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Controlled Release of 5-Fluoro-2′-deoxyuridine by the Combination of Prodrug and Polymer Matrix

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Poly(L-lactic acid) (L-PLA) microspheres containing 5-fluoro-2′-deoxyuridine (FUdR) or its ester prodrugs with saturated aliphatic acids (FUdR-C*n*, *n*=2, 3, 4, 5, 6, 8, 10 and 12) were prepared. The physicochemical and biological properties and antitumor activity of the L-PLA microspheres were studied. The lipophilicity of FUdR-C*n* was increased by prolonging its acyl-promoieties. FUdR-C5, FUdR-C6, FUdR-C8, FUdR-C10 and FUdR-C12 showed almost complete incorporation into the microspheres, while incorporation of hydrophilic FUdR and FUdR-C2 was poor. The sustained release of FUdR from the microspheres containing FUdR-C4, FUdR-C5 and FUdR-C6 was obtained in the presence of esterase, and higher antitumor activity against P388 leukemia was observed *in vivo*. On the other hand, the release rates of FUdR from the microspheres containing FUdR-C10 and FUdR-C12 were very small, and their antitumor activity was much smaller than that of the free prodrug suspension. Effects of the susceptibility to enzymatic hydrolysis and the physicochemical properties of prodrugs on the release profiles of FUdR from spheres were discussed.

Keywords poly(L-lactic acid); microsphere; 5-fluoro-2′-deoxyuridine; prodrug; sustained release; enzymatic hydrolysis; P388 leukemia

In cancer chemotherapy, it is important to control the pharmacokinetic behavior of antitumor drugs for effective treatment. Various approaches for delivering drugs to tumor tissues have been studied. Microspheres containing anticancer agents have been successfully employed for the treatment of carcinomas in the kidney, urinary bladder, and liver.[1] Though many biodegradable polymers such as gelatin,[2] albumin,[3] polyhydroxybutyric acid[4] and polylactic acid[5] have been investigated as matrices of the microspheres, their compatibility with anticancer agents have not always been sufficient. Many anticancer drugs, therefore, are poorly entrapped in microspheres and/or show rapid release from the spheres. Chemical modification of drug molecules in order to develop adequate physicochemical properties can be a promising approach to improving the drug's incorporation efficiency and release profiles. 5-Fluoro-2′-deoxyuridine (FUdR), one of the active metabolites of 5-fluorouracil (FU), shows as much as 100 times higher activity than FU against several tumor lines *in vitro*,[6–9] but it has been reported to be less effective than FU *in vivo*.[10,11] The reason for the low activity of FUdR in animal studies may be attributable to its rapid metabolism in the body,[12] since the cytotoxicity of FUdR is time-dependent or requires long retention *in vivo*.[13] To overcome the above problems, 3′,5′-diesters of FUdR with various acyl-moieties were synthesized,[8] and their antitumor activity,[8,14] susceptibility to enzymatic hydrolysis[15,16] and retention in mice[14] has been investigated. The esters were chemically very stable, so their hydrolysis rates depended strictly on their enzymatic reactivity.[15] Since these ester prodrugs of FUdR show a wide range of physicochemical properties, the compatibility, release profiles and antitumor activity of the microspheres containing the prodrugs are of interest. In the present study, eight 3′,5′-diester-FUdR with saturated aliphatic acids and poly(L-lactic acid) (L-PLA) were used to prepare the microspheres. The physicochemical and biological properties and the applicability of the L-PLA microspheres have been investigated.

Experimental

Materials FUdR was a gift from Yamasa Shoyu Co. (Chiba, Japan). 3′,5′-Diester-FUdR with aliphatic acid (FUdR-C*n*) was synthesized according to the procedure described by Nishizawa *et al.*[8]; acetyl (*n*=2), propionyl (*n*=3), butyryl (*n*=4), pentanoyl (*n*=5), hexanoyl (*n*=6), octanoyl (*n*=8), decanoyl (*n*=10), and dodecanoyl (*n*=12) esters of FUdR were so prepared. The compounds were identified by elemental analysis, nuclear magnetic resonance (NMR) and mass spectrum (MS). All the esters were more than 98% pure, as shown by one major peak by high-performance liquid chromatography (HPLC). L-PLA, with an average molecular weight of 6000, was supplied by Mitsui Toatsu Chemical Co. (Tokyo, Japan). Gelatin was a gift from Nitta Gelatin Co. (Osaka, Japan). Porcine liver esterase was purchased from Sigma Chemical Co. (St. Louis, Mo. U.S.A.). The esterase suspension (2600 units/ml) in 3.2 M $(NH_4)_2SO_4$ solution was diluted with 0.1 M phosphate buffer (pH 7.4) to give a final concentration of 100 units/ml; the resultant solution was then filtered though a membrane filter (0.45 μm, Toyo Roshi Co., Ltd., Tokyo). The esterase preparation was stored at 4 °C until use. Albumin (bovine) was purchased from Wako Pure Chemical Industries Ltd. (Osaka). All other chemicals were commercial reagent grade products.

Measurement of Melting Points Melting points of the drugs were determined on a Yanagimoto MPS-3 micro melting apparatus.

Measurement of Partition Coefficients Apparent partition coefficients of the drugs were determined in an *n*-octanol/0.1 M phosphate buffer system (pH 7.0) and a chloroform/0.1 M phosphate buffer system at 25 °C.

