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Urokinase-induced Fibrin Clot Lysis and its Inhibition by Plasma

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ABSTRACT

The kinetics of the urokinase-induced plasmin digestion of fibrin clots has been studied. Some theoretical aspects on the kinetics of the underlying enzyme reactions are presented. It is shown that the clot-lysis time may be used to measure the relative amount of fibrinolysis inhibitors in plasma.

INTRODUCTION

In order to measure the inhibitory effect of serum on the fibrinolytic system, several clot-lysis methods have been developed (7, 9, 11). They all have in common that plasmin or plasminogen together with activators such as urokinase or streptokinase are added to solutions of fibrinogen, whereafter thrombin is added to bring about clotting and the time interval between clot formation and clot lysis is measured.

Clot-lysis methods have recently been used in investigations of serum from postoperative patients (1, 2) and have been considered to be valuable tools in monitoring the increase in fibrinolysis inhibition in these patients (13). They have also been used to elucidate the influence of 6-aminohexanoic acid on plasminogen activators (12) and to measure the fibrinolytic inhibition by α_2-antiplasmin and other fibrinolytic inhibitors (3, 6).

In a study by Gallimore and Shaw (7), the influence of certain anions, ionic strength, various amounts of serum and activator on the clot-lysis time were thoroughly investigated. Paraskevas et al. (11) calculated the inhibitory effect of a serum sample from a standard curve constructed from various dilutions of control serum. Others have tried to quantitate the inhibition by comparison with a standard inhibitor, trans-4-amino-methyl--cyclohexanoic acid (AMCA) (2), but no attempts have been made to correlate the clot-lysis time with the actual amount of inhibitor in the sample.

In the present paper it is shown that the kinetics of the fibrin clot lysis induced by urokinase can, within certain limits, be treated as in a

homogenous system. It is further shown that the clot-lysis method can be used to measure the relative amount of inhibitor in a plasma sample.

THEORETICAL TREATMENT

Two enzymatic reactions are involved in the fibrinolytic process.

$$\text{Plasminogen} \xrightarrow[\text{Activator}]{k} \text{Plasmin}$$

$$\text{Fibrin} \xrightarrow[\text{Plasmin}]{k'} \text{Fibrin degradation products}$$

If plasminogen is present in excess, the first reaction can be written

$$\frac{d\,P}{d\,t} = kA$$

Integration of this expression gives

$$P = kAt \qquad \text{Eqn. 1}$$

under the condition that P = 0 when t = 0 (P = the amount of plasmin formed at time t and A = activator concentration).

The degradation of fibrin is a zero order reaction (4):

$$-\frac{d\,Fib}{d\,t} = k'P \quad \text{or} \quad -\frac{d\,Fib}{d\,t} = kAt \qquad (Fib = \text{intact fibrin}).$$

Integration of this expression gives

$$Fib_0 - Fib_t = kA(t^2 - t_0{}^2) \qquad \text{Eqn. 2}$$

where $Fib_0 - Fib_t$ is the lysed amount of fibrin at time t.

When $Fib_0 - Fib_t$ reaches a constant value F, the clot is lysed. This occurs at time T. If $t_0 = 0$, then eqn. 2 is equal to

$$F = kAT \quad \text{or} \quad \frac{1}{T^2} = \frac{kA}{F} \qquad \text{Eqn. 3}$$

which means that there is a linear dependence between $1/T^2$ and A, as was first shown by Merrills and Shaw (10).

If it is assumed that the formed plasmin is attacked by a fast-reacting irreversible inhibitor, there will be a lag period when all the formed plasmin is inhibited and, consequently, no fibrinolysis will occur. According to eqn. 1, this lag period equals IP/kA, where IP = inhibitor concentration, or actually the concentration of the inhibited plasmin. Substitution of IP/kA for t_0 in eqn. 2 gives

$$F = kA(T^2 - (\frac{IP}{k})^2) \quad \text{or} \quad F = kAT^2 - K/A$$

where $K = kIP^2/k^2$. This is equal to

$$\frac{1}{T^2} = \frac{kA}{F} - \frac{kKA}{F^2A + FK} \quad \text{or} \quad \frac{1}{T^2} = \frac{kA}{F} - \frac{1}{T'^2} \qquad \text{Eqn. 4}$$

The term $\frac{kKA}{F^2A + FK}$ or $\frac{1}{T'^2}$ in eqn. 4 expresses the inhibitors contribution to $\frac{1}{T^2}$ (cf. eqn. 3).

$$\frac{1}{T'^2} = \frac{kKA}{F^2A + FK} \Rightarrow \quad T'^2 = \frac{F}{kA} + (\frac{Fk}{kIP})^2 \qquad \text{Eqn. 5}$$

This means that there is a linear relationship between T^2 and 1/A. The inclination F/k is the same as in eqn. 3, and from the intercept with the ordinate (i.e. when A is extrapolated to infinite concentration) IP/k can be calculated. An inclination other than F/k would indicate that either the activator is affected by the inhibitor or that the inhibitor is not fast-reacting and non-competitive.

