Biochemical Pharmacology, Vol. 55, pp. 1091–1097, 1998.

ISSN 0006-2952/98/$19.00 + 0.00
PII S0006-2952(97)00682-5

Tumor Selective Delivery of 5-Fluorouracil by Capecitabine, a New Oral Fluoropyrimidine Carbamate, in Human Cancer Xenografts

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ABSTRACT. Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine) is a novel fluoropyrimidine carbamate that is converted to 5-fluorouracil (5-FUra) by three enzymes located in the liver and tumors; the final step is the conversion of 5′-deoxy-5-fluorouridine (5′-dFUrd) to 5-FUra by thymidine phosphorylase in tumors. The present study compared the efficacy of capecitabine and 5-FUra at their maximum tolerated doses in CXF280, HCT116, COLO205, and WiDr human colon cancer xenograft models, and measured subsequent 5-FUra and 5′-dFUrd levels in tumors and in the plasma and muscle. Capecitabine was effective in the first three models, whereas 5-FUra was effective only in CXF280, which is a cell line highly susceptible to fluoropyrimidines. In the three susceptible models, 5-FUra AUCs in tumors after capecitabine administration were 210 to 303 nmol · hr/g, whereas those after 5-FUra administration were 8.54 to 13.1 nmol · hr/g. In addition, capecitabine gave higher levels of 5-FUra AUC in tumors than in plasma (114- to 209-fold higher) and muscle (21.6-fold higher), whereas 5-FUra was not selectively distributed to tumors. In the refractory model, WiDr, 5-FUra AUC in tumors after capecitabine administration was only 62.8 nmol · hr/g, although the level of the intermediate metabolite 5′-dFUrd was high (AUC: 695 nmol · hr/g). The ratio of 5-FUra/5′-dFUrd levels in the WiDr tumors was 0.09, which was 23.8-fold lower than that in the HCT116 tumors. The mechanism of resistance would be the inefficient conversion of 5′-dFUrd to 5-FUra by thymidine phosphorylase in tumors. Thus, capecitabine might show its high efficacy as a result of delivering high levels of 5-FUra selectively to the tumors. BIOCHEM PHARMACOL **55**;7:1091–1097, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. capecitabine; 5-fluorouracil (5-FUra); pharmacokinetics; antitumor activity; prodrug; 5′-deoxy-5-fluorouridine (5′-dFUrd)

Cytotoxic anticancer drugs often cause severe side-effects because they do not act selectively in tumors. Many approaches have been investigated to improve tumor selectivity, including prodrug activation by enzymes located in tumor tissues. We designed capecitabine to generate the active drug 5-FUra† selectively in human tumors [1] through three sequential steps of enzyme reactions in humans: first, conversion of capecitabine to 5′-dFCyd by carboxylesterase, located in the liver; second, conversion of 5′-dFCyd to 5′-dFUrd by cytidine deaminase, located in high concentrations in the liver and solid tumors; and, finally, conversion of 5′-dFUrd to 5-FUra by dThdPase, also located in high concentrations in tumor tissues [1] (Fig. 1). Because these enzymes are so localized in the body, capecitabine is expected to generate 5-FUra selectively in tumor tissues and, consequently, to improve the efficacy and safety margins of 5-FUra, the parent drug.

In animal tumor models with human cancer xenografts, we have shown that capecitabine is indeed much safer and more effective than 5-FUra [1]. Therefore, it is of interest to investigate whether the potent antitumor activity of capecitabine results from the selective conversion of capecitabine to 5-FUra in tumors. In the present study, we measured 5-FUra levels in tumors and in the plasma and muscle after administering capecitabine to mice bearing four human cancer xenograft lines; three of these lines were susceptible *in vivo* and one line was refractory to the compound. We also measured tissue levels of the intermediate metabolite 5′-dFUrd in some of the human cancer xenograft models. These studies clearly showed that capecitabine selectively generated higher levels of 5-FUra in tumor tissues than did 5-FUra in mice bearing the three xenografts susceptible to capecitabine. Moreover, results from the line refractory to capecitabine indicated that inefficient conversion of 5′-dFUrd to 5-FUra in tumors is one possible mechanism of capecitabine resistance.