Measurements of Solubilities The solubilities of the drug in water and a saline solution containing 0.01% Tween 80 were determined by the following method. An excess amount of each drug was added to a flask filled with one of the test solvents (20 ml). The flask was immersed in a shaker bath maintained at 37 °C and shaken horizontally. After equilibrium, aliquots of the supernatant were withdrawn to determine the concentration of the drug.

Preparation of L-PLA Microspheres L-PLA microspheres were prepared by the solvent-evaporation method similar to that reported previously.[17] A weighed amount of each FUdR-C*n* (20 mg) was dissolved or dispersed (in the case of FUdR) in a 5% solution of L-PLA in methylene chloride (2 ml). The organic solution was then dispersed in a 100 ml gelatin solution (1%) under stirring at 500 rpm by means of a three-bladed propeller. The stirring was continued for 35 min at room temperature. The microspheres were collected by filtration through a glass filter (3G4, Sibata Scientific Technology Ltd., Tokyo), washed with water, and dried under reduced pressure at room temperature.

Determination of Drug Contents and Sizes of Microspheres A weighed amount of microspheres containing each FUdR-C*n* was dissolved in chloroform, and the drug concentration was determined spectrophotometrically at 268 nm. Drug contents of the microspheres (weight ratio, drug/microspheres) were then calculated. The drug content of microspheres containing FUdR was determined spectrophotometrically at 268 nm after the following extraction process. First, chloroform (2 ml) was added to a weighed amount of microspheres to dissolve the polymer. Then water (2 ml) was further added to the organic solution, which was shaken for 15 min to extract the drug. The aqueous layer was then used for the spectrophotometric determination.

The Green diameters of the microspheres were measured by an optical microscope (BH-2, Olympus Kogaku Co., Tokyo).

Release Studies Weighed amounts of microspheres in a flask were suspended in a saline solution containing 0.01% Tween 80, which works as a surfactant to complete the wetting of the spheres. The flask was immersed in a shaker bath maintained at 37 °C and shaken horizontally. At fixed time intervals, an aliquot of the solution was withdrawn and an appropriate volume of fresh medium was added to the release medium to maintain a sink condition, in which the concentration of each drug in the release medium was less than 10% of its solubility. The amount of the drug released was calculated from spectrophotometric determination of the aliquot solution at 268 nm.

Effect of Esterase and Albumin on Drug Release from Microspheres *in Vitro* In order to study the effect of esterase and albumin on drug release from microspheres *in vitro*, release studies in a 0.1 M phosphate buffer (pH 7.4) containing porcine liver esterase or albumin were carried out by the following method. Microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 were suspended in the release medium containing bovine albumin in a flask. The flask was immersed in a shaker bath maintained at 37 °C. At fixed time intervals, an aliquot of the solution was withdrawn, and an appropriate volume of fresh medium was added to the release medium to maintain the sink condition. The aliquot was subjected to an HPLC assay for both the esters and FUdR. A protein-free 0.1 M phosphate buffer (pH 7.4) was used as the release medium for the control study.

Release studies in a 0.1 M phosphate buffer (pH 7.4) containing esterase were carried out for FUdR-C4, FUdR-C5, FUdR-C6, FUdR-C8, FUdR-C10 and FUdR-C12 by the same method described above.

HPLC Assay Except for spectrophotometric determination, concentration of FUdR and FUdR-C*n* were measured by the use of an HPLC system (LC-6A, Shimadzu, Kyoto, Japan) equipped with a variable wavelength detector (SPD-6A, Shimadzu). The stationary phase was a Nucleosil $5C_{18}$ packed stainless steel column (4.6×250 mm, Macherey Nagel, Germany). The mobile phases were water : acetonitrile : acetic acid (95 : 5 : 0.1) for FUdR, a mixture of 0.02 M acetate buffer (pH 4.0) and methanol: (30 : 70) for FUdR-C4, (20 : 80) for FUdR-C5, (15 : 85) for FUdR-C6, (10 : 90) for FUdR-C8, (5 : 95) for FUdR-C10, and (2 : 98) for FUdR-C12, with a flow rate of 1.0 ml/min.

Evaluation of Antitumor Activity Female CDF_1 mice were purchased from Shizuoka Agricultural Cooperative Association for Laboratory Animals (Shizuoka, Japan). Five mice (weighing 20—23 g) for each group were inoculated intraperitoneally with 1×10^6 P388 leukemia cells. The drug suspension was given intraperitoneally either on day 1 only, or on days 1, 2, 3, 4 and 5 starting 24 h after the inoculation. Microspheres were also given intraperitoneally on day 1 only. The antitumor activities were indicated as *T*/*C* (%), the ratio of the mean survival time of the treated group (*T*) to that of the control group (*C*). To check side effects, the body weight of each group was measured on the day of onset and on day 5. The side effects were evaluated by the decrease in body weight from days 0—5.

Results and Discussion

Physicochemical Properties of Drugs and Microspheres Table I shows the physicochemical properties of the drugs and the microspheres containing each drug. The partition coefficients of 3′,5′-diester-FUdR in both systems increased when the alkyl chain length of acyl-promoieties was prolonged. On the other hand, FUdR showed very high hydrophilicity compared to the diesters. The solubility of the diesters in water and in a saline solution containing 0.01% Tween 80 tended to decrease with the prolongation of the alkyl chain length of acyl promoieties. But FUdR-C3 showed higher solubility compared to FUdR-C2, and FUdR-C5 also showed a higher solubility compared to FUdR-C4. The relatively higher solubility of FUdR-C3 and FUdR-C5 would be related to the low melting points of these compounds.

