# Potentiation of the Antitumor Effect of 5-Fluoro-2'-deoxyuridine Esters in Combination with Acyclothymidine Esters on L1210 in Mice via Oral Administration

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**Abstract** □ Fifteen pyrimidine-related compounds were evaluated for their ability to inhibit enzymatic degradation of 5-fluoro-2'-deoxyuridine (FUdR). Acyclothymidine [5-methyl-1-(2'-hydroxyethoxymethyl)uracil] showed the highest inhibitory effect on the phosphorolytic degradation of FUdR in various tissue homogenates derived from mouse, rat, and beagle organs. Both the drug (FUdR) and the inhibitor (acyclothymidine) were esterified with appropriate aliphatic acids in order to synchronize their behavior after simultaneous oral administration. The antitumor activity of orally administered FUdR esters was potentiated by the simultaneous oral administration of the acyclothymidine esters, but not by acyclothymidine.

5-Fluoro-2'-deoxyuridine (FUdR), known as an active metabolite of 5-fluorouracil (5-FU), shows high antitumor activity in vitro,[1-4] but in vivo the activity is limited and gains no advantage over that of 5-FU.[5-7] The moderate activity of FUdR has been attributed to its rapid clearance in vivo, since the cytotoxicity of FUdR is time dependent or requires long retention in vivo.[8]

We have reported the high antitumor activity and prolonged plasma levels of long aliphatic diesters of FUdR following intraperitoneal administration to mice bearing L1210.[9] The FUdR esters, however, are much less active when given by oral administration (the clinically preferable route of administration), possibly because of their rapid degradation by pyrimidine nucleoside phosphorylases on the gut wall.[10] Since the pyrimidine nucleoside phosphorylases have a high substrate specificity and several specific inhibitors have been reported,[11,12] a phosphorylase inhibitor which should be stable and nontoxic in vivo may work as a potentiator of the FUdR esters.[13] The purpose of the present study was potentiation of the antitumor activity of the FUdR esters by oral coadministration of a phosphorylase inhibitor and the FUdR esters.

## Experimental Section

**Materials**—The 3',5'-diesters of 5-fluoro-2-deoxyuridine (FUdR) were prepared according to the procedure described by Nishizawa et al.[3] The acyclonucleosides were prepared from corresponding 5-substituted pyrimidines and 2-(chloromethoxy)ethyl benzoate according to the general method described by Schroeder et al.[13] The acyclothymidine esters were obtained by acylation of acyclothymidine with acid anhydrides in pyridine. Other nucleosides and pyrimidine bases were purchased from Sigma Chemical Company (St. Louis, MO). Acetonitrile, ethanol, and *n*-hexane were HPLC grade and purchased from Nakarai Chemical Company (Kyoto, Japan). All other chemicals were of reagent grade.

**Preparation of Homogenates**—Male beagles (9.5–10 kg), Sprague Dawley rats (250–280 g), and DBA mice (25–26 g) were sacrificed to obtain livers and small intestines. Five grams of the fresh tissue were homogenized with 20 mL of ice-cooled isotonic phosphate buffer (pH 7.0) containing 0.19 M sucrose for 3–5 min in a glass homogenizer. The homogenates were centrifuged (600 × *g*, 10 min) at 5 °C to remove the nuclei, and 1 mL of the resultant homogenate (20% w/v) was transferred into 3-mL glass bottles and stored at −80 °C until use.

**Assay for Inhibition**—The stock solutions of FUdR, nucleosides, acyclonucleosides, and pyrimidine bases were prepared in ethanol to give a concentration of 2.5 mM. The assays were performed at 37 °C in the homogenates diluted with the isotonic phosphate buffer (pH 7.0) containing 0.19 M sucrose (4.5–39 μL of the stored homogenate was added to 1 mL of the buffer). The experiments were initiated by adding the stock solutions of FUdR (at a final concentration of 25 μM) and one of the inhibitors to 1 mL of the preincubated homogenates in a 3-mL glass vial. The inhibitors were typically screened at concentrations of 12.5, 25, and 50 μM; however, lower and higher concentrations were used for very potent inhibitors and certain poor inhibitors, respectively. Since the stock solutions were prepared with ethanol, an effect of ethanol concentration on the enzymatic phosphorylation rate was measured in the above system without inhibitor, and no effect was observed up to a concentration of 3% ethanol in the homogenates. The decrease in concentration of FUdR in the homogenates incubated at 37 °C was followed by periodic sampling of the reaction mixture and HPLC analysis. Samples were injected directly onto a C-18 μ-Bondapak column (300 × 4.6 mm) fitted with a guard precolumn (Lichrosorb RP-18, Brownlee). The pseudo-first-order rate constants were estimated from the slope of semilogarithmic plots of FUdR concentration against time. Percent inhibition was calculated as follows:

