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Original Paper

Design of a Novel Oral Fluoropyrimidine Carbamate, Capecitabine, which Generates 5-Fluorouracil Selectively in Tumours by Enzymes Concentrated in Human Liver and Cancer Tissue

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Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine) is a novel oral fluoropyrimidine carbamate, which is converted to 5-fluorouracil (5-FU) selectively in tumours through a cascade of three enzymes. The present study investigated tissue localisation of the three enzymes in humans, which was helpful for us to design the compound. Carboxylesterase was almost exclusively located in the liver and hepatoma, but not in other tumours and normal tissue adjacent to the tumours. Cytidine (Cyd) deaminase was located in high concentrations in the liver and various types of solid tumours. Finally, thymidine phosphorylase (dThdPase) was also more concentrated in various types of tumour tissues than in normal tissues. These unique tissue localisation patterns enabled us to design capecitabine. Oral capecitabine would pass intact through the intestinal tract, but would be converted first by carboxylesterase to 5′-deoxy-5-fluorocytidine (5′-dFCyd) in the liver, then by Cyd deaminase to 5′-deoxy-5-fluorouridine (5′-dFUrd) in the liver and tumour tissues and finally by dThdPase to 5-FU in tumours. In cultures of human cancer cell lines, the highest level of cytotoxicity was shown by 5-FU itself, followed by 5′-dFUrd. Capecitabine and 5′-dFCyd had weak cytotoxic activity only at high concentrations. The cytotoxicity of the intermediate metabolites 5′-dFCyd and 5′-dFUrd was suppressed by inhibitors of Cyd deaminase and dThdPase, respectively, indicating that these metabolites become effective only after their conversion to 5-FU. Capecitabine, which is finally converted to 5-FU by dThdPase in tumours, should be much safer and more effective than 5-FU, and this was indeed the case in the HCT116 human colon cancer and the MX-1 breast cancer xenograft models.

Key words: carboxylesterase, cytidine deaminase, thymidine phosphorylase, 5′-deoxy-5-fluorocytidine, 5′-deoxy-5-fluorouridine
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INTRODUCTION

MANY STRATEGIES for selectively delivering anticancer drugs to tumours have been reported. Specifically, prodrug activation by enzymes located in tumour tissues has been discussed. We have previously shown that 5′-deoxy-5-fluorouridine (5′-dFUrd) is not itself cytotoxic but becomes effective only after conversion to the active drug 5-fluorouracil (5-FU) by pyrimidine nucleoside phosphorylase (PyNPase), which is preferentially located in tumour tissues [1]. Since 5′-dFUrd produces higher levels of 5-FU in tumours than in normal counterparts [2], it has been found to be more effective than 5-FU and other fluoropyrimidines in various studies with mouse transplantable tumour models, particularly in terms of therapeutic indices [1, 3, 4]. 5′-dFUrd is being marketed in Japan, China and Korea (Furtulon®) for the treatment of breast, colorectal, gastric and other cancers, while it is being clinically assessed in the EU.

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The major drawback of cancer treatment with 5′-dFUrd, given orally, is its dose-limiting side-effect, diarrhoea [5]. When 5′-dFUrd passes through the intestinal mucosal membrane, 5′-dFUrd is thought to cause the intestinal toxicity of 5-FU generated in the intestine [6]. PyNPase exists predominantly as thymidine phosphorylase (dThdPase) in humans [7], while it is predominantly as uridine phosphorylase in rodents [8]. We, therefore, tried to identify a new fluoropyrimidine which could pass through the intestinal tract, by applying an approach of prodrug activation by additional enzymes. These studies created a novel fluoropyrimidine carbamate, capecitabine, which is sequentially converted to 5′-dFUrd by carboxylesterase and cytidine (Cyd) deaminase with unique tissue localisation in humans and then to 5-FU by dThdPase. In this report, we describe the tissue distribution of these enzymes for capecitabine activation. In addition, we describe that capecitabine and its intermediate metabolites were not themselves cytotoxic but became effective once they were converted to the active drug 5-FU.

MATERIALS AND METHODS

Animals and tissues

Male and female BALB/c nu/nu mice were obtained from CLEA Japan Co., Ltd, (Tokyo, Japan). Male BDF_1 mice were obtained from SLC Inc. (Hamamatsu, Japan). The mice were observed for at least 1 week and then used when 6 weeks old.

Chemicals

Capecitabine and 6-amino-5-chlorouracil (ACU, an inhibitor of dThdPase) were synthesised by methods described elsewhere [9, 10]. 5-FU and a combination drug of uracil and tegafur (UFT) were purchased from Kyowa Hakko K.K. and Taiho Pharma Co. (Tokyo, Japan), respectively, and 5′-dFUrd was obtained from Hoffmann-La Roche (Basle, Switzerland). 3, 4, 5, 6-tetrahydrouridine (THU) was purchased from Calbiochemical Co. (La Jolla, California, U.S.A.).

