IMPROVEMENT OF THERAPEUTIC EFFECT OF 5-FLUOROURACIL BY OROTIC ACID

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Therapeutic effect of FU was improved by the combination with orotic acid. This combination exhibited a marked schedule dependency. The most suitable schedule was to give orally 30 mg/kg/day of orotic acid 30 minutes before FU administration. In combination of FU and orotic acid in this schedule, ILS₃₀ was decreased to 2.1 from 3.1 mg/kg/day and the optimal dose was increased to 30 mg from 20 mg/kg/day. Maximum ILS was also increased to 81 from 54% and the therapeutic ratio was improved to 14 from 5.1.

Keywords—5-fluorouracil, orotic acid; leukemia L1210; antitumor activity; therapeutic effect; combination therapy

5-Fluorouracil (FU) has been used extensively in the treatment of several types of cancer since first synthesis in 1957. Recent studies demonstrate that maturation of ribosomal RNA in the tumor is inhibited by FU and its inhibition is considered to be the critical mechanism of antitumor effects. It was reported that orotic acid was incorporated into RNA fraction of normal tissues higher than that of hepatoma, on the other hand, the incorporation of FU was higher in hepatoma than normal tissues. But the combination chemotherapy of FU and orotic acid has not been reported. The above facts prompted us to examine the combination effect of FU with orotic acid in chemotherapy other than hepatoma. Accumulated orotic acid in host tissues such as liver probably decreases the toxicity of FU to animals without affecting its antitumor activity. In the present work, effect of orotic acid administration on antitumor activity and toxicity to host of FU was examined in L1210 system.

MATERIALS AND METHODS

Chemicals—FU was a gift from Mitsui Pharmaceuticals, Inc., and orotic acid was purchased from Sigma Chemical Co.

Tumor System and Evaluation of Antitumor Activity—Male BDF₁ mice weighing 20 ± 2 g were used. Six mice per group, both test and control, were implanted with 1 x 10⁶ cells of leukemia L1210. FU was administered intraperitoneally once daily for 5 days, starting 24 hr after transplantation. In combination groups, orotic acid was administered intraperitoneally or orally 30 min after, at the same time, and 30 min, or 120 min before FU administration.

Antitumor activity was evaluated by the increase in lifespan over controls (ILS = T/C·100%). ILS₃₀ (the dose showing 30% increase in lifespan), maximum ILS, ILS₉₀ (the dose showing maximum ILS), and therapeutic ratio (ILS₉₀/ILS₃₀) were determined as reported previously.

RESULTS AND DISCUSSION

Effect of Orotic Acid on Antitumor Activity of 5-Fluorouracil

As shown in Fig. 1, the increase in survival time with increasing dose of FU was noted until the dose reached 20 mg/kg/day, which was the optimal dose in the L1210 system and the maximum
ILS was 54%. Beyond this level there was a decrease in survival time because of its toxicity to host. To clarify the effect of orotic acid on toxicity of FU, the dose of 30 mg/kg/day was first chosen. Orotic acid (100 mg/kg/day) was not active against L1210 (Table I). Intraperitoneal administration of orotic acid at doses of 10, 30 and 100 mg/kg/day together with FU increased ILS to 59, 60 and 46%, respectively, as shown in Table I. Toxicity of FU to the host was decreased by orotic acid at various doses, therefore the dose of 30 mg/kg/day being the optimal level was used in the following experiments.

**Effects of Interval and Route of Administration of Orotic Acid on Antitumor Activity of 5-Fluorouracil**

The analysis in relation to the interval between the administrations of FU and orotic acid was performed next. Mice were treated daily for 5 days, with FU and orotic acid given simultaneously, or with orotic acid given 30 min after, or 30 or 120 min before FU administration. As shown in Fig. 2, intraperitoneal orotic acid given after FU administration decreased ILS, however, that given 30 min before FU administration increased ILS to 71%. This value was higher than that obtained in the group given orotic acid 120 min before (63%) or simultaneously (59%) with FU.

Effect of combination by oral administration of orotic acid with the intraperitoneal administration of FU was examined. Orotic acid given 30 and 120 min before FU administration increased ILSs to 81 and 79%, respectively. Optimal schedule was 30 mg/kg/day of orotic acid given orally 30 min before FU administration. Dose response curve of FU combined with orotic acid in this schedule was shown in Fig. 1.

**TABLE I. Effect of Intraperitoneal Administration of Orotic Acid on the Antitumor Activity of 5-Fluorouracil**

<table>
<thead>
<tr>
<th>Dose of 5-fluorouracil (mg/kg/day)</th>
<th>Dose of orotic acid (mg/kg/day)</th>
<th>Survival time (day)</th>
<th>Increase in lifespan over control (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>8.0</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0</td>
<td>10.0</td>
<td>25</td>
</tr>
<tr>
<td>30</td>
<td>10</td>
<td>12.7</td>
<td>59</td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td>12.8</td>
<td>60</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>11.7</td>
<td>46</td>
</tr>
</tbody>
</table>

a) 5-Fluorouracil was administered intraperitoneally once daily for five days, starting 24 hr after transplantation.

b) Orotic acid was injected with 5-fluorouracil simultaneously.

c) Survival time in control group was 8.0 days.
Combination effect of FU and orotic acid

**FIG. 2. Influences of Interval and Route of Orotic Acid Administration on Antitumor Activity of 5-Fluourourail**

All groups were given a i.p. dose of 30 mg/kg/day of FU daily for 5 days, starting 24 hr after tumor inoculation. Orotic acid (OA) was administered intraperitoneally or orally at various intervals after FU administration. Routes of orotic acid, □□ Intraperitoneal, □□ Oral.

Orotic acid increased the activity of FU, consequently, ILS₃₀ of FU was lowered to 2.1 from 3.1 mg/kg/day, further, optimal dose was increased to 30 from 20 mg/kg/day. The therapeutic effect of FU was, therefore, improved in the combination as follows; the maximum ILS was increased to 81 from 54% and therapeutic ratio was increased to 14 from 5.1. It is clear from the dose response curve (Fig. 1) that this therapeutic advantage is considered to be brought about by the combination effects of orotic acid, namely by its enhancing effect on antitumor activity of FU and decreasing effect on host toxicity of FU at a high dose. The mechanism of former remains to be examined, but the latter is considered to be caused by the higher rate of incorporation of orotic acid into RNA of normal tissues such as liver and small intestine than into tumor cells, in contrast to the high rate of incorporation of FU in tumor cells.⁷

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**REFERENCES**


