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Preferential activation of capecitabine in tumor following oral administration to colorectal cancer patients

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Abstract *Purpose*: Capecitabine (Xeloda) is a novel fluoropyrimidine carbamate rationally designed to generate 5-fluorouracil (5-FU) preferentially in tumors. The purpose of this study was to demonstrate the preferential activation of capecitabine, after oral administration, in tumor in colorectal cancer patients, by the comparison of 5-FU concentrations in tumor tissues, healthy tissues and plasma. *Methods*: Nineteen patients requiring surgical resection of primary tumor and/or liver metastases received 1,255 mg/m^2 of capecitabine twice daily p.o. for 5–7 days prior to surgery. On the day of surgery, samples of tumor tissue, adjacent healthy tissue and blood samples were collected simultaneously from each patient, 2 to 12 h after the last dose of capecitabine had been administered. Concentrations of 5-FU in various tissues and plasma were determined by HPLC. The activities of the enzymes (CD, TP and DPD) involved in the formation and catabolism of 5-FU were measured in tissue homogenates, by catabolic assays. *Results*: The ratio of 5-FU concentrations in tumor to adjacent healthy tissue (T/H) was used as the primary marker for the preferential activation of capecitabine in tumor. In primary colorectal tumors, the concentration of 5-FU was on average 3.2 times higher than in adjacent healthy tissue ($P = 0.002$). The mean liver metastasis/healthy tissue 5-FU concentration ratio was 1.4 ($P = 0.49$, not statistically different). The mean tissue/plasma 5-FU concentration ratios exceeded 20 for colorectal tumor and ranged from 8 to 10 for other tissues. *Conclusions*: The results demonstrated the preferential activation of capecitabine to 5-FU in colorectal tumor, after oral administration to patients. This is explained to a great extent by the activity of TP in colorectal tumor tissue, (the enzyme responsible for the conversion of 5′-DFUR to 5-FU), which is approximately four times that in adjacent healthy tissue. In the liver, TP activity is approximately equal in metastatic and healthy tissue, which explains the lack of preferential activation of capecitabine in these tissues.

Key words Capecitabine · Pharmacokinetics · Preferential activation · Colorectal cancer · 5-FU

Abbreviations *ANOVA* Analysis of variance · *BLQ* Below limit of quantification of the analytical assay · *CD* cytidine deaminase · *5′-DFCR* 5′-Deoxy-5-fluorocytidine · *5′-DFUR* 5′-Deoxy-5-fluorouridine · *DPD* Dihydropyrimidine dehydrogenase · *EDTA* Ethylenediamine tetra-acetate · *FBAL* α-Fluoro-β-alanine · *FdUMP* Fluorodeoxyuridine-monophosphate · *5-FU* 5-Fluorouracil · FUH_2 Dihydro-5-fluorouracil · *FUPA* 5-Fluoro-ureido-propionic acid · *HPLC* High-performance liquid chromatography · *LC* Liquid chromatography · *MS* Mass spectroscopy · QA Quality assurance · *TP* Thymidine phosphorylase

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Introduction

Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine, XELODA) is a rationally designed, orally administered fluoropyrimidine carbamate. It is tumor-

activated and preferentially generates 5-FU within the tumor by exploiting differences in TP activity in tumor and normal tissues [1]. After oral administration capecitabine passes rapidly and extensively through the intestinal membrane as an intact molecule. After absorption, it is first transformed to 5′-DFCR by hepatic carboxylesterase and then to 5′-DFUR by CD, which is found in many human tumor tissues as well as in healthy liver tissue in high concentrations [1]. The 5′-DFUR is then converted to 5-FU by the tumor-associated angiogenic factor, TP [2] which is present in tumors at higher concentrations than in healthy tissues. This reduces the exposure of healthy tissues to 5-FU [3]. The 5-FU is catabolized by DPD to FUH_2, which is then metabolized to FUPA by dihydropyrimidinase and to FBAL by β-ureido-propionase [4]. Capecitabine itself is not cytotoxic, the only cytotoxic moiety being 5-FU, which is generated preferentially in human cancer cells.

