L-Asparaginase: Acute Effects on Protein Synthesis in Rabbits With Normal and Increased Fibrinogen Production

By Barbara M. Alving, Charles F. Barr, and Douglas B. Tang

The acute effects of a single intravenous dose of L-asparaginase on protein synthesis were studied in normal rabbits and in animals that had received turpentine to stimulate fibrinogen production. Male New Zealand rabbits received L-asparaginase (500 U/kg) 16 hr before the injection of the radiolabeled amino acid [75Se]selenomethionine ([75SeM]. Incorporation of [75SeM] into fibrinogen and serum proteins in the L-asparaginase-treated rabbits was the same as for saline-treated controls, with fibrinogen representing approximately 5% of the labeled plasma proteins. In turpentine-treated rabbits, the maximal incorporation of [75SeM] into serum proteins remained unchanged, whereas [75SeM]-fibrinogen increased sixfold and accounted for 25% of the labeled proteins. Animals that received L-asparaginase at the same time as turpentine or 14 hr later showed significant decreases in synthesis of both serum proteins and fibrinogen. [75SeM]-fibrinogen that was purified from L-asparaginase-treated rabbits underwent normal catabolism when injected into normal recipient rabbits. These data indicate that L-asparaginase can acutely cause partial inhibition of both serum protein and fibrinogen synthesis when administered to rabbits shortly before or during a period of increased fibrinogen production. Fibrinogen that is synthesized in the presence of L-asparaginase does not have an abnormal rate of catabolism.

MATERIALS AND METHODS

Animals and Materials

Male New Zealand rabbits (3.2 kg, mean hematocrit value, 40%) were received from a single supplier at least 1 wk before use and were given standard laboratory food and water ad libitum.

L-asparaginase (EC-2, derived from E. coli; Merck, Sharp and Dohme, West Point, PA) was dissolved in sterile water (1,000 U/ml) and administered intravenously in the right marginal ear vein at a dose of 500 U/kg. Blood samples (2.7 ml) were obtained from the marginal ear vein and collected into polypropylene tubes that contained 0.3 ml of 2% EDTA. Plasma was obtained by centrifugation of the blood for 10 min at 4°C and 1,000 g.

Experiments for Studying the Acute Effects of L-asparaginase on Protein Synthesis in Normal and Stimulated Animals

Two groups of normal (unstimulated) rabbits received either saline or L-asparaginase; 16 hr later 40 µCi [75SeM] (218–530 Ci/g; E.R. Squibb and Sons, New Brunswick, NJ) was injected intravenously, and its percent incorporation into fibrinogen and serum proteins was determined hourly for 5 hr. Three additional groups of rabbits received subcutaneous injections of turpentine (1.5 ml/animal in 3 different sites) to stimulate fibrinogen production, and [75SeM] was injected 16 hr later. One group received no further infusions. A second group received L-asparaginase intravenously at the same time as turpentine. The third group received L-asparaginase 14 hr after the turpentine injection (2 hr before [75SeM] injection).

From the Department of Hematology and the Division of Biometrics, Walter Reed Army Institute of Research, Washington, D.C.

Submitted June 13, 1983; accepted October 13, 1983.

Address reprint requests to Dr. Barbara Alving, Department of Hematology, Walter Reed Army Institute of Research, Washington, D.C. 20307.

© 1984 by Grune & Stratton, Inc.

0006-4971/84/0304–0013$03.00/0
Experiments for Studying Catabolism of \(^{75}\)SeM-Fibrinogen Synthesized in \(L\)-Asparaginase-Treated Rabbits

One group \((n = 3)\) received turpentine and then \(^{75}\)SeM 16 hr later. A second group \((n = 7)\) received turpentine and then \(L\)-asparaginase 14 hr later (2 hr before \(^{75}\)SeM). Blood was obtained by cardiac puncture 4-5 hr after injection of \(^{75}\)SeM. Fibrinogen was purified by ammonium sulfate precipitation\(^{18}\) from plasma pooled from each of the two groups. The purified fibrinogen (94%-98% clottable) was then injected into normal recipient rabbits and its catabolism was studied for 120 hr.

Analysis of Labeled Fibrinogen and Serum Proteins

The incorporation of \(^{75}\)SeM into fibrinogen and serum proteins was measured by admixing plasma (0.3 ml) with 3 ml of Tris phosphate buffer (0.08 M Tris, 0.33% phosphoric acid, pH 6.8) and adding 20 US U of bovine thrombin (Parke-Davis, Morris Plains, NJ). After 1 hr at 22°C, the clot was wound on a glass rod, washed twice with normal saline, and dissolved in 5 ml of alkaline urea. Fibrin concentration was determined spectrophotometrically \((\varepsilon_{262} = 1.617)\). One milliliter of this solution was counted in a Searle gamma well scintillation counter.

