Pharmacological enhancement of fibrinolytic activity and ^{125}I -fibrinogen survival

J. A. HICKMAN

From the Department of Surgical Studies, The Middlesex Hospital, London

SYNOPSIS Studies with ^{125}I -fibrinogen after the administration of phenformin plus ethyloestrenol in five subjects showed no evidence of enhanced catabolism of fibrinogen despite a fall in plasma fibrinogen concentration and shortened clot lysis time. It seems probable that the fall in plasma fibrinogen concentration after the administration of these drugs is related to diminished hepatic synthesis of fibrinogen and is independent of the induction of plasminogen activator release.

It has been shown that the combined administration of phenformin and ethyloestrenol produces a sustained increase in the fibrinolytic activity of the blood both in healthy and diseased subjects. Moreover the enhancement of fibrinolysis is associated with a fall in plasma fibrinogen concentration and the temporary appearance in the serum of fibrin degradation products (Fearnley, Chakrabarti, and Hocking, 1967; Fearnley, Chakrabarti, and Evans, 1969). The association of increased fibrinolytic activity and fall in plasma fibrinogen concentration raises the question whether the lysis of fibrinogen as well as of fibrin is promoted by these drugs.

To pursue this further survival studies with ^{125}I -fibrinogen were performed in four patients and in a fifth healthy subject before and during treatment with phenformin and ethyloestrenol.

Methods

PLAN OF STUDY

^{125}I -fibrinogen survival, plasma fibrinogen concentration, dilute blood clot lysis time, and fibrin degradation products were studied in both the pretreatment and treatment periods.

In the healthy subject phenformin (sustained release capsules) 50 mg twice daily plus ethyl-

oestrenol 4 mg twice daily were given five days after the injection of 50 μC ^{125}I -fibrinogen. By the fifth day a baseline half-life ($T/2$) ^{125}I -fibrinogen had been obtained as well as estimations of fibrinogen concentration, blood lysis time, and fibrin degradation products.

In four patients treated with the same dose of phenformin and ethyloestrenol, one month was allowed to elapse before the second period of study since it is known that maximal enhancement of fibrinolytic activity and fall in plasma fibrinogen concentration does not occur until several weeks after treatment is first instituted (Fearnley, Chakrabarti, and Evans, 1966).

^{125}I -FIBRINOGEN SURVIVAL

Each subject was given 50 μC of ^{125}I -fibrinogen (Radiochemical Centre, Amersham, Bucks). The mean total clottability of this material *in vivo* was $92.4\% \pm 1.3$. The disappearance rate of ^{125}I -fibrinogen from the plasma was followed by studying the activity in the clottable fraction by the method of Regoeczi (1967). The counts were expressed as a percentage of the first sample obtained 10 minutes after injection of the fibrinogen and demonstrated graphically using a semi-logarithmic scale. The half-life ($T/2$) was obtained from regression lines to fit the values obtained more than 40 hours after injection. In case 2 sufficient data were obtained to analyse the clottable intravascular radioactivity according to

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the three-compartmental model described by Matthews (1957) and the fractional catabolic rate was calculated before and during treatment with the drugs.

PLASMA FIBRINOGEN CONCENTRATION

The gravimetric method of Fearnley and Chakrabarti (1966) was used, slightly modified in that the clot was extracted with a steel needle (Regoecki, 1967).

DILUTE BLOOD CLOT LYSIS TIME

Fearnley's method (1964) was used. Samples were taken between 9 am and 10 am on two successive days in both the pretreatment and treatment periods, each estimation being set up in triplicate.

FIBRIN DEGRADATION PRODUCTS

The Wellcome kit was used (Wellcome Research Laboratories, Beckenham, Kent) with a microtitre set (Flow Laboratories). Results were read independently by two observers; each estimation was performed in duplicate. Difficulty was experienced in removing heterophile antibodies in some specimens despite repeated absorption with sheep red cells. This method is therefore probably unreliable for detecting low levels of fibrin degradation products.

Results

The findings are shown in Table I and in Figures 1 and 2.

Table I shows the half life of ¹²⁵I-fibrinogen (T/2) before and after one month's treatment with phenformin and ethyloestrenol. Plasma fibrinogen concentration, dilute blood clot lysis times, and levels of serum fibrin degradation products are also shown.

Figure 1 shows the results from the study of the healthy male subject. ¹²⁵I-fibrinogen survival is expressed as radioactivity in the clottable

fraction as a percentage of the 10-minute sample. It will be noted that the administration of phenformin and ethyloestrenol did not cause any alteration in slope of the curve for ¹²⁵I-fibrinogen survival, although by the end of the study the blood clot lysis time had been shortened significantly. The slight rise in plasma fibrinogen level after starting the drugs has previously been observed in subjects with normal levels of fibrinogen at the outset of treatment (Fearnley, personal communication).

