The Relationship of Plasma Fibrinogen (Factor I) Level to Fibrin Stabilizing Factor (Factor XIII) Activity

By SAMIH Y. ALAMI, JAMES W. HAMPTON, GEORGE J. RACE AND ROBERT SPEER

THE RECENTLY RECOGNIZED blood coagulation factor, Factor XIII, or fibrin stabilizing factor (FSF), is considered currently to be an inactive precursor which on activation by thrombin, in the presence of calcium ions, is changed to an active enzyme, a transamidase. The active principle acts through a transamidation process at the terminal stages of blood clotting to convert a soluble fibrin into tough, cross-linked, urea-insoluble, and (presumably) a physiologically useful one.1,2,3 A hemorrhagic diathesis due to its congenital absence has been well documented.4,5 Reduced FSF activity, with or without hemorrhagic manifestations, in association with a variety of disease states has been reported.6,7

The relation of FSF activity to fibrinogen concentration was emphasized in the early studies of the mode of action of FSF.8,9 Furthermore, whereas lowered Factor XIII activity was reported in association with one case of hyperfibrinogenemia,10 the presence of normal amounts of FSF in the plasma and platelets of an afibrinogenemic individual was noted.11 The present study was undertaken to investigate the relationship of fibrinogen concentration to FSF activity in healthy subjects and patients with a variety of diseases.

MATERIALS AND METHODS

A total of 110 venous samples were tested from 20 healthy volunteers and 90 patients.

Assay of Factor XIII Activity. The one stage method followed here is essentially that described by Hampton et al.5 The two-syringe technique was used to obtain blood in most cases. Blood samples were immediately mixed in the proportion of four parts to one part of 0.1 M sodium oxalate. The test was done the same morning of blood collection, using platelet-poor plasma obtained by centrifugation of blood at 4,000 r.p.m. for 20 minutes. Using 0.15 M sodium chloride, adjusted to pH 7.4 with barbital buffer, plasma dilutions of

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This investigation was partly supported by the National Institutes of Health General Research Grant No. FR 5577-02.

First submitted April 25, 1967; accepted for publication June 18, 1967.

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BLOOD, VOL. 31, No. 1 (JANUARY), 1968
1:10, 1:20, 1:40, 1:80, 1:100, and 1:1000 were made. A 0.2 ml aliquot of a solution of lyophilized bovine fibrinogen (Warner-Chilcott, 3 mg per ml), proven to contain negligible amounts of FSF, was added to a 0.2 ml sample of undiluted plasma and to each of the plasma dilutions, contained in small tubes (75 × 10 mm), to assure sufficient fibrinogen for the formation of a visible clot. A control clot of lyophilized bovine fibrinogen, in which plasma was replaced with 0.2 ml sodium chloride, was also tested. The mixtures were clotted by the addition to each sample of 0.2 ml CaCl₂ (0.025 M) and 0.2 ml of thrombin solution (Parke-Davis, 10 U. per ml), and incubated in a 37 C water bath for 30 minutes. The clots were immersed in 3 ml of 5 M urea and were gently loosened to float in the urea solution. The tubes were stoppered and shaken briefly but gently to insure the penetration of the solvent into the clot mass. The tubes were stored at 25 C and observed for the degree of lysis at 2, 5, 24, 48, 72, and 100 hours with occasional tilting.

Grading and Grouping of Factor XIII Relative Activity. Taking into consideration clots of undiluted plasma and plasma dilutions of 1:10, 1:20, 1:40, and 1:80, the following system of grading was used:

<table>
<thead>
<tr>
<th>State of clot</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact at 100 hrs.</td>
<td>4</td>
</tr>
<tr>
<td>Dissolved in &gt; 24 - 100 hrs.</td>
<td>3</td>
</tr>
<tr>
<td>Dissolved in &gt; 5 - 24 hrs.</td>
<td>2</td>
</tr>
<tr>
<td>Dissolved within 5 hrs.</td>
<td>1</td>
</tr>
</tbody>
</table>

An upper limit of normal (100% relative activity) was represented by plasma clots that were intact in each of the five dilutions at 100 hours with a total score of 20. A total score of 5 was obtained with plasmas of relatively low activity of 25%, which in this system would be the lowest value obtained, and might be expected with deficient Factor XIII activity.

