The euglobulin lysis time test: An ineffectual monitor of the therapeutic inhibition of fibrinolysis

J. KATZ, A. LURIE, D. BECKER, AND J. METZ

From the Department of Haematology, South African Institute for Medical Research, Johannesburg

SYNOPSIS In two clinical situations associated with hyperfibrinolysis the administration of antifibrinolytic drugs resulted in clinical haemostasis. The dilute clot lysis time and fibrin plate activity test but not the euglobulin lysis time reflected this control of excessive fibrinolysis by the antifibrinolytic drugs. The inhibition by epsilon-amino-caproic acid (EACA) of hyperfibrinolysis induced *in vitro* was reflected by the dilute clot lysis time but not by the euglobulin lysis time. Paper chromatography of the supernatant and euglobulin fractions as prepared for the euglobulin lysis time test from plasma with added EACA demonstrated that some 85% of the EACA was present in the supernatant, normally discarded during the test. Similarly, cellulose-acetate electrophoresis of the supernatant and euglobulin fractions from plasma containing Trasylol demonstrated the drug in the supernatant only. These findings indicate that when acetic acid is added to plasma containing EACA or Trasylol only a small proportion of the drug is precipitated with the euglobulin fraction. The euglobulin lysis time is thus an inaccurate index of the neutralization of hyperfibrinolysis by antifibrinolytic drugs.

Hyperfibrinolysis has been associated with a variety of disease entities, occurring either secondary to disseminated intravascular coagulation (MacKay, 1965) or more rarely as a primary pathological entity (Merskey, Johnson, Kleiner, and Wohl, 1967). The therapeutic agents most frequently used to control this excessive fibrinolysis are the amino-acid derivatives, EACA and tranexamic acid, and the kallikrein inhibitor, Trasylol. Laboratory tests for measuring fibrinolytic activity include the dilute clot lysis time, the euglobulin lysis time, the fibrin plate activity test, tests for fibrinogen degeneration products, and the plasminogen assay. By virtue of its ease of performance the euglobulin lysis time has been used by Pechet, Groth, and Daloze (1969) to monitor therapy with antifibrinolytic drugs. The present report, however, indicates that this test fails to reflect changes in fibrinolysis produced by antifibrinolytic drugs. The reason for this is that only a small amount of the antifibrinolytic drugs is precipitated with the euglobulin fraction, the major portion being discarded in the supernatant during the performance of the test.

Clinical Material

Two clinical situations were studied: (1) a patient with acute promyelocytic leukaemia and increased fibrinolysis as evidenced by shortened euglobulin and dilute clot lysis time, lysis on a fibrin plate, and low levels of plasminogen and fibrinogen; (2) anhepatic baboons undergoing experimental xenograft liver transplants. Removal of the baboon liver is associated with a rapid increase in fibrinolytic activity, with shortened euglobulin and dilute clot lysis times and low levels of plasminogen.

Methods

FIBRINOLYTIC TESTS

Venous blood was collected into 3.8% trisodium citrate in a ratio of 9:1. The euglobulin lysis time (Nilsson and Olow, 1962), dilute clot lysis time

Received for publication 23 December 1969.

Fig. 1.

Fig. 2.

Fig. 1 Tests of fibrinolysis in a patient with acute promyelocytic leukaemia receiving therapy with EACA and Trasylol. The hatched areas represent the normal range.

Fig. 2 Fibrin plate with plasmas and euglobulin fractions from a patient with acute promyelocytic leukaemia receiving therapy with EACA and Trasylol and from a normal control. Excessive fibrinolysis is present in the patient's euglobulin fraction only.

Fig. 3 Tests of fibrinolysis during a liver xenograft transplant.

Fig. 3.

(Fearnley, 1960), plasminogen (Remmert and Cohen, 1949), and fibrinogen (Ellis and Stransky, 1961) assays were performed according to standard methods. Fibrin plates for determining fibrinolytic activity were prepared according to the method of Astrup and Müllertz (1952) as modified by Holström (1965).