Preparation of crude enzymes

Human tissues surgically resected from cancer patients at various hospitals in Japan were obtained and stored at − 80°C until used. For the preparation of crude carboxylesterase, these tissues were homogenised with a glass homogeniser in 4 volumes of 10 mM potassium phosphate buffer (pH 7.4) containing 1 mM β-mercaptoethanol. The homogenate was centrifuged at 700 ***g*** for 20 min. The supernatant was dialysed overnight against the same buffer, kept at − 80°C and used as a source of crude enzymes. For the preparation of crude Cyd deaminase and dThdPase, these tissues were homogenised with a glass homogeniser in 4 volumes of 10 mM Tris–HCl buffer (pH 7.4) containing 15 mM NaCl, 1.5 mM $MgCl_2$ and 50 μM potassium phosphate. The homogenate was centrifuged at 105 000 ***g*** for 90 min. The supernatant was dialysed overnight at 4°C against 20 mM potassium phosphate buffer (pH 7.4) containing 1 mM β-mercaptoethanol, and used as a source of crude enzymes. All procedures were carried out at 4°C. The protein concentration was determined using the method of Lowry and colleagues [11].

Carboxylesterase assay

The assay mixture (100 μl) contained 10 mM potassium phosphate buffer (pH 7.4), 1 mM β-mercaptoethanol, 4 mM THU (an inhibitor of Cyd deaminase), enzyme preparations, and 5 mM substrates (50 μl). The substrates were prepared by diluting 100 mM capecitabine dissolved in dimethyl sulphoxide with 10 mM potassium phosphate buffer (pH 7.0) and 4% bovine serum albumin (Fraction V, Sigma Chemical Co., St. Louis, Missouri, U.S.A.). The reaction was carried out at 37°C for 60 min and terminated by adding 300 μl of methanol. The supernatant (200 μl) containing the product 5′-deoxy-5-fluorocytidine (5′-dFCyd) was mixed with 100 μl of 0.15 mM 5-chlorouracil as the internal standard and then treated with 3 ml of chloroform. After centrifugal removal of the precipitate (10 000 rpm, 3 min), 80 μl of the aqueous solution was added to 200 μl of 10 mM sodium phosphate buffer (pH 6.8) and then 20 μl were applied to an HPLC column of ERC-ODS-1171 (ERC Inc., Kawaguchi, Japan). The solvent system used was as follows: 10 mM sodium phosphate buffer (pH 6.8) containing 5 mM 1-decane sulphonic acid: methanol (85:15 v/v). The amounts of 5′-dFCyd were calculated from the ratio of the peak area to that of an internal standard as detected with an ultraviolet monitor (265 nm). The enzyme activity was expressed as nmole 5′-dFCyd generated/mg protein/h.

Cyd deaminase assay

Enzyme activity was determined by measuring 5′-dFUrd and 5-FU generated from 5′-dFCyd, an enzyme substrate. A reaction mixture (100 μl) for the enzyme activity contained 50 mM Tris-acetate buffer (pH 7.4), 2 mM 5′-dFCyd and crude enzyme. The reaction was carried out at 37°C for 60 min and then terminated by the addition of 300 μl of methanol. After removal of the precipitate by centrifugation (10 000 rpm, 3 min), an aliquot of the reaction mixture (80 μl) was added to 200 μl of 10 mM sodium phosphate buffer (pH 6.8) containing 50 μM 5-chlorouracil as the internal standard and then applied to an HPLC column (ERC-ODS-1171). The same solvent system mentioned above was used. The amount of 5′-dFUrd and 5-FU produced was measured using an ultraviolet monitor (265 nm). Cyd deaminase activity was expressed as nmole 5′-dFCyd deaminated/mg protein/min.

dThdPase assay

The assay mixture (120 μl) contained 183 mM potassium phosphate (pH 7.4), 10 mM 5′-dFUrd and the crude enzyme. The reaction was carried out at 37°C for 60 min and then terminated by the addition of 360 μl of methanol. After removal of the insoluble material, an aliquot of the reaction mixture (100 μl) was added to 400 μl of 10 mM sodium phosphate buffer (pH 6.8) containing 20 μM 5-chlorouracil as the internal standard. The mixture was then applied to an HPLC equipped with an ultraviolet detector operated at 280 nm. The HPLC column and the elution buffer were the same as those mentioned above. 5-FU was determined from the ratios of the peak area to that of the internal standard. dThdPase activity was expressed as μg 5-FU formed/mg protein/h.

