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ORIGINAL ARTICLE

**G. J. Peters · P. Noordhuis · A. B. P. Van Kuilenburg
J. H. Schornagel · H. Gall · S. L. Turner · M. S. Swart
D. Voorn · A. H. Van Gennip · J. Wanders
U. Holwerda · K. Smid · G. Giaccone · P. Fumoleau
C. J. Van Groeningen**

Pharmacokinetics of S-1, an oral formulation of ftorafur, oxonic acid and 5-chloro-2,4-dihydroxypyridine (molar ratio 1:0.4:1) in patients with solid tumors

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Abstract S-1 is an oral formulation of ftorafur (FT), oxonic acid and 5-chloro-2,4-dihydroxypyridine (CDHP) at a molar ratio of 1:0.4:1. FT is a 5-fluorouracil (5-FU) prodrug, CDHP is a dihydropyrimidine dehydrogenase (DPD) inhibitor and oxonic acid is an inhibitor of 5-FU phosphoribosylation in the gastrointestinal mucosa and was included to prevent gastrointestinal toxicity. We determined the pharmacokinetics of S-1 in 28 patients at doses of 25, 35, 40 and 45 mg/m^2. The plasma C_{max} values of FT, 5-FU, oxonic acid and CDHP increased dose-dependently and after 1–2 h were in the ranges 5.8–13 μ*M*, 0.4–2.4 μ*M*, 0.026–1.337 μ*M*, and 1.1–3.6 μ*M*, respectively. Uracil levels, indicative of DPD inhibition, also increased dose-dependently from basal levels of 0.03–0.25 μ*M* to 3.6–9.4 μ*M* after 2–4 h, and 0.09–0.9 μ*M* was still present after 24 h. The pharmacokinetics of CDHP and uracil were linear over the dose range. The areas under the plasma concentration curves (AUC) for CDHP and uracil were in the ranges 418–1735 and 2281–8627 μmol·min/l, respectively. The $t_{1/2}$ values were in the ranges 213–692 and 216–354 min, respectively. Cumulative urinary excretion of FT was predominantly as 5-FU and was 2.2–11.9%; the urinary excretion of both fluoro-*β*-alanine and uracil was generally maximal between 6 and 18 h. During 28-day courses with twice-daily S-1 administration, 5-FU and uracil generally increased. Before each intake of S-1, 5-FU varied between 0.5 and 1 μ*M* and uracil was in the micromolar range (up to 7 μ*M*), indicating that effective DPD inhibition was maintained during the course. In a biopsy of an esophageal adenocarcinoma metastasis that had regressed, thymidylate synthase, the target of 5-FU, was inhibited 50%, but increased four- to tenfold after relapse in subsequent biopsies. In conclusion, oral S-1 administration resulted in prolonged exposure to micromolar 5-FU concentrations due to DPD inhibition, and the decrease in uracil levels after 6 h followed the pattern of CDHP and indicates reversible DPD inhibition.

Keywords Fluoropyrimidines · S-1 · Ftorafur · Oxonic acid · CDHP

Introduction

Fluoropyrimidines are still the mainstay of treatment of colorectal cancer. A number of different schedules are used [1]. Continuous infusions of 5-fluorouracil (5-FU) have a number of theoretical advantages, since 5 FU is a cell cycle-dependent drug, and with continuous exposure 5-FU would be active against all cells in a tumor during their various cycling times [2, 3, 4]. In addition, the target enzyme of 5-FU, thymidylate synthase (TS), can be inhibited continuously [5]. Continuous exposure to 5-FU in patients can be achieved by continuous infusions which have been shown to result in higher response

G. J. Peters (✉) · P. Noordhuis · H. Gall · D. Voorn
K. Smid · G. Giaccone · C. J. Van Groeningen
Department of Medical Oncology,
VU University Medical Center,
PO Box 7057, 1007 MB Amsterdam, The Netherlands
E-mail: gj.peters@vumc.nl
Tel.: +31-20-4442633
Fax: +31-20-4443844

A. B. P. Van Kuilenburg · A. H. Van Gennip
Laboratory of Genetic Metabolic Diseases,
Academic Medical Center, Amsterdam, The Netherlands

J. H. Schornagel · S. L. Turner · M. S. Swart
Department of Internal Medicine,
Netherlands Cancer Institute, Amsterdam, The Netherlands

J. Wanders
NDDO Oncology, Amsterdam, The Netherlands

U. Holwerda
Department of Clinical Chemistry,
VU University Medical Center, Amsterdam, The Netherlands

P. Fumoleau
EORTC—New Drug Development Group, Nantes, France

rates but usually no survival advantage [6]. Continuous exposure can also be achieved by frequent administration of oral 5-FU or a 5-FU prodrug. However, large variability in bioavailability for orally administered 5-FU, between 28% and 100%, has been observed [7, 8, 9], which may be related to saturable hepatic catabolism [10], but also to an additional first-pass effect. However, various oral formulations have been evaluated in patients. These are either based on a combination of 5-FU (or a prodrug) with a dihydropyrimidine dehydrogenase (DPD) inhibitor, or on a prodrug selectively activated in the tumor [11, 12]. The formulations with a DPD inhibitor include ftorafur (FT) with uracil at a molar ratio of 1:4 (UFT) [13], ftorafur with 5-chloro-2,4-dihydroxypyridine (CDHP) and oxonic acid (S-1) [14], and 5-FU with ethynyluracil [11, 15, 16]. Capecitabine is a prodrug of doxifluridine [17] which is activated by thymidine phosphorylase which has a higher activity in tumor tissues [17, 18, 19] resulting in higher tumor tissue concentrations. Plasma concentrations of these formulations vary depending on the drug combination and the frequency with which the drug is given and are generally comparable to those of continuous infusions [15, 20, 21, 22, 23, 24, 25]. Recently, it has been shown that patients prefer oral administration of a fluoropyrimidine when a similar efficacy is expected as for i.v. administration [24].

S-1 is a novel combination consisting of the 5-FU prodrug FT, the DPD inhibitor CDHP and the phosphoribosyl transferase inhibitor, oxonic acid [14]. FT (1,2-tetrahydrofuranyl-5-fluorouracil) acts as a depot form of 5-FU and produces little myelosuppression, but significant gastrointestinal toxicity and neurotoxicity. The drug is well absorbed orally in contrast to 5-FU itself [26, 27]. Conversion to 5-FU may occur predominantly in tumor cells and the liver [8] and is catalyzed predominantly by cytochrome P450 2A6 [28], although a role for thymidine phosphorylase has also been postulated [28].

DPD catalyzes the degradation of 5-FU, and is responsible for more than 80% of its elimination [10]. CDHP is a very potent inhibitor of DPD, 180-fold more potent than the natural substrate for DPD, uracil [29, 30], which competitively inhibits 5-FU degradation with an inhibition constant of 9 μM. Combining CDHP with 5-FU serves several purposes. First, CDHP is a potent reversible competitive inhibitor of DPD, preventing degradation of 5-FU [29] but it has no direct effect on 5-FU anabolism [31, 32]. Second, in combination with oral FT, CDHP enhances plasma 5-FU derived from FT. Coadministration of CDHP with FT also increases the 5-FU concentrations in the tumor five to ten times compared to FT alone or 5-FU itself [31]. After administration of S-1 to mice, plasma levels of 5-FU are substantially higher, with a 12- and 32-fold higher AUC for 5-FU derived from S-1 compared to that derived from UFT and FT, respectively [31]. The plasma C_{max} of 5-FU is about 15–30 μM after 1 h.