$$\text{percent inhibition} = \left(1 - \frac{\text{rate constant with inhibitor}}{\text{rate constant without inhibitor}}\right) \times 100 \qquad (1)$$

**Measurement of $K_i$ and $K_m$**—The stock solutions of FUdR were prepared in ethanol to give concentrations of 2.5, 10.0, and 50.0 mM. The phosphorolytic degradation rates of FUdR (velocity) were measured by disappearance of FUdR. Kinetic constants ($K_m$) of the FUdR phosphorylation were determined with five levels of FUdR (in the range 0.5–7.0 × $K_m$ of FUdR) and three concentrations of homogenates. The $K_m$ values were obtained from double-reciprocal plots of velocity versus FUdR level. Studies of inhibition kinetics were made with five levels of FUdR (0.0125, 0.025, 0.05, 0.1, and 0.2 mM) for each of three inhibitor levels and control mixtures lacking inhibitor. Ethanol concentration in the reaction mixtures did not exceed 3% in all the experiments. Inhibition constants ($K_i$) were obtained from replots of inhibitor concentrations versus slopes of double-reciprocal plots of velocity versus FUdR level. All of the latter plots were linear.

**Hydrolysis Rates of Esters**—The stock solutions of FUdR esters and acyclothymidine (AcycTdR) esters were prepared in ethanol to give a concentration of 2.5 mM. The hydrolytic rate constants of the FUdR esters and AcycTdR esters in the diluted intestinal homogenates (0.06% w/v) were measured by the method reported previously.[10]

**Hydrophobicity and Hydrophilicity of Esters**—The relative hydrophobicity and hydrophilicity of the FUdR and AcycTdR esters

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were evaluated in terms of their retention times in HPLC columns. A Lichrosorb RP-18 column (250 × 4.6 mm, having affinity for hydrophobic moieties) and a Nucleosil 5-CN column (300 × 4.6 mm, having affinity for hydrophilic moieties) were used to evaluate hydrophobicity and hydrophilicity, respectively. Mobile phases of acetonitrile:acetic acid (99.9:0.1) and *n*-hexane:ethanol (97:3) were used with the reversed-phase (Lichrosorb RP-18) and the normal-phase (Nucleosil 5-CN) columns, respectively.

**Evaluation of Antitumor Activity**—Male $BDF_1$ (C57BL/6 × DBA/2) mice (25–27 g) were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Hamamatsu, Japan), and a pellet diet (CA-1, CLEA Japan Inc.) and water were offered ad libitum. Transplantation of L1210 leukemia cells was carried out by withdrawing peritoneal fluid from donor $BDF_1$ mice bearing 7-d growth. The suspension was centrifuged for 2 min (1600 × *g*), the supernatant peritoneal fluid was decanted, and a 10-fold dilution with 0.9% NaCl solution was made. The cell number was determined with a Coulter Particle Counter and the cell population was adjusted to $1 \times 10^6$ cells/mL for the L1210 leukemia. Each of five animals in each group was inoculated intraperitoneally with the resulting cell suspension (0.1 mL containing ~$1 \times 10^5$ L1210 cells). The drugs (FUdR and its esters) and/or the phosphorylase inhibitors (acycTdR and its esters) were given orally at days 1, 3, and 5, starting 24 h after the inoculation. The tested compounds were administered as a solution or a suspension in 0.2 mL of PBS per mouse. Antitumor activity was evaluated in terms of the increase in life span relative to the controls (ILS: T/C%). The changes in weight of the mice were determined from day of onset to day 5 after inoculation, because the tumor volume increased and affected the mouse weight after day 6.