Preferential activation of capecitabine to 5-FU in the malignant tumor was demonstrated in animal models bearing human xenografts [5]. Mice were given capecitabine orally and 5-FU intraperitoneally, both at the maximum tolerated dose (MTD). In the capecitabine group, 5-FU concentrations in tumor were much higher than in plasma (127 times) or in muscle (22 times), compared with the 5-FU group where there was no tumor preferential distribution. The area under the curve (AUC) of 5-FU in tumors was up to 35 times higher after administration of capecitabine than that after intraperitoneal administration of 5-FU. This evidence of preferential generation of 5-FU in the tumor was associated with improved antitumor efficacy.

In clinical phase 1 studies, capecitabine was administered every 12 h either continuously or intermittently (2 weeks of treatment, 1 week of rest). The MTDs were 1,657 mg/m^2/day for the continuous regimen and 3,000 mg/m^2/day for the intermittent treatment. Dose-limiting toxic effects were diarrhea, nausea, vomiting, stomatitis and hand-foot syndrome. The dose levels below the MTD (1,331 mg/m^2/day and 2,510 mg/m^2/day, continuous and intermittent, respectively) were safe and well tolerated [6]. The intermittent dose regimen was particularly effective, with tumor responses in both colorectal cancer and in extensively pretreated breast cancer patients [7, 8]. In combination with low-dose orally administered leucovorin, the MTD was 2,000 mg/m^2/day for the intermittent treatment with similar dose-limiting toxicities [9]. Additional phase 1 trials exploiting the combination with paclitaxel and docetaxel demonstrated the feasibility of using dose ranges of the active single agents [10, 11].

The primary objective of this study was to demonstrate the preferential generation of 5-FU in tumor by comparing 5-FU concentrations in tumor tissue, adjacent healthy tissue and plasma, following oral administration of capecitabine to colorectal cancer patients. The concentration of 5-FU was used as the primary parameter for the activation of capecitabine because 5-FU is the cytotoxic moiety and is the capecitabine metabolite of interest. A secondary objective was to confirm the activities of the enzymes involved in the formation (CD and TP) and in the catabolism (DPD) of 5-FU, in order to interpret the 5-FU concentrations.

Materials and methods

Patients

Nineteen patients with histologically confirmed colorectal cancer, undergoing surgery for resection of primary tumor, recurrent tumor or liver metastases, or for hepatic infusion therapy, were selected for this study. Previously treated patients were allowed to enter the study but any cytotoxic chemotherapy, hormonal treatment, immuno- or radiation therapy had to have been completed at least 4 weeks prior to the start of capecitabine treatment. Patients were hospitalized in the oncology units of three different centers: the Royal Infirmary in Aberdeen, (UK), Mount Vernon Hospital in Northwood, (UK) and Hospital Rudolfstiftung in Vienna, (Austria). The participants (6 females, 13 males) ranged in age from 50 to 74 years old (mean 64.4 years), weighed 42 to 120 kg (mean 74.5 kg) and their body surface ranged from 1.4 to 2.2 m^2 (mean 1.9 m^2). Karnofsky performance status ranged from 70 to 100% (median 90%).

All of the 19 enrolled patients were evaluable for safety; 18 patients were evaluable for pharmacokinetics and one patient had to be excluded because the tissue sample (liver metastasis) was necrotic.

Study design

This was an open label, multi-center, pharmacokinetic study which was conducted in full agreement with the principles of the Declaration of Helsinki III (as amended in Tokyo, Venice and Hong Kong). The study protocol was approved prior to commencement, by the Ethical Review Boards of the participating institutions. Written informed consent was obtained from each patient before the study was begun. Screening included physical examination, recording of vital signs, ECG, laboratory safety tests and evaluation of the Karnofsky performance status. Adverse events were recorded at baseline, throughout the study and at a follow-up examination, when vital signs, physical examination and laboratory tests were repeated. Carefully selected inclusion/exclusion criteria, taking into account the stage of the disease, current medical status and the life expectancy of the patients were applied, in order to obtain evaluable clinical and pharmacokinetic data.

Patients received 1,255 mg/m^2 of capecitabine orally every 12 h for 5 to 7-consecutive days prior to surgery. All doses but the last one prior to surgery were administered within 30 min of food intake. Surgery took place 2 to 12 h after the last capecitabine dose.