Oxonic acid is an inhibitor of orotate phosphoribosyl transferase. Oxonic acid accumulates specifically in normal gut [14, 32, 33] in which it inhibits the conversion of 5-FU to 5-fluorouridine 5′-monophosphate, a precursor of 5-fluoro-2′-deoxyuridine-5′-monophosphate (FdUMP), the active metabolite of 5-FU, which inhibits the target enzyme TS [1]. Since oxonic acid does not accumulate in the tumor [14], it has a selective protective effect against gastrointestinal toxicity, but does not affect antitumor activity. Oxonic acid does not affect the pharmacokinetics of 5-FU [22]. We describe here the pharmacokinetics of the constituents of S-1 (FT, CDHP and oxonic acid) and their effects on normal pyrimidine metabolism as determined by evaluation of the concentration of uracil, the natural substrate for DPD. In addition we evaluated the effects on DPD levels as well as urinary excretion.

Patients and methods

Patients and drug administration

A total of 28 patients (15 males/13 females; 35–67 years old; median performance status 1, range 0–2) were entered into this pharmacokinetic study after providing informed consent. As prior therapy, ten patients had received chemotherapy, nine chemotherapy and radiotherapy and eight no therapy. In 12 of these patients chemotherapy included 5-FU. The major tumor types included: seven colorectal, four stomach, three lung, two kidney, two breast, three esophageal and several miscellaneous tumor types. Six patients were treated consecutively with 25 mg/m^2 S-1, six patients with 45 mg/m^2, five with 35 mg/m^2 and 11 with 40 mg/m^2. Doses were based on the amount of FT in the capsule. More details on the patient characteristics have been presented previously [23].

The patients received S-1 within 1 h of breakfast in capsules containing FT, CDHP and oxonic acid at a molar ratio of 1:0.4:1; each capsule contained 20 or 25 mg FT. For the initial pharmacokinetic study patients received S-1 once. Blood and urine were sampled over 24 h. Based on previous studies with oral 5-FU formulations, we chose to sample plasma before and at 30 min, and 1, 2, 4, 8 and 24 h after S-1 intake. Blood samples were taken by venepuncture into 9-ml heparinized tubes and transported on ice for immediate processing. Urine was collected in 6-h fractions after S-1 administration and stored at −20°C as soon as possible.

After 1 week, treatment with S-1 was initiated. One course of treatment consisted of the administration of S-1 twice daily 1 h after breakfast and dinner over a period of 28 days followed by 1 week of rest. Treatment was discontinued if disease progression or excessive toxicity occurred. Blood samples were taken every week during the first course and in the first week of subsequent courses. Two blood samples were taken by venepuncture, one before and one 2 h after S-1 administration. Blood samples were centrifuged for 5 min at 4000 rpm and 4°C. The plasma was transferred to two 2-ml Eppendorf vials and stored at −20°C. The buffy coat was diluted with phosphate-buffered saline and the white blood cells (WBC) were isolated using standard Ficoll centrifugation. After isolation and washing the WBC were counted, immediately frozen in liquid nitrogen and stored at −80°C until DPD analysis. Before freezing of the WBC, cytospins were prepared for determination of the percentage of granulocytes.

Analysis of S-1 in plasma and urine

FT, 5-FU, oxonic acid, CDHP, uracil, fluoro-β-alanine (F-β-Ala) and their internal standards β-hydroxyethyltheophyline (HOEtTP), ($^{15}N_2$)-5-FU, (^{13}C,^{15}N)-oxonic acid ($^{18}O_2$)-CDHP and ($^{15}N_2$)-uracil were supplied by TAIHO Pharmaceuticals (Tokyo, Japan). All other chemicals were of analytical grade. Water for preparation of buffers, and stock and working solutions was purified with a Millipore Q water purification system, referred to as milli-Q water. All stock solutions were prepared at a concentration of 1 m*M* in milli-Q water except for FT and HOEtTP, which were prepared in methanol. All solutions were stored in small aliquots at −20°C and thawed prior to use. The standard samples were prepared in pooled plasma from healthy volunteers, except for uracil, which was prepared in water.

The extraction of FT, oxonic acid, CDHP and uracil from plasma and urine was performed as described previously [23, 34]. After extraction the samples were stored at −20°C until analysis. F-β-Ala concentrations were determined as described previously [35].

FT extraction

To 250 µl plasma or diluted urine (1:10, v/v) were added 100 µl internal standard solution and 100 µl 0.2 *M* KH_2PO_4, pH 7.0, in a polypropylene tube and mixed. Subsequently 5 ml dichloromethane was added, vigorously mixed for 10 min and the mixture centrifuged for 10 min at 4000 rpm. The upper (water) layer was transferred to another tube, 5 ml dichloromethane was added, vigorously mixed for 10 min and the mixture centrifuged for 10 min at 4000 rpm. The upper (water) layer was transferred to another tube for 5-FU, CDHP and uracil extraction (see below). The two dichloromethane fractions were pooled (without interphase) and dried under nitrogen at 40°C. The residue was resuspended in 200 µl HPLC buffer, sonicated for 15 min, transferred to an Eppendorf tube and the suspension centrifuged 5 min at 12,000 rpm at 4°C. Insoluble material from the supernatant was removed by filtration with a Millipore-HV 0.45-µm filter in an Eppendorf tube and stored at −20°C until HPLC analysis.

FT was analyzed using an HPLC system consisting of a Gynkotek pump (M300; Dionex, Breda, The Netherlands) connected to an autosampler (Triatlon; Spark Holland, Emmen, The Netherlands) and a photodiode array detector (1000S; Separations Analytical Instruments, H.I. Ambacht, The Netherlands). The samples were analyzed by injection of 40 µl sample onto an ODS-2 column (150×4.6 mm ID, 5 µm particle size; Chrompack, Middelburg, The Netherlands) which was eluted isocratically with 10 m*M* KH_2PO_4 and 15% MeOH, pH 5.5, at a flow rate of 1 ml/min as described previously [34]. Absorbance was measured at 270 nm.

5-FU, CDHP and uracil extraction

For the 5-FU, CDHP and uracil extraction, the water layer from the FT extraction was taken, mixed with 0.4 ml 1 *M* KH_2PO_4 and subsequently vigorously mixed with 4 ml ethylacetate for 10 min and the mixture centrifuged for 5 min at 4000 rpm. The ethylacetate (upper) layer was transferred to another tube. The water layer was extracted again with 4 ml ethylacetate for 10 min and centrifuged for 5 min at 4000 rpm. The ethylacetate fractions were combined and dried under nitrogen at 40°C.

For derivatization of 5-FU, CDHP and uracil, the residue from the 5-FU, CDHP and uracil extraction was dissolved in 30 µl acetonitrile, and 10 µl triethylamine and 10 µl penta-fluoro-benzylbromide were added, mixed and the mixture kept at room temperature for 30 min. Subsequently, 50 µl ethylacetate and 500 µl hexane were added, mixed vigorously for 5 min and the mixture centrifuged for 5 min at 4000 rpm. The ethylacetate/*n*-hexane (upper) layer was transferred to an Eppendorf tube and dried under nitrogen at 40°C. The residue was reconstituted in 50 µl ethylacetate and 1 µl was analyzed by GC-MS in the electron capture negative chemical ionization mode. The GC-MS system consisted of a HP 5750 gas chromatograph, a VG30-250 mass spectrometer (VG Instruments) and a DB5MS column (15 m, 0.32 mm ID, 0.25 µm film thickness; J&W Scientific, Bester, Amstelveen, The Netherlands). The concentrations of 5-FU in tissues were assayed as described previously [36].