Tumour cells

The human cancer lines used were obtained from the following institutions: colon cancer HCT116, COLO205 and DLD-1, breast cancer ZR-75-1, bladder cancer Scaber, cervix cancer SIHA and HT-3 from the American Type Culture Collection (Maryland, U.S.A.); gastric cancer MKN45 and MKN28 from Immunobiological laboratories (Fujioka,

Japan); breast cancer MCF-7 from Y. Iino (Gunma University, Maebashi, Japan); breast cancer MX-1 from T. Tashiro (Cancer Chemotherapy Centre, Japanese Foundation for Cancer Research, Tokyo, Japan); bladder cancer T24 from H. Akaza (Tsukuba University, Japan). MX-1 was maintained by continuous passage in BALB/c nu/nu mice. The other tumours were maintained in *in vitro* cultures with the following media: HCT116 and HT-3 with McCoy's 5A containing 10% fetal bovine serum (FBS); COLO205, DLD-1, ZR-75-1, MKN45, MKN28 and T24 with RPMI1640 containing 10% FBS; MCF-7, Scaber and SIHA with Eagle's MEM containing 10% FBS, non-essential amino acids and 1 mM sodium pyruvate.

Antiproliferative activity

A single-cell suspension of tumour cells ($1–5\times10^3$ cells/well) was added to the serially diluted fluoropyrimidines with or without 200 μM THU or 100 μM ACU in the wells of a flat-bottomed 96-well microtest plate. The cells in a final volume of 200 μl/well of media were then cultured at 37°C, 5% CO_2 for 3–7 days until the cell number in the control culture increased more than 10-fold. The degree of cell growth in a monolayer was measured using the MTT method (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), as described elsewhere [12]. The IC_{50} of the fluoropyrimidines was expressed as the concentration at which cell growth was inhibited by 50% as compared with the control.

Antitumour testing

A single-cell suspension of HCT116 (6×10^6) and small pieces of MX-1 were inoculated subcutaneously into nude mice. Test compounds were dissolved or suspended in 40 mM citrate buffer (pH 6.0) containing 5% gum arabic as the vehicle and administered orally by a stomach tube daily for 3 weeks starting when the tumour size reached 100–200 mm^3. The antitumour effect of the fluoropyrimidines was evaluated by measuring the tumour size twice a week. Tumour volume was estimated by using the following equation, $v = ab^2/2$, where a and b are tumour length and width, respectively. Gastrointestinal toxicity was estimated by observing the faeces from six or seven tumour-bearing mice receiving daily drug treatment. In addition, the extent of occult blood in the faeces was measured by using a test kit (Shionogi Pharma Co., Osaka, Japan). Finally, body weight was measured twice a week and leucocytes were counted 1 day after the final drug administration.

Statistics

Statistical analysis used the Mann–Whitney's U test and the ANOVA test. Differences were considered to be significant when the probability (P) value was < 0.05.

Results

Tissue distribution of enzyme for capecitabine activation

The tissue distributions of the enzymes that sequentially metabolise capecitabine to 5-FU in human tissue extracts are shown in Figures 1–3. We measured enzyme activity levels in various types of human tumour tissues from patients and normal tissues adjacent to the tumours. Figure 1 shows that carboxylesterase, the enzyme that metabolises capecitabine to 5′-dFCyd, was almost exclusively located in the human liver and hepatoma with little individual difference. In contrast, only minute activity was detected in other tumours and organs, including the intestinal tract. In a separate experiment, the