For studies of the catabolism of \(^{75}\)SeM-fibrinogen, each 1-ml plasma sample was diluted with 3 ml of Tris phosphate buffer, pH 6.8, and clotted with 50 U of bovine thrombin for 1 hr at 22°C. The clot was then wound on a glass rod, washed with saline, and dissolved in 1 ml alkaline urea. The radioactivity of the entire volume of the remaining diluted serum supernatant and that of the dissolved clot were then measured. The clottability of the labeled fibrinogen in the samples was determined by dividing the radioactivity of the fibrin clot by the sum of the radioactivity in the clot and in the supernatant.

The titers of fibrinogen-fibrin degradation products (FDP-fdp) were determined by a tanned erythrocyte hemagglutination-inhibition immunoassay, as described previously.\(^{16}\)

Data Analysis

Values are reported as arithmetic means with standard error of the mean (SEM). Overall differences among experimental groups in mean responses (Figs. 1 and 2) and maximal incorporation (Table 1) were tested by using one-way analysis of variance.\(^{17}\) If differences were statistically significant \((p < 0.05)\), comparisons were made by using the t test for independent means (unpaired). The paired t test was used to compare changes in fibrinogen concentration (Table 2). Data were analyzed after logarithmic transformation to reduce skewness and to achieve more nearly equal variances. All reported \(p\) values are one-sided.

Maximal values for \(^{75}\)SeM incorporation were estimated by fitting the incorporation data for each rabbit to the following model:

\[
P_t = m[1 - e^{-kt}]
\]

where \(P_t\) is the observed percent \(^{75}\)SeM incorporated at a given time, \(t = \) observed time (hr) - 0.25, \(m = \) maximal (plateau) value of incorporation, and \(k\) is a constant that can be interpreted as the rate of increase in radiolabeled proteins in the circulation. This is an
Table 1. Maximal Values for Percent Incorporation of $^{75}$SeM Into Fibrinogen and Serum Proteins in Normal and Stimulated Rabbits

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Fibrinogen (%)</th>
<th>Serum Proteins (%)</th>
<th>Fibrinogen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline (6)*</td>
<td>0.6 ± 0.1†</td>
<td>9.6 ± 1.6</td>
<td>6</td>
</tr>
<tr>
<td>L-Asparaginase (5)</td>
<td>0.6 ± 0.1†</td>
<td>10.8 ± 2.0</td>
<td>5</td>
</tr>
<tr>
<td>Stimulated rabbits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Turpentine (16)</td>
<td>3.6 ± 0.2†</td>
<td>10.6 ± 0.6</td>
<td>25</td>
</tr>
<tr>
<td>Turpentine + L-asparaginase (5)</td>
<td>2.0 ± 0.2</td>
<td>7.6 ± 1.3</td>
<td>21</td>
</tr>
<tr>
<td>(14 hr later)</td>
<td>2.2 ± 0.3†</td>
<td>7.3 ± 0.4</td>
<td>23</td>
</tr>
</tbody>
</table>

*Number of rabbits in parentheses.
†Values are expressed as the mean ± SEM.
‡Total plasma proteins are the sum of fibrinogen and serum protein values.

RESULTS

Effect of L-Asparaginase on Fibrinogen and Serum Protein Synthesis in Normal and Stimulated Rabbits

In unstimulated rabbits, administration of L-asparaginase before $^{75}$SeM had no detectable effect on the time course of incorporation of $^{75}$SeM into fibrinogen (Fig 1A) or serum proteins (Fig 1B). In saline-treated controls, the mean incorporation of $^{75}$SeM into fibrinogen and serum proteins at 1 hr was 0.2% ± 0.1% (SEM) and 3.5% ± 0.5%, respectively. At the completion of incorporation, the estimated maximal values (Table 1) were 0.6% ± 0.1% and 9.6% ± 1.6%, with $^{75}$SeM-fibrinogen representing approximately 6% of the labeled plasma proteins. In rabbits that received L-asparaginase the 1-hr incorporation for fibrinogen and serum proteins was 0.2% ± 0.1% and 2.2% ± 0.2%, respectively (Fig 1). These values, as well as the corresponding maximal values and percent of total plasma proteins represented by fibrinogen (approximately 5%), were not significantly different from those of the saline-treated controls.

In animals treated with turpentine alone, the 1-hr incorporation of $^{75}$SeM into fibrinogen (Fig 2A) was 2.8% ± 0.2%, or 14 times higher than that of the saline-treated controls (Fig 1A). Incorporation into serum proteins (Fig 2B) was 6.9% ± 0.4%, representing a twofold increase ($p < 0.01$) over the saline-treated controls (Fig 1B). This increase at 1 hr for serum proteins was not reflected in significant differences in the estimated maximal levels (Table 1). In contrast, the maximal level for $^{75}$SeM-fibrinogen in turpentine-treated animals was approximately 25% of the total radioactive plasma proteins (Table 1).

Animals that received L-asparaginase at the same time as turpentine or 14 hr later showed a significant decrease ($p < 0.0001$) in the extent of incorporation of $^{75}$SeM into fibrinogen and serum proteins when compared to animals that received only turpentine (Fig 2). The partial inhibition of protein synthesis was also reflected by the decrease in maximal values in both groups for fibrinogen ($p < 0.005$) and serum proteins ($p < 0.025$, Table 1). Fibrinogen and serum protein synthesis was inhibited to a similar extent (approximately 40% and 30%, respectively).