## Results and Discussion

**Selection of a Potent Inhibitor**—Fifteen pyrimidine-related compounds (consisting of three categories: deoxynucleosides, acyclonucleosides, and pyrimidine bases) were evaluated for their ability to inhibit FUdR phosphorolytic degradation in intestinal and liver homogenates prepared from mouse, rat, and beagle tissues. The enzymatic degradation of FUdR was not observed in rat, beagle, and human plasma (data not shown). Typical time courses of disappearance of FUdR with or without inhibitors are shown in Figure 1. The slopes of semilogarithmic plots of FUdR concentration against time gave pseudo-first-order rate constants. Table I summarizes the percent inhibition calculated from the rate constants. The acyclonucleosides showed the highest effect in the three categories. The pyrimidine bases, which are supposed to work as productive (or feedback) inhibitors, showed a stronger effect than the deoxynucleosides, supposed substrative inhibitors which may compete with FUdR for the binding site. A similar influence of the 5-substituents was observed for the three categories (i.e., Me, Br, I, F > H); an exception is 5-nitroacyclothymidine which shows little effect on all homogenates studied, though 5-nitrouracil has been reported as a strong uridine phosphorylase inhibitor.[14] In naturally found substrates (i.e., thymidine, deoxyuridine, thymine, and uracil), the methyl substituent showed the higher effect; the difference was most significant in the beagle homogenates.

The $K_i$ values were measured for six inhibitors which showed a potent inhibitory effect in the percent inhibition study. Three appropriate concentrations of an inhibitor (in the range 0.3–2.0 × $K_i$) were employed to estimate the inhibition constant ($K_i$); competitive inhibition was observed in all cases. The $K_m$ values of FUdR and the $K_i$ values for the homogenates are given in Table II. Since the $K_m$ values depended on the homogenates, $K_i/K_m$ values were used for the evaluation. A higher inhibitory effect of 5-methyl-substituted compounds (i.e., thymine and its analogues) over the corresponding uracil and its analogues was observed in all the homogenates; the differences were more significant in the beagle homogenates (10–20 times) than in the rat homogenates (4–14 times). The acyclonucleosides showed much higher inhibitory effects than the normal pyrimidine metabolites, and again the tendency was more evident in the beagle (14–17 times) than in the rat (4–8 times) homogenates. The intestinal homogenates were more sensitive than the liver homogenates to the inhibition by AcycTdR (1.6 times in the beagle and 4.1 times in the rat).

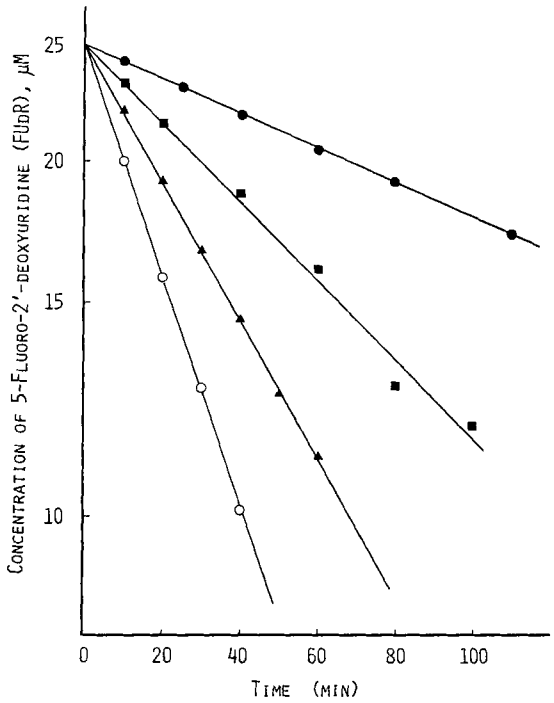


**Figure 1**—*Rat intestinal homogenate (0.09% w/v) catalyzed phosphorolytic degradation of 5-fluoro-2'-deoxyuridine (FUdR). Key:* (○) *FUdR alone, 25 μM;* (▲) *plus thymidine, 25 μM;* (■) *plus thymine, 25 μM;* (●) *plus acyclothymidine (AcycTdR), 25 μM.*

A desired potentiator is required to show biological and chemical stability and low toxicity, as well as an inhibitory effect. Acyclothymidine, which shows the strongest inhibitory effect, is stable in the homogenates studied ($t_{1/2}$ > 1000 min), though thymidine and deoxyuridine degrade rapidly ($t_{1/2}$ = 20–50 min). The low toxicity of acyclothymidine has been reported[15] and the compound itself does not show anticancer, antiviral, and antimicrobial activities.[13,15,16] Considering the above results, we have chosen acyclothymidine (AcycTdR) as a promising potentiator.