Oxonic acid

For extraction of oxonic acid, 250 µl plasma or diluted urine (1:10, v/v) was pipetted into a polypropylene tube and 50 µl internal standard solution and 500 µl 10 m*M* Tris, pH 7.4, were added and mixed. The samples were extracted using solid-phase columns. For that purpose the Bond-Elut NH_2 column was washed at g-force with 1 ml methanol and 1 ml milli-Q water and subsequently with 1 ml 10 m*M* Tris, pH 7.4. The samples were then loaded on the column, eluted and washed with a water aspirator with 4 ml 10 m*M* Tris, pH 7.4, over about 15 min ($\ll$2 psi). Subsequently the oxonic acid was eluted with a water aspirator with 1 ml 0.2 *M* Na_2CO_3, pH 10.0, over about 4 min ($\ll$2 psi). To the eluent was added 250 µl 1 *M* HCl and the resulting mixture incubated at 60°C for 1 h to decarboxylate the oxonic acid. The samples were dried under nitrogen at 60°C [34].

For derivatization the complete residue was suspended in 30 µl acetonitrile, 10 µl triethylamine and 10 µl penta-fluoro-benzylbromide were added and mixed, and the mixture was kept at room temperature for 30 min. Subsequently 50 µl ethylacetate and 500 µl hexane were added and vigorously mixed for 5 min, and the mixture was centrifuged for 5 min at 4000 rpm at 4°C. The ethylacetate/hexane (upper) layer was transferred to an Eppendorf tube, dried under nitrogen at 40°C, and reconstituted in 50 µl ethylacetate. The samples were stored at −20°C until GC-MS analysis and 1 µl was injected and analyzed as described previously [34].

Validation of the assays

The intra-assay variation for FT was between 0.5 and 20 µM with a coefficient of variation (CV) between 4.2% and 7.1% and a mean relative error between 0.5% and 2.8%. FT was not detectable at 0.1 µ*M*. The intraday variations for the assay of 5-FU, CDHP and uracil were in agreement with the acceptance criteria between 0.1 and 2 µ*M*. For 5-FU, the CV varied between −2.39% and 5%, and the mean relative error between −1.92% and 11.9%. The limit of quantification (LOQ) for 5-FU was 0.02 µ*M* (CV 2.84%, mean relative error −24.25%). For CDHP the CV varied between 3.08% and 10.05%, with a mean relative error of −7.6% and 14.4%. For uracil the CV varied between 1.4% and 6.6%, with a mean relative error of −2.6% and −7.2%. For CDHP and uracil the LOQ was between 0.02 and 0.1 µ*M* but could not be established exactly. For the interday assays, similar values were found for 5-FU, but at 0.1 µ*M* CDHP and uracil had a CV somewhat larger than 15%. The dilution assay for 5-FU, CDHP, uracil and FT showed that all CVs and mean relative errors were below 4.3%. The linearity of 5-FU, CDHP, uracil and FT showed an excellent correlation for the different parameters of the calibration curve ($r > 0.995$) and a CV $< 10\%$. The assay for oxonic acid showed a CV between 0.3% and 10.1% and was reliable between 0.05 and 1 µ*M*.

Enzyme assays

The activity of DPD in WBC was determined as described previously using a validated DPD assay regularly used for screening of DPD deficiencies in children and adults [37]. In order to prevent variations due to different DPD activities in various subcell types, care was taken to use homogeneous cellular preparations. WBC were washed using standard protocols, frozen in liquid nitrogen and extracted just before assaying. DPD was always measured in samples obtained in the morning in order to avoid circadian variations.

The activity of TS, either by the FdUMP binding assay or the catalytic assay, was measured as described previously [38], and the levels of FdUMP were also measured [36]. TS levels were measured in dissociated and non-dissociated samples. Shortly, after treatment with 5-FU, a stable ternary complex can be formed between TS, FdUMP and 5,10-methylene-tetrahydrofolate, although not all TS molecules are trapped. This part can still bind tritiated FdUMP (representing free FdUMP binding sites) or catalyze conversion of dUMP to dTMP (representing residual TS catalytic activity). Removal of FdUMP from this ternary complex [38] will allow the total number of FdUMP binding sites or total catalytic TS activity to be measured. This procedure allows TS inhibition to be estimated without the necessity to obtain pretreatment tumor samples, which is not always possible.

Data evaluation

The pharmacokinetic parameters for FT, oxonic acid, CDHP and uracil were calculated using the computer program WinNonLin (version 1.5). The data were analyzed using non-compartmental modeling. The parameters that could be established were: time point of maximum observed concentration in plasma (T_{max}), concentration in plasma corresponding to T_{max} (C_{max}), terminal half-life ($t_{1/2}$), area under the plasma concentration versus time (C-t) curve extrapolated to infinity (AUC_{INF}), volume of distribution (V_d), total body clearance (Cl), and the mean residence time extrapolated to infinity (MRT_{INF}). Data (e.g. V_d and Cl) were not corrected for the biological bioavailability of the compounds.

The terminal half-life was calculated from the terminal elimination rate constant y. This rate constant was calculated by means of linear regression of the final part of the lnC-t curve. The final half-life was calculated using the equation:

$$t_{1/2} = -\ln(2)/y = -0.693/y.$$

The elimination rate constant was calculated using the three final concentrations of the C-t curve (4, 8 and 24 h). AUC_{INF} was calculated using the logarithmic trapezoidal rule from the start to infinity. Extrapolation from the predicted last plasma concentration was carried out using the equation:

$$AUC_{INF} = AUC_{last} + C_{last}/y.$$

The volume of distribution (V_d) was calculated using the equation:

$$Vd = \text{Total dose}/(y * AUC_{INF}).$$

The total body clearance was calculated using the equation:

$$Cl = \text{Total dose}/AUC_{INF}.$$

The MRT_{INF} was calculated using the equation:

$$MRT_{INF} = AUMC_{INF}/AUC_{INF}.$$

The area under the first moment curve extrapolated to infinity ($AUMC_{INF}$) was calculated using the equation:

$$AUMC_{INF} = AUMC_{last} + t_{last} * C_{last}/y + C_{last}/y^2.$$

For the urine data the cumulative urinary excretion, CUE(%), was calculated and expressed as a percentage of the total dose. CUE(%) was calculated using the equation:

$$CUE\ (\%) = \Sigma\ (\text{Concentration} * \text{Volume})/\text{Total dose} * 100.$$

Statistical evaluation

The degree of correlation between the pharmacokinetic parameters and toxicity (diarrhea) and response using nonparametric tests (Spearman and Mann Whitney *U*-test) were evaluated using the statistical program SPSS.

Results

Toxicity and antitumor activity

Toxicity and antitumor activity of S-1 have been reported previously [23]. In brief, the dose-limiting toxicity consists of diarrhea, with grade 3–4 diarrhea in 3 of 5 patients treated at the highest dose level and in 4 of 11 patients treated at 40 mg/m^2. However, toxicity is predominantly observed in heavily pretreated patients [23]. Therefore, this dose was defined as the recommended dose for phase II studies, but was reduced to 35 mg/m^2 in a later phase of that study. At 45 mg/m^2 only one course could be completed. Toxicity was too severe to continue treatment in subsequent courses. Two patients showed a partial response, 11 stable disease and 8 disease progression. The other patients were not evaluable for response.

