AND METHODS

Venous blood samples were obtained in the nonfasting state from ten healthy young adults (seven men, three women), with application of a tourniquet for less than one minute and anticoagulation with heparin (10 U/mL). Fibrinolytic activities of paired whole blood and platelet-poor plasma samples were determined by a 125I-labeled fibrin solid-phase radiometric test tube assay, as described previously, with and without the addition of tPA (DNA recombinant tissue-type plasminogen activator rtPA, kindly provided by Genentech, Inc, South San Francisco, CA). The specificity of this preparation (lot no. H9075BX) was 142,000 IU/mg by calibration with the World Health Organization international reference standard for tPA by fibrin plate assay. The activator was added to blood or plasma in a 0.1 vol of isotonic Tris-NaCl buffer, pH 7.4, to yield the final concentrations indicated. Fibrinolytic activities were expressed as nanograms of fibrin lysed.

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The rate of increase in individual whole blood and plasma fibrinolytic activities was determined by incubation for periods from 0 to 30 minutes at a tPA concentration of 100 ng/mL. The effects of varying concentrations of tPA (5 to 50 ng/mL) were determined by assay of whole blood and plasma for 60 minutes. In addition to absolute whole blood and plasma activities determined with and without tPA for individual subjects, individual cellular-phase activities were calculated from the difference between paired whole blood and plasma activities, and tPA-induced increments in cellular-phase and plasma activities were calculated and compared.

Statistical analysis using a Statpak program was by Student's t test for paired and unpaired data.

RESULTS

The addition of tPA to whole blood or plasma from normal subjects at concentrations of 5 to 50 ng/mL resulted in the expected progressive increase in plasma fibrinolytic activity (Fig 1, open bars), with a tenfold increase in mean plasma activity at a tPA concentration of 50 ng/mL. However, there was a similar but greater increase in whole blood activity (represented in Fig 1 by open bars plus solid bars) that could not be attributed to the increase in plasma activity alone. When this cellular-phase component was estimated from the difference between individual whole blood and plasma values (Fig 1, hatched bars), it was found, on average, not to differ significantly \( (P > .1) \) from corresponding mean plasma activities at each concentration of tPA tested. There were significant increases in mean whole blood and plasma activities at 10 ng/mL \( (P < .05) \) and \( P < .005 \), respectively.

This relationship was maintained when tPA-related increments in plasma and cellular-phase activities were calculated by subtracting buffer control values determined without tPA (Fig 2). Mean individual increments in calculated cellular-phase activity were similar to but not significantly different \( (P > .1) \) from mean individual increments in plasma activity at each tPA concentration tested.

A cellular-phase response to tPA was also observed when the rate of generation of fibrinolytic activity was determined in whole blood and plasma to which a constant concentration of tPA (100 ng/mL) had been added (Fig 3). Again, there was a progressive increase in tPA-stimulated plasma fibrinolytic activity with time (open bars) and a quantitatively greater increase in whole blood activity (open bars plus solid bars), with mean individual increments in calculated cellular-phase activity (hatched bars) that were similar \( (P > .1) \) to corresponding mean individual increments in plasma activity (open bars) at each time point. Although the mean values for whole blood and calculated cellular-phase activity for the entire group of normal subjects only differed significantly from zero time values after a 15-minute assay \( (P < .01) \), which reflects the individual variation in responses

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**Fig 1.** Effects of tPA on fibrinolytic activities of whole blood and plasma in vitro. Mean activities of plasma (open bars) for ten normal adults (seven men, three women) are superimposed on corresponding mean whole blood activities (open bars plus solid bars). Mean individual calculated cellular-phase activities (whole blood activity minus plasma activity) are indicated by the hatched bars. Fibrinolytic activities were determined by a solid-phase radiofibrin assay for 60 minutes at 37°C. Vertical bars denote 1 SEM.

**Fig 2.** Mean individual increments in plasma (open bars) and calculated cellular-phase activities (hatched bars), with different concentrations of tPA added to normal blood and plasma, calculated from the data shown in Fig 1.

**Fig 3.** Mean individual increments in plasma (open bars) and calculated cellular-phase activities (hatched bars), calculated from the data shown in Fig 1.
to tPA in vitro, there was, for whole blood and plasma of each subject tested, a detectable increase in fibrinolytic activity after assay for only five minutes.

DISCUSSION

This study demonstrates that a purified preparation of tPA added to normal blood in vitro produces a cell-related fibrinolytic response that is in addition to and of the same magnitude as the well-known plasmin-mediated fibrinolytic response in plasma. These findings are relevant to the understanding of the action of tPA and other activators in physiologically and pathologically altered fibrinolysis and to the therapeutic use of tPA including the monitoring of its fibrinolytic effects.

The similarity between the increases in cellular-phase and plasma fibrinolytic activities noted here in vitro reproduce the findings with exercise, venous occlusion, and urokinase administration in vivo. The fibrinolytic responses to exercise and venous occlusion are associated with increased plasma tPA levels. The present in vitro study indicates that the large cellular-phase response to these stimuli is explicable by effects of tPA on blood components alone, without invoking nonfluid cellular elements such as endothelium or other physiological or metabolic consequences of venous occlusion or exercise distinct from the tPA-plasminogen system and its interactions with other plasma proteins and cells.

The identity of the blood cells and the mechanisms involved remain to be elucidated. The neutrophil is a candidate because of its demonstrated participation in blood fibrinolysis in other contexts including its partial explanation of the cellular responses to urokinase. However, other blood cells such as monocytes have fibrinolytic potential, although their contribution to physiological fibrinolysis in blood is uncertain. Dissection of the tPA effect will require a systematic study of interactions among tPA, plasminogen (and other plasma proteins), and purified preparations of individual blood cell types since the effect is not reproduced by simple addition of preformed plasmin to blood cells.

There is need for a rapid, sensitive, and simple method of monitoring the fibrinolytic effects of tPA and other plasminogen activators in clinical practice, particularly since optimal therapeutic dosages for plasminogen activators remain to be established. The radiometric test tube assay used in this study meets these requirements. In studies of coronary thrombolysis with various dosages of recombinant tPA preparations very similar to the one used here, mean peak values from 1,200 to 3,300 ng tPA/mL have been reported. In the present study, a relatively low concentration from the therapeutic standpoint was detectable in whole blood or plasma after only five minutes of assay (Fig 3), and even lower levels were measurable within acceptable assay times (10 to 20 ng/mL) in one hour (Fig 1), which indicates the applicability of the radiometric assay to the therapeutically relevant range of tPA concentrations. The method has the additional advantages, when applied to whole blood, of eliminating the time required for centrifugal preparation of plasma and permitting the detection and quantitation of the cellular component of the fibrinolytic response to tPA and other activators. This component, which is considerable, may constitute a variable determining individual responsiveness to tPA and other activators.

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REFERENCES

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BLOOD CELLS, TPA, AND FIBRINOLYSIS

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MW Hammouda and LA Moroz