Grouping of Factor XIII relative activity into normal, intermediate or poor, and deficient or absent was done on the basis of the following criteria:

<table>
<thead>
<tr>
<th>Group</th>
<th>Total Score and % Factor XIII Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal Factor XIII activity</td>
<td>15 - 20; 75 - 100%</td>
</tr>
<tr>
<td>2. Intermediate or poor activity</td>
<td>11 - &lt; 15; 55 - &lt; 75%</td>
</tr>
<tr>
<td>3. Deficient or absent activity</td>
<td>&lt; 11; &lt; 55%</td>
</tr>
</tbody>
</table>
RELATIONSHIP OF FIBRINOGEN LEVEL TO FACTOR XIII ACTIVITY

Table 1—Relationship of Fibrinogen Concentration to Factor XIII Activity in 110 Subjects

<table>
<thead>
<tr>
<th>Fibrinogen (mg %)</th>
<th>Number of Cases</th>
<th>Factor XIII Relative Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Healthy Patients</td>
</tr>
<tr>
<td>Less than 100</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>100-199</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>200-299</td>
<td>20</td>
<td>6</td>
</tr>
<tr>
<td>300-399</td>
<td>28</td>
<td>6</td>
</tr>
<tr>
<td>400-499</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td>500-599</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>600-699</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>700-799</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>800-899</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>900-1000</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>20</td>
</tr>
</tbody>
</table>

Fibrinogen Measurement. Plasma fibrinogen levels were determined on the same samples collected for FSF assay by the micro-Kjeldahl, or by the Biuret technique, both of which proved to be reproducible as judged by frequent duplicate determinations.

Artificial Contamination of Plasma with Bovine Fibrinogen. To investigate the relationship of FSF activity in plasma with abnormally high fibrinogen concentrations, the oxalated plasmas of normal subjects were artificially enriched with various amounts of bovine fibrinogen (Warner-Chilcott). Large amounts of fibrinogen suspended in distilled water served as an additional control.

RESULTS

Table 1 shows the relationship of fibrinogen concentration to Factor XIII relative activity in the plasmas of 110 subjects. There appears to be no consistent direct relationship between these two factors. However, cases with low or high (i.e. <200 mg or > 600 mg %) fibrinogen concentration had relatively more subjects with lowered Factor XIII activity. Regardless of FSF activity, it was noted that in plasmas with high fibrinogen concentration, stronger initial clots were formed, and remained so after resisting dissolution in urea for 100 hours. Also, some subjects (either healthy or patients) with normal ranges of fibrinogen had normal or reduced Factor XIII activity. In our series, 14 out of 90 patients had reduced FSF activity. The diseases represented here included liver disease, multiple myeloma, polycythemia rubra vera, carcinoma of the prostate, and arteriosclerotic heart disease. One subject presented with spontaneous epistaxis and a history of exposure to white phosphorus (P₄).

To study the relationship between fibrinogen concentration and Factor XIII activity, with elimination of “disease” factor, we examined plasmas of healthy subjects artificially enriched with bovine fibrinogen (Warner-Chilcott). Factor XIII activity remained essentially unchanged (normal) even in plasmas enriched with as much as 1,500 mg% fibrinogen. To rule out the possible accumulative effect of FSF contaminating fibrinogen preparations, an additional control was included. There was a quick dissolution of all clots within 1-4 hours when high concentrations of fibrinogen (1,200 mg%) suspended in distilled water rather than plasma were examined. Also, preheating (40 C for 3 hours)
of fibrinogen suspensions to inactivate possible FSF contaminants,\textsuperscript{7,8} was found to be unnecessary, since comparable results were obtained with unheated fibrinogen suspensions.