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† *Abbreviations:* 5-FUra, 5-fluorouracil; 5′-dFUrd, 5′-deoxy-5-fluorouridine; 5′-dFCyd, 5′-deoxy-5-fluorocytidine; dThdPase, thymidine phosphorylase; DPD, dihydropyrimidine dehydrogenase; and MTD, maximum tolerated dose.

Received 19 August 1997; accepted 20 November 1997.

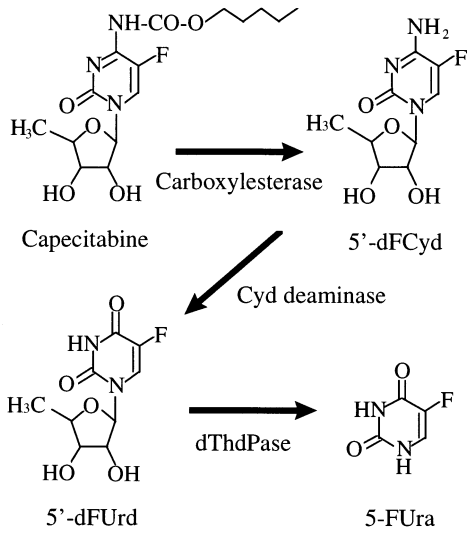

FIG. 1. Metabolic pathway of capecitabine. Cyd = cytidine.

MATERIALS AND METHODS

Animals

Male and female BALB/c nu/nu mice were obtained from SLC Inc. Following at least 1 week of observation, 6- to 7-week-old mice were used.

Tumors

The human cancer lines used were obtained from the following institutions: human colon cancer HCT116, COLO205, and WiDr, from the American Type Culture Collection, and human colon cancer CXF280 from Professor H. H. Fiebig, Freiburg University. CXF280 was maintained by continuous passages in BALB/c nu/nu mice. The other cancers were maintained in *in vitro* cultures with the following media containing 10% fetal bovine serum: McCoy's 5A for HCT116; RPMI 1640 for COLO205; and Eagle's Minimum Essential Medium for WiDr.

Human Cancer Xenograft Models

Mice were inoculated s.c. with small pieces of CXF280 xenograft tissues and a single-cell suspension (5.4 to 6 $\times$ 10^6 cells/mouse) of the other cancer cell lines. Capecitabine was dissolved or suspended in 40 mM of citrate buffer (pH 6.0) containing 5% gum arabic as the vehicle, while 5-FUra was dissolved in a saline solution. These fluoropyrimidines were administered daily for 5 or 7 days per week for 3 weeks (HCT116, CXF280, and WiDr) or 4 weeks (COLO205). To evaluate their antitumor effect, tumor size and body weight were measured twice a week. The tumor volume was estimated by using the following equation,

$$v = ab^2/2,$$

where a and b are tumor length and width, respectively. For pharmacokinetic studies, mice bearing the human cancer xenografts (mean tumor size; 748–848 mm^3) were administered the fluoropyrimidines in a single dose, either p.o. (capecitabine) or i.p. (5-FUra). Samples of blood, thigh muscle, and tumor tissue were collected from three mice at intervals of 0.25, 0.5, 1, 2, 4, 7, and 24 hr after the single administration of the test compounds. Sodium heparin (10 units/sample) was used as an anticoagulant for the blood samples. Plasma was prepared immediately by centrifuging blood at 5000 *g* for 5 min below 4°.