Tissue and blood sampling

During surgery, a tissue sample (minimum 0.8 g) of primary tumor and/or liver metastases was removed together with an equivalent sample of adjacent healthy tissue. The samples were cleaned of fat and other debris and quickly frozen, within 5 min of collection, and stored at −70 °C until analysis. Simultaneously with tissue removal, a blood sample of 10 ml was collected by vacutainers containing EDTA as anticoagulant. The specimens were centrifuged and the supernatant plasma removed and stored in plastic tubes at −20 °C until analysis.

Drug assay

Plasma concentrations of capecitabine, 5′-DFCR and 5′-DFUR were determined by HPLC with subsequent UV detection, and

5-FU, FUH_2 and FBAL were assayed by LC/MS-MS. Capecitabine and its metabolites were extracted from plasma samples following the addition of internal standards (Ro 09–1977, tegafur, [$^{15}N_2$]-5-FU, [^{13}C, $^{15}N_2$]-5-FUH_2, and β-Ala-Ala), and quantified as described previously [12, 13].

In tissue samples, concentrations of capecitabine and its five metabolites were determined by LC-MS/MS with an ion-spray interface. Tissue samples (approximately 0.5 g) were homogenized in a five-fold volume of a mixture of CH_3CN-50 mM ammonium acetate (3/1, v/v) at 0 °C. The homogenate (3 ml) was mixed with 50 µl of internal standard solution in which final concentrations of [^{13}C,$^{15}N_2$]-capecitabine, [^{13}C,$^{15}N_2$]-5′-DFCR, [^{13}C,$^{15}N_2$]-5′-DFUR, [$^{15}N_2$]-5-FU, [^{13}C,$^{15}N_2$]-FUH_2 and β-Ala-Ala were 20, 20, 20, 2, 40 and 50 µg/ml, respectively. The samples were centrifuged, after immersion in boiled water for 2 min, and the supernatant dried under nitrogen. The residue was dissolved in 250 µl of water and filtered through a disc (0.45 µm), and subsequently an aliquot (10–60 µl) was injected for analysis of capecitabine, 5′-DFCR, 5′-DFUR, 5-FU and FUH_2. Another 30 µl aliquot was mixed with 40 µl 0.1 M sodium carbonate and 10 mg/ml 2-methoxy-2, 4-diphenyl-3(2H)-furanone in 30 µl CH_3CN. The mixture was kept at 30 °C for approximately 10 min to convert FBAL and β-Ala-Ala to their diphenylfuranone derivatives. Aliquots of 20–30 µl were injected in LC-MS/MS for determination of FBAL. The limits of quantification were 0.05 µg/g tissue for FUH_2, 0.02 µg/g for capecitabine, 5′-DFUR and FBAL, 0.01 µg/g for 5′-DFCR and 0.002 µg/g for 5-FU. The overall mean recoveries of capecitabine, 5′-DFCR, 5′-DFUR, 5-FU, FUH_2 and FBAL from tissue were 57.9, 51.8, 54.9, 59.7, 64.0 and 33.2%, respectively. The corresponding overall inter-assay precisions for calibration standards and QA samples were 4.06, 2.69, 2.88, 4.04, 4.12 and 7.89%, respectively.

Enzyme activities

The activity of CD, TP and DPD was determined in tissue homogenates by modified routine catabolic assays, using 5′-DFUR, 5′-DFCR and [6-^{14}C]-5-FU as substrates [14, 15].

Statistical analysis

The primary statistical parameter was the logarithm of the 5-FU concentration ratios in tumor/healthy tissue. After logarithmic transformation of the individual concentrations, ANOVA, with the factors group, patient nested in group and tissue type, was performed to test whether the concentration of 5-FU in tumor tissue was higher than in healthy tissue, i.e. the geometric mean of the individual ratios was compared with 1.

Results

Concentrations of 5-FU in tissues and plasma

Individual concentrations of 5-FU in plasma, in colorectal tumor, healthy colorectal tissue, liver metastasis and healthy liver tissue are shown in Table 1. The plasma concentrations of 5-FU were similar to those previously reported after identical doses of capecitabine [5, 12, 13].

Colon/rectum

The concentration of 5-FU in colorectal tumors was higher than in the adjacent healthy mucosa in 10 out of 11 patients. The 5-FU concentrations as a function of time in both colorectal tissues are shown in Fig. 1.