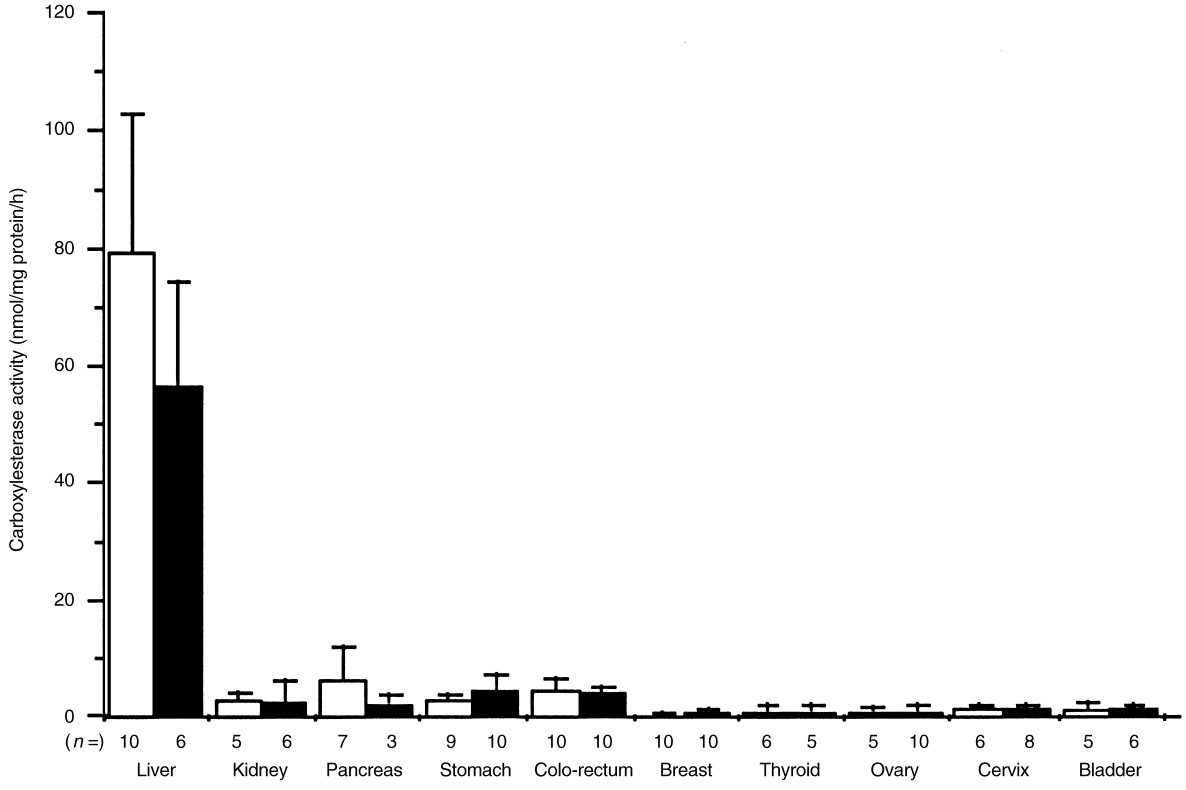

Figure 1. Tissue distribution of carboxylesterase in human normal and tumour tissues. Each column represents the mean ± standard deviation. Open bar, normal tissues; closed bar, tumour tissues.

enzyme activity was also not detected in plasma (0 versus 79 nmol/mg protein/h for the activity in the liver). Figure 2 shows the tissue distribution of Cyd deaminase, which metabolises 5′-dFCyd to 5′-dFUrd. Among normal tissues, the activity was higher in the liver than in other tumours and organs, including the gastrointestinal tract. Except in hepatoma, this activity in tumours was also higher than that of all corresponding normal tissues.

Figure 3 shows the tissue distribution pattern of dThdPase which metabolises 5′-dFUrd to 5-FU. High enzyme activity was detected in various types of tumour tissues. The activity was 3–10 times higher in all tumours than in tissues adjacent to the tumours, except for the liver, where the enzyme activity was higher than that in other normal tissues. These results suggest that capecitabine would be converted to 5′-dFCyd in the liver by carboxylesterase, to 5′-dFUrd in the liver and some tumour tissues by Cyd deaminase, and to 5-FU in various types of tumour tissues by dThdPase in humans (Figure 4).

Antiproliferative activity of capecitabine and its metabolites

Capecitabine and its metabolites were examined for their antiproliferative activity in human cancer cell lines (Table 1). 5-FU was highly cytotoxic, with IC_{50} ranging from 0.25 to 21 μM; the intermediate metabolite 5′-dFUrd was similarly cytotoxic, with IC_{50} ranging from 0.36 to 190 μM. In contrast, capecitabine and 5′-dFCyd, the first intermediate metabolite, were cytotoxic only at very high concentrations, with an IC_{50} higher than 1 mM in many cell lines. Since the activity of 5′-dFCyd was greatly reduced when the cells were cultured in the presence of the Cyd deaminase inhibitor THU (Table 2), it appears that 5′-dFCyd exerts its activity after conversion to 5′-dFUrd by Cyd deaminase, and subsequent metabolites. In contrast, the activity of 5′-dFUrd was

Table 1. *Antiproliferative activity of capecitabine and its metabolites*

Cancer cells	Culture period* (days)	IC_{50} (μM)			
		5-FU	5′-dFUrd	5′-dFCyd	Capecitabine
COLO205	5	3.1	127	> 1000	> 1000
HCT116	4	3.7	39	830	> 1000
DLD-1	4	7.6	190	> 1000	> 1000
MCF-7	4	13.0	91	> 1000	> 1000
ZR-75-1	7	0.25	0.36	207	ND
MKN45	4	3.3	38	174	994
MKN28	5	2.9	65	> 1000	> 1000
SIHA	4	7.5	67	92	578
HT-3	4	21.0	84	> 1000	> 1000
Scaber	3	0.72	3.7	9.3	97
T24	3	4.3	90	> 1000	ND

*Cells (5×10^3/well) were seeded into the wells of 96-well plates. 5-FU, 5-fluorouracil; 5′-dFUrd, 5′-deoxy-5-fluorouridine; 5′-dFCyd, 5′-deoxy-5-fluorocytidine; ND, not done.