Measuring Metabolite Levels

To measure metabolite levels, tissue samples (approximately 0.5 g) were homogenized with a 5-fold volume of acetonitrile/50 mM ammonium acetate (3:1, v/v) in ice. The homogenates were added to stable isotope-labeled internal standards (1 μg of [^{13}C, $^{15}N_2$]-5′-dFUrd and 100 ng of [$^{15}N_2$]-5-FUra) in glass tubes, boiled at 100° for 2 min, and then centrifuged. The same amounts of stable isotope-labeled internal standards were added to plasma samples (200 μL each) in ice, mixed with 400 μL of acetonitrile, and then centrifuged. The supernatant thus obtained from the tissues and the plasma was dried under nitrogen gas, and the residue was dissolved in 250 μL of water. The solution was filtered through a disc (0.45-μm pore size) and injected into a liquid chromatography/ionspray ionization tandem mass spectrometry system (LC/MS/MS) using 20 and 60 μL for measuring 5′-dFUrd and 5-FUra levels, respectively. A Shimadzu model SIL/SCL 10A autosampler with a system controller was used for sample injection. 5′-dFUrd and 5-FUra were analyzed separately on a J'sphere ODS-M80 column (150 $\times$ 4.6 mm, inner diameter; YMC). The mobile phase (methanol/5 mM of ammonium formate, 1:1, 0.8 mL/min for determination of 5′-dFUrd and 15:85, 1 mL/min for 5-FUra) was delivered by a Shimadzu model LC-10AD pump. An eluate from the liquid chromatography was split, and approximately 1/50 of it was injected into MS/MS (Sciex API-IIIplus). Selected reaction-monitoring was used to detect the compounds (negative, 245/129 and 248/131 for 5′-dFUrd and its internal standard; negative, 129/42 and 131/43 for 5-FUra and its internal standard, respectively, at a dwell time of 200 ms). Argon was used as a collision gas with gas thickness of approximately 2.7 $\times$ 10^{15} molecules/cm^2 and a collision energy of 15 eV. The retention time of 5′-dFUrd and 5-FUra was 3.1 min.

Pharmacokinetic Data Analysis

The maximum plasma concentration (C_{max}) and the maximum time to reach the C_{max} (T_{max}) were determined from the observed concentration and time. The apparent elimination rate constant (k) was estimated by a linear regression on the logarithm of the plasma concentration versus time. The apparent half-life ($T_{1/2}$) was calculated from $ln(2)/k$. The area under the plasma concentration–time

TABLE 1. Maximum tolerated doses (MTDs) of two fluoropyrimidines

		Treatment		Dose*			MBWC†		
	Drug	Route	Schedule	mmol/kg/day	(mmol/kg/week)	Drug death	g	(%)	Comment
Expt. 1	Vehicle	p.o.	qd × 7/week			0/5	−0.3	(−1)	
	Capecitabine	p.o.	qd × 7/week	1.5	(10.5)	0/5	−3.5	(−14)	MTD
			qd × 7/week	3.0	(21)	1/5	−7.1	(−31)	Toxic‡
	Capecitabine	p.o.	qd × 5/week	2.1	(10.5)	0/5	−1.8	(−8)	MTD
			qd × 5/week	4.2	(21)	1/5	−5.7	(−24)	Toxic
Expt. 2	Vehicle	p.o.	qd × 7/week			0/5	0.3	(3)	
	5-FUra	p.o.	qd × 7/week	0.15	(1.05)	0/5	0.8	(4)	MTD
			qd × 7/week	0.3	(2.1)	5/5			Toxic
	5-FUra	p.o.	qd × 5/week	0.21	(1.05)	0/5	0.3	(1)	MTD
			qd × 5/week	0.42	(2.1)	5/5			Toxic
	5-FUra	i.p.	qd × 7/week	0.15	(1.05)	0/5	0.3	(1)	MTD
			qd × 7/week	0.3	(2.1)	5/5			Toxic

Mice bearing the human cancer xenograft HCT116 were treated for 3 weeks.
*One millimole of capecitabine and 5-FUra corresponds to 359 and 130 mg, respectively.
†MBWC = mean body weight change.
‡Toxicity based on body weight loss above 20%, which is considered excessively toxic.

curve until the last measurable concentration (AUC_t) was calculated using the linear trapezoidal rule. The AUC from time 0 to infinity (AUC_{inf}) was calculated by adding the ratio of C_t/k to the AUC_t, where C_t was the final measurable concentration.