AMINO-ACID CHROMATOGRAPHY FOR EACA LOCALIZATION

Chromatography was performed using Whatman no. 1 paper. Volumes of 0.02 ml of standard and sample were spotted onto the paper. The spots were dried in an air current, and the chromatograms then developed by ascending chromatography. The solvent used was a butanol-acetic acid-water solvent (butanol 120 ml, glacial acetic acid 30 ml, distilled water 50 ml). After 12 hours the chromatograms were removed and dried in a stream of air. The chromatograms were stained with ninhydrin and then placed in an oven at 60° to 80°C for five to 10 minutes to accelerate the staining process. Amino-acid sites appeared as purplish-brown spots, and the EACA was identified from the site of the standard.

Streptokinase (μ/ml)	0	50	50	50	50	50	50	50	50	50	50	50
EACA (mg/ml)	0	0	1	5	10	15	20	25	30	40	50	60
Dilute clot lysis time (hr)	>24	3½	18	>24	>24	>24	>24	>24	>24	>24	>24	>24
Euglobulin lysis time (hr)	6	½	½	½	½	½	½	½	½	½	½	2

Table I Dilute clot and euglobulin lysis times following the addition of increasing quantities of EACA

Fig. 4 Paper chromatography of fractions, as prepared for the euglobulin lysis time test, from plasma to which EACA has been added.

EACA STD = EACA standard; ELT SPNT = supernatant; ELT PPT = euglobulin precipitate; ELT SPNT + SK = supernatant from streptokinase-activated plasma; ELT PPT + SK = euglobulin precipitate from streptokinase-activated plasma.

Fig. 5 Cellulose-acetate electrophoresis of the fractions, as prepared for the euglobulin lysis time test, from plasma to which Trasylol has been added.

CELLULOSE ACETATE ELECTROPHORESIS FOR TRASLYOL LOCALIZATION

In view of the polypeptide nature of Trasylol, electrophoresis was more suitable for identification than was chromatography. Electrophoresis was performed on cellulose acetate strips in a Tris-EDTA-borate buffer, pH 8.8. Test samples (0.02 ml) were run at 10 m amps for one hour and the electrophoretic bands identified with Ponceau S stain.

Results

CLINICAL SITUATIONS WITH INCREASED FIBRINOLYSIS

Hyperfibrinolysis in the patient with acute promyelocytic leukaemia was treated with a combination of EACA 24 g and Trasylol 1,000,000 units daily by intravenous injection. This therapy controlled the clinical bleeding and excessive fibrinolysis as evidenced by lengthening of the dilute clot lysis time, absence of fibrin plate lysis, and rise in the plasma levels of fibrinogen and plasminogen. The euglobulin lysis time, however, remained shortened (Fig. 1). Fibrin plate studies showed no fibrinolysis when whole plasma was plated, but markedly excessive fibrinolysis when the euglobulin fraction only was plated (Fig. 2). In the same way, the dilute clot, but not the euglobulin, lysis times, reflected clinical control of fibrinolysis by Trasylol in the anhepatic baboon (Fig. 3).

EUGLOBULIN LYSIS TIME AND INDUCED INCREASED FIBRINOLYSIS *in vitro*

Streptokinase¹ added to normal plasma in a concentration of 50 units per ml results in activation of fibrinolysis with marked shortening of the dilute clot and euglobulin lysis times. On the subsequent addition of increasing concentrations of EACA² (1-60 mg per ml) to aliquots of the streptokinase-activated plasma the dilute clot lysis time reverted to normal. However, the euglobulin lysis time of the activated plasma remained abnormally short in spite of the addition of EACA (Table).

¹Lederle Laboratories, USA
²Kabi, Sweden.

DISTRIBUTION OF EACA AND TRASYLOL IN THE EUGLOBULIN PRECIPITATE AND SUPERNATANT

To aliquots of normal plasma, and normal plasma in which the fibrinolytic activity was activated by the addition of streptokinase (25 units per ml), EACA (5 mg per ml) or Trasylo¹ (75,000 units per ml) was added. Aliquots of these plasmas (0.5 ml) were then diluted with 9.5 ml distilled water and the euglobulin fractions precipitated with 1% acetic acid. The precipitates and supernatants from the EACA and Trasylo¹-containing plasmas were submitted to paper chromatography and cellulose acetate electrophoresis respectively. The results indicated that the EACA and Trasylo¹ were present almost exclusively in the supernatants (Figs. 4 and 5). Control samples included euglobulin precipitates and supernatants prepared from normal plasma with and without added streptokinase (25 units per ml). The EACA and appropriate blanks on the chromatograms were eluted with 0.01 NHCl. An eluate from the EACA standard was scanned spectrophotometrically and its maximum absorption was at 250 m μ . The EACA eluted from the chromatograms of the euglobulin precipitates and supernatants were quantitated by the optical density at 250 m μ . The results indicated that 15% of the EACA was in the precipitate and 85% in the supernatant.