The drug contents in microspheres increased as a result of prolonging the alkyl chain length of acyl-promoieties of the diesters. As Bodmeier *et al.* reported,[18] the reason could be that partitioning of the drug occurred between the organic phase and the aqueous phase during the preparation process. Therefore, the successful entrapment of the prodrugs into the microspheres seems to depend on the higher lipophilicity of the prodrugs. On the other hand, the drug contents of hydrophilic drugs, such as FUdR and FUdR-C2, were very low because a large amount of the drugs were moved into the aqueous phase during the solvent-evaporation process and then removed with water during the washing step. The physicochemical properties of the drugs had little influence on the diameter of the microspheres containing the drugs. The average diameter of the microspheres was about 100 μm.

Drug Release from the Microspheres Figure 1 shows the release profiles of FUdR and diester-FUdR in a saline solution containing 0.01% Tween 80 from the microspheres.

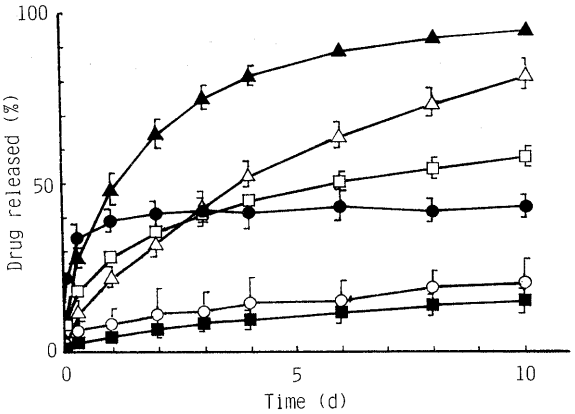

Fig. 1. Release Profiles of Drug from the Microspheres in Saline Containing 0.01% Tween 80

●, FUdR; ○, FUdR-C2; ■, FUdR-C3; □, FUdR-C4; ▲, FUdR-C5; △, FUdR-C6. Each value represents the mean ± S.D. ($n=3$). In each case of microspheres containing the prodrug, the prodrug itself was determined.

TABLE I. Physicochemical Properties of the Drugs and the Microspheres

Compd.	mp (°C)	Partition coefficient		Solubility (μg/ml)		Diameter of sphere (μm)[d]	Drug content (%)
		log P^{a}	log P^{b}	Water	Saline[c]		
FUdR	150	−1.53	−3.89	>70000	>70000	85±46	0.45
FUdR-C2	152	−0.34	1.02	3900	4300	79±34	0.17
FUdR-C3	77	0.79	2.26	5700	5100	113±45	1.60
FUdR-C4	117	1.90	3.49	100	95	101±45	9.83
FUdR-C5	59	2.72	4.19	140	115	118±39	14.2
FUdR-C6	38	4.25	5.34	20	—	104±36	15.4
FUdR-C8	Oil	6.05	5.85	1.7×10^{-2}	—	97±59	16.5
FUdR-C10	34	—	—	3.5×10^{-4}	—	108±54	15.8
FUdR-C12	63	—	—	—	—	102±50	15.9

a) *n*-Octanol: 0.1 M phosphate buffer (pH 7.0). *b*) Chloroform: 0.1 M phosphate buffer (pH 7.0). *c*) Saline containing 0.01% Tween 80. *d*) Mean ± S.D.

In the microspheres containing FUdR, the release of the drug was very slow after an initial burst. The release rates of FUdR-C2 or FUdR-C3 from their microspheres were very small, and only 10 to 30% of each drug was released during 10 d. On the other hand, the release of FUdR-C4, FUdR-C5 and FUdR-C6 from each of their microspheres was more rapid than those of FUdR-C2 and FUdR-C3. The comparative release rates were FUdR-C4 < FUdR-C6 < FUdR-C5. We reported previously that the lower the drug content in the microspheres, the slower the drug release.[19] The slow release rates of FUdR-C2 and FUdR-C3 from the spheres, though their solubility was high, would be attributable to their low drug content in the spheres. No structural changes in the microspheres' surface containing FUdR-C6 were observed after the release study in the scope of a scanning electron microscope (SEM; Type S-430, Hitachi, Tokyo) (Fig. 2). In all other microspheres, structural changes in the surface were not observed.

Effect of Esterase and Albumin on Drug Release We reported previously on the mechanism of the *in vitro* drug release from L-PLA microspheres containing diester-FUdR.[19] In the paper, we concluded that the dispersed drug in the microspheres would dissolve in an aqueous medium which had penetrated through pores formed within the microspheres, and would pass through the pores to an external medium.

