Modulation of the Pharmacokinetics of 5'-Deoxy-5-fluorouridine and 5-Fluourouracil in Rats by Oral Co-administration of Acyclothymidine

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The effect of an inhibitor of pyrimidine nucleoside phosphorylase (PyNase), acyclothymidine (AcYT), on the pharmacokinetics of 5'-deoxy-5-fluorouridine (5'-DFUR) and 5-fluourouracil (5-FU) was investigated in an oral co-administration of 5'-DFUR and AcYT in rats. AcYT increased the maximal plasma concentration (Cmax) and apparent absorption rate constant (k) of 5'-DFUR, as expected, but the increase in AUC (area under the curve) was not significant. It was expected that AcYT would only inhibit the phosphorylolytic degradation of 5'-DFUR to 5-FU, but the effect was more evident on the pharmacokinetic parameters of 5-FU than on those of 5'-DFUR. AcYT also increased AUC and Cmax of 5-FU when orally co-administered with 5-FU. An inhibitory effect of AcYT on the enzymatic degradation of 5-FU in rat liver and intestinal extract was investigated. AcYT inhibited the degradation in intestinal extract but not in the liver. The result suggests that orally administered AcYT affects the pharmacokinetics of 5-FU partly by inhibiting 5-FU degradation in the process of intestinal absorption as well as by acting as an inhibitor of PyNase.

Key words 5'-deoxy-5-fluorouridine; 5-fluourouracil; acyclothymidine; pyrimidine nucleoside phosphorylase

5'-Deoxy-5-fluorouridine (5'-DFUR) is a prodrug of 5-fluourouracil (5-FU),[1,2] and is used orally in the treatment of human malignancies in Japan. The phosphorylolytic conversion of 5'-DFUR to 5-FU by pyrimidine nucleoside phosphorylase (PyNase) is required for its activity. There are two distinct forms of PyNase[3-6]: one is thymidine phosphorylase (E.C. 2.4.2.4) in human and rabbit,[5-8] and the other is uridine phosphorylase (E.C. 2.4.2.3) in mouse and rat.[9,10] These enzymes are present in tumors and various normal tissues. Since PyNase activity is greater in tumors than in normal tissues, 5'-DFUR is effectively converted to 5-FU in tumors. PyNase activity in the intestinal tract, however, is much greater than in other normal tissues: orally administered 5'-DFUR can be converted to 5-FU in the intestinal tract before it reaches the target tumor.[11] This undesirable regeneration of 5-FU can cause gastrointestinal toxicity.[12] The inhibition of intestinal PyNase activity and hence reduction of the activation of 5'-DFUR to 5-FU in the intestinal tract may reduce the intestinal toxicity of orally administered 5'-DFUR. We showed that 5'-DFUR orally co-administered with acyclothymidine [AcYT, 5-methyl-(2-hydroxyethoxy)methyl] uracil], a potent inhibitor of PyNase,[13,14] reduced the intestinal toxicity to mice without reducing antitumor activity.[15] Since the effect of AcYT on the pharmacokinetics of 5'-DFUR is unknown, in the present study we examined the pharmacokinetics of 5'-DFUR and 5-FU after the oral administration of 5'-DFUR in combination with or without AcYT in rats, an animal which has the similar uridine phosphorylase as that in mouse. Furthermore, since AcYT may affect the pharmacokinetics of 5-FU, we examined the pharmacokinetics of 5-FU after the oral administration of 5-FU in combination with AcYT. The inhibitory effect of AcYT on 5-FU degradation in rat intestinal or liver homogenates was also investigated.

MATERIALS AND METHODS

Chemicals 5'-DFUR was generously provided by Nippon Roche Co. (Kamakura, Japan), and 5-FU was generously provided by Kyowa Hakko Co. (Tokyo, Japan). 5-Chlorouracil and (E)-5-(2-bromovinyl)uracil (BVUra) were purchased from Sigma Chemical Co. (St. Louis, MO.). AcYT was prepared from thymine and 2-chloromethoxy) ethyl benzoate according to the general method reported by Kelley et al.[16] Acetonitrile and n-hexane were HPLC grade and purchased from Wako Pure Chemicals Co. (Osaka, Japan). All other chemicals were of reagent grade.

