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Specificity of Esterases and Structure of Prodrug Esters. II. Hydrolytic Regeneration Behavior of 5-Fluoro-2′-deoxyuridine (FUdR) from 3′,5′-Diesters of FUdR with Rat Tissue Homogenates and Plasma in Relation to Their Antitumor Activity

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The bioactivation characteristics of 3′,5′-diesters of 5-fluoro-2′-deoxyuridine (FUdR) esterified with saturated aliphatic acids, including acetic, propionic, butyric, hexanoic, octanoic, decanoic, dodecanoic and trimethylacetic acids, were studied by using rat tissue homogenates and plasma. The susceptibility of the esters to hydrolysis by the biological media increased as the acyl promoiety was lengthened up to octanoyl. Further elongation resulted in decreasing susceptibility. Antitumor activity against L1210 of the esters with a longer chain or branched acyl group administered intraperitoneally and orally to tumor-bearing mice was also examined. Both the antitumor activity and the therapeutic index (ILS_{max}/ILS_{30}) of the esters improved as the acyl promoiety was lengthened from octanoyl to dodecanoyl. These results suggested that the higher antitumor activities of longer alkyl chain diesters of FUdR are due to their slow rates of FUdR regeneration with esterases.

Keywords——5-fluoro-2′-deoxyuridine; ester prodrug; acyl promoiety; enzymatic hydrolysis; antitumor activity; phosphorolytic cleavage; enzyme specificity

Much work has been done recently to improve the delivery characteristics and therapeutic value of various drugs by chemical transformation of the drug into *per se* inactive derivatives which are convertible *in vivo* to the parent drug by enzymatic or chemical regeneration, or both.[1–3] In such an approach, not only the physicochemical properties but also biological lability of the prodrug are of importance. Thus, the lability of the promoiety in various enzymic systems has been studied by many investigators[4–10] with the aim of effective design of prodrugs based on enzyme-substrate specificities.

The cytotoxicity of 5-fluoro-2′-deoxyuridine (FUdR) varies with the duration of the exposure of tumor cells to the drug,[11] and FUdR suffers from rapid phosphorolytic cleavage *in vivo*.[12,13] It was reported recently[14] that 3′,5′-dipalmitoyl-FUdR was markedly active against L1210 cells *in vivo* and suppressed tumor growth, presumably by being slowly hydrolyzed *in vivo* to FUdR. Therefore it seems possible that the cytotoxic effect of FUdR would be imporved if the FUdR level could be maintained by administering a depot form of FUdR.

In the previous study, the susceptibility of 3′,5′-diesters of FUdR with saturated aliphatic acids to hydrolysis by porcine liver esterase was found to be increased as the acyl moiety was lengthened up to octanoyl, but further elongation in the acyl moiety resulted in a sharp decrease in the susceptibility.[15] In the present paper, the hydrolysis of these esters and the regeneration of FUdR from them with rat tissue homogenates and plasma were investigated. The relationship between antitumor activity and the susceptibility of the esters is discussed.

Experimental

Chemicals——FUdR was purchased from Heinrich Mach Nachf. All other chemicals were of reagent grade quality and were obtained commercially.

The 3′,5′-diesters of FUdR with normal-chain acids were prepared according to the procedure described by Nishizawa *et al.*[16] 3′,5′-Bis(trimethylacetyl)-5-fluoro-2′-deoxyuridine (E-8) was synthesized as follows. FUdR (300 mg) in 10 ml of anhydrous pyridine was treated at 0 °C with 2.5 mol eq of trimethylacetyl chloride. The mixture was held at room temperature overnight, then added to ice water, and the esters were recovered by extraction three times with equal volumes of chloroform. The chloroform extract was then washed three times with equal volumes of 0.1 N HCl, and dried with sodium sulfate. The chloroform was evaporated off and the residue was chromatographed on a silica gel column with chloroform–ethanol (97 : 3). Evaporation of the eluate *in vacuo* gave E-8 (195 mg) as a colorless powder. mp 110—111 °C. NMR ($CDCl_3$) δ: 1.20 (18H, s, C-methyl), 2.53 (2H, m, C2′), 4.28—4.52 (3H, m, C4′C5′), 5.28 (1H, m, C3′), 6.33 (1H, t, Cl′), 7.92 (1H, d, J=6.5 Hz, CHCF). The structures and stability in aqueous solution of these prodrugs are summarized in Table I.

