Acyclothymidine Alleviates Intestinal Toxicity of 5'-Deoxy-5-fluorouridine without Loss of Antitumor Activity in Mice

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To reduce the intestinal toxicity of orally administered 5'-deoxy-5-fluorouridine (5'-DFUR) in mice, we co-administered 5'-DFUR with acyclothymidine [AcyT, 5-methyl-(2'-hydroxyethoxymethyl) uracil], a potent inhibitor of pyrimidine nucleoside phosphorylase (PyNPase). Orally administered 5'-DFUR alone caused intestinal toxicity and severe damage to the intestinal villi, while 5'-DFUR with AcyT reduced the intestinal toxicity, and prevented damage to the intestinal villi. This toxicity arising from orally administered 5'-DFUR could not be reduced by intravenous administration of AcyT, but was alleviated by oral administration. Orally co-administered AcyT showed little effect on antitumor activity of 5'-DFUR toward subcutaneously implanted Lewis lung carcinoma, though the intestinal toxicity was reduced in the tumor-bearing mice. This finding suggests that orally co-administered AcyT may prevent the undesirable conversion of 5'-DFUR to 5-FU by PyNPase during the process of absorption in the intestinal tract.

Key words 5'-deoxy-5-fluorouridine; 5-fluorouracil; pyrimidine nucleoside phosphorylase; acyclothymidine; intestinal toxicity

Fluoropyrimidines are widely used for the treatment of solid tumors, such as breast, colon, rectal and stomach cancers. 5'-Deoxy-5-fluorouridine (5'-DFUR) is an orally available cytostatic agent, but phosphorolytic conversion of 5'-DFUR to 5-fluorouracil (5-FU) by pyrimidine nucleoside phosphorylase (PyNPase) is required for its activity.1,2) This enzyme is present in tumors and in various normal tissues.3,4) Since PyNPase activity is greater in tumors than in normal tissues, 5'-DFUR is effectively converted to 5-FU in target tumors. PyNPase activity in the intestinal tract, however, is much greater than in any other normal tissues; a large part of 5'-DFUR should be converted to 5-FU in the intestinal tract before it reaches the target tumor.4,5) This undesirable conversion to 5-FU can cause symptoms of gastrointestinal toxicity, such as diarrhea, which is a dose-limiting factor of 5'-DFUR.6)

Inhibition of the intestinal PyNPase activity and the resulting reduction of conversion of 5'-DFUR to 5-FU in the intestinal tract may reduce the intestinal toxicity of orally administered 5'-DFUR. Among the nucleoside analogues, acyclopyrimidine nucleosides show a strong inhibitory effect on PyNPase activity (Fig. 1).7,8) We earlier attempted to examine the effect of acyclopyrimidine nucleosides on 5'-DFUR phosphorolysis in intestinal tissue homogenates to determine their inhibitory effect, and reported that acyclothymidine [AcyT, 5-methyl-(2'-hydroxyethoxymethyl)uracil] showed the highest inhibitory effect on the phosphorolytic conversion of 5'-DFUR.9) In the present study, we sought to learn whether the oral co-administration of AcyT with 5'-DFUR could reduce the intestinal toxicity without

Fig. 1. Metabolism of 5'-DFUR and Action Point of AcyT

The abbreviations used are: 5'-DFUR, 5'-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; AcyT, acyclothymidine; PyNPase, pyrimidine nucleoside phosphorylase.

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reducing antitumor activity in mice bearing Lewis lung carcinoma.

MATERIALS AND METHODS

Chemicals 5'-DFUR was generously provided by Nippon Roche Co. (Kamakura, Japan). 5-FU was generously provided by Kyowa Hakko Co. (Tokyo, Japan). Acyclovirhydine was prepared from corresponding 5-substituted pyrimidines and 2-(chloromethoxy)ethyl benzoate according to the general method reported by Kelley et al.10 All other chemicals were of reagent grade.

