Cancer Chemother Pharmacol (1999) 44: 453–460 

ORIGINAL ARTICLE

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# Bioequivalence of two tablet formulations of capecitabine and exploration of age, gender, body surface area, and creatinine clearance as factors influencing systemic exposure in cancer patients

Received: 26 January 1999 / Accepted: 13 May 1999

**Abstract** *Purpose*: The objective of the study was to assess the bioequivalence of two tablet formulations of capecitabine and to explore the effect of age, gender, body surface area and creatinine clearance on the systemic exposure to capecitabine and its metabolites. *Methods*: The study was designed as an open, randomized two-way crossover trial. A single oral dose of 2000 mg capecitabine was administered on two separate days to 25 patients with solid tumors. On one day, the patients received four 500-mg tablets of formulation B (test formulation) and on the other day, four 500-mg tablets of formulation A (reference formulation). The washout period between the two administrations was between 2 and 8 days. After each administration, serial blood and urine samples were collected for up to 12 and 24 h, respectively. Unchanged capecitabine and its metabolites were determined in plasma using LC/MS-MS and in urine by NMRS. *Results*: Based on the primary pharmacokinetic parameter, $AUC_{0-\infty}$ of 5′-DFUR, equivalence was concluded for the two formulations, since the 90% confidence interval of the estimate of formulation B relative to formulation A of 97% to 107% was within the acceptance region 80% to 125%. There was no clinically significant difference between the $t_{max}$ for the two formulations (median 2.1 versus 2.0 h). The estimate for $C_{max}$ was 111% for formulation B compared to formulation A and the 90% confidence interval of 95% to 136% was within the reference region 70% to 143%. Overall, these results suggest no relevant difference between the two formulations regarding the extent to which 5′-DFUR reached the systemic circulation and the rate at which 5′-DFUR appeared in the systemic circulation. The overall urinary excretions were 86.0% and 86.5% of the dose, respectively, and the proportion recovered as each metabolite was similar for the two formulations. The majority of the dose was excreted as FBAL (61.5% and 60.3%), all other chemical species making a minor contribution. Univariate and multivariate regression analysis to explore the influence of age, gender, body surface area and creatinine clearance on the log-transformed pharmacokinetic parameters $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites revealed no clinically significant effects. The only statistically significant results were obtained for $AUC_{0-\infty}$ and $C_{max}$ of intact drug and for $C_{max}$ of FBAL, which were higher in females than in males. *Conclusion*: The bioavailability of 5′-DFUR in the systemic circulation was practically identical after administration of the two tablet formulations. Therefore, the two formulations can be regarded as bioequivalent. The variables investigated (age, gender, body surface area, and creatinine clearance) had no clinically significant effect on the pharmacokinetics of capecitabine or its metabolites.

**Key words** Capecitabine · Bioequivalence · Pharmacokinetics · 5-FU

**Abbreviations** *5′-DFCR* 5′-deoxy-5-fluorocytidine · *5′-DFUR*: 5′-deoxy-5-fluorouridine · *5-FU*: 5-fluorouracil ·

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*AUC*: area under the plasma concentration versus time curve · *BSA*: body surface area · $C_{max}$: maximum concentration in plasma · $Cl_{CR}$: creatinine clearance · *CV*: coefficient of variation · *FBAL*: α-fluoro-β-alanine · $FUH_2$: dihydro-5-fluorouracil · *FUPA*: 5-fluoro-ureido-propionic acid · *LC/MS-MS*: liquid chromatography/mass spectrometry-mass spectrometry · *NMRS*: nuclear magnetic resonance spectroscopy

## Introduction

Capecitabine (Xeloda) is a novel fluoropyrimidine carbamate rationally designed as an orally administered precursor of 5′-DFUR, which is activated preferentially in the tumor to the cytotoxic agent 5-FU (Fig. 1). After oral administration, capecitabine is well absorbed and is first metabolized in the liver to 5′-DFCR. Subsequently, 5′-DFCR is converted to 5′-DFUR by cytidine deaminase, which is located in high concentrations in many human tumor tissues and in healthy liver tissue [1, 2]. Further catalytic activation of 5′-DFUR to 5-FU then occurs by the tumor-associated angiogenic factor, thymidine phosphorylase, which is present at higher concentrations in tumor than in normal tissue, thus minimizing the exposure of healthy body tissues to 5-FU [2]. The efficacy and favorable safety profiles of capecitabine have been demonstrated in patients with common solid tumors such as colorectal and breast cancer [3, 4].