Considering the above mechanism of *in vitro* drug release from the microspheres, we expected that drug release from the microspheres is influenced by various species existing in the release medium (*i.e.* enzyme, proteins and other surface-

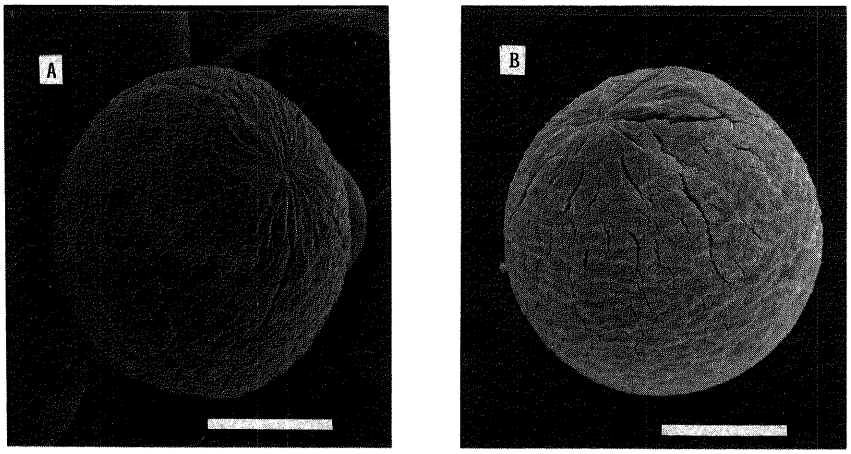

Fig. 2. Scanning Electron Photomicrographs of Microspheres Containing FUdR-C6 before and after Drug Release
A, before; B, after, Bar = 50 μm.

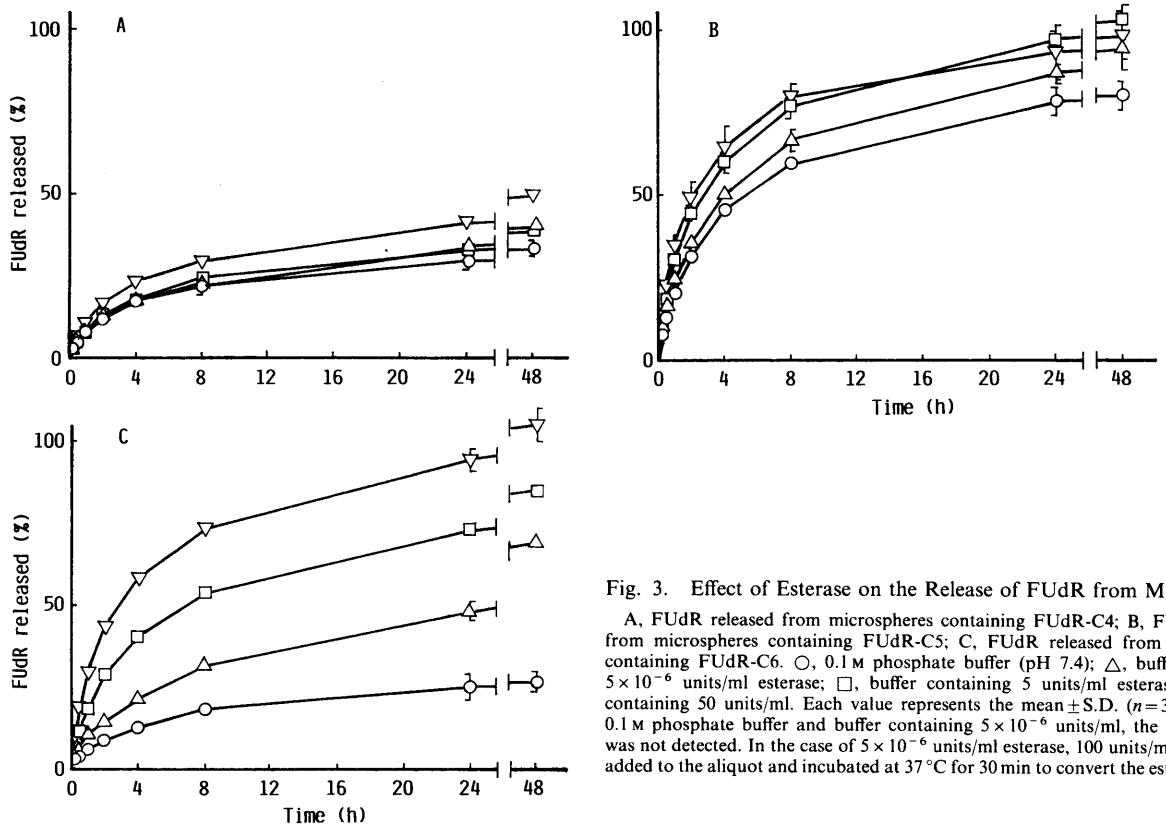

Fig. 3. Effect of Esterase on the Release of FUdR from Microspheres
A, FUdR released from microspheres containing FUdR-C4; B, FUdR released from microspheres containing FUdR-C5; C, FUdR released from microspheres containing FUdR-C6. ○, 0.1 M phosphate buffer (pH 7.4); △, buffer containing 5×10^{-6} units/ml esterase; □, buffer containing 5 units/ml esterase; ▽, buffer containing 50 units/ml. Each value represents the mean ± S.D. ($n = 3$). Except for 0.1 M phosphate buffer and buffer containing 5×10^{-6} units/ml, the prodrug itself was not detected. In the case of 5×10^{-6} units/ml esterase, 100 units/ml esterase was added to the aliquot and incubated at 37 °C for 30 min to convert the esters to FUdR.

active materials), so that release patterns of the drug from microspheres may differ from those *in vivo*. Therefore, in order to study the influence of body fluid components on the drug release from the microspheres *in vitro*, porcine liver esterase, an ubiquitous enzyme involved in the body's catalyzing the hydrolysis of diester-FUdR, to FUdR and albumin, which is expected to act as a surfactant, were selected as additives for these experiments.