Stock solutions of all the prodrugs and FUdR were prepared in ethanol to give a concentration of 4×10^{-3} M, and 10 μl of the stock solution was mixed with 1 ml of a biological preparation for enzyme assay.

Stability Measurement in Aqueous Solution——The chemical stability of 3′,5′-bis(trimethylacetyl)-FUdR as determined by the method reported previously[15] is shown in Table I along with those of other esters reported previously.

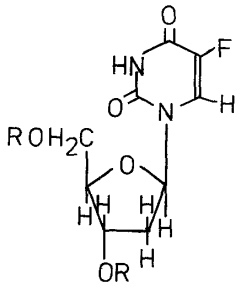
Preparation of the Enzyme Systems——Male Sprague Dawley rats weighing 250—280 g were sacrificed to obtain blood, liver, small intestine and kidney. The blood was centrifuged at 1000 × *g* for 15 min, and the resulting plasma was stored at 0—5 °C for no more than 30 h before use. The rat liver, intestine and kidney homogenates were prepared in isotonic phosphate buffer (pH 7.0) containing 0.19 M sucrose. A half gram of fresh tissue was homogenized with 5 ml of the phosphate buffer for 10—20 min in a glass homogenizer with a Teflon pestle having radial serrations. The homogenates were centrifuged at 600 × *g* for 10 min, and the supernatant was used for the experiments. The temperature was maintained at 0—5 °C during the preparation and storage of the tissue homogenates. Again these preparations were kept for no more than 30 h before use.

Stability Study in Biological Media——Bioactivation and biodegradation experiments were performed at 37 °C in the presence of various enzymic preparations diluted with isotonic phosphate buffer. The experiments were initiated by adding the stock solutions of the FUdR derivatives to the enzymic preparations to give a final concentration of 4×10^{-5} M. The decrease in concentration of the diesters and FUdR due to hydrolysis and degradation was followed by high performance liquid chromatography (HPLC) analysis of samples of the reaction mixture taken periodically to determine the pseudo-first-order rate constants. The change in concentration of FUdR released from the prodrugs with tissue homogenates and plasma was also followed by HPLC.

Analytical Method——Hydrolytic regeneration of FUdR from the diester prodrugs and degradation of FUdR were monitored by the HPLC method reported previously.[15] A mixture of methanol–0.02 M acetate buffer, pH 4.0 (60 : 40), was employed as the mobile phase for 3′,5′-bis(trimethylacetyl)-FUdR.

Evaluation of Antitumor Activity——Male BDF_1 (C57BL/6 × DBA/2) mice weighing 25—27 g were purchased from Charles River Inc. (Shizuoka, Japan) and a pellet diet (CA-1, CLEA Japan Inc.) and water were provided *ad libitum*. Each of five animals in each group was inoculated with 1×10^5 cells of murine lymphoma L1210, which was maintained in male DBA mice by weekly intraperitoneal (*i.p.*) transplantation. The drugs were given intraperitoneally

Table I. Structures and Physicochemical Properties of 3′,5′-Diesters of 5-Fluoro-2′-deoxyuridine

General structure	Compound No.	-R	$T_{1/2}$ (h) (pH = 7.0, 37 °C)	HPLC conditions (buffer[a] : methanol)
	FUdR	–H	—	95 : 5
	E-1	–CO–CH_3	5000	80 : 20
	E-2	–CO–CH_2CH_3	5000	65 : 35
	E-3	–CO–$(CH_2)_2CH_3$	5000	50 : 50
	E-4	–CO–$(CH_2)_4CH_3$	1030	35 : 65
	E-5	–CO–$(CH_2)_6CH_3$	300	25 : 75
	E-6	–CO–$(CH_2)_8CH_3$	240	11 : 89
	E-7	–CO–$(CH_2)_{10}CH_3$	300	8 : 92
	E-8	–CO–C–$(CH_3)_3$	5000	40 : 60
	E-9	–CO–$(CH_2)_{12}CH_3$	—	—

a) 0.02 M acetate buffer, pH 4.0.

on days 1, 2, 3, 4 and 5, or orally at days 1, 3 and 5, starting 24 h after the transplantation. Thereafter the survival times of the mice were recorded. Antitumor activity of the drugs was evaluated in terms of the increase in lifespan over controls (ILS: T/C, %), ILS_{30} (the dose showing 30% increase in lifespan), maximum ILS, and ILS_{max} (the dose showing maximum ILS). Further, the therapeutic ratio (ILS_{max}/ILS_{30}) was calculated to assess therapeutic advantage quantitatively.