$^{75}$SeM provides a measure of fibrinogen synthesis that is occurring only during a 1–2-hr period. To

Table 2. Effect of L-Asparaginase on Fibrinogen Concentration in Stimulated Rabbits

<table>
<thead>
<tr>
<th>Experimental Group</th>
<th>Fibrinogen Concentration (g/Liter)†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Turpentine (16)*</td>
<td>2.6 ± 0.3†</td>
</tr>
<tr>
<td>Turpentine + L-asparaginase</td>
<td>3.4 ± 0.6†</td>
</tr>
<tr>
<td>(14 hr later) (9)</td>
<td>3.4 ± 0.2†</td>
</tr>
</tbody>
</table>

*Number of rabbits in parentheses.
†Measurements were made at the time of turpentine injection and then 16 hr later at the time of $^{75}$SeM injection. Values are expressed as mean ± SEM.
obtain an indication of fibrinogen production during the 16 hr between injection of turpentine and 75SeM, we compared fibrinogen levels in the groups that received turpentine with or without L-asparaginase (Table 2). Fibrinogen levels were significantly increased in rabbits that received turpentine alone or L-asparaginase 14 hr later. However, the group that received L-asparaginase at the same time as turpentine showed no significant increase in fibrinogen production, suggesting that stimulation was at least partially inhibited throughout this time.

Effect of L-Asparaginase on Catabolism of Fibrinogen

To test the possibility that L-asparaginase may induce synthesis of an abnormal fibrinogen, we infused purified 75SeM-fibrinogen from turpentine-stimulated rabbits that had received saline or L-asparaginase into normal recipients (Fig. 3). The terminal half-life of the fibrinogen from both groups was 52 hr, with a range of 47–63 hr for fibrinogen from the control group and 49–63 hr for that from the L-asparaginase-treated donors. Furthermore, the plasma obtained from the latter donors at the time of fibrinogen purification did not show elevated titers of FDP/FP. Clottability of the purified fibrinogen did not differ between the two groups at the time of infusion or 120 hr later, at which time it was 69% (range 54–78) for the L-asparaginase-treated animals and 64% (range 55–70) for the control donors.

DISCUSSION

This study indicates that rabbits infused with L-asparaginase synthesize fibrinogen that does not have an increased rate of catabolism. Rather, L-asparaginase appears to induce altered fibrinogen levels by directly inhibiting protein synthesis, presumably through reducing the level of available L-asparagine. Its acute effect therefore depends on the rate at which amino acids are being utilized. Rabbits with normal rates of fibrinogen and serum protein synthesis showed no detectable inhibition of protein production after L-asparaginase treatment.

In contrast, the enzyme caused partial inhibition of both fibrinogen and serum protein synthesis in animals that were treated with turbentine. Kwan and Fuller have shown that after subcutaneous injection of turpentine into rats, the polysomes involved in fibrinogen production increased from 4% to 15% during the next 15–24 hr. We have found that 16 hr after the injection of turbentine into rabbits, the 75SeM-fibrinogen had increased from an initial value of 5% to 25% of the total labeled plasma proteins. We postulate that a marked increase in utilization of L-asparagine accompanies this increased synthetic activity. Under these conditions, L-asparaginase, by decreasing the quantity of L-asparagine available, can significantly diminish the biosynthesis of hepatic proteins.

L-Asparaginase reduces plasma L-asparagine to negligible levels within minutes after administration. This rapid depletion can acutely inhibit protein synthesis in animals that have received turbentine 14 hr previously and are undergoing increased fibrinogen production. The drug's plasma half-life of 20–44 hr would explain why it can inhibit protein synthesis during a 16-hr period when administered at the same time as turbentine. There was not a preferential inhibition of fibrinogen production by this enzyme, for in stimulated animals that received L-asparaginase, fibrinogen still comprised approximately 20% of the total labeled plasma proteins. Further direct evidence for the inhibitory effects of L-asparaginase on fibrinogen and albumin synthesis has been obtained in an isolated rat hepatocyte system.

The structure and amino acid composition of rabbit fibrinogen is similar to that of man and other species. Thus, the conclusions drawn from the rabbit model may well be applicable to the clinical situation. Although the hypofibrinogenemia induced by L-asparaginase rarely produces a bleeding diathesis, the decreased synthesis of other hepatic proteins, such as...
anti-thrombin III, may have clinical relevance. Increased knowledge of the conditions under which L-asparaginase causes significant inhibition of protein synthesis may allow prevention of potential side effects.

ACKNOWLEDGMENT

The authors thank Dr. J. S. Finlayson for helpful discussions and for critical review of the manuscript, and they appreciate the technical assistance of Beverly Jackson as well as the secretarial skills of Carol A. Henderson.

REFERENCES

L-asparaginase: acute effects on protein synthesis in rabbits with normal and increased fibrinogen production

BM Alving, CF Barr and DB Tang