Liver

Concentrations of 5-FU in liver metastasis and in healthy liver are presented in Table 1. The level of 5-FU was

Table 1 Individual concentrations of 5-FU in tumor, healthy tissue and plasma, following oral administration of capecitabine (1,255 mg/m^2 b.i.d.)

Patient No.	Time (h)[a]	Plasma (ng/ml)	Colon/rectum		Liver	
			Tumor tissue (ng/g)	Healthy tissue (ng/g)	Metastasis tissue (ng/g)	Healthy tissue (ng/g)
1	11.5	BLQ[b]	–	–	28.6	10.4
2	3.4	37.5	–	–	265	99.2
3	12.1	BLQ	45.8	13.7	–	–
4	6.2	5.5	127	16.2	–	–
6	4.5	3.3	198	85.3	–	–
7	3.4	78.8	1,345	418	–	–
8	5.8	10.7	–	–	252	151
9	4.6	NS[c]	1,868	233	–	–
10	5.3	BLQ	–	–	96.5	189
10	6.1	BLQ	93.1	32.4	–	–
11	3.3	29.3	–	–	290	207
12	2.2	143	–	–	1,504	1,076
13	5.1	6.4	–	–	108	63.9
14	5.7	BLQ	–	–	122	92.6
15	1.6	135	–	–	562	508
15	2.6	51.8	832	553	–	–
16	11.5	22.6	91	103	–	–
17	12.4	18.3	774	261	–	–
18	1.5	91.2	–	–	391	585
18	2.8	24.6	112	91.8	–	–
19	3.5	23.3	–	–	72.1	272
19	2.7	31.4	123	107	–	–
Mean 5-FU concentrations			510	174	336	296

[a] Time between last drug administration and plasma or tissue collection
[b] Below limit of quantification
[c] No sample

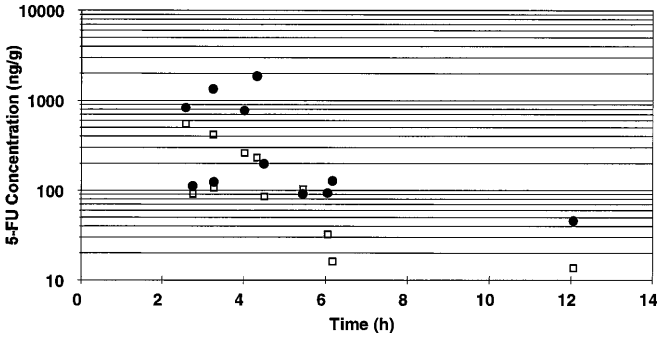

Fig. 1 Concentrations of 5-FU (ng/g) in primary colorectal tumor (●) and healthy colorectal tissue (□) after twice daily oral administration of 1,255 mg/m^2 of capecitabine. For each patient ($n = 11$) samples of tumor and healthy tissue were excised at approximately the same time. Surgery was performed 2 to 12 h after the last capecitabine dose

higher in liver metastasis than in the adjacent healthy tissue in 8 out of 11 patients.

The mean concentrations of 5-FU presented in Table 1 indicate that levels were very high (approximately 500 ng/ml) in colorectal tumor, and high in liver metastasis. On average, the 5-FU concentrations in liver metastasis were approximately twice as high than in healthy colorectal tissue, and reached approximately 70% of the amount in colorectal tumor tissue.

Ratio of 5-FU concentrations

5-FU concentration ratios for tumor, healthy tissue and plasma are shown in Table 2. The tumor/healthy tissue 5-FU concentration ratio was the primary marker for preferential activation of capecitabine in colorectal tumor and liver metastasis. Tumor tissue/plasma and healthy tissue/plasma 5-FU concentration ratios served as secondary markers of preferential activation of capecitabine.

The magnitude of the tumor/healthy tissue 5-FU ratios for colon/rectum and liver were independent of sampling time. Regression of the individual log-transformed ratios against time since last capecitabine dose showed P values of 0.217 for liver tissues and 0.337 for colorectal tissues.

The tumor/healthy tissue 5-FU ratios showed high inter-patient variability in both colon/rectum and liver (CV = 78% and 57%, respectively) (Table 2), which is not uncommon for concentrations of cytotoxic agents in tumors [16].