Discussion

The rationale for precipitating the euglobulin of plasma before measuring fibrinolytic activity is the separation of the precipitable fibrinolytic enzymes from the non-precipitable inhibitors, antiplasmin and anti-activator. Euglobulin contains the plasminogen activator and plasminogen of plasma, 25% of the fibrinogen, but only traces of the antiplasmins (Kowalski, Kopec, and Niewiarowski, 1959). The sensitivity of this test system becomes greater than that of the dilute clot lysis time in which the fibrinolytic inhibitors are not removed. This is reflected by shortening of the clot lysis time of normal plasma from 12 to 24 hours in the dilute clot lysis time to five to 10 hours in the euglobulin lysis time.

¹Bayer, Germany.

Sherry, Fletcher, Alkjaersig, and Sawyer (1959) have shown that administered EACA is unlikely to be protein bound, for the drug is readily dialyzable from plasma. By ultrafiltration techniques and protein-free filtrate assays, McNicol, Fletcher, Alkjaersig, and Sherry (1962) found recovery of the drug from the plasma to be quantitative. The present studies in addition have shown that the binding of fibrinolytic enzymes by EACA and Trasylo¹ is disrupted by euglobulin precipitation. If the euglobulin lysis time is used to monitor antifibrinolytic therapy the anti-fibrinolytic drugs are precipitated in minimal amounts in the euglobulin fraction, and the neutralization of excessive fibrinolysis is thus not reflected.

We wish to thank the Director, South African Institute for Medical Research, and Professor J. A. Myburgh, Department of Surgery, University of the Witwatersrand, for facilities to carry out these studies.

References

- Astrup, T., and Müllertz, S. (1952). The fibrin plate method for estimating fibrinolytic activity. *Arch. Biochem.*, **40**, 346-351.
- Ellis, B. C., and Stransky, A. (1961). A quick and accurate method for the determination of fibrinogen in plasma. *J. Lab. clin. Med.*, **58**, 477-488.
- Fearnley, G. R. (1960). Spontaneous fibrinolysis. *Amer. J. Cardiol.*, **6**, 371-377.
- Holmström, B. (1965). Streptokinase assay on large agar diffusion plates. *Acta chem. scand.*, **19**, 1549-1554.
- Kowalski, E., Kopec, M., and Niewiarowski, S. (1959). An evaluation of the euglobulin method for the determination of fibrinolysis. *J. clin. Path.*, **12**, 215-218.
- MacKay, D. G. (1965). *Disseminated Intravascular Coagulation: An Intermediary Mechanism of Disease*. New York and London, Harper Row.
- McNicol, G. P., Fletcher, A. P., Alkjaersig, N., and Sherry, W. (1962). The absorption, distribution and excretion of ϵ -aminocaproic acid following oral or intravenous administration to man. *J. Lab. clin. Med.*, **59**, 15-24.
- Merskey, C., Johnson, A. J., Kleiner, G. J., and Wohl, H. (1963). The defibrination syndrome: clinical features and laboratory diagnosis. *Brit. J. Haemat.*, **13**, 528-549.
- Nilsson, I. M., and Olow, B. (1962). Fibrinolysis induced by streptokinase in man. *Acta chir. scand.*, **123**, 247-266.
- Pechet, L., Groth, C. G., and Daloze, P. M. (1969). Changes in coagulation and fibrinolysis after orthotopic canine liver homotransplantation. *J. Lab. clin. Med.*, **73**, 91-102.
- Remmert, L. F., and Cohen, P. P. (1949). Partial purification and properties of a proteolytic enzyme of human serum. *J. biol. Chem.*, **181**, 431-448.
- Sherry, S., Fletcher, A. P., Alkjaersig, N., and Sawyer, W. (1959). ϵ -Aminocaproic acid. 'A potent anti-fibrinolytic agent'. *Trans. Ass. Amer. Physns.*, **72**, 62-70.