Cytostatics

5-FUra and [$^{15}N_2$]-5-FUra were purchased from the Kyowa Hakko Co. and CDN Isotopes Inc., respectively. 5′-dFUrd (doxifluridine) and [^{13}C, $^{15}N_2$]-5′-dFUrd were obtained from F. Hoffmann-La Roche, Basle, Switzerland, and Hoffmann-La Roche, Nutley, NJ, U.S.A., respectively, while capecitabine was synthesized through a method described elsewhere [2].

Statistical Analysis

Tumor size was analyzed using the ANOVA test. Differences were considered significant when $P < 0.05$.

RESULTS

Antitumor Activity

The effectiveness of capecitabine (p.o.) and of 5-FUra (i.p. and p.o.) was compared in athymic mice bearing the human colon cancer xenograft lines HCT116, COLO205, CXF280, and WiDr. Among the four cancer lines, CXF280 was highly susceptible to the *in vitro* antiproliferative activity of 5-FUra with an inhibitory concentration (IC_{50}) of 36 nM, while HCT116, COLO205, and WiDr were moderately susceptible to 5-FUra with IC_{50} values of 1600, 189, and 1316 nM, respectively. The compounds were given for 3–4 weeks to the mice at their MTDs; the MTD in mice bearing the HCT116 cancer was determined to be 10.5 and 1.05 mmol/kg/week (daily × 5 or × 7/week) for capecitabine and 5-FUra, respectively (Table 1). As Table 2 shows, capecitabine was the most effective. Capecitabine inhibited tumor growth by more than 50% in the HCT116, COLO205, and CXF280 xenograft models, but was not effective in the WiDr model. In contrast, 5-FUra was effective in only the CXF280 model.

TABLE 2. Antitumor activity of two fluoropyrimidines at their MTDs against four human cancer xenografts

			Tumor volume change, mm³ (% tumor growth inhibition)							
			HCT116		CXF280		COLO205		WiDr	
Drug	Route	Dose (mmol/kg/week)	qd × 7/week	qd × 5/week	qd × 7/week	qd × 5/week	qd × 7/week	qd × 5/week	qd × 7/week	qd × 5/week
Vehicle	p.o.		1373	1188	1395	913	1413	1259	1373	887
Capecitabine	p.o.	10.5	191 (86)	−9 (101)	−25 (102)	36 (96)	320 (77)	495 (61)	745 (46)	740 (17)
5-FUra	i.p.	1.05	1153 (16)	ND*	228 (84)	ND	862 (39)	ND	907 (34)	714 (20)
5-FUra	p.o.	1.05	1056 (23)	728 (39)	ND	393 (57)	ND	813 (35)	927 (33)	ND

Drugs were administered qd × 5/week or × 7/week for 3 (HCT116, CXF280, and WiDr) or 4 (COLO205) weeks. Similar results were obtained in at least one additional experiment with the same protocol.
*ND, not done.