Table 1 Pharmacokinetic parameters of S-1. Values are means ± SD of the number of patients given in parentheses

Drug	Dose (mg/m^2) (*n*)	T_{max} (min)	C_{max} (μ*M*)	$T_{1/2}$ (min)	AUC (μ*M*·min)	V_d (l)	Cl (l/min)	MRT (min)
FT	25 (6)	91 ± 75	6.5 ± 3.0	462 ± 95	3329 ± 1233	54 ± 16	0.09 ± 0.04	675 ± 163
	35 (5)	101 ± 32	7.5 ± 1.6	414 ± 187	3710 ± 929	48 ± 13	0.09 ± 0.04	572 ± 210
	40 (11)	81 ± 34	9.5 ± 2.2	366 ± 118	5002 ± 1259	40 ± 17	0.08 ± 0.02	559 ± 173
	45 (6)	98 ± 76	11.3 ± 2.7	389 ± 93	4861 ± 894	49 ± 4	0.10 ± 0.02	564 ± 127
5-FU[a]	25 (6)	131 ± 61	0.9 ± 0.4	203 ± 94	272 ± 117			319 ± 100
	35 (5)	171 ± 66	1.4 ± 0.3	160 ± 56	523 ± 153			295 ± 46
	40 (11)	181 ± 117	1.7 ± 0.4	212 ± 152	689 ± 193			328 ± 118
	45 (3)	160 ± 58	2.1 ± 0.2	195 ± 101	757 ± 110			384 ± 116
CDHP	25 (6)	121 ± 67	1.4 ± 0.5	354 ± 199	442 ± 144	113 ± 32	0.25 ± 0.12	479 ± 265
	35 (5)	101 ± 33	2.9 ± 0.6	343 ± 135	1056 ± 195	60 ± 21	0.13 ± 0.03	446 ± 164
	40 (11)	112 ± 47	2.6 ± 0.7	256 ± 118	913 ± 145	60 ± 26	0.16 ± 0.03	365 ± 133
	45 (6)	110 ± 65	2.4 ± 0.5	403 ± 397	1092 ± 466	82 ± 46	0.17 ± 0.05	539 ± 442
Oxonic acid	25 (6)	96 ± 31	0.1 ± 0.1	114 ± 17	28 ± 23	2898 ± 2890	18.7 ± 20.6	204 ± 19
	35 (5)	112 ± 27	0.5 ± 0.5	290 ± 222	174 ± 183	3000 ± 4622	11.5 ± 18.8	420 ± 189
	40 (11)	128 ± 42	0.4 ± 0.2	396 ± 352	163 ± 129	1572 ± 1080	4.0 ± 3.4	483 ± 378
	45 (6)	126 ± 59	0.3 ± 0.2	362 ± 195	122 ± 126	3490 ± 2389	7.6 ± 6.2	487 ± 115
Uracil	25 (6)	279 ± 89	5.5 ± 1.1	301 ± 86	3160 ± 1150			578 ± 136
	35 (5)	247 ± 5	6.1 ± 1.7	345 ± 60	4827 ± 1164			568 ± 61
	40 (11)	283 ± 100	6.2 ± 1.8	291 ± 50	4871 ± 1642			543 ± 79
	45 (6)	311 ± 107	7.6 ± 1.8	338 ± 132	5972 ± 2160			661 ± 152

[a] From reference 23

[b] V_d and Cl were not corrected for biological bioavailability

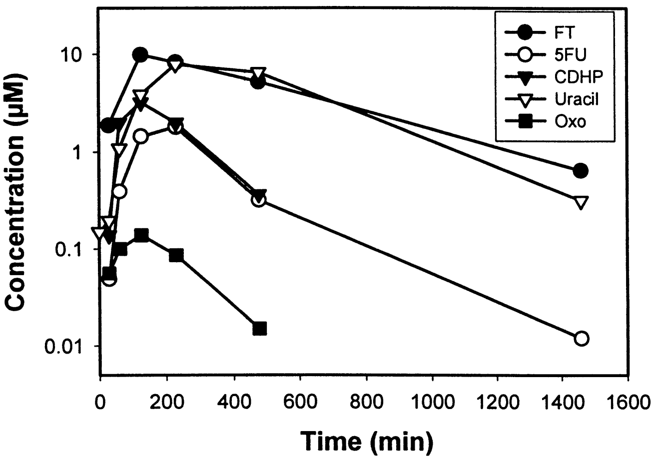

Fig. 1 Representative plasma concentration-time curves of FT, 5-FU, oxonic acid (*Oxo*), CDHP and uracil at 40 mg S-1/m^2

Pharmacokinetics of FT

FT, the parent compound, showed a rapid uptake with a T_{max} ranging from 80.7 to 101.4 min and a mean C_{max} ranging from 6.5 to 11.3 μM (Table 1). A representative sample plasma vs concentration curve at a dose of 40 mg/m^2 is shown in Fig. 1. The mean V_d ranging from 40.1 to 54.0 l indicates an incomplete distribution of the drug through the body. The total body clearance was low (0.077 to 0.094 l/min) which was also reflected by a high MRT (558.6 to 675.3 min). The correlation between the dose of S-1 and C_{max} was 0.58 including every data point and 0.95 for the mean value of FT per dose of S-1 (Fig. 2). For the AUC these correlation coefficients were 0.51 including every data point and 0.90 for the mean value for FT per dose of S-1.

FT showed a mean level (after 2 h) ranging from 8.54 to 12.53 μM during course 1 with a coefficient of variation from 14.7% to 45.0% (Table 2). During subsequent courses the mean FT concentration ranged from 5.70 μM at 25 mg/m^2 to 11.25 μM at 40 mg/m^2 with a coefficient of variation between 22.1% and 44.6%. The correlation between the FT levels and dose was excellent for the first course as well as the subsequent courses: 0.82 and 0.94, respectively (Fig. 2).

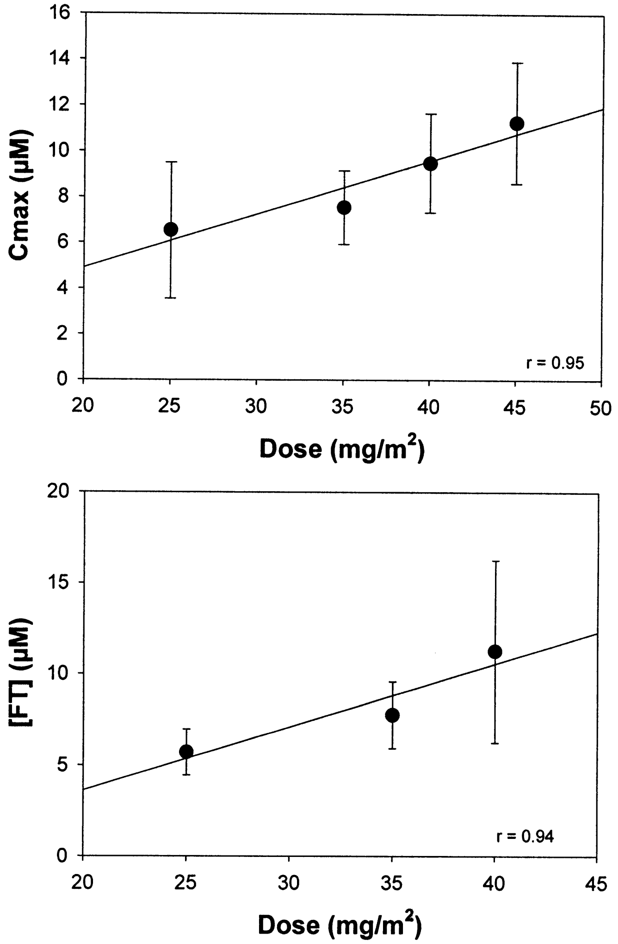

Fig. 2a, b Relationship between FT plasma levels and the dose of S-1. **a** C_{max} of FT after single administration; **b** FT levels 2 h after S-1 administration in the first week of subsequent courses

Pharmacokinetics of oxonic acid

Oxonic acid showed a T_{max} ranging from 60 to 241 min and a corresponding mean C_{max} from 0.1 to 0.5 μM (Table 1). The $t_{1/2}$ varied from 83 to 1133 min. The V_d was very high and varied from 210 to 7663 l, indicating a strong compartmentalization in the body. The total body clearance ranged from 0.6 to 53.9 l/min and the MRT from 192 to 1356 min. The correlation between the dose of S-1 and C_{max} was low: 0.18 including every data point and 0.46 for the mean level of oxonic acid per dose of S-1 (Fig. 3). For the AUC, these correlation coefficients were similar: 0.16 including every data point and 0.67 for the mean level for oxonic acid per dose of S-1.