Pharmacokinetics in the Rat Male Domyyu rats were used, 4 to 5 weeks of age and weighing 190—210 g. To minimize the effect of diet, animals were fasted overnight. Each rat was administered a dose of 50 mg/kg 5'-DFUR (25 mg/ml in 0.9% NaCl) in combination with or without 4 mg/kg AcYT (2 mg/ml in 0.9% NaCl) via the oral route; the molar ratio of 5'-DFUR to AcYT was 1:0.1. 5-FU was administered by the oral route at a dose of 20 mg/kg (20 mg/ml in 0.9% NaCl) together with or without a equimolar dose of AcYT (35.5 mg/kg). Blood samples were collected in heparinized tubes at 10, 20, 30, 40, 60, 90, 120, 240, and 360 min. The samples were centrifuged at 1000 x g to collect plasma, and all plasma samples were stored at -80 °C until analysis. These samples were analyzed by the method reported previously.[17]

In Vitro 5-FU Degradation The rats were sacrificed to obtain the intestine and liver, and these extracts were prepared as described previously.[14] Assays of 5-FU degradation were carried out at 37 °C using 1 ml of the tissue extract (500 µg protein/ml) diluted with isotonic

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phosphate buffer (pH 6.8) containing 2 μM dithiothreitol as a protector of enzyme activity. The assay mixture contained 12.5 μM 5-FU, 250 μM NADPH, 500 μM EDTA, and 5 mM MgCl₂ in a final volume of 1 ml. After incubation for 10 min, the reaction was stopped by adding acetonitrile (200 μl) containing 1.0 μg/ml of 5-chlorouracil as the internal standard to the sample (100 μl). Precipitated proteins were eliminated by centrifugation. The supernatant fraction was then analyzed by HPLC. 17)

Inhibition studies were carried out in the presence of 12.5 μM AcyT or BVUra. Protein concentration in the preparation was determined by the method of Lowry et al. 18)

Data Analysis For the calculation of AUC, the trapezoidal rule on all experimental points with extrapolation to infinity was applied. 19) These data were fitted to a one-compartment model using the MULTI pharmacokinetic analysis program 20) and pharmacokinetic parameters were estimated.

Statistical Analysis Results were expressed as means ± S.D. The Student’s t test was applied to evaluated the significance of differences among each group. A p value of 0.05 or less was considered to be significant.

RESULTS AND DISCUSSION

Pharmacokinetics of 5-FU and 5′-DFUR after the Oral Administration of 5′-DFUR with or without AcyT Figure 1 shows the plasma concentration–time profiles of 5′-DFUR in rats after its oral administration in combination with or without AcyT. Pharmacokinetic parameters were obtained by compartment model dependent analysis (one-compartment model) and are summarized in Table 1. The Cmax of 5′-DFUR after its oral administration alone was 8.10 μg/ml, and was obtained at around 30 min. The AUC (from 0 to infinity) of 5′-DFUR following the oral administration was 1423.24 μg·min/ml.

It is believed that 5′-DFUR is converted to 5-FU in the normal intestinal tract during the absorption process after oral administration. AcyT, an inhibitor of PyNase, may prevent this conversion during the intestinal absorption process. It is assumed that this inhibition results in an increase of Cmax and the apparent absorption rate constant (ka) of 5′-DFUR; Cmax increased to 13.60 μg/ml from 8.10 μg/ml (p < 0.05), and ka increased to 0.160 min⁻¹ from 0.059 min⁻¹ (p < 0.05). The effect of AcyT on the AUC of 5′-DFUR after the oral co-administration was not as significant as that on ka, nor was the apparent elimination half-life (T1/2) significantly changed. Similarly, the AUC and T1/2 of 5′-DFUR were not significantly changed following the intravenous administration of 5′-DFUR in combination with AcyT (data not shown). This suggests that orally co-administered AcyT may have little effect on the elimination behavior of 5′-DFUR in the systemic blood, and may exclusively show this effect on PyNase during the process of absorption in the intestinal tract.