The effects of esterase on the drug release from the microspheres containing FUdR-C4, FUdR-C5 or FUdR-

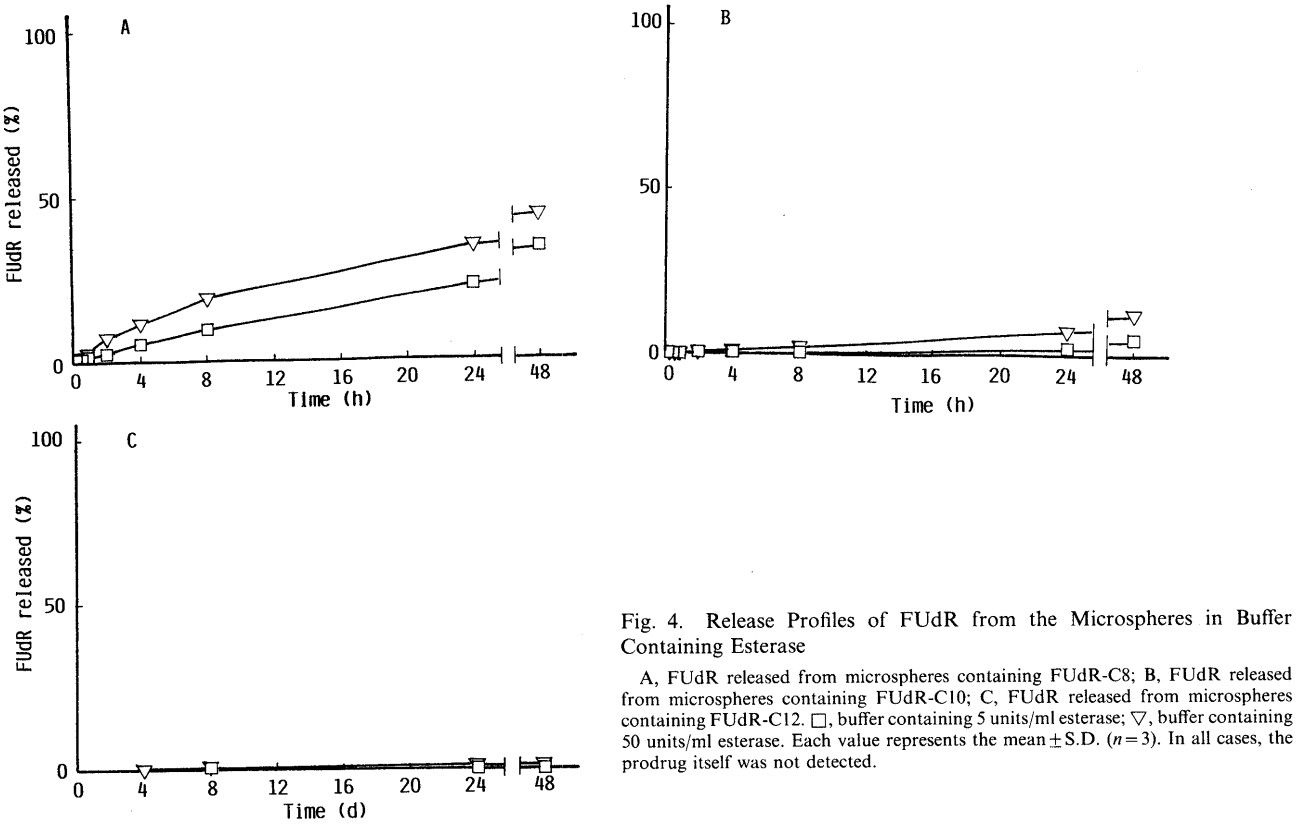

Fig. 4. Release Profiles of FUdR from the Microspheres in Buffer Containing Esterase

A, FUdR released from microspheres containing FUdR-C8; B, FUdR released from microspheres containing FUdR-C10; C, FUdR released from microspheres containing FUdR-C12. □, buffer containing 5 units/ml esterase; ▽, buffer containing 50 units/ml esterase. Each value represents the mean ± S.D. ($n=3$). In all cases, the prodrug itself was not detected.