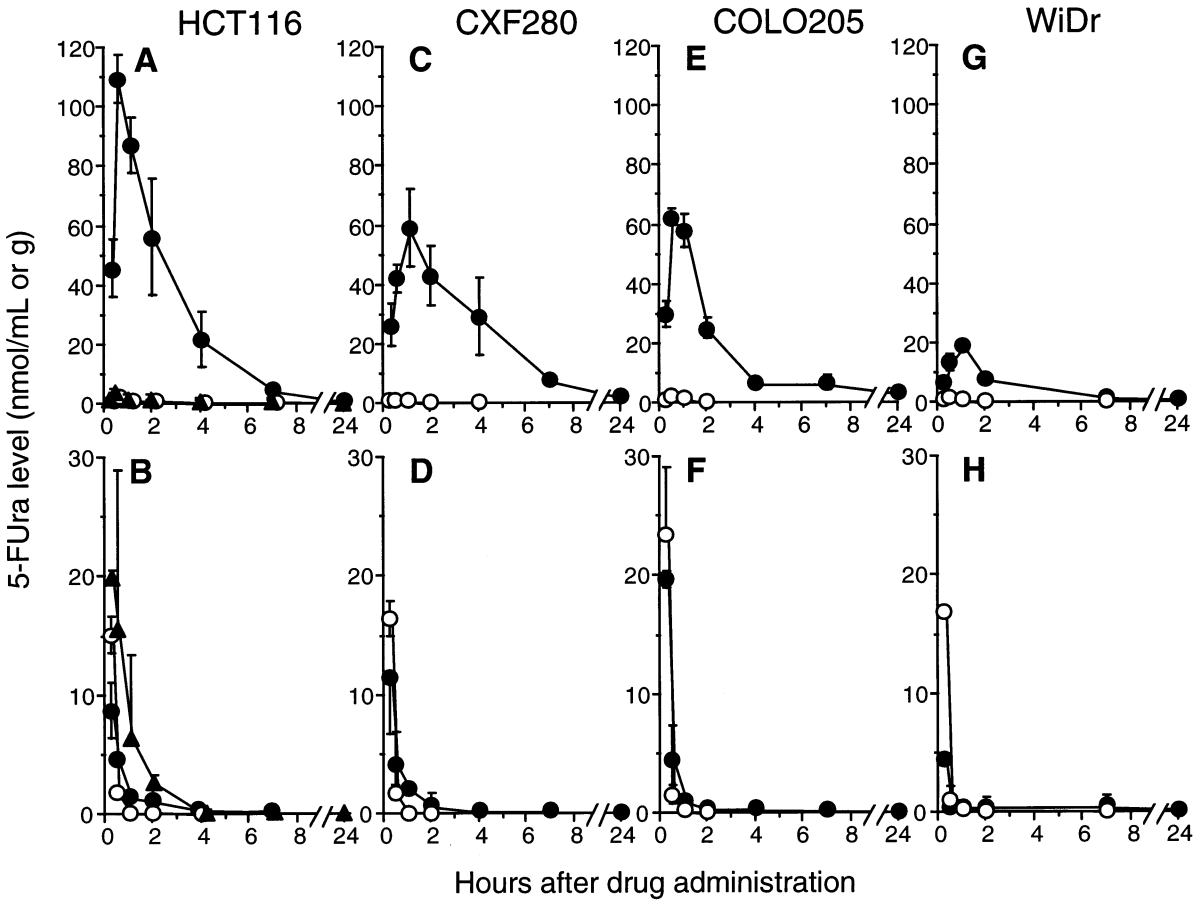

FIG. 2. Pharmacokinetic profiles of 5-FUra after the single administration of fluoropyrimidines at their MTDs to mice bearing human cancer xenografts. Capecitabine (1.5 mmol/kg, p.o.) (A, C, E, and G) and 5-FUra (0.15 mmol/kg, i.p.) (B, D, F, and H) were given to mice bearing human cancer HCT116 (A and B), CXF280 (C and D), COLO205 (E and F), and WiDr (G and H). Symbols: averages of 5-FUra concentrations in the plasma (○), the tumor (●), and the muscle (▲). Values are means ± SD, N = 3 per each time point.

5-FUra Levels

Panels A and B of Fig. 2 display 5-FUra levels in tumors, plasma, and muscle after a single administration of capecitabine (p.o.) and 5-FUra (i.p.) at their MTDs to mice bearing the HCT116 human cancer xenograft. Capecitabine (p.o.) produced levels of 5-FUra AUC in tumors that were 127 and 22 times higher than those in plasma and muscle, respectively. In contrast, 5-FUra itself generated similar levels of 5-FUra in tumor, plasma, and muscle, and so showed no tumor selectivity. The 5-FUra AUC in tumors after capecitabine administration (303 nmol · hr/g tissue) was much higher than that after 5-FUra administration (8.54 nmol · hr/g tissue) (Table 3).

