The Synergistic Effect of Hypoglycemia and L-Asparaginase upon Transplantable Hepatomas of Varying Growth Rates

F. F. Becker and K. M. Klein

Department of Pathology, New York University, School of Medicine, New York, New York 10016

SUMMARY

L-Asparaginase inhibits the growth of several animal and human tumors and delays the onset of mitotic activity that occurs in the residual liver following 70% hepatectomy. In the present study the effect of this agent upon DNA synthesis in Morris hepatomas of varying growth rates was determined. A single, small dose of L-asparaginase produced a prolonged inhibition of a very-slow-growing tumor, 9618A. The DNA synthesis of this tumor was also inhibited by 70% hepatectomy alone. Neither a fast-growing tumor, 3924A, nor an intermediate tumor, 9121, was affected by either procedure alone. However, when L-asparaginase administration was combined with 70% hepatectomy, a prolonged suppression of DNA synthesis of hepatoma 9121 ensued while that of hepatoma 3924 remained unaffected.

Seventy % hepatectomy of rats bearing hepatoma 9121 resulted in a severe and prolonged hypoglycemia. The inhibition of DNA synthesis of hepatoma 9121 induced by the combined procedure of 70% hepatectomy and enzyme administration was reversed by the administration of glucose. It would appear, therefore, that the hypoglycemia induced in hepatoma 9121-bearing rats by operation sensitizes the tumor to the effect of L-asparaginase.

INTRODUCTION

L-Asparaginase of high substrate avidity is cytotoxic for certain animal and human tumors of lymphoid origin (6, 12). This enzyme also delays the mitotic activity of normal hepatocytes after 70% hepatectomy for 10 to 12 hr (2, 3). No information has been published regarding the effect of L-asparaginase upon hepatocellular carcinomas (9). In our continuing study of the interaction of this enzyme with normal tissues and malignant tumors, we have examined its effect upon the DNA synthesis of 3 transplantable hepatomas of widely varying growth rates (9).

The therapeutic implication of a combined procedure, 70% hepatectomy with L-asparaginase administration, was also examined.

MATERIALS AND METHODS

Morris hepatomas 3924A and 9121 were carried in ACI rats while hepatoma 9618A was carried in Buffalo rats. A single 2-mm fragment of tumor was injected into the hamstring muscles of each leg with a 13-gauge trochar and obturator. Tumors were tested when they achieved a diameter of 4 ± 1.5 cm. For hepatoma 3924A this was approximately 4 weeks; for hepatoma 9121, it was 8 weeks; and for 9618A, the time was 28 weeks. Escherichia coli-derived L-asparaginase (a gift from Merck, Sharpe and Dohme, Rahway, N. J.) was suspended in 0.9% NaCl solution and administered i.p. (Other experiments demonstrated that equivalent results were achieved if the enzyme was administered either s.c. or i.v.) Although as little as 100 i.u./kg body weight produced all of the described effects, the standard dose used was 250 i.u./kg body weight administered at the time of operation.

A 70% hepatectomy was performed by removing the median and left lobes as previously described. The sham operation was identical except for amputation of the liver (1).

After stated intervals each rat received a single s.c. injection in the interscapular area of thymidine-3H at a dose of 50 μCi/100 g body weight and was sacrificed by exsanguination 2.5 hr later (thymidine-3H was from Schwarz BioResearch, Orangeburg, N. Y.; specific activity, 6.0 Ci/mmole). Identical results were achieved with i.p. injection.

At sacrifice, the tumor was carefully trimmed of any necrotic tissue or clot. The liver of each tumor-bearing rat was also removed and treated as described below.

Tissues were minced in distilled water and the DNA was extracted and hydrolyzed by the trichloroacetic acid method of Schneider (4). The final hydrolysates were analyzed for DNA by the diphenylamine colorimetric method of Burton (8) and for radioactivity with a Beckman TLA-BBS III system in a Beckman LS-250 scintillation counter.

Serum glucose was determined on a Beckman Model ERA-2001 glucose analyzer. This instrument determines true glucose levels with the use of a glucose oxidase reaction with a reported accuracy of ±2%.

1This work was supported by a Grant from the Ruth Estrin Goldberg Memorial and Grant BC-53 from the National Cancer Institute, NIH. This work was presented in part at the Hepatoma Symposium, Philadelphia, Pa., May 17 to 18, 1971.

2Career Scientist, Health Research Council of the City of New York.

3USPHS Fellow in Experimental Pathology.