During treatment, oxonic acid showed a mean level (after 2 h) from 0.041 to 1.112 μM in course 1 with a coefficient of variation from 41.5% to 58.4% (Table 2). During subsequent courses the mean oxonic acid concentration ranged from 0.046 to 0.866 μM with a coefficient of variation between 37.9% and 61.6%. Surprisingly, the correlation between the oxonic acid levels and the dose level for the first course was higher than during the pharmacokinetic part of the study: 0.52 including every data point and 0.99 for the mean values per dose level (Fig. 3). For the subsequent courses these coefficients were 0.53 and 0.96, respectively. During the first course there was an accumulation of oxonic acid. At 25 mg/m^2 oxonic acid increased almost twofold from 0.1 μM in the first week to 0.2 μM in the third week. At 35 mg/m^2 the pattern was not clear. At 40 mg/m^2 oxonic acid reached its maximum also in week 3 (from 0.335 to 0.487 μM). The highest accumulation was found at 45 mg/m^2 and increased from 0.334 μM in week 1 to 0.893 μM in week 4, which is an increase of more than 250%. When for every patient the 2-h value for week 1

Table 2 Plasma concentrations (μM) of S-1 compounds during course 1, before and 2 h after S-1 administration. Values are means ± SD of the number of patients given in parentheses

Drug	Time	Dose (mg/m^2)			
		25 (n=6)	35 (n=5)	40 (n=11)	45 (n=6)
FT	Before	4.00 ± 3.35	3.61 ± 0.58	4.70 ± 1.96	4.83 ± 0.75
	After	8.54 ± 3.84	9.66 ± 1.42	12.53 ± 4.27	11.17 ± 2.51
5-FU[a]	Before	0.07 ± 0.04	0.10 ± 0.05	0.11 ± 0.08	0.23 ± 0.14
	After	0.90 ± 0.31	1.10 ± 0.28	1.51 ± 0.39	1.71 ± 0.30
CDHP	Before	0.17 ± 0.04	0.47 ± 0.27	0.33 ± 0.18	0.43 ± 0.30
	After	1.35 ± 0.32	2.53 ± 0.80	2.58 ± 0.72	2.41 ± 0.80
Oxonic acid	Before	0.06 ± 0.04	0.12 ± 0.10	0.11 ± 0.17	0.13 ± 0.09
	After	0.15 ± 0.09	0.33 ± 0.19	0.43 ± 0.18	0.49 ± 0.33
Uracil	Before	1.80 ± 1.09	2.65 ± 0.62	2.20 ± 1.28	3.64 ± 1.47
	After	3.56 ± 1.09	4.45 ± 1.24	4.28 ± 1.62	5.08 ± 1.12

[a] From reference 23

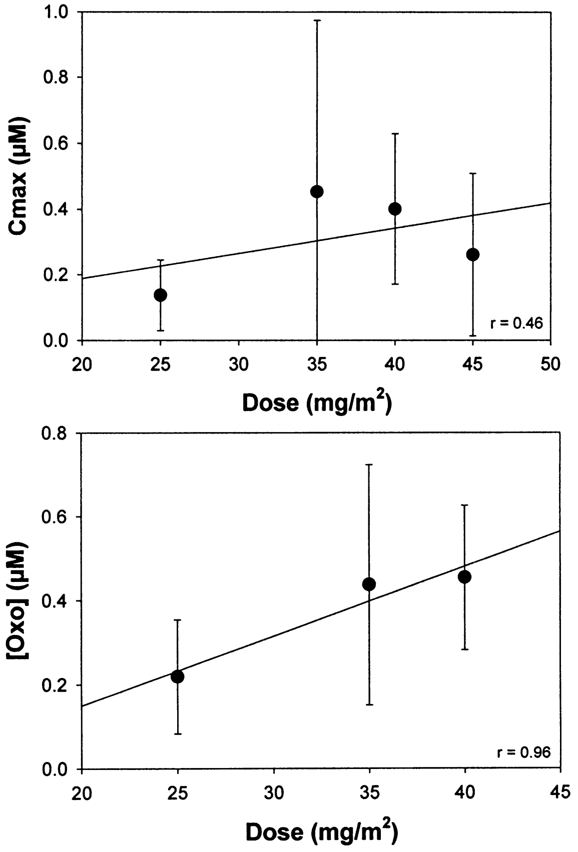

Fig. 3a, b Relationship between oxonic acid (*Oxo*) plasma levels and the dose of S-1. **a** C_{max} of oxonic acid after a single administration; **b** Oxonic acid levels 2 h after S-1 administration in the first week during subsequent courses

was set to 100% and the relative increase in oxonic acid concentration was calculated, the mean values of all patients per dose level and per week showed an even more pronounced accumulation.

Pharmacokinetics of CDHP

CDHP showed its T_{max} from 101.0 to 122.3 min, which is somewhat slower than FT (Fig. 1; Table 1). The mean C_{max} ranged from 1.4 to 2.9 μM, which is above the Ki of DPD inhibition [29]. The $t_{1/2}$ of CDHP ranged from 256 to 403 min and therefore CDHP is capable of inhibiting DPD for a long period, although this inhibition was not complete. The V_d was 60 to 113 l which means that there was a better distribution of CDHP in the body than of FT. The total body clearance was twice as fast as FT: 0.125 to 0.253 l/min. The MRT ranged from 365 to 539 min. The correlation between the dose of S-1 and the C_{max} was 0.62 including every data point and 0.87 for the mean values. For the AUC the values were 0.62 and 0.87, respectively (Fig. 4). The correlation coefficients were high but the data points were not homogeneously distributed around the correlation curves, indicating saturation at the higher doses.

CDHP concentrations ranged from 1.35 to 2.58 μM in the first course and from 1.42 to 2.86 μM in subsequent courses (Table 2). The coefficient of variation ranged from 23.8 to 33.3% in the first course and from 12.4 to 17.2% in subsequent courses. Although there was an excellent correlation between the dose of S-1 and the mean CDHP levels during the first course ($r = 0.83$), the graph indicated that there was saturation at higher dose levels. This was also seen after the single administration of S-1. During subsequent courses no saturation was observed because no patients were treated with multiple courses of 45 mg/m^2. The correlation coefficient was 0.99.

Pharmacokinetics of uracil

Uracil is an important endogenous metabolite responding to S-1 treatment since inhibition of DPD by CDHP will increase the concentration of uracil. Therefore uracil levels are an indirect determinant of DPD inhibition. The T_{max} of uracil ranging from 247.0 to 311.2 min was about twice as long as the T_{max} of CDHP (Table 1; Fig. 1). At the T_{max} inhibition of DPD was maximal. The mean C_{max} was between 5.5 and 7.6 μM, while the normal level of uracil is about 0.2 μM. The $t_{1/2}$ was between 291.1 and 344.5 min because of the prolonged inhibition of DPD by CDHP. The mean MRT was 543.3 to 660.8 min. This increase in uracil led to a second desired effect: inhibition of DPD by uracil itself. The correlation between the dose of S-1 and the C_{max} was 0.36 including every data point and 0.89 for the

