Reversal of Antileukemic Action and Toxicity of 1-Aminocyclopentanecarboxylic Acid in Mice by L-Valine

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The agent 1-aminocyclopentanecarboxylic acid (ACPC) possesses marked antitumor activity in rodents (4, 6, 9, 11) and has been found clinically useful in the treatment of plasmocytic myeloma (2, 7, 14). However, the toxicity of the drug is highly variable from species to species (11).

Although the precise mode of action of ACPC in the tumor-bearing host has not yet been described, several reports have appeared in the literature which suggest that ACPC is a valine antagonist (1, 3, 8). Thus, Berlinguet et al. (3) demonstrated that ACPC antagonizes the in vitro and in vivo incorporation of valine into proteins of the rat and that the likely mode of action is the prevention of the attachment of valine to transfer RNA. Machlin et al. (8) reported that the toxicity of ACPC in one strain of Escherichia coli could be readily reversed by L-valine. On the basis of these interesting observations, and acting on the inference that amino acids may reverse the ACPC action, we attempted to reduce the toxicity of ACPC without suppressing the antitumor action by providing an environment of great amino acid surplus.

The line of Leukemia L1210 was obtained from Cancer Chemotherapy National Service Center as an ascites tumor in DBA/2 mice, and serial passage was maintained in DBA/2 mice. Chemotherapy and toxicity experiments were carried out in 20-gm BDF1 mice [(C57BL X DBA/2)F1 hybrid]. In all therapy trials against L1210, mice were implanted i.p. with a leukemic cell inoculum prepared by dilution of hemocytometer-counted ascites fluid. The cell suspension was such that each mouse received 10⁷ tumor cells. ACPC was administered i.p. Amino acids were administered either orally by gavage or i.p. in a volume of 1 ml. Physiologic saline served as the diluent for the amino acids and ACPC.

In a study made to compare the lethality of ACPC alone with that of valine-ACPC combinations, the mice were given a single i.p. injection of 200 to 2000 mg/kg of ACPC, followed by 3 consecutive days of treatment with a single i.p. dose of 2000 mg/kg/day of L- or D-valine on Days 0, 1, and 2, or 4000 mg/kg/day of the racemic mixture. On Day 0 dosing with ACPC was followed with the doses of the appropriate amino acid within 30 minutes. The ACPC-L-valine combination was markedly less toxic; in all groups this combination produced much less weight loss than ACPC alone and gave almost complete protection against toxic death with 5 of 6 mice surviving doses of 2000 mg/kg of ACPC. In contrast, the animals receiving 600 mg/kg or greater of ACPC all died from drug toxicity, as did those that received a similar amount of ACPC plus D-valine. In the animals receiving the racemic mixture (4000 mg/kg), there was evidence of marked toxicity, as measured by weight loss, but the mortality was greatly reduced. Four of 6 mice died at 2000 mg/kg, and 2 of 6 mice died at 600 mg/kg of ACPC.

A fixed dose of ACPC (approximately five times the LD₅₀) was also employed in conjunction with various amounts of L-valine. In this study, toxicity data were obtained using groups of ten animals per single i.p. dose of 2000 mg/kg ACPC in combination with 3 successive daily doses of L-valine on Days 0, 1, and 2 ranging from 0 to 2000 mg/kg i.p. The results showed that the critical dose of L-valine for obtaining complete reversal of ACPC toxicity is 2000 mg/kg. Protection against toxic death was still apparent after a 50-day period. Some slight protection was achieved with 1000 mg/kg and 500 mg/kg.

In tumor (L1210 leukemia)-bearing animals the reversal of ACPC toxicity mediated by L-valine was accompanied by a corresponding loss of antitumor activity when the amino acid was administered by the oral or intraperitoneal route (Table 1). Orally administered DL-valine behaved like the natural amino acid. This is to be expected since only the L-form is absorbed from the intestine rapidly and to an appreciable extent (5). D-valine has no activity by either route. Dosing of L-valine on each of 3 successive days is required for reversal of the antitumor activity of ACPC. Animals dosed with 2000 mg/kg of D- or L-valine or 4000 mg/kg of DL-valine in the absence of ACPC showed a weight gain and obvious development of tumor. There was no evidence of toxicity in these groups. The effects of these drug combinations against subcutaneously implanted rodent tumors are currently being evaluated.

Despite the limitations of the current data, certain implications can be drawn which are of importance toward understanding the mechanism of action of ACPC as an antitumor agent. In the first place, a significant association exists between L-valine and ACPC, resulting in a reversal of the toxicity.
Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>ACPC</th>
<th>L-Valine</th>
<th>Average weight change, (gm)</th>
<th>T/C (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>No. of doses</td>
<td>Dose (mg/kg)</td>
<td>No. of doses</td>
<td>Route of Administration</td>
</tr>
<tr>
<td>80</td>
<td>daily</td>
<td>2000</td>
<td>daily</td>
<td>i.p.</td>
</tr>
<tr>
<td>60</td>
<td>daily</td>
<td>2000</td>
<td>daily</td>
<td>i.p.</td>
</tr>
<tr>
<td>40</td>
<td>daily</td>
<td>2000</td>
<td>daily</td>
<td>i.p.</td>
</tr>
<tr>
<td>200</td>
<td>1</td>
<td>2000</td>
<td>daily</td>
<td>i.p.</td>
</tr>
<tr>
<td>300</td>
<td>1</td>
<td>2000</td>
<td>daily</td>
<td>i.p.</td>
</tr>
<tr>
<td>300</td>
<td>1</td>
<td>2000</td>
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<td>200</td>
<td>1</td>
<td>2000</td>
<td>daily</td>
<td>p.o.</td>
</tr>
<tr>
<td>300</td>
<td>1</td>
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</tr>
</tbody>
</table>

Reversal by L-valine of 1-aminocyclopentanecarboxylic acid (ACPC) activity against L1210 leukemia in mice.

* Intraperitoneal ACPC treatment was followed by L-valine treatment within 30 minutes.

\[ T/C \geq 125\% \] is considered active by CCNSC specifications.

and antitumor activity of ACPC. This relationship is quite specific in view of our inability to produce similar results with other amino acids, e.g., leucine, alanine, histidine, proline, lysine, glycine, asparagine, or methionine. Secondly, whole-animal toxicity and chemotherapeutic activity of ACPC are intimately related. These considerations suggest that the basis for toxicity of ACPC on both tumor and host may be the same. Studies designed to further define the ACPC-valine interaction are in progress.

REFERENCES

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