Animals Male BDF1 mice with a body weight of 21-23 g (Japan SLC, Inc., Hamamatsu) were housed in plastic cages with woodchip bedding, and received a CA-1 pellet diet (Clea Japan, Inc., Tokyo) and water ad libitum. All experiments were performed in an animal laboratory at the controlled temperature of 25°C.

Effect of Oral Co-administration of AcyT on Intestinal Toxicity of 5'-DFUR Non-tumor-bearing mice were used and were divided into groups with approximately equal mean body weights. 5'-DFUR was administered at the dose of 2.0 mmol/kg/d based on the results of intestinal toxicity of 5'-DFUR alone.11,12 The molar ratio of 5'-DFUR and AcyT was 1:0.5, 1:1, 1:2, and 1:4 (5'-DFUR:AcyT). For the oral co-administration study, AcyT was simultaneously administered with 5'-DFUR and the drugs were dissolved in the same saline solution. For intravenous administration of AcyT, the drug was injected into the tail vein immediately after the oral administration of 5'-DFUR. Drugs were administered every day for 10 d.

Measurement of Toxicity to the Intestinal Tract The toxicity of the drugs was estimated by two different methods. The feces from the mice were observed every day. The intestinal toxicity was based on the character of the feces or diarrhea and was scored as follows: —, normal feces; ±, slightly loose; +, loose; ++, diarrhea; ++++, severe diarrhea. In the other method for evaluating the intestinal toxicity, the intestinal tract (duodenum) was removed after the above-described therapeutic experiments, and the contents were washed out by a syringe. The tract was then fixed with phosphate-buffered saline solution containing 10% formalin. Specimens for pathological observation were stained with hematoxylin and eosin. Tissue damage was observed pathologically under a microscope.

Antitumor Effect on Lewis Lung Carcinoma BDF1 mice were inoculated subcutaneously (s.c.) on Day 0 with Lewis lung carcinoma (5 x 10⁵ cell/mouse); this carcinoma had been maintained by s.c. transfer every 2 weeks into C57BL/6 mice. Beginning on Day 10 after inoculation of the tumors, 5'-DFUR (1.0 mmol/kg/d) in combination with or without AcyT was orally administered daily until the mice died. The molar ratio of 5'-DFUR and AcyT was 1:0.5, 1:1, and 1:2 (5'-DFUR: AcyT). The body weight of the mice and the longest (a) and shortest (b) tumor diameters were measured every day using calipers. Tumor volume (v) was calculated using the following formula:

\[ v = \frac{a \times b^2}{2} \]

Statistical Analysis The Student's t test was used to evaluate the significance of differences among each group. A P value of 0.05 or less was considered to be significant.

RESULTS

Effect of Co-administered AcyT on Intestinal Toxicity To determine whether orally co-administered AcyT could reduce the intestinal toxicity of 5'-DFUR, we orally administered 5'-DFUR in combination with AcyT to non-tumor-bearing BDF1 mice. As shown in Table 1, the incidence of intestinal toxicity was 100% after oral administration of 5'-DFUR at the dose of 2.0 mmol/kg/d for 10 d with the result of loose feces (+) in 2 of 5 mice, and diarrhea (+++) in 3 of 5 mice. Intestinal toxicity was markedly reduced by AcyT (1.0 to 4.0 mmol/kg/d) and completely prevented by 8.0 mmol/kg/d of AcyT; normal feces (—) were observed in all mice. As shown in Fig. 2, body weight remarkably decreased when 5'-DFUR alone was administered at the dose of 2.0 mmol/kg/d. AcyT co-administered orally at the dose of 8.0 mmol/kg/d reduced the body weight loss. Figure 3 shows sections of the duodenum of mice treated with 5'-DFUR alone (2.0 mmol/kg/d) and in combination with AcyT (8.0 mmol/kg/d) for 10 d. 5'-DFUR alone caused severe damage to the intestinal villi and the overall appearance of the villi was rough. 5'-DFUR co-administered with AcyT was less toxic, and almost completely prevented damage to the intestinal villi. Intravenous administration of AcyT showed little effect on the reduction of intestinal toxicity when compared with oral administration (Table 1). These data showed that intestinal toxicity owing to orally administered 5'-DFUR could not be reduced by the intravenous administration of AcyT, but was improved by oral administration.