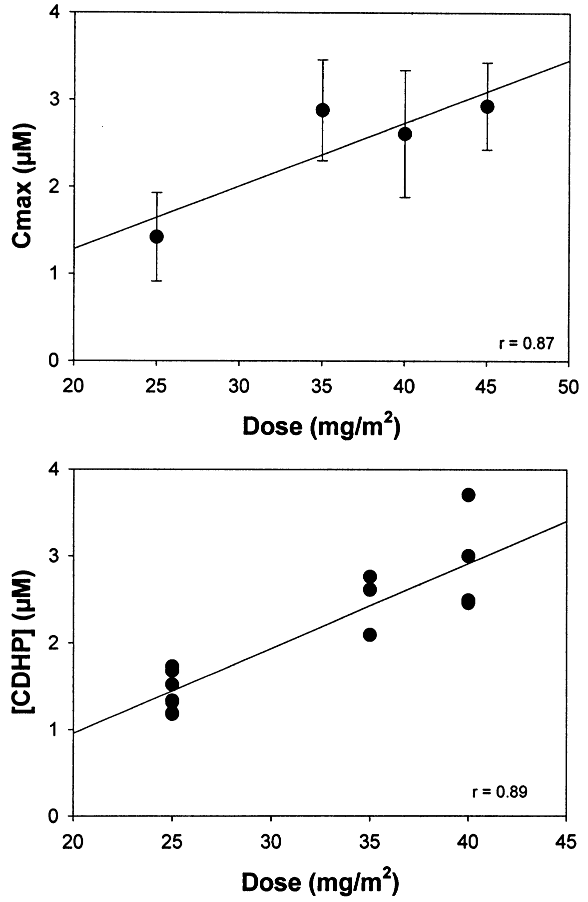

Fig. 4a, b Relationship between CDHP plasma levels and the dose of S-1. **a** C_{max} of CDHP after a single administration; **b** CDHP levels 2 h after S-1 administration in the first week during subsequent courses

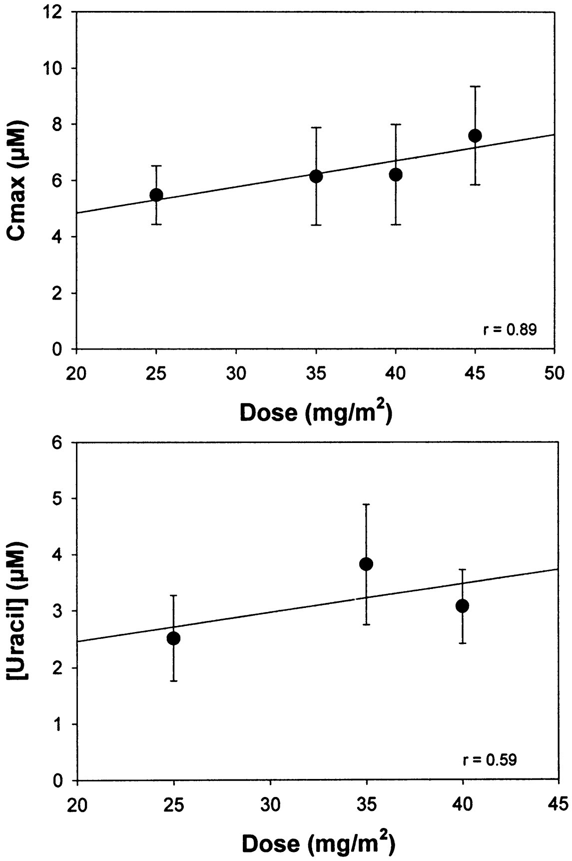

Fig. 5a, b Relationship between uracil plasma levels and the dose of S-1. **a** C_{max} of uracil after a single administration; **b** uracil levels 2 h after S-1 administration in the first week during subsequent courses

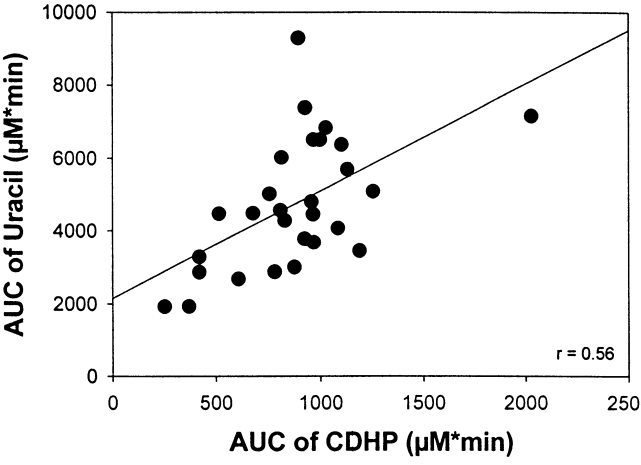

Fig. 6 Relationship between uracil and CDHP levels indicating a correlation between the accumulation of CDHP and inhibition of DPD shown as uracil accumulation ($r = 0.56$)

mean values of uracil per dose of S-1. These values for the AUC were 0.50 and 0.97, respectively (Fig. 5).

Since CDHP resulted in inhibition of DPD, we determined the degree of correlation between the concentrations of CDHP and uracil. The correlation between the C_{max} of CDHP and the C_{max} of uracil showed a heterogeneous pattern whereas the correlation between the AUC of CDHP and the AUC of uracil was stronger with a correlation coefficient of 0.56 (Fig. 6). The correlation between the C_{max} of CDHP and the AUC of uracil was also minimal (correlation coefficient 0.24).

Uracil concentrations varied in the first course from 3.6 to 5.1 μM and in subsequent courses from 2.5 to 3.8 μM (Table 2). The coefficient of variation ranged from 22.1 to 37.8% for the first course and from 21.2 to 30.0% for subsequent courses. The correlation between the mean uracil levels and the dose of S-1 was 0.93 for the first course and somewhat lower (0.59) for subsequent courses.

Urinary excretion

The CUE could not be determined for FT because it was below the detection limit. However, the concentrations of 5-FU, oxonic acid, CDHP and F-β-Ala were evaluable (Table 3). After 24 h, 6.5 to 11.2% of the administered total dose of FT was excreted as 5-FU and 42.2 to 48.5% as F-β-Ala. The presence of high levels of F-β-Ala in urine showed that the inhibition of DPD was far

Table 3 Cumulative urinary excretion of S-1 compounds. Values are means ± SD of the number of patients

Dose (mg/m^2)	No. of patients	Urinary excretion (%)			
		5-FU	CDHP	F-*β*-Ala	Oxonic acid
25	6	6.5 ± 3.5	31.3 ± 17.1	42.2 ± 10.4	0.5 ± 0.3
35	5	9.4 ± 3.1	48.7 ± 26.7	46.8 ± 4.5	2.0 ± 3.0
40	11	11.2 ± 7.2	43.9 ± 25.4	48.5 ± 14.5	2.1 ± 3.4
45	6	9.1 ± 3.1	38.8 ± 12.9	44 ± 8.1	0.7 ± 0.7

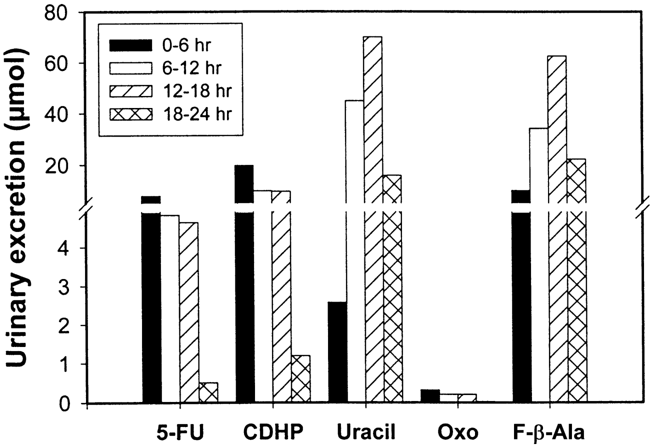

Fig. 7 Urinary excretion of S-1 compounds. Data from one patient (same as in Fig. 1) treated at 40 mg/m^2 are shown

from complete. In most patients the largest amount of F-*β*-Ala was excreted between 6 and 18 h (Fig. 7), similar to uracil and following the recovery of DPD activity. The relatively high levels in F-*β*-Ala in the urine was in contrast to generally low or undetectable levels of this compound in the plasma. After 24 h, 31.3 to 48.7% of the administered dose was excreted as CDHP.