Figure 2 shows the plasma concentration–time profiles of 5-FU in rats after the oral administration of 5′-DFUR in combination with or without AcyT. The Cmax of 5-FU rose to 0.23 μg/ml when orally co-administered with AcyT; this was about 2.1 times higher than Cmax following the administration of 5′-DFUR alone (p < 0.05). The AUC of 5-FU increased about 2.3 times to 41.85 μg·min/ml (p < 0.01). Since the increase in Cmax and AUC of 5-FU in the presence of AcyT was more evident than that of 5′-DFUR, the increased plasma concentration of 5-FU cannot be fully attributed to the increased plasma concentration of 5′-DFUR.

Pharmacokinetics of 5-FU after Its Oral Administration in Combination with or without AcyT The pharmacokinetics of 5-FU after its oral co-administration with AcyT was examined to learn the direct effect of the latter. Since AcyT showed a stronger inhibitory effect on 5′-DFUR phosphorolysis than 5-FU degradation in vitro, 14) each rat

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Table 1. Pharmacokinetic Parameters of 5′-DFUR and 5-FU in Rats after the Oral Administration of the Former with or without AcyT

<table>
<thead>
<tr>
<th></th>
<th>AUC (μg·min/ml)</th>
<th>Cmax (μg/ml)</th>
<th>Tmax (min)</th>
<th>T1/2 (min)</th>
<th>ka (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU</td>
<td>18.40 ± 10.82</td>
<td>0.11 ± 0.02</td>
<td>34.0 ± 16.7</td>
<td>53.3 ± 29.5</td>
<td>0.059 ± 0.021</td>
</tr>
<tr>
<td>5′-DFUR</td>
<td>1423.24 ± 350.5</td>
<td>8.10 ± 2.05</td>
<td>30.0 ± 7.1</td>
<td>70.1 ± 22.7</td>
<td>0.059 ± 0.021</td>
</tr>
<tr>
<td>5′-DFUR + AcyT</td>
<td>41.85 ± 8.72**</td>
<td>0.23 ± 0.05*</td>
<td>28.0 ± 13.0</td>
<td>104.2 ± 40.7</td>
<td>0.160 ± 0.085*</td>
</tr>
</tbody>
</table>

Values represent means ± S.D. of five experiments. A rat was orally administrated a dose of 50 mg/kg 5′-DFUR in combination with or without 4 mg/kg AcyT; the molar ratio of 5′-DFUR to AcyT was 1:0.1. These lines represent the fitted curves by MULTI pharmacokinetic analysis program using one-compartment model. Values represent means ± S.D. of five experiments. * p < 0.05, ** p < 0.01. The difference between 5′-DFUR alone and 5′-DFUR in combination with AcyT was statistically significant.
was administered 5-FU in combination with an equimolar dose of AcyT, while the molar ratio of 5'-DFUR to AcyT was 1:1 when 5'-DFUR was co-administered with AcyT. Figure 3 shows the plasma concentration–time profiles of 5-FU in rats after its oral administration in combination with or without AcyT. The pharmacokinetic parameters are listed in Table 2. The $C_{\text{max}}$ and $k_{\text{e}}$ of 5-FU increased when AcyT was orally co-administered: $C_{\text{max}}$ increased to 1.50 $\mu$g/ml ($p<0.01$), and $k_{\text{e}}$ increased to 2.421 min$^{-1}$ ($p<0.05$). The $AUC$ of 5-FU following oral co-administration with AcyT was also increased significantly to 34.84 $\mu$g·min/ml ($p<0.05$). This result suggested that AcyT might affect not only the kinetics of 5'-DFUR, but also that of 5-FU. Intravenously co-administered AcyT increased $AUC$ and prolonged $T_{1/2}$ of 5-FU (data not shown), though the effect was not evident compared with that following the oral co-administration of 5-FU in combination with AcyT.