Effect of Orally Co-administered AcyT on the Antitumor

Table 1. Intestinal Toxicity after Oral or Intravenous Administration of 5'-DFUR alone or with AcyT in Mice

<table>
<thead>
<tr>
<th>Intestinal toxicity</th>
<th>—</th>
<th>±</th>
<th>+</th>
<th>++</th>
<th>+++</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>5'-DFUR alone⁶</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>5'-DFUR with AcyT (p.o.)¹</td>
<td>1:0.5</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>1:1</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1:2</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5'-DFUR with AcyT (i.v.)⁷</td>
<td>1:0.5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>1</td>
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<td>0</td>
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<tr>
<td>1:4</td>
<td>0</td>
<td>0</td>
<td>1</td>
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</tr>
</tbody>
</table>

a) Intestinal toxicity was evaluated on Day 10 and scored as follows: —, normal feces; ±, slightly loose; +, loose; ++, diarrhea; ++++, severe diarrhea. b) 5'-DFUR alone (2.0 mmol/kg/d). c) 5'-DFUR (2.0 mmol/kg/d) by the p.o. route with AcyT by the p.o. route at the molar ratio of 1:0.5, 1:1, 1:2 and 1:4. d) 5'-DFUR (2.0 mmol/kg/d) by the p.o. route with AcyT by the i.v. route at the molar ratio of 1:0.5, 1:1, 1:2 and 1:4.
Fig. 2. Body Weight Changes of Non-tumor-Bearing Mice after the Oral Administration of 5'-DFUR or in Combination with AcyT

Values represent means ± S.E. of five experiments. Drug was as follows: □ — control; ◆ — 5'-DFUR alone (2.0 mmol/kg/d); ▲ — 5'-DFUR with AcyT (2.0 mmol/kg/d) at the molar ratio of (1:1); ▼ — 5'-DFUR with AcyT (8.0 mmol/kg/d) at the molar ratio of (1:4).

Fig. 3. Morphology of the Small Intestinal Villi of Mice Administered 5'-DFUR (2.0 mmol/kg/d) Alone or in Combination with AcyT (8.0 mmol/kg/d) at the Molar Ratio of (1:4)

Drugs were administered to non-tumor-bearing BDF₁ mice daily for 10 d by the p.o. route.

Activity To determine whether 5'-DFUR-induced intestinal toxicity could be selectively reduced by AcyT without affecting its antitumor effect, the antitumor activity of 5'-DFUR alone or in combination with AcyT was examined in BDF₁ mice bearing Lewis lung carcinoma. 5'-DFUR was administrated daily to the mice, which had received the implanted tumor 10 d before administration, and the treatment was continued as long as the mice survived. Intestinal toxicity was evaluated by the character of the feces. Figure 4 shows tumor volume following the administration of 5'-DFUR alone (1.0 mmol/kg/d) or in combination with AcyT at the molar ratios of 1:0.5, 1:1, and 1:2, and Fig. 5 shows the respective survival rates and intestinal toxicity. AcyT itself, at the dose of 2.0 mmol/kg/d, had no effect on the tumor volume and survival. 5'-DFUR alone or combined with AcyT significantly increased the survival of the mice (p < 0.05). There was no significant difference in tumor volume or the survival of the mice among the group administered 5'-DFUR alone and 5'-DFUR with AcyT. The intestinal toxicity (loose feces) caused by 5'-DFUR alone was observed approximately 10 d after the start of the treatment, and 5'-DFUR alone caused diarrhea (+ +) in 3 of 5 mice, and loose feces (+) in 2 of 5 mice until they died. On the other hand, 5'-DFUR combined with AcyT was slightly toxic, and did not cause any toxicity; normal feces (−) was observed in all mice at the molar ratio of 1:2. These results showed that orally administered 5'-DFUR in combination with AcyT alleviated the intestinal toxicity without reducing the antitumor activity.
of 5'-DFUR in BDF1 mice bearing implanted Lewis lung carcinoma.