Colon/rectum

The mean of colorectal tumor/healthy tissue 5-FU concentration ratios was 3.21 (range = 0.88 to 8.02). The tumor/healthy tissue 5-FU ratios were similar in colon tissues (mean = 3.18, $n = 5$) and in rectal tissues (mean = 3.23, $n = 6$). Statistical comparison of the log-transformed individual 5-FU levels showed that the concentrations of 5-FU in colorectal tumors were greater than in adjacent healthy tissue ($P = 0.002$).

Liver

The mean liver metastasis/healthy tissue 5-FU concentration ratio was 1.41 (range = 0.26 to 2.67). Statistical comparison of log-transformed levels showed that those of 5-FU in liver metastasis were not different from those in adjacent healthy liver tissue ($P = 0.49$).

The tissue/plasma 5-FU concentration ratios provided further support for the preferential activation of capecitabine in colorectal tumors. The colorectal tumor tissue/plasma 5-FU level was 21.4. The three other tissue/plasma 5-FU ratios ranged from 8 to 10 (Table 2).

Concentration ratios of capecitabine, 5′-DFCR, 5′-DFUR, FUH_2 and FBAL

The tumor/healthy tissue, tumor/plasma and healthy tissue/plasma concentration ratios of capecitabine, 5′-DFCR, 5′-DFUR, FUH_2 and FBAL are shown in Table 3.

Colon/Rectum

The median colorectal tumor/healthy tissue concentration ratios for capecitabine and its non-5-FU metabolites ranged from 0.6 to 1.3, which is lower than the median 5-FU ratio for these tissues (2.9). The median colorectal tumor/plasma concentration ratios for capecitabine and the non-5-FU metabolites ranged from 0.2

Table 2 Concentration ratios of 5-FU in tumor, healthy tissues and plasma

Parameter	Colon/rectum			Liver		
	Tumor tissue to healthy tissue	Tumor tissue to plasma	Healthy tissue to plasma	Metastasis to healthy tissue	Metastasis to plasma	Healthy tissue to plasma
Mean	3.21	21.4	8.85	1.41	9.94	7.89
CV%	78	95	90	57	72	49
Median	2.87	16.6	4.94	1.40	8.48	7.30
Minimum	0.88	3.92	2.95	0.26	3.09	2.65
Maximum	8.02	59.9	25.9	2.67	23.6	14.1
n	11	8	8	11	8	8

Table 3 Median (range) concentration ratios of capecitabine and its metabolites in tumor, healthy tissues and plasma

Compound	Colon/rectum			Liver		
	Tumor tissue/ healthy tissue	Tumor tissue/plasma	Healthy tissue/plasma	Metastasis tissue/ healthy tissue	Metastasis tissue/plasma	Healthy tissue/plasma
Capecitabine	0.8 (0.2–6.0)	1.3 (0.7–2.0)	2.0 (0.8–9.2)	1.6 (0.8–6.9)	0.9 (0.4–2.2)	0.5 (0.3–0.6)
5′-DFCR	0.6 (0.01–1.3)	0.2 (0.01–1.0)	0.5 (0.3–1.4)	3.1 (2.0–6.1)	0.3 (0.07–0.7)	0.05 (0.04–0.1)
5′-DFUR	0.6 (0.4–1.1)	0.2 (0.2–0.5)	0.5 (0.2–1.2)	4.7 (1.9–8.9)	0.3 (0.2–0.8)	0.1 (0.05–0.2)
FUH_2	1.0 (0.8–1.6)	1.9 (0.6–4.6)	1.5 (0.6–3.7)	1.8 (0.7–9.3)	1.5 (0.4–4.7)	0.3 (0.2–2.2)
FBAL	1.3 (0.7–5.4)	1.7 (0.5–5.0)	1.0 (0.6–2.1)	0.4 (0.2–3.6)	2.3 (0.7–3.3)	3.4 (0.3–12)
5-FU	2.9 (0.9–8.0)	16.6 (3.9–60)	4.9 (3.0–26)	1.4 (0.3–2.7)	8.5 (3.1–24)	7.3 (2.7–14)

to 1.9 and the median colorectal healthy tissue/plasma ratios from 0.5 to 2.0. These are also lower than the respective values for 5-FU (colorectal tumor/plasma ratio = 16.6 and colorectal healthy tissue/plasma ratio = 4.9), and demonstrate that 5-FU is the only metabolite of capecitabine with a higher median concentration in colorectal tumors than in healthy tissue or plasma.