Results

Degradation of FUdR in Rat Tissue Homogenates

The enzymatic degradation of FUdR was studied *in vitro* with rat tissue homogenates and plasma at 37 °C. Table II shows the pseudo-first-order rate constants calculated from the slope of semilogarithmic plots of FUdR concentration against time. The degradation of FUdR in rat intestinal, liver and kidney homogenates was observed even at high dilution (0.1—0.4%), while FUdR was shown to be stable in rat plasma.

Degradation of 3′,5′-Diesters of FUdR in Rat Homogenates and Plasma

The relative susceptibility of the diester prodrugs to enzymatic hydrolysis was examined *in vitro* with rat tissue homogenates and plasma at 37 °C. For all the enzyme preparations studied, the decrease in concentration of the diester prodrugs followed pseudo-first-order kinetics, the rate constants being summarized in Table III. These degradation rate constants were significantly dependent on tissue and plasma concentration (data not shown). As shown in Table III, the susceptibility of the prodrug varied with its ester promoiety. Hexanoate (E-4) and octanoate (E-5) showed a high susceptibility to tissue homogenates and plasma, while dodecanoate (E-7) was relatively stable in all enzymic samples. Acetate (E-1) and trimethylacetate (E-8) showed specific resistance to enzymatic degradation in intestinal and kidney homogenates, respectively.

Table II. Degradation of FUdR with Rat Tissue Homogenate and Plasma

Tissue		Rate constants ± S.D. (h^{-1})
0.1% intestinal	$n=5$	2.6568 ± 0.2011
0.4% liver	$n=5$	0.5016 ± 0.0841
0.4% kidney	$n=4$	0.4200 ± 0.0288
20% plasma	$n=4$	<0.001

Table III. Hydrolytic Rate Constants of 3′,5′-Diesters of FUdR with Rat Tissue Homogenate and Plasma

Compound	0.2% Rat intestinal ($n=3$)	0.4% Rat liver ($n=3$)	20% Rat plasma ($n=3$)	0.4% Rat kidney ($n=3$)
E-1	0.0557 ± 0.0253	0.3384 ± 0.0048	0.3324 ± 0.1296	0.3936 ± 0.0504
E-2	4.176 ± 0.588	0.9216 ± 0.5424	1.620 ± 0.132	0.4416 ± 0.0432
E-3	208.8[a] ± 1.2	7.125 ± 3.432	4.956 ± 1.656	0.9696 ± 0.2256
E-4	178.7[a] ± 76.1	64.56 ± 15.36	33.24 ± 7.68	14.93 ± 3.22
E-5	189.5[a] ± 74.3	69.6 ± 43.9	34.32 ± 8.04	3.168 ± 0.082
E-6	1.075 ± 0.125	0.499 ± 0.101	0.420 ± 0.100	0.2129 ± 0.0768
E-7	0.0531 ± 0.0173	0.0370 ± 0.0170	0.0632 ± 0.0198	0.0362 ± 0.0072
E-8	2.232 ± 0.276	0.3120 ± 0.00564	0.1512 ± 0.0580	0.0238 ± 0.0096

a) Because of the higher reactivity, the measurements were carried out in 0.02% homogenate. The values were estimated by extrapolation of the results.

FUdR Concentrations in Rat Tissue Homogenates and Plasma

Time courses of FUdR regeneration from the prodrugs (E-1—8) and its subsequent degradation in enzymic preparations are shown in Figs. 1—8. All experiments were initiated at a prodrug concentration of 4×10^{-5} M, which is equivalent to 9.85 μg/ml in FUdR concentration. Though the regeneration behavior differed considerably among the prodrugs, the concentrations of the enzymic preparations (0.2% intestinal, 0.4% liver and kidney, 20%

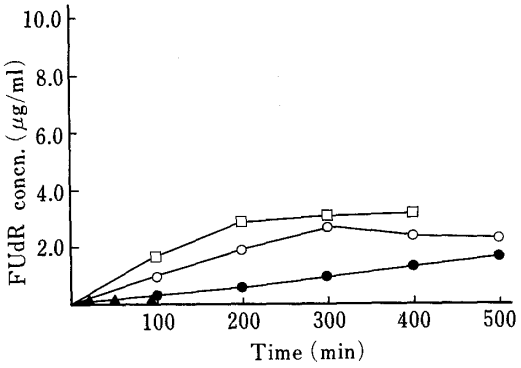


Fig. 1. FUdR Regeneration from 3′,5′-Diacetyl-FUdR (E-1) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