**Discussion**

Previous clinical impressions, and biochemical observations on the relationship between fibrinogen and FSF activity,\textsuperscript{8,9,10,12} suggested that FSF activity may be dependent on fibrinogen concentration and that there might be lowering of FSF activity in the presence of high fibrinogen concentration. A recent report,\textsuperscript{11} indicated that the circulating level of FSF is not dependent upon the fibrinogen concentrations. This latter conclusion is supported by our results which reveal that there is no consistent, predictable relationship between these two factors. Our observations further suggest that in association with certain pathological conditions characterized by hyper- or hypofibrinogenemia, there may be an associated lowering of FSF activity. This, in reality, may reflect a deficiency or a decrease in Factor XIII synthesis, rather than the effect of the fibrinogen-FSF ratio. Lowered FSF activity was encountered with low, normal, and high fibrinogen concentrations.

With rare exceptions, no serious hemorrhagic manifestations are evident in patients with the acquired form of FSF deficiency. Recently, Baggett et al.\textsuperscript{18} described two cases with an acquired bleeding disorder, evidence of abnormal hepatic functions and reduced FSF. Both patients had laboratory evidence of circulating fibrinolysins. Only four of our patients exhibited mild hemorrhagic manifestations and none with serious complications. Two patients had liver disease, and one had carcinoma of the prostate, with a relatively low fibrinogen level. One other patient was a young adult male who noted the sudden onset of spontaneous epistaxis and petechiae and gave a history of exposure to white phosphorus (P\textsubscript{4}) for about four months. He had normal fibrinogen levels, and his reduced FSF activity was transient. Both the abnormal bleeding and the reduced FSF disappeared after he changed his occupation.

As suggested by Sigg,\textsuperscript{14} the simultaneous measurement of FSF activity and fibrinogen determination would be advisable, especially when the patient has a bleeding disorder. The FSF assay method used here, reflects the overall result of Factor XIII activity, which depends on its presence and proportionally on its concentration. It proved to be reliable and reproducible as judged by frequent duplicate determinations. It is important, however, to include proper controls, to use platelet-poor plasma, and to perform the assay on the same morning the blood is collected, unless the plasma is stored in a frozen state.

It appears reasonable to assume that the liver is the site of Factor XIII synthesis. However, its synthesis may not necessarily be related to fibrinogen synthesis, since there may be increased fibrinogen concentration with concurrent normal or even decreased FSF activity. Thus, certain disease conditions which affect the liver may cause depression of FSF synthesis and activity, without affecting the rate or quantity of fibrinogen synthesis. On the other hand, as indicated by others,\textsuperscript{11} normal Factor XIII activity may be encountered in association with afibrinogenemia or low fibrinogen concentration.
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SUMMARY

The relationship of fibrinogen concentration to Factor XIII activity in the plasmas of 110 subjects (90 patients and 20 healthy volunteers) was studied. No definite, consistent, predictable relationship could be established. However, in certain pathological conditions, with hyper- or hypofibrinogenemia, a moderate reduction in Factor XIII activity may be present, and usually occurs without hemorrhagic manifestations. On the other hand, examination of normal plasmas artificially enriched with large amounts of bovine fibrinogen revealed no changes in Factor XIII activity. In the light of these observations, the suggestion is made that reduced Factor XIII activity may be encountered in certain disease conditions without simultaneous changes in fibrinogen concentration. The site of synthesis of these two proteins, although most likely the liver, would appear to not influence their plasma concentrations simultaneously.

SUMMARIO IN INTERLINGUA

Esseva studiate le relation inter le concentration de fibrinogeno e le activitate de factor XIII in specimen de plasma ab 110 subjectos, incluse 90 patientes e 20 voluntarios in bon stato de sanitate. Nulle definite, uniforme. e predicibile relation poteva esser establite. Tamen, in certe conditiones pathologic—con hyper- o hypofibrinogenemia—un moderate declino in le activitate de factor XIII pote esser presente e occurre alora sin manifestationes hemorrhagic. Del altere latere, le examine de plasmas normal artificialmente inricchite con grande quantitates de fibrinogeno bovin revelava nulle alterationes in le activitate de factor XIII. In le lumine de iste constatationes, le these es formulate que reducite activitate de factor XIII es incontrate in certe conditiones pathologic sin simultanee alterationes in le concentration de fibrinogeno. Il pare que le sito del synthese del duo mentionate proteinas (le qual es le plus probablemente le hepate) exerce nulle influentia simultanee super lor concentrationes in le plasma.

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