Liver

The median liver metastasis/healthy tissue concentration ratios for capecitabine and the non-5-FU metabolites were higher (1.6–4.7) than for 5-FU (1.4). Median liver tissue/plasma ratios for capecitabine and non-5-FU metabolites were similar to those for colorectal tissue/plasma, ranging from 0.3 to 2.3 for metastasis tissue/plasma and from 0.05 to 3.4 for healthy liver tissue/plasma. These are considerably lower than the respective median ratios for 5-FU (8.5 in metastasis and 7.3 in healthy liver), and identify 5-FU as the capecitabine metabolite with the highest liver tissue/plasma concentration ratio.

Activity of enzymes responsible for formation and catabolism

Cytidine deaminase

The enzyme CD is located principally in the liver and tumor tissues, and metabolizes 5′-DFCR to 5′-DFUR, the precursor of 5-FU. Its activity was two to three times greater in colorectal tumor tissue than in healthy colorectal tissue, but similar in liver metastasis and healthy liver tissue (median of ratios = 0.81). In general, activity of CD was considerably higher in liver tissue than in colorectal tissue (Table 4).

Thymidine phosphorylase

The enzyme TP is responsible for the biotransformation of 5′-DFUR to 5-FU, and is located predominantly in tumors and in the liver. The activity of TP in tumor and healthy tissues, and the tumor/healthy tissue activity ratios are shown in Table 4. The median colorectal tumor/healthy tissue TP activity ratio was 3.66. In contrast, TP activity was similar in liver metastasis and in healthy liver tissue (median activity ratio = 0.99).

Dihydropyrimidine dehydrogenase

The enzyme DPD mediates the metabolic degradation of 5-FU to FUH_2 in liver and tumors. The activity of DPD was similar in colorectal tumor tissue and in healthy colorectal tissue (median activity ratio = 1.39). Median DPD activity was approximately three times higher in liver metastasis and six times higher in healthy liver tissue than in colorectal tumors.

Table 4 Activity of cytidine deaminase, thymidine phosphorylase and dihydropyrimidine dehydrogenase in tumor and healthy tissues

	Cytidine deaminase (nmol/min/mg protein)			Thymidine phosphorylase (nmol/min/mg protein)			Dihydropyrimidine dehydrogenase (pmol/min/mg protein)		
	Median	Min	Max	Median	Min	Max	Median	Min	Max
Colon/rectum (n = 8)									
Tumor tissue	2.29	0.55	6.36	12.1	4.80	24.1	9.22	4.80	18.2
Healthy tissue	0.95	0.25	1.35	2.35	1.50	6.24	5.72	3.70	82.5
Ratio tumor tissue/healthy tissue	2.42	1.00	9.88	3.66	2.40	6.41	1.39	0.20	2.63
Liver (n = 8)									
Metastasis	4.92	0.39	15.5	11.0	4.87	17.9	30.5	5.66	73.1
Healthy tissue	5.03	3.72	8.15	10.6	5.17	16.0	59.4	3.87	98.1
Ratio metastasis/healthy tissue	0.81	0.08	3.32	0.99	0.71	3.46	0.81	0.09	8.07

Discussion

The primary objective of this study was to demonstrate the preferential activation of capecitabine in colorectal tumors. Following administration of capecitabine to colorectal cancer patients, 5-FU concentrations in the colorectal tumor were 3.2 times higher than in adjacent healthy tissue and 21 times higher than in plasma.

Previous studies in mice bearing human cancer xenografts were qualitatively predictive of the preferential activation of capecitabine shown in this study. However, the mouse studies showed higher 5-FU concentrations ratios in tumor compared with healthy tissue and plasma. The difference in the magnitudes of the 5-FU concentration ratios between animals and humans is probably due to different tissue distribution of 5-FU and to differing activity of thymidine and uridine phosphorylases in humans and in animals. Literature data indicate that uridine phosphorylase is the predominant enzyme in rodents for the phosphorolysis of 5′-DFUR to 5-FU, whereas in humans, the predominant enzyme is TP [22].