Similar results were obtained in mice bearing the CXF280 (Fig. 2, C and D) and COLO205 (Fig. 2, E and F) cancer xenograft lines, which are also susceptible to capecitabine. However, in mice bearing the WiDr cancer xenograft line (Fig. 2, G and H), which is the line refractory to the two fluoropyrimidines, capecitabine produced 5-FUra AUC in tumors of only 62.8 nmol · hr/g tissue (Table 3); this concentration is 3.3 to 4.8 times lower than that observed in the susceptible tumor lines.

5′-dFUrd Levels

To illuminate the mechanism behind the resistance of the WiDr xenograft line to capecitabine and 5-FUra, we compared tumor levels of 5′-dFUrd in the WiDr tumor with those in the susceptible line, HCT116, after administering capecitabine (Table 4 and Fig. 3). 5′-dFUrd levels in the plasma differed only slightly in these two xenografts, whereas 5′-dFUrd levels in tumor tissues were quite different. In mice bearing the HCT116 xenograft, the levels of 5′-dFUrd in tumor were slightly lower than those of 5-FUra after administering capecitabine. In contrast, in mice bearing the WiDr xenograft, the levels of 5′-dFUrd in tumors were much higher (11.1-fold) than those of 5-FUra, indicating that the conversion of 5′-dFUrd to 5-FUra in tumors was not efficient.

DISCUSSION

We designed capecitabine to be selectively converted to 5-FUra in tumor tissues [1]. The present study demonstrated that capecitabine did indeed produce significantly higher levels of 5-FUra in tumors than in plasma or muscle in

TABLE 3. Pharmacokinetic parameters of 5-FUra in mice bearing human cancer xenografts after the single administration of two fluoropyrimidines

		Pharmacokinetic parameters of 5-FUra									
		Capecitabine administration					5-FUra administration				
Cancer xenograft	Tissue	C_{max}*	T_{max} (hr)	AUC_t†	AUC_{inf}†	$T_{1/2}$ (hr)	C_{max}	T_{max} (hr)	AUC_t	AUC_{inf}	$T_{1/2}$ (hr)
HCT116	Tumor	109	0.5	303	307	4.5	8.69	0.25	8.54	9.54	2.3
	Plasma	1.54	0.5	2.38	2.38	1.0	15.1	0.25	4.69	4.85	1.6
	Muscle	3.46	0.5	14.0	14.2	4.3	20.0	0.25	23.5	23.8	6.6
	Ratio$_{(Tumor/Plasma)}$‡			*127*	*129*				*1.8*	*2.0*	
	Ratio$_{(Tumor/Muscle)}$			*21.6*	*21.6*				*0.4*	*0.4*	
CXF280	Tumor	58.6	1	289	302	5.5	11.5	0.25	13.1	15.5	12.4
	Plasma	9.23	0.5	1.38	1.46	1.0	16.4	0.25	4.77	4.77	0.30
	Ratio$_{(Tumor/Plasma)}$			*209*	*207*				*2.7*	*3.2*	
COLO205	Tumor	61.8	0.5	210	284	17.4	19.7	0.25	12.9	15.9	15.1
	Plasma	1.84	0.5	1.85	2.08	0.54	23.5	0.25	6.77	6.85	0.40
	Ratio$_{(Tumor/Plasma)}$			*114*	*137*				*1.9*	*2.3*	
WiDr	Tumor	18.8	1	62.8	65.5	5.5	4.46	0.25	11.4	12.4	7.8
	Plasma	1.00	0.5	1.69	1.77	1.5	17.0	0.25	5.08	5.23	0.68
	Ratio$_{(Tumor/Plasma)}$			*37.1*	*37.0*				*2.2*	*2.4*	

Mice were administered with two fluoropyriniidines at their MTDs. Pharmacokinetic details of these data are in Fig. 2.