**Selection of Ester Combinations**—The in vitro properties of acyclothymidine (strong inhibitory effect, stability, and low toxicity) seemed promising. In vivo, however, the distributions of the inhibitor and FUdR after simultaneous oral administration must be synchronized in order to achieve the expected potentiation. Therefore, long aliphatic esters of acyclothymidine, which are expected to be highly lipophilic and to regenerate acyclothymidine by enzymatic hydrolysis in the same manner as FUdR esters, were synthesized. The hydrolysis rates in rat and mouse intestinal homogenates of the FUdR esters (octanoate ~ hexadecanoate) and the acyclothymidine esters (octanoate ~ eicosanoate) were determined to select a suitable combination of the esters in terms of their susceptibility to esterases. The relative hydrophobicity and hydrophilicity of the esters, which can affect the water–lipid partition coefficient or distribution in the GI tract, were also determined by measuring their retention times in HPLC columns. Table III shows the hydrolytic susceptibility to the rat and mouse enzyme systems and the physicochemical properties of the selected esters. The com-

**Table I—Inhibition of 5-Fluoro-2′-deoxyuridine (FUdR) Phosphorylation in Rat, Beagle, and Mouse Homogenates by Pyrimidine-Related Compounds**

| Inhibitor, μM | % Inhibition (Mean ± SD) | | | | |
|---|---|---|---|---|---|
| | Rat Intestine[a] | Rat Liver[b] | Beagle Intestine[c] | Beagle Liver[d] | Mouse Intestine[e] |
| None | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Thymidine | | | | | |
| 12.5 | — | 34.1 ± 2.8 | — | 48.7 ± 4.5 | — |
| 25 | 44.4 ± 7.9 | 29.4 ± 2.6 | 52.2 ± 6.7 | 67.7 ± 4.1 | 45.0 ± 3.1 |
| 50 | 42.0 ± 3.5 | — | 69.7 ± 6.9 | — | — |
| Deoxyuridine | | | | | |
| 12.5 | — | 7.3 ± 0.9 | — | — | — |
| 25 | — | 14.1 ± 2.1 | 8.8 ± 2.8 | 9.4 ± 3.8 | 8.4 ± 2.9 |
| 50 | 23.4 ± 5.3 | — | 18.0 ± 6.8 | 17.9 ± 5.3 | — |
| 100 | 27.0 ± 3.8 | — | — | — | — |
| 5-I-Deoxyuridine | | | | | |
| 12.5 | 33.0 ± 2.1 | — | 67.9 ± 2.4 | 70.3 ± 9.8 | — |
| 25 | 45.9 ± 1.9 | 27.4 ± 1.9 | 80.9 ± 10.1 | 81.1 ± 3.4 | — |
| 50 | — | 42.3 ± 2.0 | — | — | — |
| 5-Br-Deoxyuridine | | | | | |
| 12.5 | 38.4 ± 3.5 | — | 77.4 ± 4.7 | 78.7 ± 3.2 | — |
| 25 | 52.3 ± 2.4 | 29.5 ± 2.1 | 87.3 ± 1.2 | 86.3 ± 5.5 | — |
| 50 | — | 45.0 ± 3.3 | — | — | — |
| Acyclothymidine | | | | | |
| 2.5 | — | — | 67.0 ± 4.2 | 71.4 ± 13.1 | — |
