Feasibility of Recording Clot Lysis with the Thermometric Clot Detector

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We investigated the possibility of continuously recording the process of clot lysis with a thermometric clot detector. Experiments in which fibrinolytic activators and inhibitors were used demonstrate that the detection system, without any mechanical modifications, is suitable for monitoring the complete coagulation process from clot formation to the ultimate lysis of the clot itself.

Additional Keyphrases: fibrinolysis • streptokinase therapy • clot lysis inhibition • thermistor

Currently, the study of fibrinolysis is beset by problems of methodology (1). Disadvantages of many of the present methods for measurement of clot lysis—including lysis time for the dilute blood clot, thromboelastography, euglobulin clot lysis, fibrin plate method, and caseinolytic assay (1)—are imprecision of results and time required to perform the test (2). The electromechanical lysis timer of Folus and Kramer (2) appears to be the only readily automated device, but so far it has not proven entirely satisfactory for measuring lysis time (3). Among the continuous-recording devices, the thromboelastograph reportedly indicates fibrinolysis (4); however, it is expensive and insufficiently sensitive (4).

In this communication, we report the feasibility of using a thermometric clot timer (5, 6) to study fibrinolysis.

Materials and Methods

Instrumentation

The thermometric clot detector and associated circuitry are identical to those described previously (5, 6). The transducer is a 2000-Ω glass probe thermistor (Model 32A222; Victory Engineering Corp., Springfield, N.J. 07081). The power applied to the thermistor is approximately 20 mW, and the device is vibrated at 60 Hz with an amplitude of 0.06 cm.

Reagents

“Simplastin A” (General Diagnostics Division, Warner-Lambert Co., Morris Plains, N.J. 07950) was used as the clotting agent in the fibrinolytic studies. This reagent contained thromboplastin and calcium with additional fibrinogen and factor V.

The fibrinolytic activator used was “Varidase” (Lederle Laboratories, Inc., Pearl River, N.Y. 10965). When reconstituted according to the manufacturer’s specifications, the stock solution contained 10,000 and 2500 units of streptokinase and streptodornase activity, respectively, per milliliter.

The fibrinolytic inhibitor we used was 6-amino-caproic acid (Sigma Chemical Co., St. Louis, Mo. 63178).

The plasma reagent used was “Verify Normal Oxalate” (General Diagnostics).

Procedure

Fibrinolytic studies: A one-stage prothrombin time test, in which “Simplastin A” was used as the
thromboplastin reagent, was performed on a dilution of the plasma reagent. The plasma was diluted four-fold with saline (9 g of NaCl per liter). The saline diluent also contained the streptokinase and (or) the fibrinolytic inhibitor. Before analysis the diluted sample was incubated for 20 min in a water bath set at 37.0 ± 0.1 °C, which converts plasminogen to plasmin by the action of streptokinase.

Results and Discussion

One must understand that the signal observed at point A in Figure 1a reflects a change in the internal temperature of the thermistor caused by a radical change in the removal of heat from the transducer by forced convection (6). A firm clot—e.g., one obtained from plasma containing more than 100 mg of fibrinogen per deciliter—shows a large, distinct break (Figure 1a). However, if the plasma is so greatly diluted that there is insufficient fibrinogen available to give a mechanically firm clot, the tenuous, diffuse gel can no longer completely eliminate convective removal of heat from the thermistor and an indistinct end point of decreased magnitude (Figure 1b) will be obtained.

We postulated that fibrinolysis could be measured if an initially firm clot could be formed in the presence of activated plasmin; a distinct clotting end point will occur. Ultimately the plasmin will dissolve the fibrin web, and consequently the signal from the vibrated, self-heated thermistor will relax toward the baseline.

Figure 2 qualitatively indicates the effect of fibrinolysis on the thermometric record of the coagulation process. When neither fibrinolytic activators or inhibitors are present a well-defined end point (as in curve a of Figure 2) is observed, and in similar experiments the steady-state self-heating persists without significant decrease for several hours. In the experiment illustrated by curve b of Figure 2, the incubation mixture contained about 125 units of streptokinase per milliliter and about 20 mmol of 6-aminocaproic acid, a potent fibrinolytic inhibitor, per liter (1, 2). The net effect of the presence of both the enzyme and its inhibitor is that there was no degradation of the steady-state signal during the 10-min period the sample was observed. A similar experiment, in which the inhibitor was deleted but the amount of enzyme kept constant (see curve c, Figure 2), shows that the signal reaches a maximum and decreases significantly within 4 min. The fibrin clot did in fact dissolve. Curve d of Figure 2 was obtained with about 250 units of streptokinase per milliliter. The clot lysis rate increased, as shown by the significant decrease in the signal at 2.5 min. When streptokinase activity is low, the lysis time decreases as the concentration of streptokinase increases (2). For present purposes we have defined clot lysis time as the time from the prothrombin end point (see point A in Figure 3) to the point at which the signal decreases to half its maximum value. Three replicate runs for a sample containing 150 units of streptokinase per milliliter are shown in Figure 3; the coefficient of variation for these was 10%.

The preliminary results presented here may only be compared to the technique of Folus and Kramer (2), for which there are adequate published data. The incubation time we used (20 min) is reasonably short and can be done on several samples simultaneously, as can the method of Folus and Kramer. The instrument can be loaded with a sample and the appropriate reagents added in 1 min. If either plasminogen or
streptokinase are present or added in very low amounts, clot lysis will be quite prolonged and will determine the analysis time. The runs reported in Figure 3 required about 5 min per sample, excluding the 20-min incubation. Folus and Kramer reported a reproducibility of 10% for six replicate runs for which the lysis time varied from 100 to 3000 s. We believe that analysis time and precision for the instrument described here is comparable to that of Folus and Kramer, but is superior to that of other methods mentioned above.

A sample of plasma incubated with streptokinase to generate plasmin will have a decreased amount of Factor V and fibrinogen, because plasmin can digest these factors as well as fibrin (9). For this reason we used "Simplastin A," a concentrated source of these factors, to clot the incubation mixture, so as to pro-

vide a distinct initial end point. If an aliquot of plasmin reagent is added to a test plasma, as in the procedure of Folus and Kramer for assay of streptokinase (2), thrombin may be used to clot the sample.

Plasminogen is a protein that is converted to the fibrinolytic enzyme, plasmin, in the presence of an activator such as streptokinase (1, 2). Plasminogen, like fibrinogen, acts as an acute-phase reactant, and is either increased or decreased in various diseases (7). Fibrinolytic activators such as streptokinase have been used therapeutically to dissolve thrombi in vivo (7, 8).

With modification of the procedure, the ther-

metric detector could be used to assay for plasminogen, plasmin, streptokinase, or anti-streptokinase (streptokinase is antigenic).

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