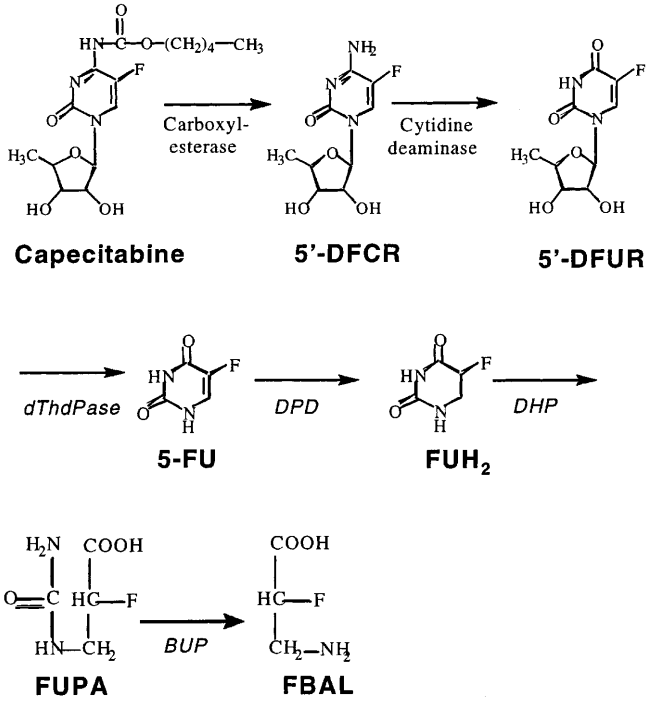


**Fig. 1** Metabolic pathway of capecitabine in humans (*5′-DFCR* 5′-deoxy-5-fluorocytidine, *5′-DFUR* 5′-deoxy-5-fluorouridine, *5-FU* 5-fluorouracil, $FUH_2$ dihydro-5-fluorouracil, *FUPA* 5-fluoro-ureidopropionic acid, *FBAL* α-fluoro-β-alanine, *dThdPase* thymidine phosphorylase, *DPD* dihydropyrimidine dehydrogenase, *DHP* dihydropyrimidinase, *BUP* β-ureidopropionase)

The main objective of this study was to compare the bioavailability of 5′-DFUR after administration of two tablet formulations of capecitabine. The first formulation (formulation A), which was used in clinical phase I studies, was not suited for large scale production. The second formulation (formulation B) is the proposed formulation for marketing. As the pharmacokinetics of 5′-DFUR do not change during multiple dosing [5], a single-dose administration was appropriate to compare the two capecitabine formulations. Capecitabine is classified as a cytotoxic drug and therefore it was decided to perform this study in cancer patients rather than in healthy subjects. The secondary objective of the study was to explore the effect of selected variables such as age, gender, body surface area (BSA), and creatinine clearance on AUC and $C_{max}$ of capecitabine and its metabolites. The aim of this exploratory analysis was to identify possible subgroups of patients (e.g. the elderly) with different pharmacokinetic characteristics.

## Material and methods

### Patients

Enrolled in this study were 25 patients (9 females and 16 males) with histologically or cytologically confirmed solid tumors. All completed the study and were evaluable for safety and pharmacokinetics. These patients had failed standard oncologic therapy and had not received cytotoxic chemotherapy or radiation therapy within 4 weeks prior to the start of the study. The patients were aged between 41 and 80 years (mean 62.6 years), weighed 42–103 kg (mean 72.5 kg) and their Karnofsky performance status ranged between 70% and 100% (mean 90%).

The BSA, (in meters squared) of the patients was taken from the nomogram of Du Bois and Du Bois [6]:

$$BSA = 0.007184 \times BW^{0.425} \times H^{0.725}$$

where BW is body weight in kilograms and H is height in centimeters. Creatinine clearance (in milliliters per minute) was calculated using the formulas of Cockroft and Gault [7]: [$Cl_{CR}$= BW × (140 – age in years)/SCR]/72 for males and [$Cl_{CR}$ = 0.85 × BW × (140 – age in years)/SCR]/72 for females, where SCR is serum creatinine concentration in milligrams per deciliter.

