Hypofibrinogenemia Due to L-Asparaginase: Studies of Fibrinogen Survival Using Autologous $^{131}$I-Fibrinogen

By R. E. Betigole, E. S. Himelstein, H. F. Oettgen and G. O. Clifford

E. coli L-ASPARAGINASE has been given to a large number of patients with neoplastic disease during the past two years. Apart from its therapeutic effects, mainly in patients with acute lymphoblastic leukemia, this enzyme preparation has produced a number of side effects. When we began to measure plasma fibrinogen in patients receiving L-asparaginase in our institution, falling levels were noted in 32 of the first 33 patients in whom fibrinogen levels were followed. Some levels were 50 mg. per cent or less. In order to determine whether this decrease in plasma fibrinogen was due to increased consumption or decreased synthesis, we investigated the survival in vivo of autologous $^{131}$I-fibrinogen before and during treatment with L-asparaginase.

Methods*

Patients known to be bleeding or likely to bleed were excluded to avoid external loss of labeled fibrinogen. Thyroid uptake of $^{131}$I was blocked by daily administration of 10 drops of saturated solution of potassium iodide. Fibrinogen was precipitated in dilute solution at 56°C for 15 min. and measured turbidometrically, except in case 2 where fibrinogen was measured by the micro-Kjeldahl technique. Factor V and Factor VIII were measured by one-stage methods. Euglobulin lysis time was measured by a modification of the method of Sherry. The patients' fibrinogen was prepared and tagged as follows: An equal volume of saturated $(\text{NH}_4)_2\text{SO}_4$ was added to 4 ml. of freshly obtained citrated plasma and the resultant precipitate was washed three times with quarter-saturated $(\text{NH}_4)_2\text{SO}_4$. The remaining fibrous precipitate was dissolved in 0.75 ml. of citrate buffer and labeled with carrier-free $^{131}$I according to the method of McFarlane. One-half milliliter of the patient's citrated plasma was then added, plus a volume of saturated $(\text{NH}_4)_2\text{SO}_4$ equal to the total volume of the tagging mixture plus the plasma. The resulting precipitate was washed three times with quarter-saturated $(\text{NH}_4)_2\text{SO}_4$ and redissolved in one ml. of citrate buffer as above. Again, 0.5 ml. of the patient's citrated plasma was added, plus 1.5 ml. of saturated $(\text{NH}_4)_2\text{SO}_4$. The precipitate was washed three times with quarter-saturated $(\text{NH}_4)_2\text{SO}_4$, dissolved in citrate buffer, 0.5 ml. of the patient's citrated plasma was added, and the solution was sterilized by Zeitz filtration. The total radioactivity in the solution was measured by an ionization chamber with a vibrating reed electrometer.

*The patients studied all gave their informed consent both for the labeling and reinjection of their own fibrinogen and for therapy with L-asparaginase.
and approximately 20 μCi injected intravenously into the patient. For the survival curve, two-ml. aliquots of simultaneously obtained serum and EDTA plasma were counted in a Nuclear-Chicago well-type scintillation counter with 2-in. NaI, thallium-activated crystal, and fibrinogen radioactivity was considered to be plasma radioactivity minus serum radioactivity. Using this technique approximately 95 per cent of the radioactivity was clottable before injection and this per cent clottability remained fairly constant in the specimens obtained during the studies. In our laboratory the survival of fibrinogen prepared by this technique in subjects with cancer who are neither bleeding nor experiencing clinical consumption coagulopathies is in the range of a halftime (T/2) of 50–90 hr.

Results

Case 1

The first patient studied, in what was actually the “pilot study” for this project, was a 54-year-old man with myelomonocytic leukemia. Figure 1 illustrates the fall in plasma fibrinogen while he received L-asparaginase 1000 I.U./kg./day intravenously. The fall in plasma fibrinogen continued to a low of 155 mg per cent after 20 days of L-asparaginase therapy. The rate of disappearance of the patient's labeled fibrinogen does not appear to have been altered by L-asparaginase therapy.