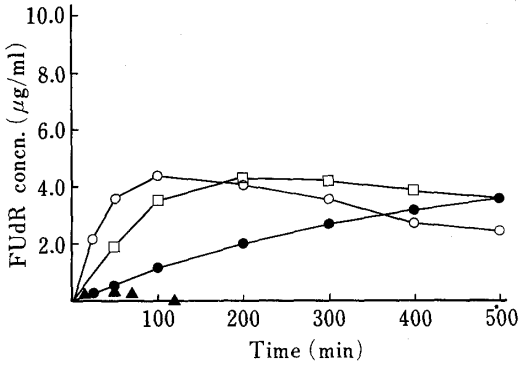


Fig. 2. FUdR Regeneration from 3′,5′-Dipropionyl-FUdR (E-2) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver, □, 0.4% Kidney) and 20% Plasma (●)

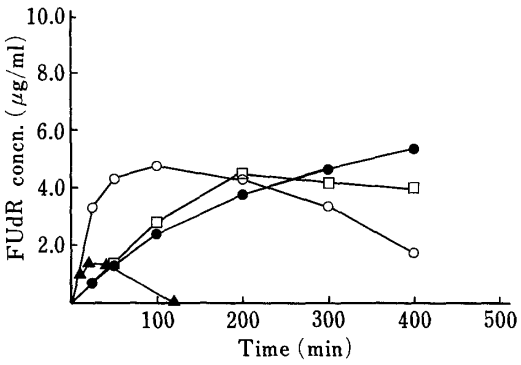


Fig. 3. FUdR Regeneration from 3′,5′-Dibutyryl-FUdR (E-3) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

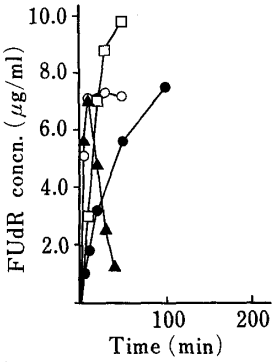


Fig. 4. FUdR Regeneration from 3′,5′-Dihexanoyl-FUdR (E-4) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

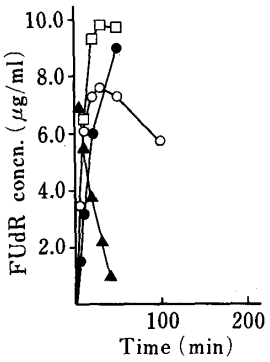


Fig. 5. FUdR Regeneration from 3′,5′-Dioctanoyl-FUdR (E-5) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

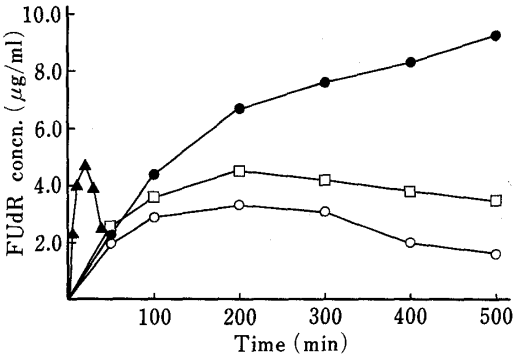


Fig. 6. FUdR Regeneration from 3′,5′-Didecanoyl-FUdR (E-6) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

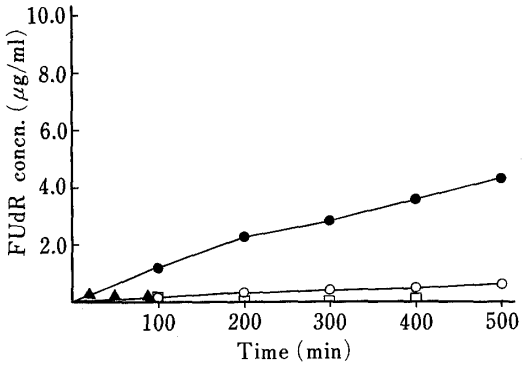


Fig. 7. FUdR Regeneration from 2′,5′-Didodecanoyl-FUdR (E-7) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