Twenty patients had colorectal cancer and the remaining five patients had breast cancer, lung cancer, thyroid cancer, leiomyosarcoma and adenocarcinoma of unknown primary site.

### Clinical procedure

This open-label, randomized, two-way crossover study was performed in full agreement with the guidelines for Good Clinical Practice and according to the Revised Declaration of Helsinki. Prior to the start of the study , informed consent was obtained from all patients and the protocol was approved by the local ethical review boards. Screening at study start included physical examination, medical history, vital signs, ECG, laboratory safety tests (hematology, serum biochemistry, urinalysis) and evaluation of the Karnofsky performance status. In addition, safety parameters (adverse events, laboratory tests, and vital signs) were assessed on treatment days and at follow-up. All patients had to meet carefully selected inclusion/exclusion criteria taking into account the stage of their disease, current medical status, and life expectancy.

The patients were randomly assigned to the treatment sequences A/B or B/A. The patients had a standard breakfast, and 5 min later received a single dose of 2000 mg capecitabine as four tablets of formulation A (reference treatment) or a single dose of 2000 mg capecitabine as four tablets of formulation B (test treatment). The washout period between the two treatments was 2 to 8 days.

The film-coated 500-mg formulation A tablet which was used in clinical phase I studies, is not suitable for large-scale production. The film-coated 500-mg formulation B tablet which has been proposed for marketing was used in clinical phase II and III trials. Formulation B differed from formulation A by an increased magnesium stearate concentration (6 and 9 mg/tablet in formulation A and B, respectively), by the addition of microcrystalline cellulose (24 mg/tablet) to the core, by the removal of plasticizers from the film coat and by a decrease in the hydroxypropyl methylcellulose content of the coat. Both tablet formulations showed comparable dissolution profiles in water at 37 °C (USP Apparatus 2, paddle speed 50 r.p.m.). In clinical phase I–III studies, capecitabine was administered at a dose of 1250 mg/m$^2$ twice daily in cycles of 3 weeks (2 weeks on treatment, 1 week off). For a patient with a BSA of 1.6 m$^2$, the capecitabine dose would correspond to 2000 mg. This fixed dose was considered to be appropriate to test for bioequivalence between the two formulations.

For pharmacokinetic assessment, 5-ml blood samples were collected in Vacutainers containing EDTA as anticoagulant at the following time-points: predose, and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8 and 12 h after capecitabine intake. Blood samples were stored immediately on ice prior to centrifugation (10 min at 600 *g*, room temperature). The supernatant plasma was removed and stored in plastic tubes at −20 °C until analysis. Urine was collected predose and for the periods 0–12 h and 12–24 h after dosing. All urine samples were stored refrigerated at 4 °C throughout the collection periods. Aliquots of 30 ml were removed from these fractions and stored in plastic tubes at −20 °C until analysis.

## Analytical assay

The analytical assay for capecitabine and its metabolites has been described previously [8]. Plasma samples were analyzed for capecitabine, 5′-DFCR, 5′-DFUR, 5-FU, and FBAL by LC/MS-MS. The plasma concentration calibration ranges were 0.01–5.00 μg/ml for capecitabine and 5′-DFCR, 0.05–25.0 μg/ml for 5′-DFUR, 0.002–1.00 μg/ml for 5-FU, and 0.020–10.1 μg/ml for FBAL. The overall between-day variabilities (%RSD) of the quality control (QC) samples were less than 4% for capecitabine, 5′-DFCR, and 5′-DFUR, < 5% for 5-FU, and < 6% for FBAL. The QC deviations from nominal concentrations (%DEV) were within 3% for capecitabine and 5-DFCR, within 9% for 5′-DFUR, within 3% for 5-FU, and within 5% for FBAL. The overall between-day variabilities of the calibration standards were less than 4% for capecitabine, < 2% for 5′-DFCR, < 5% for 5′-DFUR, < 8% for 5-FU and FBAL. The calibration standard deviations from nominal concentrations were within 4% for capecitabine, within 3% for 5′-DFCR and FBAL, within 11% for 5′-DFUR, and within 5% for 5-FU. The coefficients of determination for the calibration curves for capecitabine and its metabolites ranged from 0.9901 to 0.9996.