Case 2

This 38-year-old woman with advanced metastatic breast cancer showed no obvious shortening of her 131I-fibrinogen survival (T/2 = 60 hr.) while receiving L-asparaginase, although her fibrinogen fell to less than one fifth of its initial level in 10 days. The plasma fibrinogen remained at about 100 mg per cent thereafter while she continued to receive L-asparaginase. Coagulation studies were done on the morning of the day she began to receive
L-asparaginase and nine days later. During this period the fibrinogen fell from 800 mg. per cent to 143 mg. per cent. Factor V levels were 132 per cent and > 200 per cent, Factor VIII > 100 per cent and 100 per cent, and the euglobulin lysis time remained at > 120 min.*

Case 3

This 26-year-old woman with metastatic hypernephroma (Fig. 2) had a fall in plasma fibrinogen to less than one third of the initial values during the first 12 days of L-asparaginase therapy, without any apparent effect on the rate of disappearance of her $^{131}$I-fibrinogen ($T/2 = 80$ hr.). After five days of L-asparaginase therapy the fibrinogen was 370 mg. per cent, Factor V was 103 per cent, Factor VIII 244 per cent, and the euglobulin lysis time 120 min. Six days later the fibrinogen was 190 mg. per cent, Factor V 104 per cent, Factor VIII 400 per cent, and the euglobulin lysis time > 120 min.

Case 4

This 56-year-old man with metastatic malignant melanoma had a fall in plasma fibrinogen from 440 mg. per cent to 120 mg. per cent without any obvious change in the slope of his $^{131}$I-fibrinogen survival ($T/2 = 72$ hr.). Coagulation studies were done on the morning of the first day he received L-asparaginase, and on day 6 and day 13. The euglobulin lysis time was > 120

*We are indebted for the coagulation studies on this patient to Dr. Ralph Nachman of the New York Hospital-Cornell Medical Center.
min. on all three studies. Factor V was 123 per cent, 90 per cent and 78 per cent, and Factor VIII was 198 per cent, 151 per cent and 170 per cent on the three studies.

Case 5

This 21-year-old man with metastatic neuroblastoma had a fall in his fibrinogen level from 1000 mg. per cent to 155 mg. per cent without any evident change in the slope of his $^{131}$I-fibrinogen survival ($T/2 = 55$ hr.). Coagulation studies were done four days before L-asparaginase therapy was begun, and again three days and 10 days after therapy was started. Factor V was 135 per cent, 105 per cent and 73 per cent, Factor VIII was 232 per cent, 152 per cent and 200 per cent, and euglobulin lysis times were $> 120$ min., $> 120$ min. and 105 min., respectively.

Case 6

This 40-year-old woman had disseminated reticulum cell sarcoma. Therapy with L-asparaginase was delayed so that there was sufficient fibrinogen radioactivity to follow for only three more days. However, during that time there was no change in the rate of fibrinogen disappearance ($T/2 = 51$ hr.), although its level fell from 560 mg. per cent to 345 mg. per cent. The fibrinogen level continued to fall, reaching a plateau at a level of 220–250 mg. per cent. Coagulation studies were done three days before L-asparaginase therapy was started, and four days and 11 days after L-asparaginase was begun. Factor V was 123 per cent, 96 per cent and 105 per cent, and Factor VIII 178 per cent, 316 per cent and 400 per cent, respectively. Euglobulin lysis time was $> 120$ min. on the first two occasions and was not done on the third. The pertinent laboratory data in this group of patients is summarized in Table 1.