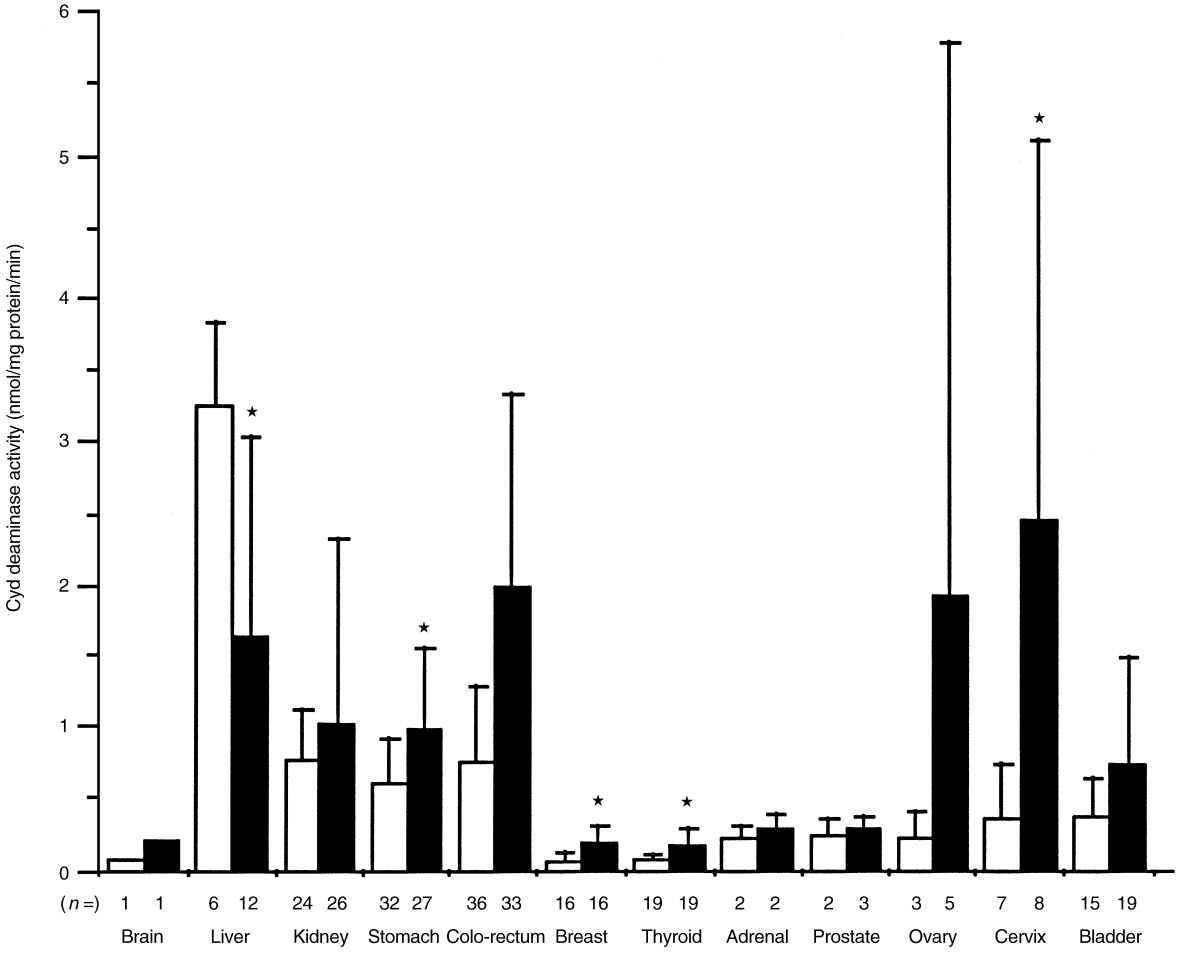

Figure 2. Tissue distribution of cytidine deaminase in human normal and tumour tissues. Each column represents the mean ± standard deviation. Open bar, normal tissues; closed bar, tumour tissues. *$P < 0.05$.