In plasma, the lower limit of quantification for capecitabine, 5′-DFCR and 5′-DFUR was 0.05 μg/ml using 0.5-ml aliquots. For 5-FU and FBAL, the lower limits of quantification were 0.003 and 0.02 μg/ml, respectively.

Urine samples were analyzed for capecitabine, 5′-DFCR, 5′-DFUR, 5-FU, $FUH_2$, FUPA, and FBAL using NMRS. Interassay precision from QC samples (%CV) was 3.8% for capecitabine, 3.4% for 5′-DFCR, 2.5% for 5-FU, and 4.7% for FBAL.

In urine, the lower limit of quantification for capecitabine, 5′-DFCR, 5′-DFUR, 5-FU, $FUH_2$, and FBAL was 0.02 μmol/ml using 5-ml aliquots. For FUPA, the lower limit of quantification was 0.05 μmol/ml.

## Pharmacokinetic evaluation

The pharmacokinetic parameters of capecitabine and its metabolites (5′-DFCR, 5′-DFUR, 5-FU, and FBAL) were estimated from the concentration-time data using noncompartmental methods [9]. Maximum plasma concentrations ($C_{max}$) and time to reach this value ($t_{max}$) were determined from the observed highest concentration and time of its occurrence, respectively. Apparent elimination half life ($t_{1/2}$) was estimated from ln(2)/k, where the apparent rate constant of elimination, k, was estimated by linear regression of the logarithm of the plasma concentration versus time data. Area under the plasma concentration time curve from zero to infinity ($AUC_{0-\infty}$) was estimated from the sum of $AUC_{0-t}$ and $C_{t\ last}/k$. $AUC_{0-t}$ is the area under the plasma concentration time curve from zero to the last sampling time-point ($t_{last}$) at which a concentration was measurable ($C_{t\ last}$). $AUC_{0-t}$ was estimated using the linear trapezoidal rule. From the urine concentrations of capecitabine, 5′-DFCR, 5′-DFUR, 5-FU, $FUH_2$, FUPA, and FBAL, and the excreted urine volumes, the percentage of dose for each chemical species recovered in urine was determined.

## Statistical analysis

### *Bioequivalence testing*

Descriptive statistics were used to summarize the pharmacokinetic parameters. Geometric mean and geometric CV are reported for $C_{max}$ and $AUC_{0-\infty}$, arithmetic mean and CV for $t_{1/2}$ and median, minimum and maximum for $t_{max}$. The primary parameter for bioequivalence testing was the $AUC_{0-\infty}$ of 5′-DFUR. Equivalence for AUC can be concluded if the 90% confidence interval for the ratio of the effects of formulation B to the effects of the reference formulation A is in the reference region 0.80–1.25. For $C_{max}$, this measure of relative bioavailability is inherently more variable than the AUC ratio [10] and therefore a wider acceptance range (0.70–1.43) was used based on safety and efficacy considerations. A two-period ANOVA (PROC GLM, SAS 6.12) with the factors 'patient', 'period', and 'treatment' was applied to lnAUC using the main effects model [11]. The same analysis was performed for $C_{max}$ of 5′-DFUR, but was interpreted in an exploratory sense only. Other pharmacokinetic parameters were regarded as secondary.

### *Covariable selection*

To explore the influence of the selected parameters age, gender, BSA, and $Cl_{CR}$ on the log-transformed pharmacokinetic parameters $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites following treatment with formulation B, single and multiple regression analyses were performed using the log-transformed covariables (PROC GLM and PROC MIXED, SAS 6.11). This is equivalent to a multiplicative model for the original variables (i.e. the covariables are raised to some power). Since the class variable 'gender' was coded 0 for males and 1 for females, it was treated like a log-transformed covariable. Because the analyses had to be carried out for two pharmacokinetic parameters on five analytes and for four variables, an $\alpha$ value of 0.01 was used to determine influential effects. This value of $\alpha$ will reduce the probability of false-positive results but is much higher than any formal adjustment for multiple comparisons. To hold a multiple significance level of $\alpha = 0.05$, a Bonferoni adjustment for the analysis of influential variables would require an $\alpha$ value of 0.00125.