DISCUSSION

Our own studies and those of others show that labeled fibrinogen survival, when plotted on semilog paper, approximates a straight line at least after the first 12–24 hr. Since in the patients studied the rate of disappearance of the tagged fibrinogen remained essentially unaltered, the fall in their fibrinogen levels was not due to accelerated catabolism or utilization. It must, therefore, have been a result of decreased fibrinogen synthesis. These studies do not,

<table>
<thead>
<tr>
<th>Patients</th>
<th>$^{131}$I-Fibrinogen T/2 (hours)</th>
<th>Before L-Asparaginase (mg. %)</th>
<th>At End of $^{131}$I-Fibrinogen Study (mg. %)</th>
<th>L-Asparaginase Dose (I.U./Kg./day)</th>
<th>Duration of $^{131}$I-Fibrinogen Study (days)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76</td>
<td>490</td>
<td>245</td>
<td>1000</td>
<td>11 (6)</td>
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<tr>
<td>2</td>
<td>60</td>
<td>780</td>
<td>145</td>
<td>1000</td>
<td>13 (10)</td>
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<tr>
<td>3</td>
<td>80</td>
<td>690</td>
<td>190</td>
<td>1000</td>
<td>18 (13)</td>
</tr>
<tr>
<td>4</td>
<td>72</td>
<td>440</td>
<td>120</td>
<td>1000</td>
<td>13 (10)</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>1000</td>
<td>155</td>
<td>1000</td>
<td>13 (10)</td>
</tr>
<tr>
<td>6</td>
<td>51</td>
<td>560</td>
<td>345</td>
<td>1000</td>
<td>10 (3)</td>
</tr>
</tbody>
</table>

* Number in parenthesis indicates number of days on L-asparaginase during $^{131}$I-fibrinogen study.
of course, prove that no patient receiving L-asparaginase could develop a
defibrination syndrome, but their uniformity suggests that this latter must at
least be relatively rare if it occurs at all. A few patients receiving L-aspara-
ginase were found to have low levels of fibrinogen even before starting therapy;
in these it might be inferred that a consumption coagulopathy was present
before L-asparaginase therapy was begun. In this latter instance, of course,
the slow-down of fibrinogen synthesis produced by L-asparaginase results in
a more rapid fall of fibrinogen (and to lower levels) than in other patients,
because fibrinogen survival is already shorter than normal.

Other plasma proteins have been observed to be decreased in patients
receiving L-asparaginase.7,8 It seems likely that these proteins, like fibrinogen,
are decreased because of a decrease in their synthesis. It is of interest there-
fore, that Factor V and Factor VIII, both of which have a biologic half life
of less than a day, were not depressed by L-asparaginase. It is not known
whether these Factors contain asparagine.

Only the first of the patients reported above had any objective or subjective
clinical improvement coincident with L-asparaginase therapy. We have not
yet had the opportunity to study fibrinogen metabolism by means of labeled
fibrinogen when a remission is induced in a patient with asparaginase-sensitive
acute leukemia with a very high white blood cell count and a “packed”
marrow. It is possible that rapid destruction of large numbers of leukemic
cells might produce a temporary consumption coagulopathy.

Since completion of these studies “abnormal coagulation and fibrinolysis”
have been described in 15 patients with acute leukemia treated with L-aspara-
ginase, with falls in the levels of Factors V and VIII to less than 50 per cent
of pretreatment levels.9 Another group reports decreased fibrinogen levels in
13 of 14 patients treated with L-asparaginase; the lowest fibrinogen level was
47 mg. per cent, and Factor V and VIII levels were normal in the four patients
in whom they were measured.10 Both these reports suggest decreased synthesis
as a possible mechanism for the abnormalities observed. Our studies suggest
that decreased fibrinogen synthesis is probably a very common if not universal
effect of L-asparaginase. The frequency of fibrinolytic episodes or “consump-
tion coagulopathies” as a result of L-asparaginase therapy remains to be shown.

SUMMARY

Plasma fibrinogen levels fall, often dramatically, in patients receiving
L-asparaginase. Studies of six patients using autologous 131I-fibrinogen showed
that at a dose of 1000 I.U./kg./day L-asparaginase had no effect on their rates
of fibrinogen disappearance. The fall in their plasma fibrinogen levels, there-
fore, must have been a result of decreased synthesis since it could not be
ascribed to increased utilization or destruction of fibrinogen.

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REFERENCES


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