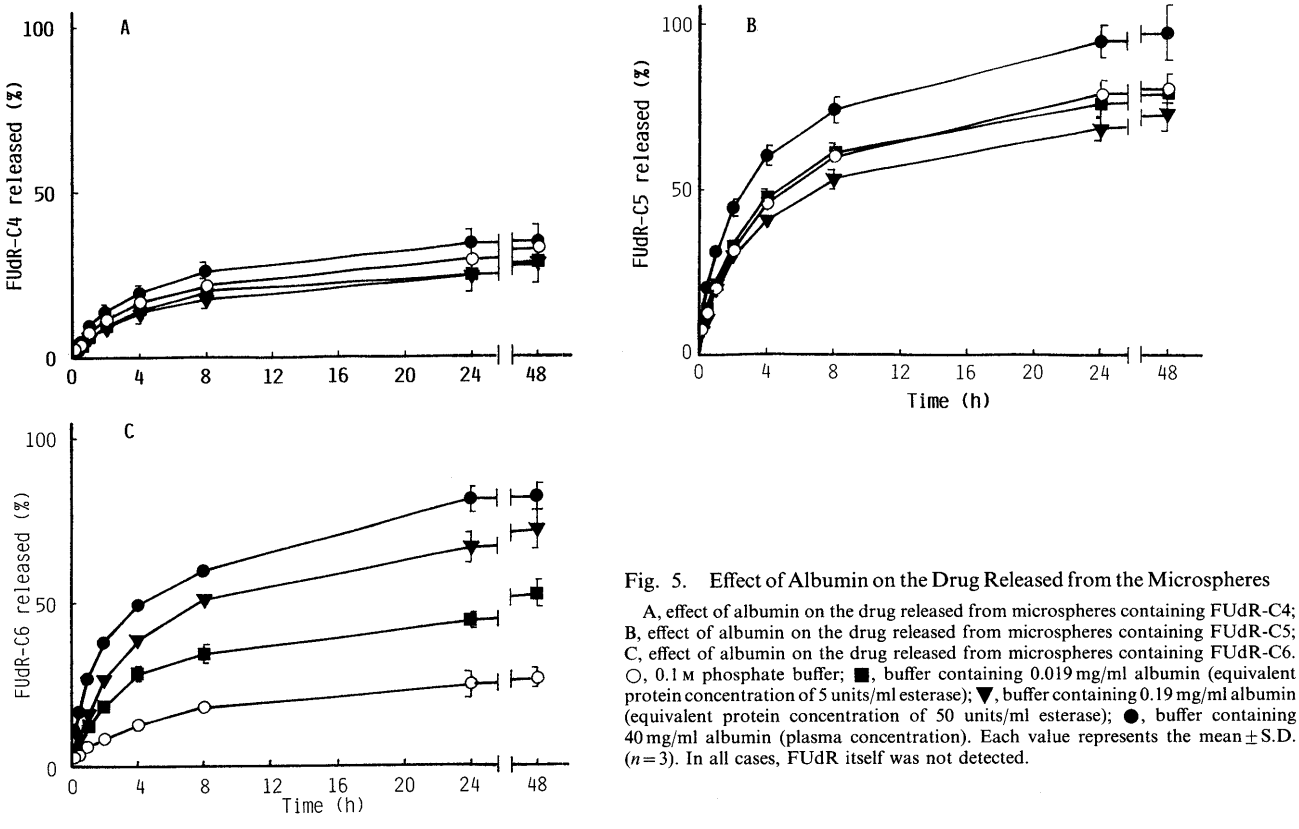

Fig. 5. Effect of Albumin on the Drug Released from the Microspheres

A, effect of albumin on the drug released from microspheres containing FUdR-C4; B, effect of albumin on the drug released from microspheres containing FUdR-C5; C, effect of albumin on the drug released from microspheres containing FUdR-C6. ○, 0.1 M phosphate buffer; ■, buffer containing 0.019 mg/ml albumin (equivalent protein concentration of 5 units/ml esterase); ▼, buffer containing 0.19 mg/ml albumin (equivalent protein concentration of 50 units/ml esterase); ●, buffer containing 40 mg/ml albumin (plasma concentration). Each value represents the mean ± S.D. ($n=3$). In all cases, FUdR itself was not detected.

C6 are shown in Fig. 3. Although the release study for microspheres containing FUdR-C8, FUdR-C10 or FUdR-C12 could not be carried out because of their very low solubility in saline, the release in the presence of esterase could be carried out because the lipophilic prodrugs were converted to hydrophilic FUdR by enzymatic hydrolysis, and the results are shown in Fig. 4. In the case of the microspheres containing FUdR-C6, it is evident that the greater the concentration of esterase in the release medium, the more rapidly the drug is released. On the other hand, the effect on drug release from microspheres containing FUdR-C5, FUdR-C8 and FUdR-C10 was smaller than those of FUdR-C6. The release enhancing effect of the esterase was not evident in microspheres containing FUdR-C4 and FUdR-C12. The enhancing effect of esterase on drug release from the microspheres containing diester-FUdR may be related to their reactivity to porcine liver esterase, because insoluble diester-FUdR would be converted to very soluble FUdR by the enzymatic hydrolysis during the drug release process from the microspheres. As reported previously,[15] the reactivity of diester-FUdR to porcine liver esterase increased as the acyl chains were lengthened up to dioctanoyl (-C8), but a further increase in the acyl chain length resulted in a sharp decrease in reactivity. Therefore, a larger enhancing effect by esterase could be observed in the highly reactive FUdR-C6 and FUdR-C8, and the effect of esterase on the drug release from microspheres was small in the less reactive FUdR-C10 and FUdR-C12. Although the reactivity of FUdR-C8 to porcine liver esterase was higher than that of FUdR-C6, the release of FUdR-C8 from the microspheres was slower than that of FUdR-C6 in the same concentration of esterase. This discrepancy may be partly attributable to the very low solubility of FUdR-C8.

The effect of albumin on the drug release from the microspheres is shown in Fig. 5. A similar effect to that of esterase on drug release was observed. In the case of microspheres containing FUdR-C6, it is evident that the greater the concentration of albumin in the release medium, the more rapidly was the drug released. The effect of albumin on the microspheres containing FUdR-C4 or FUdR-C5 was smaller than that for FUdR-C6. Since FUdR-C4 and FUdR-C5 show considerable intrinsic solubilities, the effect of albumin as a surfactant to increase apparent solubility of the esters can be limited. Under the same protein concentration (0.019 and 0.19 mg/ml), the effect of esterase on FUdR-C6 release from the microspheres (Fig. 3) was greater than that of albumin, which shows no esterase activity for the FUdR esters. No structural changes in the microspheres' surface were observed during the course of this study in the scope of SEM analysis (data not shown). These results suggest that the release rate of the drug, especially FUdR-C6 and FUdR-C8, depends on the concentration of esterase, albumin and other materials, and that

