Practical Method for Thrombolytic Therapy with Streptokinase

Maintenance of Nearly Complete Plasminogen Depletion Associated with High Circulating Activator Activity

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In a previous paper we reported our clinical experience with purified streptokinase as a thrombolytic agent in patients with peripheral arterial occlusions (Verstraete *et al.*, 1963). In that study the need of angiographic control in evaluating the success of the thrombolytic treatment was emphasized. More recently we have demonstrated that in 17 recent occlusions treated during at least 48 hours, arteriographic evidence of clot dissolution was obtained in 10 instances (59%) (Amery *et al.*, 1963c). It is considered that a causal relationship with the administered streptokinase is at least a fair assumption.

Further research was directed toward the discovery of a satisfactory parameter, whose modifications would have a definite relationship with thrombolytic success. Previous experiments have demonstrated that the fibrinolytic activity of circulating blood on heated or unheated fibrin film, the streptokinase reactivity, and the blood-fibrinogen level fail to be such parameters (Amery *et al.*, 1963 bis). Subsequently our attention was drawn to the plasma-plasminogen level as a possible parameter whose variations could be related to thrombolysis: this molecule is not only the precursor of plasmin activity, but is also closely related to the formation of activator activity. If plasminogen variation is to be studied as a parameter, the thrombolytic effect of various degrees of streptokinase-induced plasminogen depletion should be evaluated. In this paper results obtained with nearly complete plasminogen exhaustion are presented.

Materials and Methods

The streptokinase (AB Kabi, Stockholm) was dissolved in 5% glucose and administered intravenously at a controlled rate. Initial doses of streptokinase were titrated by and calculated from the streptokinase reactivity test (Amery *et al.*, 1963a). Fibrinogen, as assayed by the fibrin polymerization time test (Vermylen *et al.*, 1963), was expressed in mg./100 ml. normally reactive fibrinogen in whole blood. The plasminogen level was determined as activator according to the method of Christensen (1949), modified by one of us (De Vreker, 1964). Human plasminogen, in highly diluted plasma, is allowed to react with excess streptokinase; the formed activator converts bovine plasminogen into plasmin, which is measured in terms of a dilution lysing within 30 minutes a standard bovine clot. Briefly, clots constituted at 0° C. by 1 ml. plasma serial dilutions in borate buffer (*p*H 7.5, I 0.2) containing 1600 units of streptokinase, 2.5 mg. of lyophilized bovine fibrinogen (batch No. 57—Poviet Producten, N.V., Amsterdam), and 2 units of bovine thrombin, are incubated at 37° C. until trapped air bubbles spontaneously move into the meniscus. From a log. lysis time versus log. reciprocal of plasma dilution straight plot, the dilution causing lysis within 30 minutes is interpolated and considered, by definition, to contain one human plasminogen unit. Unheated fibrin plates were prepared with Astrup's method (Astrup and Müllertz, 1952); heat denaturation of plasminogen was performed according to Lassen (1952). The success of thrombolytic therapy was assessed by comparing serial arteriograms (six exposures) before and after therapy.

Results

Figs. 1 and 2 describe some biochemical modifications induced by a continuous intravenous infusion of high amounts of streptokinase. The rapid administration of the priming dose of streptokinase was associated with a drastic decrease of

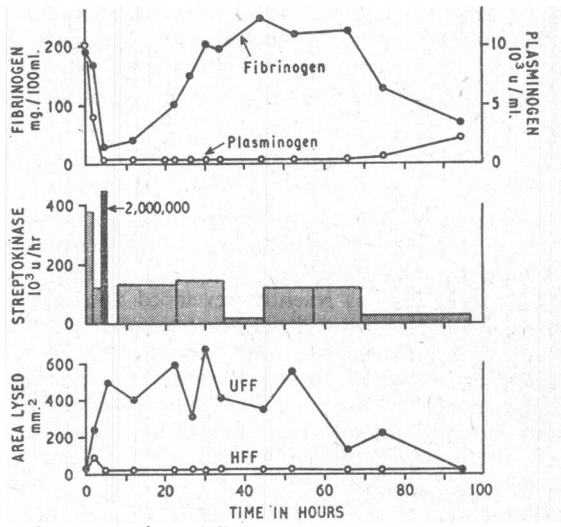

FIG. 1.—Patient V. D. M. Biochemical parameters during thrombolytic therapy with very high levels of streptokinase. Initial fibrinogen: 192 mg./100 ml. blood. Initial plasminogen: 10,000 u./ml. plasma. 20-min. dose: 3,745,000 u. streptokinase.

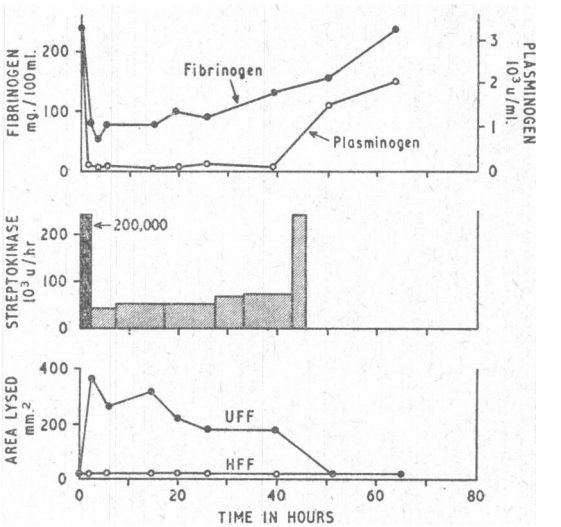

FIG. 2.—Patient B. R.M. Biochemical parameters during thrombolytic therapy with high levels of streptokinase. Initial fibrinogen: 250 mg./100 ml. blood. Initial plasminogen: 3,700 u./ml. plasma. 20-min. dose: 200,000 u. streptokinase.

the circulating plasminogen to very low levels (below 150 units/ml. plasma). Simultaneously a severe decrease of fibrinogen was noted. At this stage an increased plasma fibrinolytic

activity can be measured not only on unheated but also on heated fibrin film.