Preferential activation in the tumor in colorectal tissue was observed after administration of capecitabine, but not after administration of intravenous 5-FU to colorectal cancer patients. Kovach and Beart [17] examined 5-FU concentrations in colorectal tumor, healthy tissue and plasma of colorectal cancer patients following administration of 5-FU as an intravenous bolus (500 mg/m^2) and as an intravenous infusion (1,000 mg/m^2 over 24 h). From the data reported in their publication, the 5-FU concentration ratios for tumor/healthy tissue, tumor/plasma and healthy tissue/plasma were calculated to be near 1.0, indicating a lack of preferential distribution in the tumor following administration of 5-FU. These findings are confirmed by another study in colorectal cancer patients performed by Peters et al. [16]. Following administration of 5-FU as an intravenous bolus (500 mg/m^2), they determined the concentrations of 5-FU in tumor tissues and normal mucosa. Neither mean values of 5-FU concentrations in tissues, nor ratios for 5-FU concentrations were presented in that publication, but it was reported that "the average variation between 5-FU concentrations in mucosa and colon tumor did not significantly differ from zero". Therefore one can conclude that the tumor/healthy tissue 5-FU concentration ratio was close to one, and tumor preferential distribution following administration of 5-FU can thus be excluded.

Despite the lack of preferential activation of capecitabine in liver metastasis, the high concentrations of 5-FU in liver metastasis following capecitabine administration are expected to elicit the necessary antitumor activity in these cells. Clinical results have confirmed these expectations. In a clinical study of the effects of paclitaxel in 162 patients with refractory metastatic breast cancer, the overall response rate was 20%, and 12 responses were observed in liver metastases [18]. Although high concentrations of 5-FU were measured in normal liver tissue, they were not of clinical concern, because hepatic toxicity is not dose limiting following chronic administration of capecitabine to cancer patients.

The activity of the 5-FU-generating enzyme TP was four times higher in colorectal tumor tissue than in healthy colorectal tissue. This result provides the most likely explanation for the preferential activation of capecitabine in the colorectal tumor. Similar results had been reported previously by Peters et al. [19] and Nio et al. [20], who reported a three to five times higher TP activity in colorectal tumor tissue than in healthy mucosa. There may be a pathophysiological mechanism behind the high levels of TP in solid tumors. In addition to its role in nucleic acid metabolism, TP is also an angiogenic factor. Recent studies have shown that that TP is identical in amino acid sequence and activity to platelet-derived endothelial cell growth factor (PD-ECGF) [2, 21].

In contrast to that in colorectal tissue, TP activity in liver metastasis and healthy liver was similar, and this result might explain the lack of preferential activation of capecitabine in liver tissues. The qualitative and quantitative TP pattern of the liver is unique and is not representative of other tissues. For example, TP activity is higher in healthy liver than in many other healthy tissues (e.g., breast, stomach, colorectum, cervix and ovary) [20]. Therefore, lack of preferential activation of capecitabine in liver metastasis dose not preclude preferential activation in other tumors.

The CD activity was two to three times greater in colorectal tumors than in adjacent healthy tissue. This result indicated that the conversion of 5′-DFCR to 5′-DFUR also occurs preferentially in the tumor and therefore, the higher CD activity in tumor may contribute to the overall preferential activation of capecitabine in colorectal tumors.

There was no clear difference in DPD activity between colorectal tumor and healthy tissue. The DPD mediates the metabolic degradation of 5-FU to FUH_2. This finding suggests that there is no preferential degradation of 5-FU in tumor or healthy tissue, regardless of whether it is given intravenously as 5-FU or is generated after oral administration of capecitabine. The DPD activity in colorectal tumors was markedly lower than in liver metastasis and healthy liver tissue. This result might partially explain the lower metabolic clearance of 5-FU in colorectal tissue and thus the high 5-FU concentrations in colorectal tumors.

In conclusion, the results of this study demonstrated the preferential activation of capecitabine in colorectal tumors. The preferential activation of capecitabine can be explained to a great extent by the higher TP activity in the colorectal tumor compared with that in adjacent healthy tissue. Since TP converts 5′-DFUR into the active 5-FU, these results show that capecitabine is preferentially activated to 5-FU in the primary colorectal tumor.

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