| 5.0 | — | — | 81.6 ± 3.3 | 86.6 ± 6.5 | — |
| 12.5 | 70.2 ± 3.1 | 48.2 ± 3.4 | — | — | — |
| 25 | 85.4 ± 4.5 | 59.1 ± 6.0 | — | — | 78.2 ± 5.9 |
| Acyclouridine | | | | | |
| 2.5 | 14.0 ± 2.2 | — | 28.3 ± 2.2 | — | — |
| 5.0 | 20.9 ± 3.4 | — | 31.5 ± 2.2 | 45.0 ± 4.3 | — |
| 12.5 | 38.2 ± 4.4 | −0.4 ± 14.6 | 49.4 ± 5.3 | 58.9 ± 3.1 | — |
| 25 | 61.6 ± 3.3 | 30.9 ± 10.2 | 61.7 ± 3.7 | 69.8 ± 7.7 | 52.7 ± 2.3 |
| Acyclo-5-F-uridine | | | | | |
| 5.0 | — | — | 40.6 ± 7.8 | 50.2 ± 9.8 | — |
| 12.5 | 53.1 ± 4.7 | 39.4 ± 4.1 | 56.7 ± 2.9 | 63.1 ± 3.7 | — |
| 25 | 65.6 ± 3.9 | 42.2 ± 3.2 | — | — | 59.7 ± 4.2 |
| Acyclo-5-Br-uridine | | | | | |
| 2.5 | — | 31.6 ± 7.9 | 55.5 ± 2.3 | 65.1 ± 5.4 | — |
| 5.0 | 32.6 ± 3.5 | 33.3 ± 4.9 | 71.2 ± 3.6 | 75.3 ± 3.1 | — |
| 12.5 | 47.7 ± 2.3 | — | — | — | — |
| Acyclo-5-$NO_2$-uridine | | | | | |
| 5.0 | 0.9 ± 2.2 | −2.1 ± 3.0 | 7.6 ± 3.2 | 1.8 ± 2.1 | — |
| 12.5 | 12.2 ± 6.3 | −1.1 ± 2.4 | 8.8 ± 6.6 | 11.6 ± 2.0 | — |
| 25 | 6.9 ± 5.3 | — | — | — | — |
| Thymine | | | | | |
| 12.5 | 55.3 ± 5.0 | 46.6 ± 4.1 | 65.3 ± 7.2 | 69.0 ± 8.0 | — |
| 25 | 69.0 ± 3.0 | 36.4 ± 7.2 | 72.5 ± 5.6 | 71.3 ± 9.1 | 61.2 ± 3.5 |
| Uracil | | | | | |
| 12.5 | 23.7 ± 3.1 | 18.8 ± 4.4 | 3.6 ± 3.1 | 24.5 ± 2.1 | — |
| 25 | 32.3 ± 2.0 | 23.1 ± 4.0 | 26.0 ± 8.7 | 25.4 ± 2.1 | 21.3 ± 1.7 |
| 5-Aminouracil | | | | | |
| 25 | 11.9 ± 5.4 | 12.1 ± 3.3 | −3.2 ± 2.2 | 9.3 ± 6.1 | — |
| 250 | 11.6 ± 5.7 | 9.9 ± 7.5 | 22.8 ± 10.8 | 16.0 ± 7.7 | — |
| 5-Bromouracil | | | | | |
| 12.5 | 47.2 ± 1.2 | — | 85.3 ± 4.4 | 83.9 ± 2.8 | — |
| 25 | 62.9 ± 4.3 | 36.4 ± 2.8 | 91.6 ± 2.0 | 91.2 ± 4.8 | — |
| 50 | — | 48.9 ± 3.0 | — | — | — |
| 5-Fluorouracil | | | | | |
| 25 | 37.0 ± 3.2 | 37.7 ± 6.1 | 53.6 ± 3.7 | 52.2 ± 3.8 | — |
| 50 | 54.9 ± 3.1 | 51.8 ± 5.0 | 71.1 ± 5.8 | 70.2 ± 7.0 | — |
| 5-Iodouracil | | | | | |
| 12.5 | — | 20.9 ± 2.0 | 71.5 ± 7.7 | 72.9 ± 4.2 | — |
| 25 | 5.14 ± 3.2 | 33.6 ± 1.8 | 84.3 ± 4.9 | 83.5 ± 3.0 | — |
| 50 | 68.1 ± 2.8 | — | — | — | — |

[a] 0.09% w/v; rate constant for FUdR (25 μM) degradation was 0.0233 ± 0.0009 $min^{-1}$.
[b] 0.50% w/v; rate constant for FUdR (25 μM) degradation was 0.0096 ± 0.0003 $min^{-1}$.
[c] 0.40% w/v; rate constant for FUdR (25 μM) degradation was 0.0249 ± 0.0007 $min^{-1}$.
[d] 0.40% w/v; rate constant for FUdR (25 μM) degradation was 0.0140 ± 0.0005 $min^{-1}$.
[e] 0.75% w/v; rate constant for FUdR (25 μM) degradation was 0.0363 ± 0.0008 $min^{-1}$.