DISCUSSION

5'-DFUR has been reported to have a tumor selective action, but at higher doses it was demonstrated to cause intestinal toxicity in mice, and diarrhea was found to be the dose-limiting factor in clinical trials. 5'-DFUR is generated from 5'-DFUR by PyNPase, mainly by thymidine phosphorylase (EC 2.4.2.4) in humans, and uridine phosphorylase (EC 2.4.2.3) in mice. Both in humans and mice, PyNPase is more abundant in tumors than in normal tissues, except for the intestinal tract. Consequently, 5'-DFUR is effectively converted to 5-FU in the intestinal tract as well as tumors following oral administration. This tissue-specific conversion of 5'-DFUR to 5-FU accounts for the tumor selective action and the dose-limiting factor of diarrhea.

It was reported that 5-FU, which was formed from 5'-DFUR by PyNPase in the intestine, caused the intestinal toxicity. The inhibition of intestinal PyNPase and hence the reduction of the undesirable regeneration of 5-FU may reduce this intestinal toxicity. In this study, following the oral administration of 5'-DFUR, we attempted to co-administer 5'-DFUR with AcyT, a potent inhibitor of PyNPase, to reduce intestinal toxicity. Although it is assumed that the inhibition of the phosphorolytic conversion of 5'-DFUR results in an increase in its plasma concentration, the area under the curve (AUC) of 5'-DFUR after its oral co-administration with AcyT was not significantly different from that after 5'-DFUR alone. Orally administered AcyT had no effect on the myelotoxicity of 5'-DFUR; nor was there any significant difference in mean (± S.D.) white blood cell counts (WBC) after the oral administration of 5'-DFUR (2.0 mmol/kg/d) or in combination with AcyT at the molar ratio of 1:1 for 10 d to non-tumor-bearing...
BDF₁ mice among the group administered 5'-DFUR alone (WBC = 2900 ± 659 mm³) and combined with AcyT (WBC = 3300 ± 803 mm³). Since 5'-DFUR is effectively converted to 5-FU by PyNPase in both the intestinal tract and tumors, it is possible that AcyT reduces not only the intestinal toxicity, but also the antitumor activity. In non-tumor-bearing mice, orally administered AcyT reduced body weight loss and/or intestinal toxicity of 5'-DFUR. Intravenously administered AcyT, in contrast, did not sufficiently reduce the toxicity. These results suggest that only AcyT which is simultaneously absorbed with 5'-DFUR through the intestinal tract can inhibit the conversion of 5'-DFUR to 5-FU by PyNPase in the intestinal tract. This minimal effect of intravenously administered AcyT is consistent with the finding that orally co-administered AcyT does not reduce the antitumor activity of 5'-DFUR on subcutaneously implanted Lewis lung carcinoma.

Since there are species differences in PyNPase, it cannot be assumed that these findings from animal experiments would hold true in humans. AcyT showed the highest inhibitory effect on the phosphorolytic conversion of 5'-DFUR by both uridine phosphorylase in mice and thymidine phosphorylase in humans. Accordingly, it is expected that orally co-administered AcyT will reduce the intestinal toxicity of 5'-DFUR without loss of the antitumor effect in humans. Further studies using animals in which the action of thymidine phosphorylase must be investigated for clinical trials, and studies on the effect of AcyT on the therapeutic selectivity of 5'-DFUR as well as 5-FU are currently in progress in our laboratory.

REFERENCES