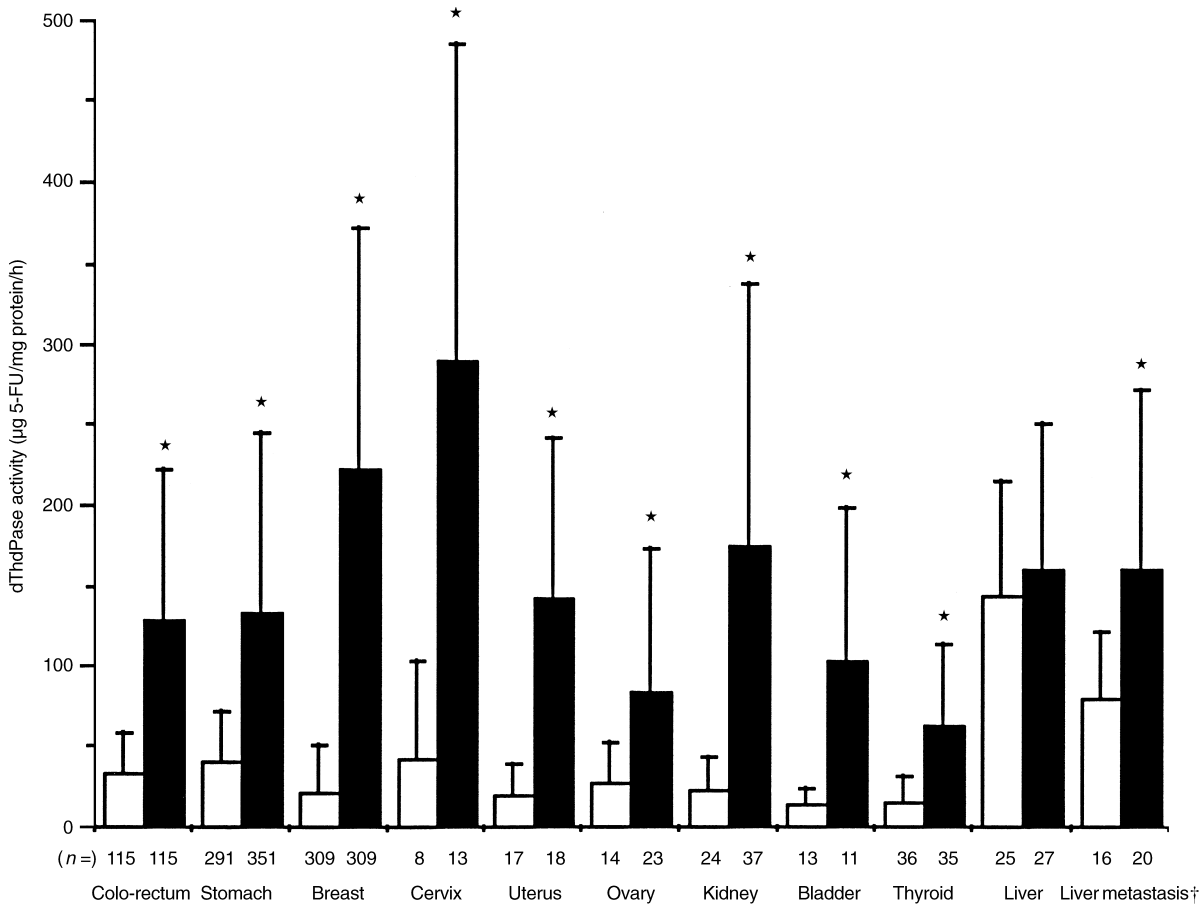

Figure 3. Tissue distribution of thymidine phosphorylase in human normal and tumour tissues. Each column represents the mean ± S.D. Open bar, normal tissues; closed bar, tumour tissues. *$P < 0.05$, †metastasis of colorectal cancer.

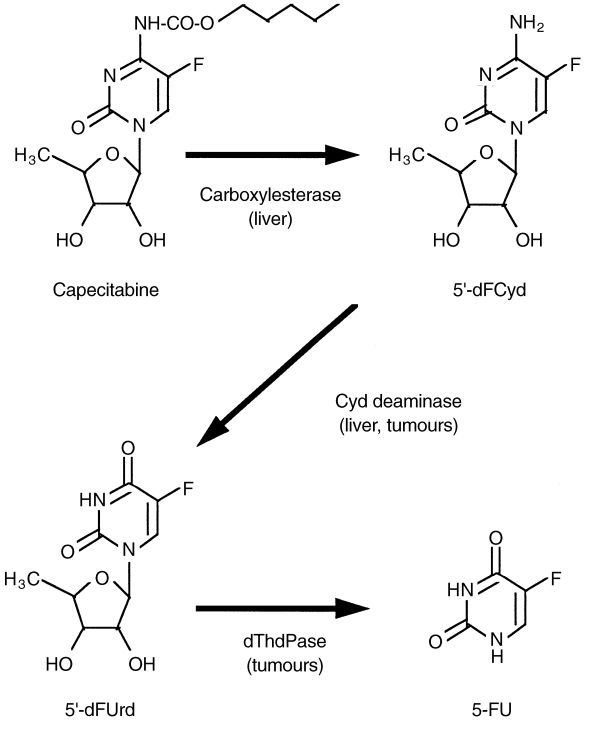

Figure 4. Metabolic pathway of capecitabine.

not changed. The dThdPase inhibitor, ACU, suppressed the activity of 5′-dFUrd, although the suppression was not complete (Table 2). The intermediate metabolites 5′-dFCyd and 5′-dFUrd would become effective only after conversion to 5-FU.

Antitumour testing

The antitumour activity of capecitabine and other fluoropyrimidines given orally were compared in the HCT116

Table 2. Effect of cytidine deaminase and thymidine phosphorylase inhibitors on the antiproliferative activity of capecitabine metabolites

	Scaber*			SIHA*			ZR-75-1*		
	IC_{50} (μM)			IC_{50} (μM)			IC_{50} (μM)		
Drug	–	THU†	Ratio	–	THU†	Ratio	–	ACU‡	Ratio
5′-dFCyd	54	> 1000	> 19	42	929	22	ND	ND	–
5′-dFUrd	14	19	1.4	9.7	13	1.3	1.5	40	27
5-FU	ND	ND	–	ND	ND	–	0.78	3.9	5.0

*Tumour cells (1×10^3 cells/well) were cultivated for 5 days (Scaber) or 8 days (SIHA, ZR-75-1). †Cytidine deaminase inhibitor (200 μM). ‡Thymidine phosphorylase inhibitor (100 μM). THU, 3,4,5,6-tetrahydrouridine; ACU, 6-amino-5-chlorouracil; 5′-dFCyd, 5′-deoxy-5-fluorocytidine; 5′-dFUrd, 5′-deoxy-5-fluorouridine; 5-FU, 5-fluorouracil; ND, not done.