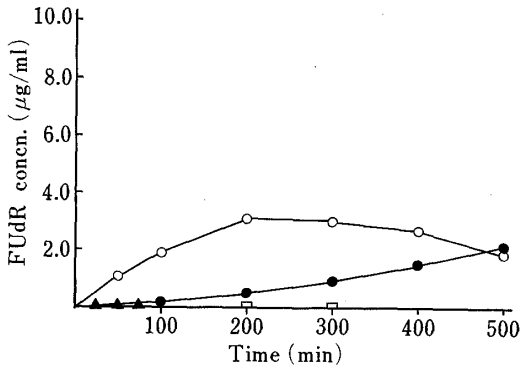


Fig. 8. FUdR Regeneration from 3′,5′-Ditrimethylacetyl-FUdR (E-8) in Rat Tissue Homogenates (▲, 0.2% Intestinal; ○, 0.4% Liver; □, 0.4% Kidney) and 20% Plasma (●)

plasma) were kept constant for each prodrug so that the relative susceptibility of the prodrugs could be compared.

Intestinal Homogenate——FUdR regeneration was hardly observed from E-1, E-2, E-7 or E-8 in intestinal homogenate (Figs. 1, 2, 7 and 8). FUdR was released instantaneously from E-4 and E-5, but the liberated FUdR rapidly decomposed. This fast disappearance of FUdR was expected in view of the instability of FUdR in rat intestinal homogenate (Table II).

Liver and Kidney Homogenates——Both homogenates showed similar relative activity with all prodrugs having a normal alkyl chain. The susceptibility of the esters increased as the alkyl chain of the promoiety lengthened up to octanoyl, but further elongation of the alkyl chain resulted in decreased susceptibility to hydrolysis. The susceptibility of dodecanoate (E-7) was lower than that of acetate (Figs. 1 and 7). In the case of trimethylacetate (E-8) (Fig. 8), the liver homogenate showed considerable specificity, while a very small amount of FUdR was released with kidney homogenate.

Plasma——Although the specificity of rat plasma for the hydrolytic regeneration of FUdR varied with the prodrugs as in the cases of the liver and kidney homogenates, the plasma preparation appeared to retain substantial activity to release FUdR from dodecanoate (E-7) (Fig. 7).

Antitumor Activity of Diesters of FUdR

As reported previously, the antitumor activity of the diester prodrugs with shorter alkyl chains (E-1—4) against L1210 leukemia cells following *i.p.* administration was no greater than that of unesterified FUdR.[14] The present experiments were, therefore, limited to the prodrugs with a longer chain or branched chain acid residue.

Survival times of mice bearing L1210 cells following *i.p.* administration of the prodrugs (E-5—9) or FUdR are shown in Fig. 9. Esters with octanoyl and trimethylacetyl groups were less active than FUdR. However, the antitumor activity of prodrugs with a longer chain was much higher than that of FUdR. The ILS_{30} values of prodrugs with decanoyl and dodecanoyl esters were 10 and 1.0 $mg \cdot kg^{-1} \cdot d^{-1}$, which were less than one-fifth and one-fiftieth of that of FUdR, respectively. Furthermore, the difference between the dose giving ILS_{max} and that giving ILS_{30} was greater than that of FUdR, the therapeutic ratio being improved to 10 for E-6 and 30 for E-7 compared to 2.0 for FUdR (Table IV).

ILS and weight change of mice bearing L1210 cells following *p.o.* administration of the prodrugs (E-5—7, 9) or FUdR are shown in Table V. No antitumor activity was observed with E-5 at doses up to 300 $mg \cdot kg^{-1} \cdot d^{-1}$, while toxicity appeared at higher doses. E-6 and FUdR gave positive ILS values which, however, were not dose-dependent. In spite of its

TABLE IV. Antitumor Activity of 3′,5′-Diacyl-5-fluoro-2′-deoxyuridine and FUdR against L1210 Following *i.p.* Administration

Compound No.	ILS_{30} ($\mu mol \cdot kg^{-1} \cdot d^{-1}$)	ILS_{max} ($\mu mol \cdot kg^{-1} \cdot d^{-1}$)	Max. ILS (%)	Therapeutic ratio
FUdR	200	400	54	2.0
E-6	18	180	54	10.0
E-7	1.5	45	62	30.0
E-8	25	60	35	2.4

