Effect of 1-hexylcarbamoyl-5-fluorouracil on spontaneous mammary adenocarcinoma of mice*1

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The antitumor activity of 1-hexylcarbamoyl-5-fluorouracil (HCFU) in various schedules of long-term oral administration was examined in spontaneous mammary adenocarcinoma of SHN mice, an autochthonous tumor system. In the control group, the average time to local recurrence and average longevity after surgical intervention were 21 and 48 days, respectively. Oral administration of HCFU at 200–300 mg/kg/day, 3 times a week or for 5 consecutive days every 2 or 3 weeks was markedly effective against the adenocarcinoma. The optimal schedule was 20 administrations of HCFU at 300 mg/kg/day, 3 times a week. The average time to local recurrence after the operation was increased to 200% and average postoperative survival was also prolonged to 150%. Growth of the tumors was slower and lung metastases at autopsy were found to be suppressed by HCFU. The effect of HCFU in delaying local recurrence and prolonging longevity was slightly affected by the schedule of administration.

Key words: Antitumor activity — 1-Hexylcarbamoyl-5-fluorouracil — Spontaneous mammary adenocarcinoma — SHN mouse — Autochthonous tumor

1-Hexylcarbamoyl-5-fluorouracil was reported from our laboratory in 1976 as a new antitumor agent.2 This compound shows a very strong antitumor activity in mice against various transplantable tumors such as leukemias, carcinomas and sarcomas, and is less toxic to the host than 5-fluorouracil upon oral administration. The range of optimal dosage is 100–300 mg/kg daily for 5 days or three times a week until death.3,4,5 1-Hexylcarbamoyl-5-fluorouracil has a wider tumor spectrum than 5-fluorouracil and its therapeutic index is always greater than that of the latter upon oral administration.2

In this paper, the antitumor activity of 1-hexylcarbamoyl-5-fluorouracil in various schedules of long-term oral administration was examined in spontaneous mammary adenocarcinoma of mice by a method established for autochthonous tumor-host systems.9,10,11

Materials and Methods
Spontaneous mammary adenocarcinomas were taken from a strain of SHN female mice showing high incidence of mammary tumors. The mice, about 5 to 9 months old, have been established in this Institute from Swiss albino mice.7 Tumors of about 1 cm in diameter were surgically removed as completely as possible and the tumor autograft, 1 mm in diameter (10⁶ cells) was placed under the skin at the site of the operation. This insured 100% local recurrence of the tumor at the grafted site, as we have already shown.10,11 When there was more than one tumor in a mouse, all tumors

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*1 This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare.

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were removed at the same time but the autograft was placed at the site of only one tumor. All the tumors removed were histologically examined and confirmed to be mammary gland adenocarcinomas.

1-Hexylcarbamoyl-5-fluorouracil (HCFU) was suspended with 0.5% carboxymethyl cellulose in physiological saline and administered orally in a volume of 0.01 ml/g body weight, starting 24 hr after the operation. HCFU was not decomposed under these conditions. Five mice were used in each group. Various schedules of oral administration were examined and finally 7 experimental groups were used, with one as a control.

The experimental treatments were: Group 1 received no drug (control); group 2 received single daily oral administrations of 200 mg/kg/day, 3 times a week (on every other day) until death; group 3 received 300 mg/kg/day as a single dose 3 times a week (on every other day) until death; group 4 received 300 mg/kg/day 3 times a week (on every other day) until 20 administrations; group 5 received single administrations 300 mg/kg/day twice a week (every three days) until death; group 6 received single administrations 300 mg/kg/day on 5 consecutive days biweekly until death; group 7 received 300 mg/kg/day for 5 consecutive days every three weeks.

5-Fluorouracil was used as a reference compound and administered intraperitoneally as a single dose of 40 mg/kg/day, 3 times a week (on every other day) until death.

All the mice were carefully examined weekly and palpable tumor nodules at and/or away from the site of operation were noted. The tumor growth was measured in two perpendicular diameters with a caliper, and the size was calculated using the following formula as described in the protocol:

$$\text{Tumor size (mm}^3\text{)} = \frac{ab^2}{2}$$

where a = length and b = width in mm. All the mice were autopsied when they died.

For evaluation of the effect of treatments the following four parameters were used: (1) number of days before the first detection of local recurrence at the site of operation, (2) postoperative longevity, (3) appearance of new tumors elsewhere in the mammary gland, and (4) frequency of lung metastases found at autopsy. Geometric means were used in the cases of local recurrences and longevities, because their distributions were log normal, as reported previously.

**Results and Discussion**

Fig. 1 is an example of the growth curves for the autografts of spontaneous mammary tumors after the operation with or without HCFU administration. The volume doubling time of the tumors in the control group was 2 to 3 days, whereas that in the treated group was 5 to 6 days. The growth of the tumors...
Table I.  Effect of HCFU on Spontaneous Mammary Adenocarcinoma of Mice upon Oral Administration