TABLE II. Antitumor Activity of Single Injection of FUdR and Its Diesters

Compd.	Dose[a] (mg/kg)	Weight change (0—5 d, g/mouse)	Survival time ($n=5$, d±SEM)	T/C (%)	Control[b]
FUdR-C3	250	+0.9	10.8±0.4	102	A
	50	+1.8	10.2±0.2	96	A
	25	+0.8	11.0±0.8	104	A
FUdR-C4	250	+0.4	11.2±0.4	115	B
	50	+1.4	10.2±0.2	105	B
	25	+1.7	10.6±0.2	106	C
FUdR-C5	250	+1.6	10.2±0.4	104	D
	50	+2.6	9.8±0.2	100	D
	25	+1.5	10.2±0.2	104	D
FUdR-C6	250	+1.1	11.0±1.0	113	B
	50	+2.9	10.2±0.2	105	B
	25	+1.9	9.6±0.2	104	E
FUdR-C8	250	−1.4	11.8±0.5	116	F
	50	−0.4	10.8±0.6	106	F
	25	−0.1	11.8±0.4	118	C
FUdR-C10	250	−3.2	11.2±1.3	106	A
	50	−1.8	13.8±0.7	130	A
	25	−1.2	13.4±0.5	126	A
FUdR-C12	250	−3.1	10.0±0.8	100	C
	50	−1.9	13.0±0.0	130	C
	25	−1.4	13.6±0.5	136	C
	5	+0.4	13.8±1.4	147	G
	2.5	+1.4	9.6±0.4	114	G
FUdR	250	+1.3	11.2±0.4	114	D
	50	+1.7	10.2±0.6	105	B

a) Dose of diester-FUdR mg/kg or FUdR mg/kg. *b*) Control groups were not treated. The survival time of control groups; A, 10.6±0.2; B, 9.7±0.2; C, 10.0±0.3; D, 9.8±0.2; E, 9.2±0.2; F, 10.2±0.5; G, 9.4±0.4. The weight changes of control groups were A = +1.7, B = +2.3, C = +2.5, D = +1.6, E = +2.4, F = +1.2 and G = +2.7.

TABLE III. Antitumor Activity of Five Injections (day 1, 2, 3, 4 and 5) of FUdR and Its Esters.

Compd.	Dose[a] (mg/kg/d)	Weight change (0—5 d, g/mouse)	Survival time ($n=5$, d±SEM)	T/C (%)	Control[b]
FUdR-C3	225	0	12.2±0.2	122	A
	75	−0.5	11.2±0.2	112	A
	22.5	+0.4	10.4±0.2	104	A
FUdR-C4	225	−6.7	12.4±2.3	123	B
	75	−2.7	13.2±1.6	131	B
	22.5	−2.0	12.6±0.4	125	B
FUdR-C5	750	−2.4	9.2±0.2	94	C
	225	−2.5	14.6±0.2	146	A
	75	−1.2	13.4±0.2	134	A
	22.5	−0.7	12.4±0.4	124	A
FUdR-C6	750	−1.5	10.0±0.5	102	C
	225	−1.5	13.6±0.9	135	B
	75	−1.5	12.2±0.9	121	B
	22.5	−0.6	12.0±1.3	119	B
FUdR-C8	750	−2.8	9.0±0.0	92	C
	225	−2.0	12.6±0.2	125	B
	75	−2.9	12.2±1.7	121	B
	22.5	−2.7	11.8±1.6	117	B
FUdR-C10	225	−2.6	11.8±0.2	118	A
	75	−2.9	13.4±1.0	134	A
	22.5	+0.4	14.4±0.5	144	A
	7.5	+0.8	12.3±0.9	131	D
	2.25	+1.2	10.5±0.9	112	D
FUdR-C12	225	−2.9	10.4±0.5	102	E
	75	−1.6	13.2±1.0	129	E
	22.5	−0.1	14.6±0.4	143	E
	7.5	−0.7	14.4±0.2	144	A
	2.25	+1.3	12.8±0.6	128	A
FUdR	750	−3.0	9.4±0.4	96	C
	225	−2.1	15.4±0.4	157	C
	75	−0.1	13.0±0.0	133	C
	22.5	+0.8	11.8±1.1	120	C

a) Dose of diester-FUdR mg/kg or FUdR mg/kg. *b*) Control groups were not treated. The survival time of control groups; A, 10.0±0.0; B, 10.1±0.2; C, 9.8±0.2; D, 9.4±0.4; E, 10.2±0.2. The weight changes of the control groups were A = +2.0, B = +2.9, C = +1.6, D = +2.7 and E = +2.3.

these are ubiquitous in the injection sites of microspheres. Thus, the release pattern of drugs from microspheres containing FUdR-C4, FUdR-C5, FUdR-C10 or FUdR-C12 may not undergo such a large change in the body.

***In Vivo* Antitumor Activity** The antitumor effects of FUdR or diester-FUdR were measured by a single injection of a saline solution or suspension to mice bearing P388 leukemia (Table II).

Diester-FUdR, with shorter alkyl chains (FUdR-C3—FUdR-C6), showed a poor effect ($T/C=96$—115%), and only an increase in the body weight was observed. In contrast, diester-FUdR with longer alkyl chains showed a significant effect (130 and 147% at 50 mg/kg FUdR-C10, 5 mg/kg FUdR-C12, respectively) compared to that of unesterified FUdR (114 and 105% at 250, 50 mg/kg, respectively).