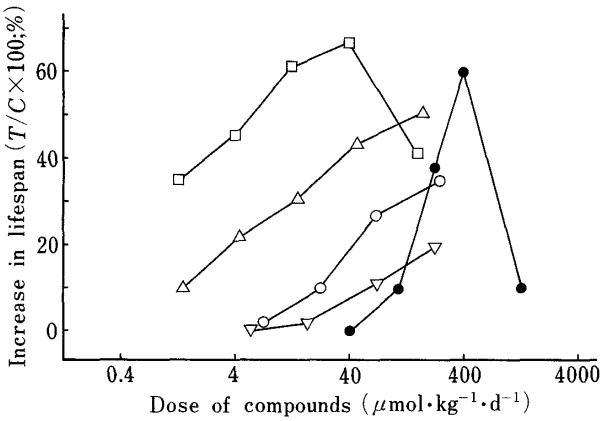


Fig. 9. Dose-Response Curves of Diesters of 5-Fluoro-2′-deoxyuridine (FUdR) against L1210

(—●—), FUdR; (—○—), ditrimethylacetyl (E-8); (—▽—), dioctanoyl (E-5); (—△—), didecanoyl (E-6); (—□—), didodecanoyl (E-7).

TABLE V. Antitumor Activity of 3′,5′-Diacyl-5-fluoro-2′-deoxyuridines and FUdR against L1210 Following *p.o.* Administration

Compound	Dose ($mg \cdot kg^{-1} \cdot d^{-1}$)	ILS (%)	Weight change (1—4 d, g/mouse)
Control	—	0	+0.4
FUdR	10	15	+0.6
	30	10	+1.8
	100	15	−4.2
	300	10	−5.2
E-5 (octanoyl)	10	−5	+1.8
	30	3	+1.6
	100	−5	−2.8
	300	5	−2.2
E-6 (decanoyl)	10	7	−0.8
	30	26	−1.4
	100	15	−2.4
	300	20	−3.8
E-7 (dodecanoyl)	10	0	+1.0
	30	5	+1.0
	100	15	0
	300	40	−2.6
E-9 (tetradecanoyl)	1	3	+2.2
	3	3	+2.0
	10	27	−0.8
	30	33	−1.4
	100	17	−2.4

poor antitumor activity on *p.o.* administration, FUdR showed severe toxicity at 100 and 300 mg·kg^{-1}·d^{-1}. Survival time was prolonged by *p.o.* administration of prodrugs with dodecanoyl (E-7) and tetradecanoyl (E-9) esters in a dose-dependent manner. E-7 showed a 40% increase in life span at 300 mg·kg^{-1}·d^{-1} with 2.6 g weight loss and E-9 showed a 33% increase at 30 mg with 1.4 g weight loss.

Discussion

In *in vitro* studies, FUdR revealed up to 100 times higher activity than 5-fluorouracil (FU) against several tumor lines.[17,18] In *in vivo* studies, however, FUdR has not appeared to be as active as FU, possibly because of the rapid degradation of FUdR to FU by pyrimidine nucleoside phosphorylases.[12,13] In designing 3′,5′-diester prodrugs of FUdR resistant to phosphorolytic cleavage, the esters of long-chain aliphatic acid were found to give high plasma levels of FUdR after *i.p.* administration and were highly active against L1210 leukemia in mice following *i.p.* administration.[14]

As the esters release FUdR *in vivo*, presumably being metabolized by esterases, the susceptibility of the esters with various saturated aliphatic acids to porcine liver esterase was investigated, and it was found previously that the susceptibility of the esters increased as the alkyl chain was lengthened up to octanoyl but decreased thereafter.[15] In the present study, the susceptibility of 3′,5′-diesters to tissue homogenates including rat intestinal, rat liver, rat kidney and rat plasma was investigated. Although the susceptibility to the rat tissue homogenates and plasma was substantially identical, in relative values, to that to the porcine liver esterase (Fig. 1—8), a relatively high susceptibility of the esters with longer chains (decanoate and dodecanoate) to plasma (Fig. 6, 7) and poor susceptibility of the trimethylacetate to kidney homogenate (Fig. 8) were noteworthy.

Enzymatic degradation of the parent compound, FUdR, also seems to play an important role in the delivery characteristics of the prodrugs. FUdR was degraded by the rat tissue homogenates, in particular intestinal homogenate where the half-life of FUdR was around 15 min at a homogenate concentration of 0.1% w/v. On the other hand, FUdR was quite stable in rat plasma (Table II). These results suggest that FUdR released in the intestine would scarcely be absorbed as intact FUdR, but in plasma it would be stable until it was metabolized in other tissues such as the liver and kidney.

