Pathophysiology of Plasmin System

II. Fibrinolysis and Plasmin (Activator)—Inhibitor Ratio

By

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It is generally believed, as mentioned by Sherry, Fletcher and Alkjaersig¹⁾, that the enhancement of fibrinolysis is associated with an increased level of plasminogen activator in the circulating blood, but not with significant changes in plasmin, antiplasmin or plasminogen. However, the following unexpected findings have been occasionally encountered during the course of our experiments. Failure of fibrinolysis by the method of diluted plasma clot has been observed even in a case having a high fibrinolytic activity in its euglobulin fraction. On the other hand, positive fibrinolysis has been found even in a cases showing only a low fibrinolytic activity in the euglobulin fraction. Therefore, it may be possible to presume that not only plasmin and or its activator, but also its inhibitor may play important roles in the occurrence of fibrinolysis in plasma.

This paper will deal with a correlation between the activity of plasmin (activator) and its inhibitor in relation to the occurrence of fibrinolysis, with special reference to the quantitative expression of their activity ratio.

MATERIALS AND METHODS

Blood samples were collected at random from healthy and diseased males and females in 3.8% sodium citrate in the usual manner.

Plasmin (activator) assay: The standard fibrin solution method described in the first report²⁾ was used for this purpose. Up to the present time, there have been no methods for selective estimation of individual activity of plasmin and its activator separately. The method employed was capable of determining simultaneously both plasmin and its activator. Therefore, it was not clear whether the results obtained by this method were due to a preformed free plasmin, or

that secondarily produced by the activator. For these reasons, the term 'plasmin(activator)' was intentionally applied.

Inhibitor assay: Inhibitor was estimated as trypsin inhibitor instead of plasmin inhibitor by the method of Maki described in the first report.²⁾ Although, it is known that all of the plasmin inhibitors in sera are not completely identical with the trypsin inhibitor, there has been experimental evidence of a close relation between fibrinolysis in sera and trypsin inhibitor. For this reason and because of the difficulty encountered in obtaining pure plasmin preparation, trypsin was used in this assay.

Assay of fibrinolysis in plasma: Fibrinolysis in plasms was measured by a slight modification of Macfarlane's and Kuroyanagi's methods described in a previous report.²⁾

Calculation of plasmin(activator)—inhibitor ratio (PIR): The PIR was calculated by the following formula;

$$\text{PIR} = \frac{\text{P}}{\text{I}}$$

P = Amounts of tyrosine ($\mu\text{g.}$) released in the filtrate.

I = Inhibitory activity expressed as %.

RESULTS

1. Relation between fibrinolysis in plasma and proteolytic activity of euglobulin fraction

The relation between fibrinolysis in plasma and the same plasma's caseinolytic activity in 111 cases (Table I and Fig. 1) and the fibrinolytic activity in 115 cases (Table II and Fig. 2) according to the euglobulin fraction method is shown. In spite of possessing high euglobulin activity, the plasma showed, in some cases, no evidence of fibrinolysis. Conversely, in some other cases having low euglobulin activity, definite fibrinolysis was noted. Any relationship was statistically not significant. It was supposed, therefore, that the high euglobulin activity was not always associated with the enhancement of fibrinolysis in plasma.

TABLE I. Relation between Fibrinolysis Level in Plasma and Caseinolytic Activity of Euglobulin Fraction

Level of fibrinolysis	No. of cases	Caseinolytic activity ([PU])	
		Average	Range
—	55	12.9	0.3-48.7
+	20	11.9	0.6-58.3
++	18	9.1	0.9-24.2
+++	18	9.9	1.8-53.9

Fig. 1. Relation between fibrinolysis level in plasma and caseinolytic activity of euglobulin fraction.

TABLE II. Relation between Fibrinolysis Level in Plasma and Fibrinolytic Activity of Euglobulin Fraction

Level of fibrinolysis	No. of cases	Fibrinolytic activity (μ g.)	
		Average	Range
-	68	61.4	14-122
+	20	67.1	30-136
++	16	63.5	40-98
+++	11	59.1	27-90

Fig. 2. Relation between fibrinolysis level in plasma and fibrinolytic activity of euglobulin fraction.

2. Relation between fibrinolysis in plasma and trypsin inhibitor

A significant correlation was found between the occurrence of fibrinolysis in plasma and the same plasma's inhibitory power on trypsin (Table III and Fig. 3). Namely, there was a tendency for fibrinolysis to occur with lesser activity levels

TABLE III. Relation between Fibrinolysis Level in Plasma and Trypsin Inhibitor

Level of fibrinolysis	No. of cases	Trypsin inhibitor (%)	
		Average	Range
-	60	71.6	25-94
+	6	58.1	43-78
++	22	40.7	26-68
+++	15	42.8	25-65

Fig. 3. Relation between fibrinolysis level in plasma and trypsin inhibitor.

of trypsin inhibition, and for failure of fibrinolysis to occur with higher levels of inhibitory activity. It may be much more closely related to the occurrence of fibrinolysis rather than to the activity of plasmin(activator). Of course, some exceptional cases were found. This is in striking contrast to the general belief that fibrinolysis is related directly to the activity of activator, but only partly with that of antiplasmin. These facts suggested the importance of the activity equilibrium between plasmin(activator) and inhibitor as an indicator of fibrinolysis in plasma.