**Table II—Kinetic Constants ($K_m$) and Inhibition Constants ($K_i$) for Rat and Beagle Tissue Homogenates**

| Inhibitor | Rat Liver[a] | | Rat Intestine[b] | | Beagle Liver[c] | | Beagle Intestine[d] | |
|---|---|---|---|---|---|---|---|---|
| | $K_i$, mM | $K_i/K_m$ | $K_i$, mM | $K_i/K_m$ | $K_i$, mM | $K_i/K_m$ | $K_i$, mM | $K_i/K_m$ |
| AcycTdR | 0.021 | 0.22 | 0.0037 | 0.053 | 0.00043 | 0.013 | 0.00046 | 0.0079 |
| AcycUdR | 0.17 | 1.77 | 0.014 | 0.20 | 0.0048 | 0.15 | 0.0090 | 0.16 |
| TdR | 0.019 | 0.20 | 0.024 | 0.34 | 0.0072 | 0.23 | 0.010 | 0.17 |
| UdR | 0.27 | 2.81 | 0.13 | 1.86 | 0.10 | 3.13 | 0.19 | 3.28 |
| Thymine | 0.044 | 0.46 | 0.011 | 0.16 | 0.0046 | 0.14 | 0.0058 | 0.10 |
| Uracil | 0.16 | 1.67 | 0.056 | 0.80 | 0.048 | 1.50 | 0.063 | 1.09 |

[a] A 0.5% (w/v) homogenate was used for $K_i$ measurement; three levels of the homogenate (0.25, 0.5, and 1.0% w/v) were used to obtain $K_m$ of FUdR (0.096 mM). [b] A 0.09% (w/v) homogenate was used for $K_i$ measurement; three levels of the homogenate (0.02, 0.045, and 0.09% w/v) were used to obtain $K_m$ of FUdR (0.070 mM) [c] A 0.8% (w/v) homogenate was used for $K_i$ measurement; three levels of the homogenate (0.2, 0.4, and 0.8% w/v) were used to obtain $K_m$ of FUdR (0.032 mM). [d] A 0.8% (w/v) homogenate was used for $K_i$ measurement; three levels of the homogenate (0.2, 0.4, and 0.8% w/v) were used to obtain $K_m$ of FUdR (0.058 mM).

patible values in the two combinations [i.e., dodecanoyl-FUdR(C12-FUdR):octadecanoyl-acyclothymidine(C18-AcycTdR) and tetradecanoyl-FUdR (C14-FUdR):eicosanoyl-acyclothymidine(C20-AcycTdR)] suggested close similarity of the susceptibility and the physicochemical properties in the combinations.

**Potentiation of Antitumor Activity**—The effect of AcycTdR and its esters (C18-AcycTdR and C20-AcycTdR) on the antitumor activity of FUdR esters (C12-FUdR and C14-FUdR) was evaluated by simultaneous oral administration to mice bearing L1210 (Table IV). Though the oral coadministration of AcycTdR (molar ratio of 2.0 to C12-FUdR or C14-FUdR) showed no potentiation on the activity of the FUdR esters, significant potentiation was obtained with the C14-FUdR:C20-AcycTdR combination over C14-FUdR alone in the wide range of doses (3–100 mg/kg/d), and the C12-FUdR:C18-AcycTdR combination showed higher antitumor effect at a lower dose (34% ILS at 10 mg + 15 mg/kg/d) than C12-FUdR alone (15% ILS at 10 mg/kg/d). These results

**Table III—Susceptibility to Enzymatic Hydrolysis and Physicochemical Properties of the Esters of 5-Fluoro-2'-deoxyuridine (FUdR) and Acyclothymidine (AcycTdR)**

| Ester[b] | Hydrolysis Rate Constants in Intestinal Homogenates, $h^{-1}$[a] | | Retention Time in HPLC Column, min | |
|---|---|---|---|---|
| | Rat | Mouse | Column A[c] | Column B[d] |
| C12-FUdR | 0.159 ± 0.016 | 0.0698 ± 0.0018 | 9.2 | 14.9 |
| C18-AcycTdR | 0.215 ± 0.011 | 0.142 ± 0.01 | 10.5 | 14.6 |
| C14-FUdR | 0.0698 ± 0.0025 | 0.033 ± 0.009 | 15.5 | 13.9 |
| C20-AcycTdR | 0.0881 ± 0.0031 | 0.0305 ± 0.011 | 15.3 | 14.0 |

[a] 0.06% (w/v). [b] Initial concentration of 0.025 mM. [c] Lichrosorb RP-18, having affinity for hydrophobic moieties. [d] Nucleosil 5-CN, having affinity for hydrophilic moieties.