To select influential covariables, a backward selection was used. Separately for each selected pharmacokinetic parameter of capecitabine and its metabolites, a multiple regression analysis was performed with all selected log-transformed covariables included. The covariables with the highest *P*-value were dropped from the model and the analysis was repeated until all the remaining variables were significant at the 0.01 level. The effects of variables in the final model were estimated from this model. The effects of class variables were given as the backtransformed least squares means. For covariables, both the estimated regression coefficients and the estimated effect of

a 50% decrease in the covariable on the untransformed pharmacokinetic parameters AUC and $C_{max}$ were given. For an estimated regression coefficient $\gamma$, an $x$% increase in the covariable results in an increase in the parameter of $(1 + x\%/100)^{\gamma} \times 100 - 100$ (%). In addition, separate analyses were performed for each covariable. The coefficients of determination ($R^2$) are reported.

## Results

### Pharmacokinetics and bioequivalence assessment

The mean plasma concentration versus time profiles obtained after single oral administration of 2000 mg capecitabine in the form of tablet A and tablet B are shown for capecitabine and the primary metabolite 5′-DFUR in Figs. 2 and 3, respectively. Except for a somewhat higher $C_{max}$ after intake of tablet B, similar concentration profiles were obtained after administration of both tablet formulations.

The mean peak plasma concentrations of intact drug were 38% higher after intake of formulation B than after intake of formulation A (Table 1). The time to reach these peak concentrations was identical for both formulations (2.0 h). Plasma concentrations then declined exponentially with half-lives of 0.48 h and 0.58 h for formulations A and B, respectively. The $AUC_{0-\infty}$ was similar after treatment with both formulations and reached 4.94 μg · h/ml for formulation A and 5.62 μg · h/ml for formulation B. The pharmacokinetic parameters $C_{max}$, $t_{max}$, $AUC_{0-\infty}$ and $t_{1/2}$ of the metabolites 5′-DFCR, 5′-DFUR, 5-FU, and FBAL were comparable following administration of formulation A or B. There was, however, a trend for slightly higher $C_{max}$ values after treatment with formulation B (Table 1).

The primary parameter for the assessment of bioequivalence in this study was the $AUC_{0-\infty}$ of 5′-DFUR. Following administration of formulation B, $AUC_{0-\infty}$ of 5′-DFUR was 102% of that following administration of formulation A. The relative 90% confidence intervals were 97% to 107% (Table 2). Since the 90% confidence intervals were within the reference region of 80% to 125%, equivalence of the two formulations can be concluded. No statistically significant period effect was found ($P = 0.77$). The within-subject coefficient of variation was estimated as 10.5%.

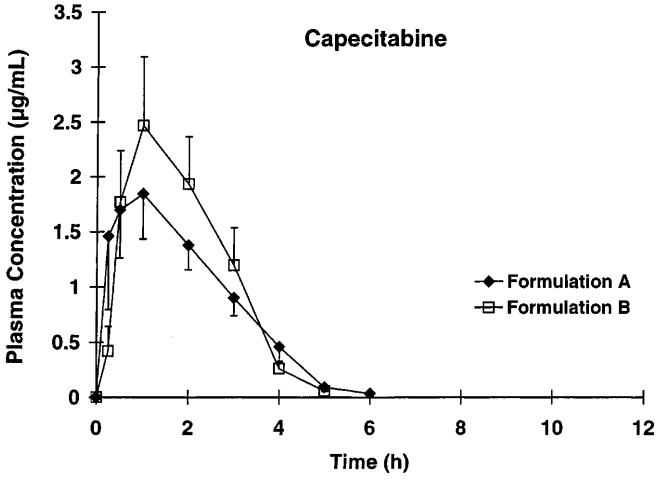


**Fig. 2** Mean plasma concentration profiles of capecitabine after a single oral administration of 2000 mg as formulation A (◆) or formulation B (□) ($n = 25$)