3. Relation between fibrinolysis and PIR

The results of 106 cases are illustrated in Table IV and Fig. 4. It is apparent from the experiments that a significant relation exists. The negative fibrinolysis

TABLE IV. Relation between Fibrinolysis Level in Plasma and PIR

Level of fibrinolysis	No. of cases	PIR	
		Average	Range
—	63	0.17	0.11-1.63
+	8	1.43	1.02-1.96
++	18	1.72	0.76-3.16
##	17	1.69	1.09-3.11

Fig. 4. Relation between fibrinolysis level in plasma and PIR.

group showed the ratio to be usually below 1.0, and the positive group showed the ratio generally to be above 1.0. Thus, it may be emphasized that the PIR is a critical indicator in the occurrence of fibrinolysis in plasma.

DISCUSSION

Theoretically, fibrinolysis in blood or plasma may occur under the condition of an excess of fibrinolytic activity in the euglobulin fraction as compared with the inhibitory activity of plasma. And failure of fibrinolysis may occur under the reversed situation as illustrated in Fig. 5. Taking account of the complicated factors involved, the fact that no significant relationship was found between fibrinolysis in plasma and proteolytic activity of euglobulin fraction may be explained by the increased activity state of its inhibitor. That is, a lack of fibrinolysis in the presence of high euglobulin activity must be attributed to an extraordinary excess of inhibitory power over and above the elevated euglobulin activity. In the same way, fibrinolysis with low euglobulin activity must be due to an extremely diminished activity of the inhibitor. It must be emphasized, therefore, that the measurement of the PIR will be important in order to obtain

Fig. 5. Scheme of positive and negative occurrence of fibrinolysis.

complete recognition of the state of the various individual factors involved in the fibrinolysis mechanism. In fact, the value of PIR has been revealed to be closely related to whether fibrinolysis occurs or not as shown in Fig. 4. As the PIR values depend on the properties of the used fibrinogen preparation, as noted before, it is necessary to use the same fibrinogen preparation throughout any series of experiments in order to compare and judge the results obtained.

Although many results have been obtained by measurement of the plasmin system, there have been some inevitable weak points throughout these experiments. One was that the authors were obliged to use a preparation of trypsin instead of plasmin, and the other was that casein was used as substrate for the plasmin inhibitor assay. Although a more satisfactory method of antiplasmin assay has become available by the recent work of Alkjaersig, Fletcher and Sherry³⁾ and Norman⁴⁾, trypsin has been necessarily used in this clinic because of the failure to obtain such a pure preparation of plasmin without contamination of activator. Up to the present time, a number of reports have been presented on the plasmin inhibitor and trypsin inhibitor in sera; however, no conclusive results have been available as to their properties and the differences between them.

Grob⁵⁾ fractionated plasma by the Cohn method six and found both plasmin and trypsin inhibitor in Fraction IV, and plasmin inhibitor largely free from trypsin inhibitor in Fraction V. Jacobsson⁶⁾ reported that antiplasmin activity largely resided in the alpha-2 globulin fraction, whereas the large part of antitrypsin activity was found in the alpha-1 fraction, the fraction devoid of antiplasmin activity. Schulman⁷⁾ concluded that some 10% of the plasma trypsin inhibitor was also capable of inhibiting plasmin by using his differential titration method. Recently, Gray, Priest, Blatt, Westphal and Jensen⁸⁾ obtained an essentially homogeneous preparation from bovine blood capable of both antitryptic and antiplasmin activity. However, we presumed, and based our experiments on the consideration that the trypsin substitution is permissible within the limitations of clinical use.

SUMMARY

The following results were obtained by the estimation of fibrinolysis in plasma, proteolytic activity of euglobulin fraction and trypsin inhibitor.

1. Plasma did not always evidence fibrinolysis in the presence of high euglobulin activity. Nor did it evidence failure of fibrinolysis in the presence of low euglobulin activity.

2. Trypsin inhibitor was more closely related to the occurrence of fibrinolysis in plasma than the proteolytic activity of euglobulin fraction.

3. The sera showed fibrinolysis by the diluted plasma clot method when the PIR values were more than 1.0. Contrariwise, no fibrinolysis occurred when PIR values were less than 1.0.

References

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