Received February 10, 1972; accepted June 12, 1972.
RESULTS

Livers of Hepatoma 9121-bearing Rats (Table 1). After 70% hepatectomy, DNA synthesis in the residual livers of the rats that bore this tumor was comparable to that which we have reported in non-tumor-bearing animals (2). Following the operation, DNA synthesis began at 18 hr and achieved maximal rates between 24 and 28 hr. When L-asparaginase was administered at the time of operation, the onset of DNA synthesis in the liver was delayed 10 to 12 hr. Peak synthetic rates were achieved at 36 hr (2).

Hepatoma 9121 (Table 1). The level of DNA synthesis, as determined in 35 control tumors, remained constant throughout the study at a mean of 356 ± 27 cpm/μg of DNA.

Every major manipulation of the tumor-bearing rats caused a depression in tumor DNA synthesis of approximately 25 to 40%, 6 to 10 hr later. After either sham operation, 70% hepatectomy or a single dose of L-asparaginase of 250 i.u./kg this depression was transient, and DNA synthesis returned to control levels by 14 to 18 hr.

However, when a single dose of L-asparaginase of 250 i.u./kg body weight was administered at the time of 70% hepatectomy, DNA synthesis in hepatoma 9121 was reduced from baseline levels by 90% at 10 hr and remained significantly depressed through the 26th hr. Since doses of L-asparaginase as high as 1250 i.u./kg, when administered without 70% hepatectomy, did not significantly prolong the inhibition of hepatoma 9121 DNA synthesis produced by the standard dose of 250 i.u./kg, the prolonged depression that resulted from the combined procedure did not appear to be the result of an alteration in enzyme clearance. Furthermore, the slightly prolonged inhibition of DNA synthesis achieved by administering a 2nd dose of 250 i.u./kg body weight 8 hr after the 1st dose was statistically insignificant when compared with that of the combined procedure.

The effect of L-asparaginase administration at the time of sham operation was no greater than that of each procedure separately.

At 28 hr after combined 70% hepatectomy and L-asparaginase administration, tumor DNA synthesis began rising, achieving values as large as 2 times that of the controls between 36 and 42 hr and returning to control levels by 48 hr.

Hepatoma 3924A (Table 2). Neither 70% hepatectomy, nor L-asparaginase administration, nor both in combination altered the DNA synthetic activity of this fast-growing hepatoma.

Hepatoma 9618A (Table 2). A single dose of L-asparaginase induced a 42% decrease of hepatoma 9618A DNA synthesis which persisted for as long as 22 hr. The 70% hepatectomy alone reduced the rate of tumor DNA synthesis by 90%.

Blood Glucose Levels of Hepatoma 9121-bearing Rats. Following 70% hepatectomy the blood glucose levels of rats have been reported to be reduced, especially in fasted rats (11). We set out to determine whether postoperative hypoglycemia might participate in the synergistic depression of tumor DNA synthesis that resulted from 70% hepatectomy and L-asparaginase administration.

Following 70% hepatectomy of fed, nontumor-bearing rats, serum glucose levels fell from a basal, mean value of 141 mg/100 ml to a mean value of 85 mg/100 ml at 8 hr, approximately 60% of normal. A slow rise in serum glucose then took place achieving normal range between the 20th and 24th hr.

The basal serum glucose value for fed, tumor-bearing rats was 119 mg/100 ml or 84% that of normal rats. At 4 hr after 70% hepatectomy this level had fallen as low as 49 mg/100 ml or 35% of normal. The glucose level remained between 35 and 50% of normal basal levels through the first 12 hr after 70% hepatectomy. As late as 22 hr after operation the mean serum glucose level was only 89 mg/100 ml or 63% of normal.

Glucose Administration to Hepatoma 9121-bearing Rats. Glucose was administered to hepatoma 9121-bearing rats in quantities known to alleviate postoperative hypoglycemia.