Subsequently, streptokinase was administered in sufficiently high amounts to *maintain* the plasminogen below 150 units/ml. plasma throughout treatment (117,000 and 52,000 units of streptokinase/hour in Cases 1 and 2 respectively). In both cases a sustained rise of the normally reactive fibrinogen level was observed ; 50 mg./100 ml. blood was obtained within 24 hours, which in our experience meets haemostatic requirements. Throughout, high levels of plasma fibrinolytic activity could be found on unheated fibrin film, with complete absence of fibrinolytic activity on heated fibrin film.

In Case 1 the administration of plasminogen-exhausting amounts of streptokinase was continued during 55 hours, followed by streptokinase administration at a lower rate (37,000 units/hour). This resulted in a progressive rise of the plasminogen level ; simultaneously the blood fibrinogen concentration decreased. Strong fibrinogen fall was already observed with a plasma-plasminogen level of 600 units/ml. (6% of the initial plasminogen level). This demonstrates that during streptokinase therapy fibrinogenolysis is avoided only by maintenance of a very low plasminogen concentration.

In both of these patients thrombolysis was demonstrated.

Discussion

The interpretation of the fibrinogen changes obtained with the proposed mode of streptokinase administration requires some additional details about the assay in use. The fibrin polymerization time test, as recently developed in our laboratory, expresses the fibrinogen available for haemostasis in milligram of normally clottable protein per 100 ml. When the fibrin polymerization time is prolonged owing to changes in the fibrinogen molecule or inhibition of fibrinogen conversion by polymerization inhibition, the value given refers to the amount of normally reactive fibrinogen clotting in the same time.

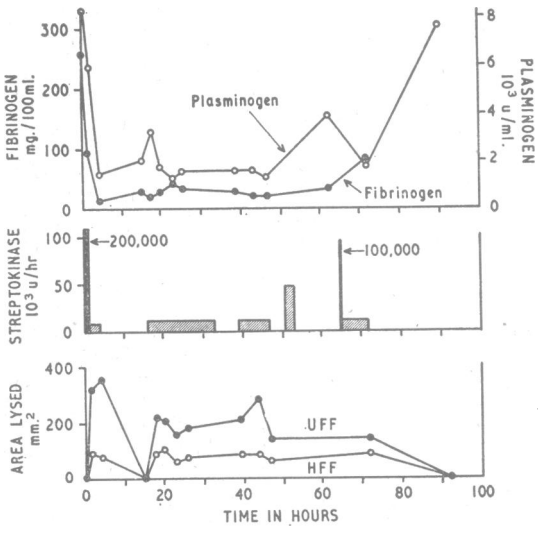

FIG. 3.—Patient V. P. Biochemical parameters during thrombolytic therapy with moderate levels of streptokinase. Initial fibrinogen: 320 mg./100 ml. blood. Initial plasminogen: 8,300 u./ml. plasma. 20-min. dose: 200,000 u. streptokinase. UFF= unheated fibrin film. HFF=heated fibrin film.

It is considered a major advantage to be able to assess in one test the fibrinogen which is relevant for haemostasis. The drastic fibrinogen depletion encountered during streptokinase therapy is at present the major hazard of streptokinase administration and so far unavoidable. Nevertheless, the results described in this paper show that a rapid increase of the initially lowered fibrinogen level can be obtained during administration of plasminogen-exhausting amounts of streptokinase. This can be explained by the fact that with an extremely low plasminogen and high circulating streptokinase level mainly activator and almost no circulating plasmin is formed (Kline and Fishman, 1961). Therefore the plasma fibrinogen, no longer subjected to proteolytic activity, is gradually replenished.

This rapid restoration of the plasma fibrinogen with plasminogen-exhausting doses of streptokinase is in striking contrast to what is observed when less large amounts of streptokinase are administered (Fig. 3). In this case, in which the patient is receiving 12,000 units/hour, the plasminogen is not exhausted (lowest value 1,250 units/ml.) and the fibrinogen level remains dangerously low throughout (less than 50 mg./100 ml. blood). In addition repeated evidence of circulating plasmin is obtained on heated fibrin film.

Besides the rapid fibrinogen repletion, the high dosage scheme also leads to continuously high levels of circulating activator, available for action on the occluding thrombus. Our experience, although limited, proves that thrombolysis can be obtained with this administration scheme provided the streptokinase was perfused for at least 48 hours, or until clot-dissolution occurred.

Summary

Continuous intravenous infusion of large amounts of streptokinase results in a dramatic decrease of the circulating plasminogen and of the fibrinogen available for haemostasis. It is demonstrated that a rapid increase of the initially lowered fibrinogen level can be obtained in pursuing the administration of plasminogen-exhausting amounts of streptokinase. In these circumstances mainly circulating activator and almost no plasmin is obtained. A high incidence of thrombolysis evidenced by arteriograms has been obtained, however, in a limited number of cases treated for 48 hours.

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