*C_{max}: nmol/mL plasma or g tissue.

†AUC: nmol · hr/mL plasma or g tissue.

‡Ratio of the AUC in the tumor to that in the plasma or muscle.

human cancer xenograft models. In contrast, 5-FUra itself produced similar levels of 5-FUra in tumor and muscle tissue and in plasma. In addition, 5-FUra levels in tumors after capecitabine administration were much higher than those after 5-FUra was administered at its MTD. In separate experiments, we observed that capecitabine was only slightly cytotoxic and its intermediate metabolites, 5′-dFCyd and 5′-dFUrd, themselves became cytotoxic only after conversion to 5-FUra. Therefore, we concluded that capecitabine should be much safer and more effective than 5-FUra. In fact, capecitabine was much more effective at a wider dose range than 5-FUra in the human colon cancer xenograft models used in the present study.

Presant *et al.* [3] reported that the retention of 5-FUra in tumors, which was measured by NMR, correlated with the efficacy of 5-FUra in patients. The present study indicated that the antitumor efficacy of capecitabine would correlate with the extent of its conversion to 5-FUra in tumors. Among the four human colon cancer xenograft models studied, capecitabine produced high levels of 5-FUra in tumor tissues of the three xenograft lines susceptible to this compound (CXF280, COLO205, and HCT116). In addition, capecitabine appeared to efficiently generate 5-FUra from the intermediate metabolite 5′-dFUrd by dThdPase in the HCT116 tumors, because the 5′-dFUrd level in the tumor was lower than the 5-FUra level. In contrast, 5-FUra levels in the WiDr xenograft (the line refractory to capecitabine) were several times lower than those in the three susceptible xenograft lines, while the level of 5′-dFUrd was several times higher than that in HCT116 tumors, indicating that 5′-dFUrd might not be efficiently converted to 5-FUra in the WiDr tumors. The inefficient conversion of 5′-dFUrd to 5-FUra by dThdPase in tumor tissues would be one mechanism of resistance to capecitabine. Since it has

TABLE 4. Pharmacokinetic parameters of 5′-dFUrd in mice bearing human cancer xenografts after the single administration of capecitabine

		Pharmacokinetic parameters of 5′-dFUrd				
Cancer xenograft	Tissue	C_{max}*	T_{max} (hr)	AUC_t†	AUC_{inf}†	$T_{1/2}$ (hr)
HCT116	Tumor	76.8	0.5	141	142	3.9
	Plasma	86.6	0.5	113	114	4.5
	Ratio$_{(Tumor/Plasma)}$			*1.2*	*1.2*	
WiDr	Tumor	219	1	695	699	3.6
	Plasma	72.0	0.25	181	186	5.3
	Ratio$_{(Tumor/Plasma)}$			*3.8*	*3.8*	

Mice were administered capecitabine at its MTD. Pharmacokinetic details of these data in tumors are in Fig. 3.

*C_{max}: nmol/mL plasma or g tissue.

†AUC: nmol · hr/mL plasma or g tissue.

‡Ratio of the AUC in the tumor to that in the plasma.