METHODS

The clots were prepared from 1 mg fibrinogen ($3 \cdot 10^{-9}$ moles, AB KABI), 0.3 caseinolytic units (CU) plasminogen (10^{-10} moles, AB KABI), 0-70 mg human plasma (previously stored at -20 °C) or $3 \cdot 10^{-8}$ moles AMCA, $2.4\text{-}36 \cdot 10^{-13}$ moles (1-15 Ploug units) urokinase (UK, Leo, Denmark) (in the control clots without plasma 0.36, 0.72, 1.08 and $1.44 \cdot 10^{-12}$ moles urokinase were used) and finally 2 National Institute of Health units of thrombin (Topostasine^R, Hoffman-La Roche), which was added a few seconds after the urokinase. The final volume was 0.8 ml. All reagents were dissolved in 0.02 M phosphate, 0.15 M NaCl, pH 7.4. The lysis time in minutes was taken to be the interval from the addition of the urokinase until the visual absence of fibrin. For numerical reasons and to avoid effect of enzyme degradation, only lysis times in the range 15-60 minutes were concidered.

Calculations. T^2 was calculated according to eqn. 4: $1/T^2 = kA/F - 1/T^2$, where T is the actually measured clot-lysis time, and k/F was calculated from the non-inhibited standard curve by plotting $1/T^2$ (in this case, T is the clot-lysis time of the non-inhibited sample) versus A = urokinase concentration {UK} (eqn. 3). T^2 was plotted versus $\{UK\}^{-1}$ (see Figs. 1 and 2), and IP/k was calculated from the intercept with the ordinate, or $(Fk/kIP)^2$ (eqn. 5).

RESULTS AND DISCUSSION

A good linear relationship between $1/T^2$ and A was always obtained in the non-inhibited control experiments, which confirms the results of Merrills and Shaw (10). F/k varied between 145 and $240 \cdot 10^{-12}$ moles·min^2, probably depending on the quality of the urokinase.

When the lysis was inhibited by plasma the relationship between T^2 and 1/A (Fig. 1) never diverged significantly from a straight line with the inclination F/k, as long as only lysis times lower than 60 minutes were concidered. This indicates that the assumptions made in the theoretical treatment are valid, i.e. plasma acts as an apparently irreversible inhibitor of plasmin, and urokinase in the concentrations used is unaffected by plasma. This was expected, since the main plasmin inhibitor in plasma is α_2-antiplasmin, which has the qualities mentioned (8).

When low urokinase activities were used and the lysis time exceeded one hour, the T^2 values had a tendency to be too low, perhaps because the urokinase

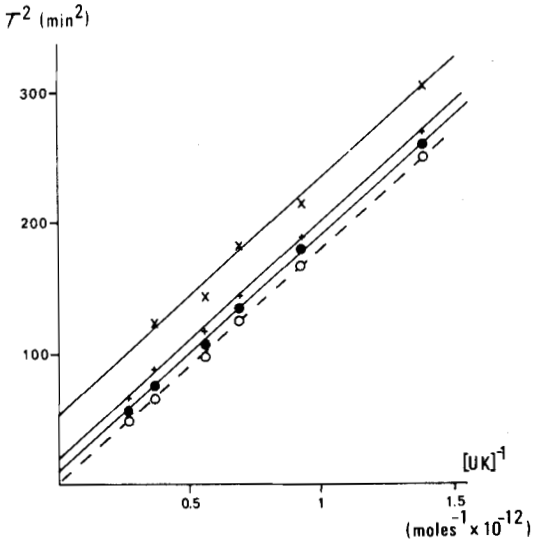

Fig. 1. Effect of various amounts of plasma on the T^2 versus $\{UK\}^{-1}$ -plot. See text for details. x=15, +=25 and •= 37.5 l. o---o = uninhibited $T^2=F/(kA)$.