In the case of *i.p.* administration, both the antitumor activity and the therapeutic index of the prodrugs were improved as the acyl promoiety was lengthened from octanoyl to dodecanoyl. Since the susceptibility of these esters to rat tissue homogenates and plasma changed in an opposite manner, the higher antitumor activity of longer chain diesters of FUdR should be partly due to slow release of FUdR *in vivo*.

The improved antitumor activity of E-6 and E-7 should be related to the low susceptibility to esterase, but the esters with shorter chain and branched chain moieties (E-1—3, 9), which did not show any advantage over FUdR in antitumor effect,[14] were also observed to release FUdR slowly through enzymatic hydrolysis. As mentioned in the previous paper, the low hydrophobicity of these esters with shorter chains may affect the interaction with the tumor cells or the kinetic behavior of the prodrugs *in vivo*.

Although the susceptibility of E-9 to the enzymic systems was not investigated because of the low aqueous solubility, both E-9 and E-7 can be assumed to be strongly resistant to enzymatic hydrolysis in the intestine. This low susceptibility to the intestinal preparation may have contributed to the better antitumor activity of the esters following oral administration (Table III). On the other hand, E-5, which showed the highest susceptibility to the intestinal preparation, showed no antitumor activity on *p.o.* administration.

Although the observations in this series of studies cover only FUdR esters, they suggest

that more specific delivery and better therapeutic values can also be built into other nucleoside-related drugs by suitable esterification.

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References and Notes

1) V. Stella and T. Higuchi, "Prodrugs as Novel Drug Delivery Systems," Am. Chem. Soc., Washington, DC, 1975, p. 1.
2) A. A. Sinkula and S. H. Yalkowsky, *J. Pharm. Sci.*, **64**, 181 (1975).
3) A. A. Sinkula, Perspective on prodrugs and analogs in drug design, In E. B. Roche (Ed.), "Design of Biopharmaceutical Properties through Prodrugs and Analogs," Am. Pharm. Assoc., Washington, DC, 1977, pp. 1—17.
4) M. Johansen, H. Bundgaard and E. Falch, *Int. J. Pharmaceut.*, **13**, 89 (1983).
5) V. H. L. Lee, R. E. Stratford, Jr. and K. W. Morimoto, *Int. J. Pharmaceut.*, **13**, 183 (1983).
6) S. Babhair and A. Hussain, *Int. J. Pharmaceut.*, **13**, 273 (1983).
7) V. H. L. Lee, *J. Pharm. Sci.*, **72**, 239 (1983).
8) D. C. Baker, S. D. Kumar, W. J. Waites, G. Arnett, W. M. Shanonn, W. I. Higuchi and W. J. Lambert, *J. Med. Chem.*, **27**, 270 (1984).
9) H. Sasaki, E. Mukai, K. Hashida, T. Kimura and H. Sezaki, *Int. J. Pharmaceut.*, **15**, 61 (1983).
10) H. Sasaki, M. Fukumoto, M. Hashida, T. Kimura and H. Sezaki, *Chem. Pharm. Bull.*, **31**, 4083 (1983).
11) F. Kanzawa, A. Hoshi and K. Kuretani, *Eur. J. Cancer*, **16**, 1087 (1980).
12) G. D. Birnie, H. Kroeger and C. Heidelberger, *Biochemistry*, **2**, 566 (1962).
13) R. G. Moran and C. Heidelberger, *Bull. Cancer* (Paris), **66**, 79 (1979).
14) F. Kanzawa, A. Hoshi, K. Kuretani, M. Saneyoshi and T. Kawaguchi, *Cancer Chemother. Pharmacol.*, **6**, 19 (1981).
15) T. Kawaguchi, Y. Suzuki, Y. Nakahara, N. Nambu and T. Nagai, *Chem. Pharm. Bull.*, **33**, 301 (1985).
16) Y. Nishizawa, J. E. Casida, S. W. Anderson and C. Heidelberger, *Biochem. Pharmacol.*, **14**, 1605 (1965).
17) J. D. Laskin, E. F. Jordan, L. N. Kenny, D. Sugg, A. N. Divekar and M. T. Hakala, *Proc. Am. Assoc. Cancer Res.*, **17**, 71 (1976).
18) M. A. Rich, J. L. Bolaffi, J. E. Knoll, L. Cheong and M. L. Eidinoff, *Cancer Res.*, **18**, 730 (1958).