Table 3. Antitumour activity of fluoropyrimidines in BALB/c nu/nu mice bearing HCT116 human colon carcinoma

		Antitumour effect		Toxic effect			
Drug†	Dose (mmol/kg)	Tumour vol. change (mm^3)	% growth inhibition	Survival on day 34	Body weight increase (g)	Faecal obs.‡	Occult blood‡
Vehicle	–	1700	–	12/12	1.5	N	–
5-FU	0.067	1663	2	6/6	1.2		
	0.1	1562	8	6/6	0.9		
	0.15	1365*	20	6/6	0.3		
	0.225	(681)§	(60)	4/6	(−3.7)	N	±
UFT	0.044	1794	−6	6/6	0.0		
	0.067	1312*	23	6/6	1.3	N	–
	0.1	1027*	40	6/6	0.4	N	–∼±
	0.15	–	–	0/6	–	(L_2)	(++)
5′-dFUrd	0.2	1165*	31	6/6	0.3		
	0.3	1280*	25	6/6	1.5		
	0.44	909*	47	6/6	−0.9	N	–∼±
	0.67	361*	79	6/6	−3.2	N∼L_1	+
	1.0	(71)	(96)	2/6	(−6.4)	L_1	+
	1.5	–	–	0/6	–	D	+++
Capecitabine	0.2	956*	44	5/5	0.0		
	0.3	964*	43	6/6	0.0		
	0.44	653*	62	6/6	−0.8		
	0.67	529*	69	6/6	−1.0		
	1.0	298*	82	6/6	−1.4		
	1.5	−44*	103	6/6	−3.1	N	–∼±
	2.25	(−173)	(110)	1/6	(4.8)	N	±

*$P < 0.05$. †Administered daily from day 13 to day 33 orally. ‡The parameters of gastroinestinal toxicity were observed on day 22. The degree of loose passage or diarrhoea of the faeces was estimated as follows: normal faeces (N), slightly loose passage (L_1), loose passage (L_2) and diarrhoea (D). §Data put in parentheses when survival rate is less than 80%. 5-FU, 5-fluorouracil; UFT, combination of uracil and tegafur; 5′-dFUrd, 5′-deoxy-5-fluorouridine.

Table 4. Antitumour activity of fluoropyrimidines in BALB/c nu/nu mice bearing MX-1 human mammary carcinoma

		Antitumour effect		Toxic effect			
Drug†	Dose (mmol/kg)	Tumour vol. change (mm^3)	% growth inhibition	Survival on day 33	Body weight increase (g)	Faecal obs.‡	Occult blood‡
Vehicle	–	5507	–	12/12	4.0	N	±
5-FU	0.067	5826	−6	6/6	5.2		
	0.1	4889	11	6/6	3.5		
	0.15	5291	4	6/6	2.9	N	+
	0.225	(1832)§	(67)	3/6	(2.9)	L_1	+
UFT	0.044	4584	17	6/6	3.5		
	0.067	5103	7	6/6	3.5	N	+
	0.1	3966*	28	6/6	3.8	N	±∼+
	0.15	–	–	0/6	–		
5′-dFUrd	0.3	3331*	40	6/6	2.2		
	0.44	2157*	61	6/6	2.3		
	0.67	1993*	64	6/6	2.7	N	+
	1.0	(898)	(84)	4/6	(2.5)	L_1	++
	1.5	(593)	(89)	1/6	(2.6)	(N)	(±)
Capecitabine	0.3	4431	20	6/6	3.5		
	0.44	3172*	42	6/6	3.4		
	0.67	2432*	56	6/6	2.6		
	1.0	2259*	59	6/6	1.8		
	1.5	1511*	73	6/6	1.7	N	±
	2.25	(1060)	(81)	1/6	(0.6)	N	+∼++
	3.38	–	–	0/6	–	(L2)	(++)

*$P < 0.05$. †Administered daily from day 12 to day 32 orally. ‡The parameters of gastrointestinal toxicity were observed on day 26. The degree of loose passage or diarrhoea of the faeces was estimated as follows: normal faeces (N), slightly loose passage (L_1), loose passage (L_2) and diarrhoea (D). §Data put in parentheses when survival rate is less than 80%. 5-FU, 5-fluorouracil; UFT, combination of uracil and tegafur; 5′-dFUrd, 5′-deoxy-5-fluorouridine.