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg/day)</th>
<th>Treatment schedule</th>
<th>Local recurrence(^a) (days)</th>
<th>T/C (%)</th>
<th>Longevity(^a) (days)</th>
<th>T/C (%)</th>
<th>Lung metastases (%)</th>
<th>Incidence of new tumor (%)</th>
<th>Paralysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td></td>
<td>20.7 (14.7 − 29.2)</td>
<td>100</td>
<td>48.4 (36.0 − 65.0)</td>
<td>100</td>
<td>2/5 (40)</td>
<td>3/5 (60)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>2</td>
<td>200</td>
<td>3/week</td>
<td>30.6 (18.4 − 50.9)</td>
<td>148</td>
<td>62.3 (51.8 − 75.0)</td>
<td>129</td>
<td>0/5 (0)</td>
<td>4/5 (80)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>3</td>
<td>300</td>
<td>3/week</td>
<td>46.9** (34.1 − 64.5)</td>
<td>227</td>
<td>69.8* (58.9 − 82.7)</td>
<td>144</td>
<td>0/5 (0)</td>
<td>4/5 (80)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>4</td>
<td>300</td>
<td>3/week × 20</td>
<td>42.6** (34.7 − 60.5)</td>
<td>206</td>
<td>79.3* (52.9 − 107.4)</td>
<td>164</td>
<td>1/5 (20)</td>
<td>5/5 (100)</td>
<td>4/5 (80)</td>
</tr>
<tr>
<td>5</td>
<td>300</td>
<td>2/week</td>
<td>35.2 (17.3 − 71.4)</td>
<td>170</td>
<td>57.7 (38.5 − 86.5)</td>
<td>119</td>
<td>0/5 (0)</td>
<td>4/5 (80)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>6</td>
<td>300</td>
<td>5/2 week</td>
<td>39.5* (25.7 − 60.9)</td>
<td>191</td>
<td>65.1 (50.7 − 83.5)</td>
<td>135</td>
<td>1/5 (20)</td>
<td>5/5 (100)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>7</td>
<td>300</td>
<td>5/3 week</td>
<td>25.4 (17.2 − 37.6)</td>
<td>123</td>
<td>61.8 (47.6 − 80.2)</td>
<td>128</td>
<td>0/5 (0)</td>
<td>5/5 (100)</td>
<td>0/5 (0)</td>
</tr>
<tr>
<td>5-FU(^b)</td>
<td>40</td>
<td>3/week</td>
<td>43.0** (27.8 − 66.3)</td>
<td>208</td>
<td>63.6 (53.3 − 75.9)</td>
<td>131</td>
<td>1/5 (20)</td>
<td>5/5 (100)</td>
<td>0/5 (0)</td>
</tr>
</tbody>
</table>

\(^a\) The values in parentheses are 95% confidence intervals of the means.

\(^b\) Intraperitoneal injection.

* Significantly greater than the control ($P<0.05$).

** Significantly greater than the control ($P<0.01$).
EFFECT OF HCFU ON SPONTANEOUS MAMMARY TUMOR

was slowed by HCFU administration. Similar retardation of tumor growth was also observed in the Lewis lung carcinoma system. All the mice, treated or control, eventually died with tumors.

As shown in Table I, the effects of HCFU (delay of local recurrence and the prolongation of longevity) were slightly affected by the schedule of oral administration. In group 1 (control), the average time to local recurrence and average longevity after the operation were 21 days and 48 days, respectively. Administration of HCFU at 200 mg/kg/day, 3 times a week (group 2), was as effective as 300 mg/kg/day, twice a week (group 5). Upon oral administration of HCFU at 300 mg/kg/day, 3 times a week until death (24–36 times; group 3), the average postoperative time to local recurrence and average longevity were 47 and 70 days, respectively. This treatment was the most effective as regards delay of the onset of local recurrence in all the experiments, but the postoperative longevity was shorter than in group 4. The reason might be cumulative toxicity due to the prolonged and continuous administration, because the postoperative survival was prolonged to about 79 days by limitation of the administration to 20 doses (group 4).

That was the best schedule as regards postoperative longevity. The effects of 5 consecutive administrations at 300 mg/kg/day every two weeks (group 6) and every three weeks (group 7) were similar to those of other treatments.

The rate of lung metastases in SHN mice is usually 30 to 40% at autopsy. In this experiment, the rate of lung metastases was 2/5 in the control (group 1) and 0/5 in most of the treated groups, but 1/5 in groups 4 and 6 (Table I). This suppression of lung metastases is consistent with that in the Lewis lung carcinoma system. The suppression of lung metastases has not previously been reported in this spontaneous mammary adenocarcinoma system.

In SHN mice, new tumors often appeared apart from the site of operation. The rate of appearance of new tumors in the control (group 1) was 60% and that in treated groups was 80 to 100%. The reason for this high rate of appearance in treated groups is not clear, but may be a result of the longer survival of treated mice, because many of the SHN females have multiple tumors (up to 4 per mouse). HCFU is effective in suppressing local recurrence, but not the appearance of new tumors. All the mice, 35 mice in 7 groups, finally died with tumors.

In group 3, all the mice showed paralysis of the hind legs after 18 to 22 administrations. On the other hand, in group 4, 4 out of 5 mice showed such paralysis after 20 administrations of HCFU, but the paralysis disappeared within 1 month after the last administration. In group 6, 1 out of 5 mice showed similar symptoms. Mice in the other groups (2, 5 and 7) did not show paralysis. Paralysis of the hind legs was thus one of the side effects of long-term administration of HCFU.

5-Fluorouracil has been used clinically for the treatment of various tumors by parenteral administration, but it is clinically rather toxic upon oral administration. In the case of intraperitoneal injection of 5-fluorouracil at 40 mg/kg/day, 3 times a week until death, the average time to local recurrence and average longevity were 43 and 64 days, respectively. The antitumor effect of 5-fluorouracil in this system was similar to that of HCFU.

Thus, HCFU was found to be active against spontaneous mammary adenocarcinoma as an autochthonous tumor-host system. Upon oral administration of HCFU, the average time to local recurrence after the operation was increased to 200% and the average postoperative survival was prolonged to 150%. The lung metastases found at autopsy were also suppressed by oral administration of HCFU.
We are indebted to Mitsui Pharmaceuticals, Inc., Tokyo, for kindly supplying the test compound.

(Received Feb. 23, 1980/Accepted July 26, 1980)

REFERENCES