**Table IV—Antitumor Activity of Oral 5-Fluoro-2'-deoxyuridine (FUdR) Esters Coadministered with Acyclothymidine (AcycTdr) Esters**

| Drug/Potentiator | Dose mg/kg/d[a] | MDS ± SE, d[b] | ILS, % | Weight Change (0–5 d), g/mouse |
|---|---|---|---|---|
| Control | — | 8.2 ± 0.2 | 0 | +2.6 |
| FUdR | 100 | 9.4 ± 0.4 | 15 | −4.2 |
| | 300 | 9.0 ± 0.2 | 10 | −5.2 |
| C12-FUdR | 10 | 9.4 ± 0.2 | 15 | +0.8 |
| | 30 | 10.8 ± 0.7 | 32 | +0.6 |
| | 100 | 12.0 ± 0.4 | 46 | −0.8 |
| | 300 | 12.6 ± 0.5 | 54 | −1.0 |
| C12-FUdR | 30 + 20 | 10.8 ± 0.6 | 32 | +1.0 |
| + AcycTdR | 100 + 66 | 11.2 ± 0.5 | 37 | +0.6 |
| | 300 + 200 | 12.0 ± 0.4 | 46 | −0.4 |
| C12-FUdR | 10 + 15 | 11.0 ± 0.2 | 34[c] | −0.2 |
| + C18-AcycTdR | 30 + 46 | 12.0 ± 0.4 | 46 | 0.0 |
| | 100 + 153 | 12.0 ± 0.4 | 46 | −1.0 |
| | 300 + 450 | 12.0 ± 0.3 | 46 | −1.0 |
| AcycTdR | 200 | 9.2 ± 0.2 | 12 | +1.2 |
| C18-AcycTdR | 450 | 9.2 ± 0.2 | 12 | +1.6 |
| Control | — | 8.2 ± 0.2 | 0 | +2.1 |
| C14-FUdR | 3 | 8.4 ± 0.2 | 2 | +1.8 |
| | 10 | 9.4 ± 0.2 | 15 | +1.6 |
| | 30 | 9.6 ± 0.4 | 17 | +1.2 |
| | 100 | 10.6 ± 0.5 | 29 | +1.0 |
| C14-FUdR | 3 + 2 | 8.2 ± 0.4 | 0 | +1.7 |
| + AcycTdR | 10 + 6 | 8.6 ± 0.2 | 5 | +1.2 |
| | 30 + 18 | 9.0 ± 0.3 | 10 | +1.2 |
| | 100 + 61 | 10.2 ± 0.2 | 24 | +1.2 |
| C14-FUdR | 3 + 5 | 9.6 ± 0.4 | 17[d] | +1.4 |
| + C20-AcycTdR | 10 + 15 | 10.6 ± 0.2 | 29[e] | +0.2 |
| | 30 + 45 | 12.0 ± 0.3 | 46[e] | 0.0 |
| | 100 + 148 | 13.2 ± 0.7 | 60[e] | −0.6 |
| AcycTdR | 100 | 8.2 ± 0.2 | 0 | +2.3 |
| C20-AcycTdR | 150 | 8.4 ± 0.2 | 2 | +2.6 |

[a] The molar ratio of the potentiators to the drugs was fixed at 2:1, e.g., C12-FUdR (30 mg, 49 μmol) + AcycTdR (20 mg, 98 μmol) and C14-FUdR (30 mg, 45 μmol) + C20-AcycTdR (45 mg, 90 μmol). [b] Five mice per group. [c] $p < 0.05$, compared with C12-FUdR (10 mg/kg/d, 15% ILS) for statistical analysis. [d] $p < 0.05$, compared with C14-FUdR (3–100 mg/kg/d). [e] $p < 0.01$, compared with C14-FUdR (3–100 mg/kg/d).