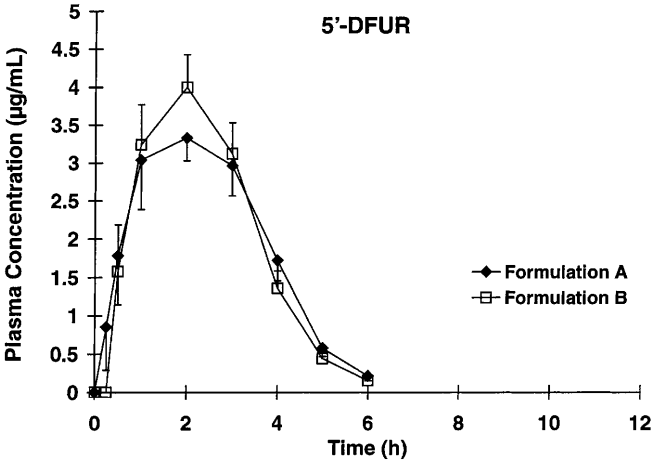


**Fig. 3** Mean plasma concentration profiles of 5′-DFUR after a single oral administration of 2000 mg capecitabine as formulation A (◆) or formulation B (□) ($n = 25$)

Following treatment with formulation B, the $C_{max}$ of 5′-DFUR (secondary parameter) was 111% of that following treatment with formulation A with a confidence interval of 95% to 136% (Table 2). As for the primary parameter $AUC_{0-\infty}$, there was no significant period effect for $C_{max}$ of 5′-DFUR ($P = 0.90$).

The $AUC_{0-\infty}$ of capecitabine after administration of formulation B was 114% of that following administration of formulation A. The relative 90% confidence intervals were 102% to 127% (Table 2).

Additional analyses performed for metabolites 5′-DFCR, 5-FU, and FBAL revealed that following treatment with formulation B, the $AUC_{0-\infty}$ was 106%, 102% and 99% of that following treatment with formulation A and the confidence intervals were all within the equivalence range 80–125%.

Total urinary recoveries (percentage of dose) following single administration of capecitabine as formulation A and as formulation B were 86.0% and 86.5%, respectively. The percentage of dose and the proportion recovered as each metabolite were similar for the two formulations A and B (Table 3). The majority of the dose was recovered as FBAL (61.2% after treatment with formulation A and 61.5% after treatment with formulation B). All other chemical species made only a minor contribution to urinary excretion (Table 3).

### Single factor analysis

Single factor analyses were used to assess the influence of selected parameters (age, gender, BSA, and creatinine clearance) on the log-transformed pharmacokinetic

**Table 1** Pharmacokinetic parameters of capecitabine and its metabolites following oral administration of 2000 mg as formulation A or formulation B ($n = 25$). $C_{max}$ and $AUC_{0-\infty}$ values are geometric means (CV) $t_{max}$ values are medians (min–max) and $t_{1/2}$ values are arithmetic means (CV)

| Metabolite | Parameter (unit) | Formulation A | Formulation B |
|---|---|---|---|
| Capecitabine | $C_{max}$ (μg/ml) | 2.85 (71%) | 3.96 (74%) |
| | $t_{max}$ (h) | 2.00 (0.27–4.05) | 2.00 (0.27–3.02) |
| | $AUC_{0-\infty}$ (μg · h/ml) | 4.94 (41%) | 5.62 (44%) |
| | $t_{1/2}$(h) | 0.48 (46%) | 0.58 (57%) |
| 5′-DFCR | $C_{max}$ (μg/ml) | 3.98 (74%) | 4.71 (74%) |
| | $t_{max}$(h) | 2.00 (0.27–4.05) | 2.00 (0.50–4.13) |
| | $AUC_{0-\infty}$ (μg · h/ml) | 9.60 (70%) | 10.1 (62%) |
| | $t_{1/2}$ (h) | 0.81 (21%) | 0.81 (33%) |
| 5′-DFUR | $C_{max}$ (μg/ml) | 5.18 (44%) | 5.73 (33%) |
| | $t_{max}$ (h) | 2.10 (0.50–4.05) | 2.00 (0.50–4.13) |
| | $AUC_{0-\infty}$ (μg · h/ml) | 11.9 (26%) | 12.1 (23%) |
| | $t_{1/2}$ (h) | 0.70 (30%) | 0.69 (25%) |
| 5-FU | $C_{max}$ (μg/ml) | 0.204 (52%) | 0.232 (45%) |
| | $t_{max}$ (h) | 2.10 (0.27–4.05) | 2.00 (0.50–4.13) |
| | $AUC_{0-\infty}$ (μg · h/ml) | 0.453 (40%) | 0.461 (32%) |
| | $t_{1/2}$ (h) | 0.70 (22%) | 0.75 (29%) |
| FBAL | $C_{max}$ (μg/ml) | 4.26 (28%) | 4.48 (30%) |
| | $t_{max}$ (h) | 4.02 (2.00–5.03) | 3.02 (2.00–4.25) |
| | $AUC_{0-\infty}$ (μg · h/ml) | 22.9 (32%) | 22.9 (34%) |
| | $t_{1/2}$ (h) | 2.67 (16%) | 2.71 (18%) |