The CUE of oxonic acid was very low. After 24 h, 0.13 to 12.0% of the administered total dose of oxonic acid was excreted in the urine. In only 6 patients out of 28 was the CUE higher than 1%. In the majority of patients the largest amount of oxonic acid was excreted between 0 and 6 h. The low urinary excretion is also indicative of the high compartmentalization of oxonic acid in the gut.

DPD levels

The activity of DPD was determined before and during treatment. The initial reason for determining pretreatment levels was to prevent DPD-deficient patients (or those with very low DPD) being entered into the study, which would potentially have led to lower DPD levels during treatment than would be considered acceptable. However, the pretreatment DPD levels were within the expected range in all patients and varied between 3.6 and 15.9 nmol/h per mg protein (mean ± SD 9.8 ± 2.7, *n* = 20). The second reason for determining DPD levels was to determine whether DPD would remain inhibited during treatment, possibly providing an additional risk during subsequent treatment with a fluoropyrimidine similar to that seen with ethynyluracil [15, 20, 39]. However, in contrast to ethynyluracil, CDHP is not a suicide inhibitor but its action is reversible, and during the pharmacokinetic study no indication of a decrease in DPD levels was observed. The findings also demonstrate that CDHP is not bound to DPD and was washed away from the cells completely during the isolation and extraction procedure, since during treatment DPD even increased (Fig. 8). In the dose range used there was no indication that this increase was dose-dependent. Therefore, it can be concluded that it is unlikely that a cumulative decrease in DPD levels will occur.

TS levels during treatment

In one patient with a skin metastasis of esophageal cancer it was possible to obtain multiple biopsy specimens during and after treatment. Since it is usually complicated to get sequential tumor samples in a phase I study, these data were included because they could be compared with previously obtained data after a 5-FU bolus [38]. The nature of TS inhibition (formation of a ternary complex) allowed the extent of inhibition to be measured, since dissociation of the ternary complex yields the total enzyme. This patient had a partial response, and TS was inhibited by more than 50% (Table 4); however TS levels were increased two- to threefold when the patient progressed and went off study. The FdUMP and 5-FU levels in the tumor were relatively high.

Relationship between pharmacokinetics and toxicity and antitumor activity

In contrast to the correlation between 5-FU pharmacokinetics and the grade of toxicity [23], we could not find any correlation between toxicity and the pharmacokinetic parameters of CDHP, uracil, oxonic acid and FT. There was also no significant correlation for any of the parameters tested with the response to treatment with S-1.

Discussion

In this study, we demonstrate that oral administration of S-1 resulted in a high bioavailability of FT and CDHP

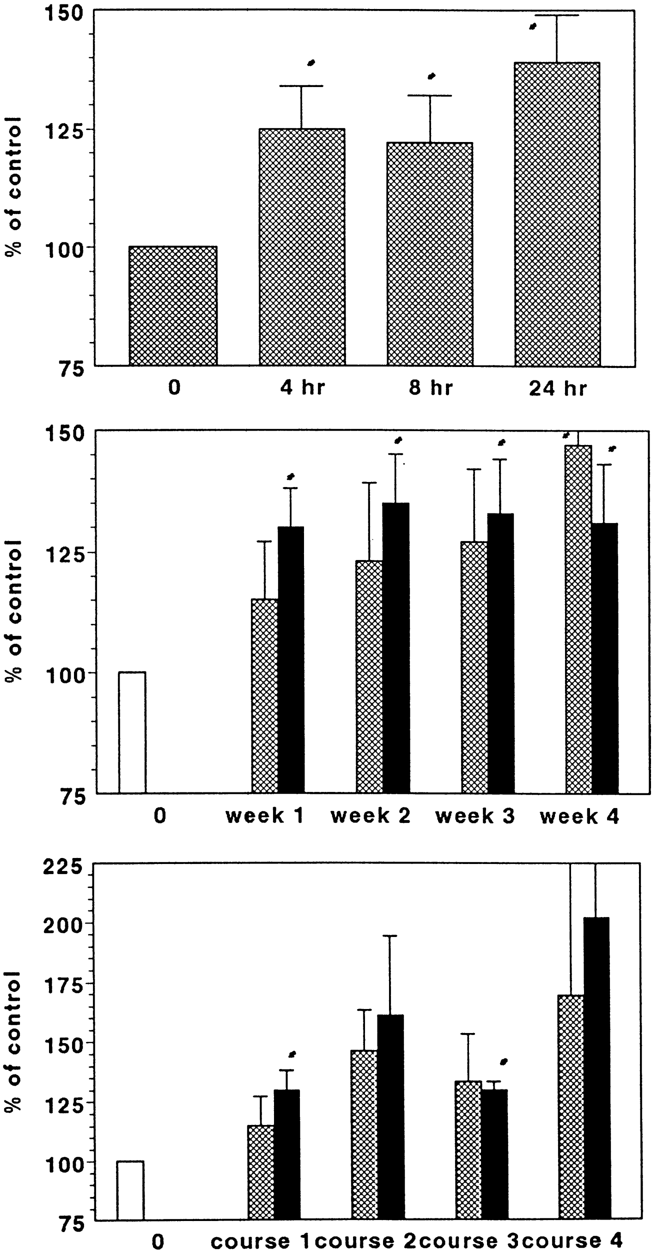

Fig. 8a–c Effect of S-1 treatment on the activity of DPD in WBC. DPD levels before treatment were set at 100% for each patient and values represent means ± SE. **a** Relative DPD levels in the first 24 h during the pharmacokinetics part of the study ($n = 16–18$ patients). **b** DPD levels during the first course of S-1 treatment, just before and 2 h after the morning S-1 administration ($n = 15–17$ patients). **c** DPD levels in the first week of the first four consequent courses of S-1 administration ($n = 3–9$ patients for courses 2–4). In later courses of S-1 administration, not enough samples were obtained to measure DPD levels. $^*P < 0.05$ vs DPD levels before treatment, paired *t*-test

Table 4 Inhibition and induction of TS, and the concentrations of 5-FU and FdUMP, in a skin metastasis of a patient with esophageal cancer who responded to treatment with S-1 (at 40 mg/m^2; same patient as in Figs. 1 and 7). Residual and free TS were measured before dissociation of the ternary complex and represent the actual catalytic TS activity and the number of free FdUMP binding sites available during treatment, respectively. Total TS was measured by dissociation of the ternary complex resulting in removal of bound FdUMP and represents the total catalytic activity and the total number of FdUMP binding sites. During treatment means that samples were obtained during S-1 treatment. After treatment represents tumor samples obtained 3 months after the initial samples, when the patient was not receiving S-1 treatment because of disease progression. In these samples dissociation would not have resulted in any FdUMP release and therefore residual TS is similar to total TS. No FdUMP or 5-FU were measurable because the patient was not receiving treatment

Parameter	During treatment	After treatment
FdUMP binding (fmol/mg protein)		
Free TS	47	183
Total TS	105	
Catalytic TS activity (pmol/h/mg protein)		
Residual TS	32	251
Total TS	68	
5-FU (pmol/mg w.w.)	0.81	
FdUMP (fmol/mg w.w.)	157	

and that FT was rapidly converted to the active drug 5-FU (Table 1), but initially not to its catabolite F-β-Ala. The dose of S-1 was linearly correlated with the C_{max} and AUC of FT, CDHP and uracil. DPD was efficiently inhibited since uracil concentrations increased rapidly and were maintained for a long period, while F-β-Ala had predominantly been eliminated at the end of the 24-h urine collection period. However, the relatively high F-β-Ala urinary excretion showed that the inhibition of DPD recovered after several hours, and is therefore not complete and is reversible.