Table III shows the antitumor effect of FUdR and diester-FUdR given five times at 1, 2, 3, 4 and 5 d after inoculation.

Higher effects have been observed compared to those by single injection, in the shorter esters, which were expected to show a short retention in the body.[17] These results reconfirm the fact that the cytotoxicity of FUdR is time-dependent or requires long retention *in vivo*.

The effects of a single injection of the microspheres containing diester-FUdR on the survival of mice bearing P388 leukemia are shown in Table IV.

TABLE IV. Antitumor Activity of Single Injection of Microspheres

Compd.	Dose[a] (mg/kg)	Weight change (0—5 d, g/mouse)	Survival time ($n=5$, d±SEM)	T/C (%)	Control[b]
FUdR-C3	24 (1500)[c]	−3.8	11.2±1.9	110	A
	8.0 (500)	−3.3	13.0±0.8	127	A
	2.4 (150)	−2.1	11.2±0.3	110	A
	0.80 (50)	−1.3	11.4±0.9	112	A
FUdR-C4	145 (1500)	−3.8	11.6±0.9	120	B
	49.0 (500)	−3.3	14.2±0.8	146	B
	14.5 (150)	−0.5	11.4±0.2	118	B
	4.90 (50)	+0.6	10.8±0.4	111	B
FUdR-C5	225 (1500)	−3.1	10.4±0.5	106	C
	75 (500)	−3.3	14.2±0.8	146	C
	22.5 (150)	−1.3	12.4±0.4	127	C
	7.5 (50)	−0.1	12.0±0.0	122	C
FUdR-C6	225 (1500)	−3.4	12.0±1.2	124	B
	75 (500)	−4.0	14.8±0.3	153	B
	22.5 (150)	−3.3	13.6±0.3	140	B
	7.5 (50)	−0.2	11.4±0.2	118	B
FUdR-C8	225 (1500)	−4.1	11.8±2.1	118	A
	75 (500)	−3.3	13.6±0.9	133	A
	22.5 (150)	−1.2	12.0±0.6	111	A
	7.5 (50)	+0.8	11.0±0.4	108	A
FUdR-C10	225 (1500)	−2.6	9.8±1.2	97	D
	75 (500)	−3.5	12.0±1.3	119	D
	22.5 (150)	−0.1	11.2±0.4	111	D
	7.5 (50)	−1.9	11.2±0.5	111	D
FUdR-C12	225 (1500)	−0.4	12.0±0.3	119	D
	75 (500)	+1.2	10.6±0.4	105	D
	22.5 (150)	+3.1	10.2±0.2	101	D
	7.5 (50)	+3.2	10.0±0.3	99	D
Spheres[d]	(900)	+1.8	9.2±0.2	94	C

a) Dose of content drug in the microspheres. *b*) Control groups were not treated. The survival time of control groups; A, 10.2±0.5; B, 9.7±0.2; C, 9.8±0.2; D, 10.1±0.2. The weight changes of the control groups were A = +1.2, B = +2.3, C = +1.6 and D = +2.9. *c*) In parentheses, dose of microspheres mg/kg. *d*) The microspheres did not contain the drug.

The optimal dose of microspheres was in the range of 8—75 mg/kg except for the FUdR-C12-sphere, and the microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 showed significant effects ($T/C>140\%$). The microspheres containing FUdR-C6 showed the maximal effect ($T/C=153\%$) at the dose of 75 mg/kg. The higher activity of microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 may be attributable to their moderate release rates of the drug from the microspheres (Fig. 1, 3 and 5). Although the suspension of free FUdR-C12 showed high activity, and its optimal dose was low (Tables II and III), the microspheres containing FUdR-C12 showed a poor effect. This may be because the release rate of FUdR-C12 from the microspheres is too small (Fig. 4), the microspheres at the highest dose, therefore, showed the highest effect as well as a decrease in body weight.

As compared to the single administration of the suspensions at about the same dose (Table II), the effect of microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 was evidently higher. The effects of microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 were at least equal to or greater than those of the drug suspensions given by consecutive injection (5 times). These results suggested that in spite of the single administration and low dose of drugs, microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 are more potent than the suspensions of free drugs.

In conclusion, the combination of chemical modification and polymeric carrier devices seems to be a promising means to control the *in vivo* fate and increase the therapeutic efficacy of anticancer agents. In order to accomplish this purpose, the prodrug should be designed to be efficiently incorporated into carriers and to supply the drug sufficiently at an adequate rate. In this study, diester-FUdR with saturated aliphatic acids were shown with various physicochemical properties, and the drug contents in L-PLA microspheres were improved by prolonging the alkyl chain length of acyl groups of diester-FUdR. The microspheres containing FUdR-C4, FUdR-C5 or FUdR-C6 showed higher antitumor activity, though the prodrug suspensions did not show the advantage of prodrug over the parent drug, compared to those containing FUdR-C10 or FUdR-C12, which showed high activity when administered as free drug suspensions.

In the future, the determination of release of FUdR from the microspheres *in vivo* and the kinetic behavior of the released FUdR are required to clarify the mechanism of the action. Since the microspheres also are more useful than an aqueous solution or suspension in targeting therapy, the microspheres containing diester-FUdR may be applicable to cancer chemotherapy.

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