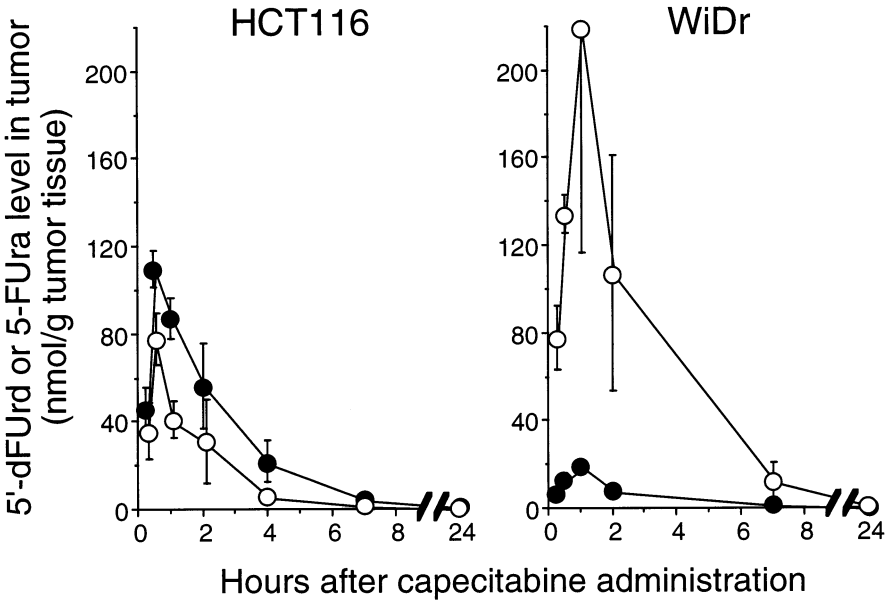

FIG. 3. Pharmacokinetic profiles of 5′-dFUrd and 5-FUra in tumors after the single administration of capecitabine to mice bearing human cancer xenografts. Capecitabine (1.5 mmol/kg, p.o.) was given to mice bearing human cancer HCT116 (left panel) and WiDr (right panel). Symbols: average concentrations of 5′-dFUrd (○) and 5-FUra (●) in tumors. Values are means ± SD, N = 3 per each time point.

been reported that dThdPase activity is higher in tumor tissues from patients than in normal tissues adjacent to the tumors [4, 5], the intermediate metabolite 5′-dFUrd would be efficiently converted to 5-FUra in tumors in patients.

Our separate studies with 24 human cancer xenograft models also indicated that 5-FUra levels in tumors are likely to correlate with the efficacy of capecitabine. We observed that the *in vivo* efficacy of capecitabine correlated well with the ratio of dThdPase to DPD levels in the tumor [6]. dThdPase is the enzyme that produces 5-FUra by phosphorolyzing the intermediate metabolite 5′-dFUrd [4, 5], whereas DPD is the enzyme catabolizing 5-FUra to the inactive molecule dihydrofluorouracil [7, 8]. We also observed that the ratio in the refractory lines, including WiDr, was low, while that of the susceptible lines, including HCT116, COLO205, and CXF280, was high. 5-FUra levels in tumors would be a main factor affecting the efficacy of capecitabine. It is therefore warranted that methods enhancing 5-FUra levels in tumors be investigated, including those increasing the ratio of dThdPase to DPD in tumors, for optimizing capecitabine therapies. In addition, such factors affecting 5-FUra levels would be determinants of sensitivity to capecitabine.

However, the levels of 5-FUra and the enzymes affecting it in tumors are not the only factors bearing on the efficacy of capecitabine. In the present study, 5-FUra itself gave very low levels of 5-FUra in tumors and did not show efficacy in the tumor models tested except for the CXF280 model, where 5-FUra was effective to some extent. Since CXF280 is highly susceptible to the antiproliferative activity of 5-FUra, such low levels of 5-FUra would be sufficient to inhibit the growth of this tumor. Although the mechanisms of the high susceptibility of this tumor line to 5-FUra have not been elucidated yet, 5-FUra might be efficiently anabolized to the active metabolites FdUMP and FUTP. This possibility is likely because deletion of, or diminished activity of, various enzymes that activate 5-FUra has been described in tumors resistant to 5-FUra, such as those of enzymes anabolizing 5-FUra to FUrd, 2′-dFUrd, FUMP, and FdUMP [9–11]. The levels of such enzymes and possibly those involved in the incorporation of 5-FUra into RNA and DNA would also be crucial for the efficacy of 5-FUra and, consequently, for that of capecitabine in some tumor lines.

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