Oxonic acid is intended to accumulate specifically in gut tissue after oral administration of S-1 [14, 23]. The high compartmentalization of oxonic acid indeed suggests a high tissue accumulation. Consequently, it is not favorable to have high plasma levels of oxonic acid because they may affect the activity of 5-FU. High plasma concentrations of oxonic acid may lead to an increased undesired uptake in tumor tissue, preventing 5-FU phosphoribosylation in the tumor, which is considered to be one of the main activation pathways for 5-FU [1, 5]. The potential difference in oxonic acid absorption should be taken into account when evaluating the efficacy of the formulation.

After a single administration of S-1, there was no correlation between the C_{max} or AUC of oxonic acid and the dose of S-1, but there was a linear correlation between oxonic acid at 2 h and the dose of S-1 during the first course and during subsequent courses. In addition, there was an up to fourfold accumulation of plasma oxonic acid during treatment. In a Japanese study [22], no indication of an accumulation in plasma oxonic acid was observed. Possibly in Japanese patients oxonic acid is retained better in the tissue, which would provide a better protection against diarrhea, which was observed to a lesser extent in the Japanese patients. In European patients some plasma accumulation of oxonic acid occurred during the twice-daily administration of S-1, but accumulation of oxonic acid in the gut was not sufficient to provide adequate protection against damage to the gut mucosa, which is considered to be responsible for diarrhea. This may be related to the different diet of European patients. Retention of oxonic acid in the gut of these patients does not seem to be optimal because there is a large interpatient variation; however, this did not preclude the correlation between dose and oxonic acid plasma accumulation. Since a high compartmentalization of oxonic acid was observed, it was not unexpected that the urinary excretion of oxonic acid was very low and that the pharmacokinetic parameters of oxonic acid were not correlated with either toxicity or response.

The uptake and metabolism of FT are determined by a number of different parameters. First, after oral intake, FT has to be absorbed from the gastrointestinal tract, after which it is rapidly distributed [22] (Table 1). Subsequently, it has to be cleaved to 5-FU, for which the cytochrome P450 isozyme CYP 2A6 is responsible [26, 28]. This enzyme is widely distributed in Caucasians [28, 40], but has a lower expression in Japanese. Consequently, when given a similar dose of S-1 (based on body surface), the concentration of FT is higher in Japanese, while that of 5-FU is lower [22, 23]. The subsequent fate of 5-FU is determined by its distribution and elimination. The latter process is influenced by the extent of inhibition of 5-FU degradation by DPD. The more potent this inhibition, the longer will be the retention of 5-FU in plasma. So, theoretically one would expect a relatively short half-life of 5-FU derived from FT in the UFT combination compared to that in the S-1 combination. This has indeed been found, since 5-FU derived from UFT has a shorter half-life of 40 min [24, 25] compared to 2–4 h for FU derived from S-1 [22, 23]. Ethynyluracil is a more potent inhibitor of DPD leading to an even longer retention of 5-FU, despite the fact that in this formulation, 5-FU itself was given and not FT [15, 20]. The initial half-life of 5-FU derived from FT given as an i.v. infusion is 10 min [26], and is comparable to that of 5-FU given as an i.v. infusion [24, 41] and orally [9], but is significantly shorter than that with each DPD inhibitor, including the relatively weak DPD inhibitor uracil. Thus, it can be concluded that the increased half-life of 5-FU derived from UFT and S-1 is due to the inhibition of DPD [42] and that the difference between the S-1 formulation and ethynyluracil/5-FU seems to be related to the better efficacy of the suicide DPD inhibitor, ethynyluracil.

The latter aspect, the reversible inhibition by CDHP and the suicide inhibition by FU, can be evaluated by the difference in retention of DPD inhibition. Humerickhouse et al. [20] have reported that no DPD activity is detectable shortly after administration of the ethynyluracil/5-FU combination and that the enzyme remains undetectable during various courses, while Ahmed et al. [39] have shown that the ethynyluracil-induced inhibition persists until several weeks after withdrawal of the drug. This is in contrast to our data on S-1, which clearly demonstrated that DPD inhibition by CDHP was reversible. DPD levels in WBC of treated patients even increased during treatment, although this did not seem to affect the efficacy of DPD inhibition, based on accumulation of uracil. This reversibility of CDHP can also be judged from the appearance of F-β-Ala in the urine, which increased when the concentration of CDHP decreased. This gives CDHP several advantages compared to, for example, uracil in UFT and ethynyluracil in the ethynyluracil/5-FU combination. The inhibition by CDHP is quite potent but easily reversible upon withdrawal. Therefore, no severe toxicity is expected when patients who discontinue S-1 treatment receive another 5-FU-based chemotherapy.

Although in only one patient TS levels could be evaluated in the tumor, the data support the crucial role of TS inhibition in the activity of fluoropyrimidines. In addition, 5-FU and FdUMP reach levels sufficient to exert cytotoxicity [36, 38]. At relapse, TS levels in this patient increased, in agreement with previous data on the role of TS induction in 5-FU resistance [43].

Currently, a number of oral fluoropyrimidine formulations are being evaluated in the clinic. UFT and the thymidine phosphorylase-dependent prodrug capecitabine have been approved in a number of countries, while S-1 is also approved in Japan. Marked differences exist between the formulations. In contrast to the tumor-specific activation of capecitabine [17], it also shows excessive degradation as shown by the appearance of degradation products [21], such as plasma F-β-Ala, which was very low in the plasma of S-1-treated patients. Since this is the major difference between long-term continuous infusions of 5-FU and capecitabine on the one hand and DPD inhibitor-containing formulations on the other hand, this led us speculate that breakdown products of 5-FU might contribute to the occurrence of the hand-foot syndrome [44]. This is typical of 5-FU continuous infusions [6, 45] and capecitabine, but is not found with 5-FU bolus injections or with formulations with a DPD inhibitor [11, 15, 22, 23]. This supports the evidence [46, 47] that breakdown products of 5-FU are responsible for some 5-FU side effects, possibly including the hand-foot syndrome. Since all oral formulations of fluoropyrimidines achieve plasma 5-FU concentrations in the same range [42], although somewhat higher in the 5-FU/ethynyluracil formulation [15], it is evident that it is not the continuous presence of 5-FU levels between 1 and 10 μM that is responsible for the hand-foot syndrome, but a metabolite.

Oral administration of anticancer drugs has long been considered unreliable, either because of unpredictable bioavailability or because of the likelihood that oral drugs will cause gastrointestinal toxicity. However, prodrugs such as FT have a relatively high bioavailability [26, 27] by themselves, which is enhanced by the use of a DPD inhibitor. S-1 has as an additional advantage—the inclusion of oxonic acid, which in preclinical models selectively protects against gastrointestinal toxicity [14], but in humans the effect is variable [22, 23], possibly related to ethnic differences [28, 40, 48].

Therefore, future clinical studies on oral drugs should identify the causes for dose-limiting toxicities such as the hand-foot syndrome for capecitabine, and the diarrhea in S-1-treated patients. Since patients prefer an oral drug when a similar efficacy is expected [24], development of a safe S-1 formulation should be focused on control of gastrointestinal toxicity, either by different scheduling or possibly by addition of more oxonic acid. Such modifications would make these formulations suitable for future combination therapy with other novel drugs active against gastrointestinal malignancies such as irinotecan, oxaliplatin and possibly the EGFR-dependent tyrosine kinase inhibitor iressa. An additional advantage of this latter drug is that it is also an oral drug.

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