In Vitro Inhibition of 5-FU Degradation by AcyT. AcyT was evaluated for its ability to inhibit 5-FU degradation at a concentration of 12.5 $\mu$m in rat liver and intestinal extract in vitro. BVUra was used as a positive control compound for an inhibitor of dihydropyrimidine dehydrogenase, which is responsible for the enzymatic degradation of 5-FU. Table 3 summarizes the percent inhibition calculated from the ratio of 5-FU degradation without inhibitor to that with AcyT.

In the intestine extract, AcyT showed moderate inhibition (26.5%) ($p<0.05$). In liver extract, little inhibitory effect (3.2%) was observed, though BVUra showed higher inhibitory effect on the enzymatic degradation of 5-FU in both the intestine and liver. Although the inhibitory effect of AcyT on the degradation of 5-FU was not as strong as BVUra even in the intestinal homogenate, the inhibition in intestinal tract may affect the kinetics of 5-FU administered orally. It may be possible that AcyT is not only an inhibitor of phosphorylation of 5'-DFUR in the process of intestinal absorption, but also an inhibitor of the degradation of 5-FU. This assumption, however, cannot fully explain the observation that the increase in $C_{\text{max}}$ and $AUC$, and prolongation in $T_{1/2}$ of 5-FU was more evident than those of 5'-DFUR after its oral co-administration with AcyT. Because of the considerable differences in the inhibitory effect among species, we should not simply assume that the findings from animal experiments would be true in human. However, the complex inhibitory effects of AcyT on pyrimidine related compounds in rat promote further interest in its combined effect with other pyrimidine nucleosides such as 5-fluoro-2-deoxyuridine and 5-fluourouridine.

| Table 2. Pharmacokinetic Parameters of 5-FU in Rats after Its Oral Administration with or without AcyT |
|-------------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|
| $AUC_{\text{tr}0-\infty}$ (mg·min/ml) | $C_{\text{max}}$ (mg/ml) | $T_{1/2}$ (min) | $k_{\text{e}}$ (min$^{-1}$) |
| 5-FU alone | 13.29 ± 8.35 | 0.51 ± 0.25 | 8.9 ± 2.1 | 0.33 ± 0.078 |
| 5-FU + AcyT | 34.84 ± 22.85* | 1.50 ± 1.21** | 13.9 ± 2.6* | 2.421 ± 0.678* |

Values represent means ± S.D. of five experiments. A rat was orally administered a dose of 20 mg/kg 5-FU in combination with or without 38.5 mg/kg AcyT; the molar ratio of 5'-DFUR to AcyT was 1:1. $T_{1/2}$ and $k_{\text{e}}$ were analyzed by computer-fitting to a one-compartment model using MULTI program. * $p<0.05$, ** $p<0.01$. The difference between 5-FU alone and 5-FU in combination with AcyT was statistically significant.

| Table 3. Percent Inhibition of 5-FU Degradation in Rat Intestine or Liver Tissue Homogenates by AcyT and 5-BVUra |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Ratio of 5-FU degradation (Percent of inhibition) | Intestine | Liver |
| Control | 31.68 ± 1.58 | 31.62 ± 1.70 |
| BVUra | 4.50 ± 0.63 (85.8)* | 11.15 ± 0.24 (68.4)* |
| AcyT | 23.28 ± 4.58 (26.5)* | 30.64 ± 1.12 (3.2) |

Values represent means ± S.D. of three experiments. 5-FU (12.5 $\mu$m) was incubated in rat tissue homogenates with or without AcyT (12.5 $\mu$m) and BVUra (12.5 $\mu$m) for 10 min. The ratio of 5-FU degradation was determined from the relative amount of remaining 5-FU. The percent inhibition of 5-FU degradation without inhibitor was taken as 0%, and the reduction in degradation rate was expressed as percent of inhibition. * $p<0.05$ for the control group.
REFERENCES