**Table V—Antitumor Activity of Oral 5-Fluor-2-deoxyuridine (FUdR) Esters Coadministered with Acyclothymidine (AcycTdr) Esters in Various Molar Ratios**

| Dose, mg/kg/d | | Molar Ratio | MDS ± SE, d[c] | ILS, % | Weight Change (0–5 d), g/mouse |
|---|---|---|---|---|---|
| C12-FUdR + C18-AcycTdR | | | | | |
| 0 | 0 | — | 8.4 ± 0.2 | 0 | +3.0 |
| 30[a] | 0 | — | 10.4 ± 0.4 | 24 | +1.6 |
| 30 | 3 | 1:0.125 | 11.2 ± 0.2 | 33 | 0.0 |
| 30 | 6 | 1:0.25 | 10.8 ± 0.4 | 29 | +0.4 |
| 30 | 11 | 1:0.5 | 10.4 ± 0.4 | 24 | +0.6 |
| 30 | 22 | 1:1.0 | 11.3 ± 0.3 | 35 | +0.2 |
| 30 | 45 | 1:2.0 | 11.6 ± 0.3 | 38 | 0.0 |
| 30 | 68 | 1:3.0 | 11.9 ± 0.4 | 42 | −0.6 |
| 30 | 90 | 1:4.0 | 10.9 ± 0.4 | 30 | −0.2 |
| 0 | 90 | — | 8.5 ± 0.2 | 1 | +1.6 |
| C14-FUdR + C20-AcycTdR | | | | | |
| 0 | 0 | — | 8.0 ± 0.2 | 0 | +1.2 |
| 30[b] | 0 | — | 9.4 ± 0.2 | 17 | −0.6 |
| 30 | 2.2 | 1:0.1 | 11.4 ± 0.2 | 43 | −0.2 |
| 30 | 6.6 | 1:0.3 | 11.2 ± 0.2 | 40 | −0.4 |
| 30 | 22 | 1:1.0 | 12.4 ± 0.4 | 55 | −1.0 |
| 30 | 44.5 | 1:2.0 | 12.2 ± 0.4 | 52 | −1.0 |
| 0 | 44.5 | — | 7.8 ± 0.2 | −2 | +1.1 |

[a] Equivalent of 49 μmol. [b] Equivalent of 45 μmol; [c] Five mice in each group were used.

suggest that appropriate esterification or optimization of the hydrolytic reactivity and the physicochemical properties of both esters is important to achieve the expected inhibitory effect of AcycTdR in vivo. The poor effect of C18-AcycTdR on the potentiation of C12-FUdR activity may be due to their less compatible properties (e.g., large difference in the susceptibility to the mouse intestinal homogenate, Table III).

Since the molar ratio of the AcycTdR esters to the FUdR esters was arbitrarily fixed at 2:1 in the study described above, separate experiments were carried out to evaluate the effect of the combination ratio of the AcycTdR esters and the FUdR esters under a fixed FUdR ester dose (30 mg/kg/d, Table V). The C20-AcycTdR ester potentiates the activity of C14-FUdR even at the molar ratio of 0.1 (43% ILS versus 17% ILS). The C20-AcycTdR ester showed the highest effect at a molar ratio of 1.0 (55% ILS), though the dose dependency on the potentiator was not evident.

The effect of C18-AcycTdR on the activity of C12-FUdR in the various molar ratios was less significant. Higher antitumor effect was observed at molar ratios of 2.0 and 3.0, but these values were not statistically significant ($p > 0.05$) and showed no dose dependency on C18-AcycTdR. This result can be consistent with the poor effect of C18-AcycTdR on C12-FUdR in the fixed molar ratio study and may be attributed to the less compatible characteristics of the two esters.

Potentiation of the antitumor activity of FUdR by using phosphorylase inhibitors has been suggested by many investigators[14,17–20] and several successes have been reported in cultured cells.[21,22] In the present study, AcycTdR was selected as a promising phosphorylase inhibitor to potentiate the activity of FUdR esters. Simultaneous oral administration of AcycTdR and the FUdR esters, however, showed no potentiation of the survival of mice. The expected potentiation was achieved by the oral coadministration of C20-AcycTdR and C14-FUdR, which have compatible susceptibility to enzymatic hydrolysis and physicochemical properties. Though many studies are required to elucidate the mechanism of the potentiation, these results may be explained as follows: C20-AcycTdR and C14-FUdR release their parent compounds (AcycTdR and FUdR) at similar points (possibly in the GI tract) and rates in vivo, and then the released AcycTdR inhibits the phosphorylase activity or potentiates the antitumor activity of FUdR.

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