Table 5. Therapeutic indices of fluoropyrimidines in BALB/c nu/nu mice bearing human cancer xenografts

	Therapeutic index			
	(TD*/ED_{50}†)		(TDi‡/ED_{50})	
Drug	HCT116	MX-1	HCT116	MX-1
5-FU	N.E.	N.E.	N.E.	N.E.
UFT	N.E.	N.E.	N.E.	N.E.
5′-dFUrd	2.3	2.6	1.5	1.8
Capecitabine	6.4	3.4	> 6.4	3.4

*Lethal toxic dose: toxicity based on more than 33% death of mice. †ED_{50} values were calculated from Tables 3 or 4. ‡Toxic dose-intestine: the minimum dose causing intestinal toxicity. 5-FU, 5-fluorouracil; UFT, combination of uracil and tegafur; 5′-dFUrd, 5′-deoxy-5-fluorouridine; N.E., not effective.

human colon cancer and MX-1 human mammary cancer xenografts in athymic mice. Capecitabine, which is designed to generate 5-FU selectively in tumours through non-cytotoxic intermediate metabolites, was more effective at a wider dose range than 5′-dFUrd, 5-FU and UFT in both xenograft models (Tables 3, 4). Capecitabine was obviously superior to the others when their therapeutic indices, the ratio of lethal toxic doses (TD) and the minimum doses causing intestinal toxicity (TDi) to ED_{50}, were compared (Table 5). In addition, capecitabine caused less intestinal toxicity, measured by occult blood test and faecal observation.

DISCUSSION

The present study showed the unique localisation of the enzymes, which would selectively convert capecitabine to 5-FU in human tumours. Carboxylesterase is concentrated in the liver; Cyd deaminase is located in the liver and various types of tumour tissues, while dThdPase is concentrated in tumour tissues. To take advantage of the unique localisation of these enzymes, we synthesised capecitabine to improve the efficacy and safety profiles of 5-FU and 5′-dFUrd [9]. When given orally, it was designed to pass intact through the intestinal tract without causing local toxicity and then sequentially convert to 5′-dFCyd in the liver by carboxylesterase, then to 5′-dFUrd mainly in the liver and tumours by Cyd deaminase and finally to 5-FU in tumours by dThdPase. In both animals and patients, capecitabine produced only the above metabolites in addition to those observed after 5-FU administration [13, 14]. This finding supports the metabolic pathway indicated for capecitabine in the present study.

In a separate experiment, we purified the enzyme that metabolises capecitabine to 5′-dFCyd from human liver extract. The sequence of the N-terminal 28 amino acids was 89% identical to that disclosed for human carboxylesterase (data not shown). We also observed that a commercially available rabbit carboxylesterase converted capecitabine to 5′-dFCyd and that human recombinant Cyd deaminase [15] converted 5′-dFCyd to 5′-dFUrd. We have previously observed that 5′-dFUrd is phosphorolised to 5-FU by human dThdPase [16], which is now known to be identical to PD-ECGF, a platelet-derived endothelial cell growth factor with angiogenic activity [17] and the levels of which are associated with the density of micro-vessels in tumour tissues [18, 19]. The enzymes mentioned above should be those essential for the conversion of capecitabine to 5-FU. The fact that inhibitors of Cyd deaminase and dThdPase suppressed the cytotoxicity of the intermediate metabolites 5′-dFCyd and 5′-dFUrd, respectively, suggests that these enzymes are indeed involved in the conversion to 5-FU.

The cytotoxicity study with the inhibitors of Cyd deaminase and dThdPase indicated that capecitabine and its intermediate metabolites, 5′-dFCyd and 5′-dFUrd, are not themselves cytotoxic and become active once they have been converted to 5-FU. These results indicate that capecitabine is delivered as inactive molecules to tumour tissues, where the active drug 5-FU is efficiently generated by dThdPase. If capecitabine is metabolised to 5-FU in patients as designed, it should be safe and tumour selective. In the HCT116 human colon and the MX-1 human breast cancer xenograft models, capecitabine was indeed safer and more effective than 5-FU and other fluoropyrimidines, although tissue distribution of Cyd deaminase is different in humans than in mice, where enzyme activity is high in the kidney followed by gastrointestinal tracts [20]. In extended efficacy studies, capecitabine was much more effective than 5-FU, 5′-dFUrd or UFT; capecitabine inhibited tumour growth by more than 90% in seven of 24 human cancer xenograft models, whereas 5′-dFUrd inhibited tumour growth by > 90% in one of 24 models [21]. The approach for drug discovery based on the localisation of enzymes for prodrug activation would be useful for identifying tumour selective anticancer drugs.

The three enzymes, carboxylesterase, Cyd deaminase and dThdPase, are essential for the efficacy of capecitabine. Among normal tissues, the liver has high levels of all these enzyme activities, so that capecitabine may cause liver toxicity. However, we have not yet observed any hepatotoxicity specific to capecitabine in toxicology studies in mice and monkeys (data not shown). Localisation of these enzymes in the liver may be different. In human cancer tissues, the activities of Cyd deaminase and dThdPase were variable among patients, suggesting that interpatient variation in the response to capecitabine treatment would be large. In human cancer xenografts, cancers with high dThdPase activity were susceptible to capecitabine treatment, whereas those with low dThdPase activity appeared to be less susceptible [21]. If this turns out to be the case in clinical trials, the efficacy of capecitabine could be predicted by measuring the enzyme activity in tumours before treatment starts. This approach of selecting patients should be pursued to optimise the efficacy of capecitabine.

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