Figure 2 shows the results from case 2. The ¹²⁵I-fibrinogen survival is shown before and after one month's treatment. The T/2 has fallen from 116 hours to 100 hours but mathematical analysis of the curves reveals that the fractional catabolic rate is virtually unchanged at 21.2% per day compared with 19.5% per day. Furthermore, in view of the fall in the plasma fibrinogen pool in the second study, the absolute amount of fibrinogen catabolized per day has actually fallen from 3.27 g to 2.31 g per day, a fall of 29%.

Discussion

The values obtained for the half-life of ¹²⁵I-fibrinogen in this study are similar to those obtained in healthy subjects by Takeda (1966), by Regoecki and Stannard (1969), and in control subjects with miscellaneous disorders by Baker, Rubenberg, Dacie, and Brain (1968). This suggests that the ¹²⁵I-fibrinogen preparation used here was adequate for metabolic purposes.

In this study fibrinogen catabolism was not significantly enhanced after the administration of phenformin and ethyloestrenol despite increased fibrinolytic activity in all the subjects and a fall in the plasma fibrinogen concentration in three of the four patients treated with the drugs for one month.

Similar findings of increased fibrinolysis without change in fibrinogen catabolism were obtained by Regoecki and Walton (1967) in a study of eight monkeys whose fibrinolytic mechanisms were stimulated by injections of nicotinic acid or

Patient No.	Sex	Age (yr)	Diagnosis	T/2 ¹²⁵ I-Fibrinogen (hr)		Fibrinogen Concentration (mg % ± S.D.)		Blood Clot Lysis Time (hr) ¹		Fibrin Degradation Products (μg/ml)	
				Before	After	Before	After	Before	After	Before	After
1	M	54	Arteriosclerosis	104	90	618 ± 68	558 ± 56	6	3½	5	0
2	M	58	Ischaemic heart disease	116	100	438 ± 52	311 ± 29	7	2½	5	2.5
3	F	52	Cutaneous vasculitis	87	116	555 ± 33	441 ± 40	8	5	5	5
4	M	46	Erythema multiforme ²	72	72	267 ± 58	337 ± 37	8	6½	0	0

Table I Summary of data from four patients before and after treatment with phenformin and ethyloestrenol

¹Mean of two daily observations.

²This patient relapsed during the treatment period.

Fig. 1 The effect of administration of ethyloestrenol and phenformin upon ¹²⁵I-fibrinogen survival, fibrinogen concentration, clot lysis time, and serum levels of fibrin degradation products in a healthy male.

Fig. 2 Comparison of ¹²⁵I-fibrinogen survival and fractional catabolic rate of fibrinogen before and after one month's treatment with phenformin and ethyloestrenol (patient no. 2).

Patient No. 2	Pl. Vol.	Plasma Fibrinogen			Catabolic Rate		
		Concen- tration ± S.D.	Pool (g)	T½	i.v. %	%/ day	g/day
Before	3500	479 ± 49	16.8	116	70	19.5	3.27
After	3400	311 ± 29	10.9	100	80	21.2	2.31

by the consumption of phenformin. However, no change in the plasma fibrinogen concentration was produced in these short-term experiments.

If the fall in plasma fibrinogen concentration after the administration of phenformin and ethyloestrenol is not a reflection of increased catabolism then it would seem likely that the hepatic synthesis of fibrinogen is decreased. Such a decrease is unlikely to be related to the fibrinolytic activity of these drugs, an effect that is thought to be due to the induction of plasminogen activator release. The independence of these two activities is borne out by the fact that no correlation has ever been observed between enhancement of fibrinolysis and fall in plasma fibrinogen concentration. This dissociation is illustrated in the study of the healthy subject reported here who showed significant shortening of the lysis time despite a rise in the plasma fibrinogen concentration.

Interest in phenformin and ethyloestrenol is chiefly centred on their effect upon the fibrinolytic mechanism. However, alterations in the plasma fibrinogen concentration will affect those tests of fibrinolysis which use the subject's own fibrinogen as the stroma for the test. Thus Gallimore and Shaw (1969) have demonstrated that the addition of purified fibrinogen to dilute blood clots prolongs the lysis time in direct proportion to the amount of fibrinogen added. Therefore the shortening of the dilute blood clot or euglobulin lysis times after phenformin and ethyloestrenol is probably a reflection in part of the fall in plasma fibrinogen concentration rather than the total result of an increase in plasminogen activator release.

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