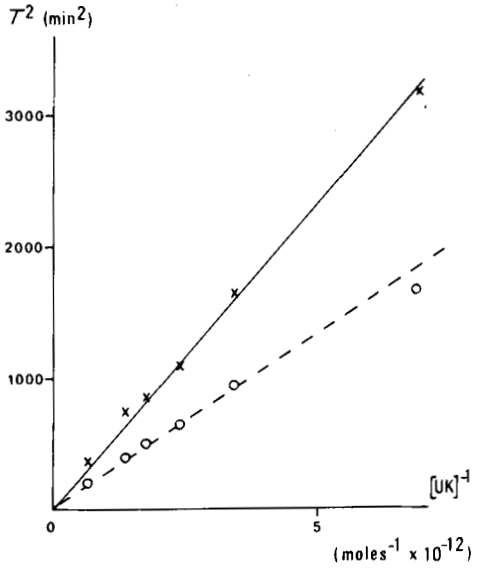

Fig. 2. Effect of $3 \cdot 10^{-8}$ moles AMCA on the T^2 versus $\{UK\}^{-1}$-plot. o---o = uninhibited $T^2=F/(kA)$.

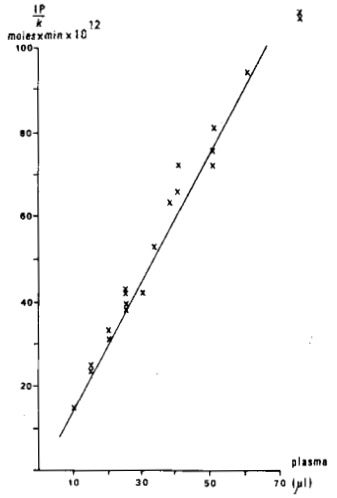

Fig. 3. Inhibition activity, IP/k, in various amounts of plasma.

activity was not constant but decreased during the lysis. This does not affect the calculation of IP/k, where the activator concentration is extrapolated to infinity.

The inhibitory effect of AMCA is different from that of plasma. The concentration used was chosen to give an inhibition of the same magnitude as did plasma. As seen in Fig. 2, AMCA did not behave in the same way as plasma, since the inclination of the graph differs conciderably from the theoretical F/k. The effect of AMCA and other ω-amino acids on the fibrinolysis is complex (5, 12),

and AMCA is therefore unsuitable as a standard substance in the assay of physiological fibrinolysis inhibitors.

Fig. 3 illustrates the linear relationship between the inhibition factor IP/k and the amount of plasma added to inhibit the clot lysis. Unfortunately it is not possible to measure the actual amount of inhibitor, IP, since the constant k is unknown in the clot system.

REFERENCES

1. Åberg, M. and Nilsson, I.M.: Fibrinolytic activity of the vein wall after surgery. Br J Surg 65: 259-262, 1978.
2. Bagge, L. and Saldeen, T.: The primary fibrinolysis inhibitor and trauma. Thromb Res 13: 1131-1136, 1978.
3. Bagge, L.: Studies on posttraumatic fibrinolysis inhibition with special reference to the primary fibrinolysis inhibitor (α_2-antiplasmin). Acta Universitatis Upsaliensis No. 342, 1979.(Dissertation.)
4. Berg, W., Korsan-Bengtsen, K. and Ygge, J.: Plasminogen assay by means of the lysis time method. Thromb Diath Haemorrh 14: 127-144, 1965.
5. Christensen, U.: Allosteric effects of some antifibrinolytic amino acids on the catalytic activity of human plasmin. Biochim Biophys Acta 526: 194-201, 1978.
6. Collen, D., Billiau, A., Edy, J. and DeSomer, P.: Identification of the human plasma protein which inhibits fibrinolysis associated with malignant cells. Biochim Biophys Acta 499: 194-201, 1977.
7. Gallimore, M.J. and Shaw, J.T.B.: Some aspects of fibrin clot lysis and its inhibition by human serum. Thromb Diath Haemorrh 16: 101-113, 1967.
8. Highsmith, R.F.: Evidence that α_2-antiplasmin is the primary physiological inhibitor of plasmin. In: The Physiological Inhibitors of Coagulation and Fibrinolysis (ed: D. Collen, B. Wiman and M. Verstraete), pp 103-113.
9. Mann, R.: Standardization of physiological variables of the fibrinolytic system in drug and other controlled studies. Progress in Chemical Fibrinolysis and Thrombolysis 4: 143-148, 1979.
10. Merrills, R.J. and Shaw, J.T.B.: Kinetics of fibrin clot lysis. Biochem J 106: 101-105, 1968.
11. Paraskevas, M., Nilsson, I.M. and Martinsson, G.: A method for determining serum inhibitors of plasminogen activation. Scand J Clin Lab Invest 14: 138-144, 1962.
12. Thorsen, S.: Influence of fibrin on the effect of 6-aminohexanoic acid on fibrinolysis caused by tissue plasminogen activator or urokinase. Progress in Chemical Fibrinolysis and Thrombolysis 3: 269-283, 1977.
13. Wallin, R., Bagge, L. and Saldeen, T.: Viewpoints on the determination of fibrinolysis inhibition activity in blood. Forens Sci 10: 154-156, 1977.

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