Table 1

<table>
<thead>
<tr>
<th>Hr</th>
<th>Sham</th>
<th>Asp</th>
<th>Aspx2</th>
<th>PHep</th>
<th>PHepe-Asp</th>
<th>PHep</th>
<th>PHepe-Asp</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>356 ± 27 (35)</td>
<td>218 ± 14 (4)</td>
<td>362 ± 81 (7)</td>
<td>23 ± 2 (3)</td>
<td>23 ± 2 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>108 ± 6 (2)c</td>
<td>98 ± 8 (5)b</td>
<td>221 ± 25 (6)c</td>
<td>17 ± 6 (5)</td>
<td>17 ± 6 (5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>401 ± 83 (4)</td>
<td>392 ± 38 (6)</td>
<td>408 ± 62 (10)</td>
<td>241 ± 4 (4)</td>
<td>241 ± 4 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>480 ± 111 (4)</td>
<td>349 ± 64 (7)</td>
<td>410 ± 56 (5)</td>
<td>38 ± 3 (6)b</td>
<td>38 ± 3 (6)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td>364 ± 43 (4)</td>
<td>350 ± 17 (3)</td>
<td>305 ± 22 (4)</td>
<td>60 ± 7 (10)b</td>
<td>60 ± 7 (10)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>350 ± 17 (3)</td>
<td>331 ± 18 (4)</td>
<td>290 ± 37 (3)</td>
<td>194 ± 113 (3)</td>
<td>194 ± 113 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>350 ± 17 (3)</td>
<td>331 ± 18 (4)</td>
<td>259 ± 18 (2)</td>
<td>14 ± 2 (2)b</td>
<td>14 ± 2 (2)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>32</td>
<td>409 (1)</td>
<td>290 ± 37 (3)</td>
<td>204 ± 77 (3)</td>
<td>25 ± 6 (3)b</td>
<td>25 ± 6 (3)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>241 ± 77 (2)</td>
<td>640 ± 20 (4)b</td>
<td>214 ± 10 (2)</td>
<td>25 ± 6 (3)b</td>
<td>25 ± 6 (3)b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>180 ± 20 (4)c</td>
<td>374 ± 32 (2)</td>
<td>106 ± 20 (2)</td>
<td>228 ± 20 (2)</td>
<td>228 ± 20 (2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Sham, sham operation; Asp, L-asparaginase, single dose; Aspx2, L-asparaginase, 2 doses at zero and 8 hr; PHep, 70% hepatectomy; PHep-Asp, L-asparaginase administered at operation.

b p < 0.001.
c p < 0.01.
F. F. Becker and K. M. Klein

Dissolved in 0.45% NaCl solution at a concentration of 100 mg/ml, 0.5 ml glucose was administered in each of 2 s.c. sites at the time of 70% hepatectomy and L-asparaginase administration. A 2nd administration of 0.25 ml in each of 2 s.c. sites was given at 8 hr.

The inhibition of tumor DNA synthesis normally produced by the combined treatment at 22 hr was totally reversed by glucose administration. Tumors exposed to a combined operative-enzyme and glucose regimen demonstrated incorporation at a level of 267 ± 53 cpm/µg DNA as against 70 ± 10 for those that did not receive glucose (p < 0.01).

**DISCUSSION**

The results of these experiments demonstrate a relationship between the growth rate of 3 transplantable hepatomas and their response to either the administration of L-asparaginase or 70% hepatectomy. The sensitivity of at least 1 of these solid tumors, hepatoma 9618A, to the inhibitory activity of the enzyme is interesting in view of the generally unfavorable response of most parenchymal tumors tested thus far.

The sensitivity of hepatoma 9618A to the effect of L-asparaginase is similar to that of the normal dividing hepatocyte (2). It has been generally accepted that the major effect of this enzyme is to reduce available exogenous amino acid to withstand this action of the enzyme. However, the growing hepatoma is unable to synthesize sufficient amino synthetase (7, 10). It appears likely, therefore, that this slowly growing hepatoma is unable to synthesize sufficient amino acid to withstand this action of the enzyme. However, the differences in effect from one tumor to another could result from differences in the handling of the enzyme by the tumors. Further study should reveal whether the well-differentiated tumor cells of 9618A, like the normal hepatocyte, can escape from L-asparaginase inhibition by the synthesis of L-asparagine synthetase (7, 10).

It appears from the synergistic result of the combined procedures upon hepatoma 9121 that the severe postoperative hypoglycemia of tumor-bearing rats greatly increased the sensitivity of the tumor to L-asparaginase. The degree of hypoglycemia is an accentuation of that seen after 70% hepatectomy in normal rats (5, 11) and may be related to the glucose demands of the tumor itself and/or the nutritional state of the rat. The mechanism by which hypoglycemia increased the effectiveness of L-asparaginase in inhibiting tumor DNA synthesis is unclear. Glucose administration did not reverse the inhibitory effect of L-asparaginase upon regenerating livers.

Although the mechanism of synergism between induced hypoglycemia and L-asparaginase is as yet unclear, the therapeutic implications must be considered of interest.

**REFERENCES**

The Synergistic Effect of Hypoglycemia and L-Asparaginase upon Transplantable Hepatomas of Varying Growth Rates

F. F. Becker and K. M. Klein


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/32/10/2082

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.