**Table 2** Comparative statistical analysis of the primary pharmacokinetic parameter $AUC_{0-\infty}$ of 5′-DFUR and of the secondary parameters of 5′-DFUR ($C_{max}$) and capecitabine ($AUC_{0-\infty}$ and $C_{max}$)

| Metabolite | Pharmacokinetic variable | Estimate | | Formulation B relative to formulation A | | |
|---|---|---|---|---|---|---|
| | | Reference formulation A | Test formulation B | Relative estimate (%) | Relative 90% confidence interval (%) | Conclusion |
| 5′-DFUR | $AUC_{0-\infty}$ | 11.9 | 12.1 | 102 | 97–107 | Equivalence[a] |
| | $C_{max}$ | 5.18 | 5.73 | 111 | 95–136 | Equivalence[b] |
| Capecitabine | $AUC_{0-\infty}$ | 4.94 | 5.62 | 114 | 102–127 | – |
| | $C_{max}$ | 2.85 | 3.96 | 138 | 111–173 | – |

[a] Acceptance region for $AUC_{0-\infty}$: 80–125%
[b] Acceptance region for $C_{max}$: 70–143%

**Table 3** Summary of the recovery in urine (mean percentages of the dose) of capecitabine administered as each chemical species after administration of 2000 mg capecitabine as formulation A or formulation B to 23 cancer patients (patients 4 and 15 were excluded from the analysis – urine volume not recorded for the 0–12 h collection period and 150 ml of the 0–12 h urine sample discarded in error, respectively)

| | Formulation A ($n = 23$) | | Formulation B ($n = 23$) | |
|---|---|---|---|---|
| | Recovery (%) | Coefficient of variation (%) | Recovery (%) | Coefficient of variation (%) |
| Capecitabine | 2.68 | 39 | 2.69 | 32 |
| 5′-DFCR | 7.43 | 42 | 7.47 | 30 |
| 5′-DFUR | 9.83 | 44 | 10.0 | 34 |
| 5-FU | 0.707 | 63 | 0.597 | 38 |
| $FUH_2$ | 0.278 | 89 | 0.304 | 60 |
| FUPA | 3.89 | 40 | 3.94 | 43 |
| FBAL | 61.2 | 28 | 61.5 | 24 |
| Total | 86.0 | 28 | 86.5 | 21 |

parameters $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites (Table 4). No statistically significant effect of age ($P > 0.15$) was observed in the study population (age range 41–80 years) for $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites (Figs. 4 and 5).

The effect of gender (9 females and 16 males) on $AUC_{0-\infty}$ and $C_{max}$ on capecitabine and its metabolites is shown in Table 4 and Fig. 6. A statistically significant effect was obtained for $AUC_{0-\infty}$ and $C_{max}$ of intact drug and $C_{max}$ of FBAL and borderline significant effects ($P = 0.01$–$0.05$) were observed for $AUC_{0-\infty}$ of all other metabolites. The AUC of intact drug is 87% greater in females than in males, whereas for the AUC of FBAL, the increase was 37% in females (Table 4).

There was borderline significance ($P = 0.01$–$0.05$) for the $AUC_{0-\infty}$ of capecitabine and 5-FU and a trend for the $C_{max}$ of FBAL to increase with increasing BSA (Table 4 and Fig. 7).

Creatinine clearance (range 42–129 ml/min) had no statistically significant effect on $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites, except for a trend in the case of $AUC_{0-\infty}$ of FBAL (Table 4).

## Multifactor analysis

To select influential covariables, a significance level of $\alpha = 0.01$ was used. Only the variable 'gender' is in the final model as an influential variable. It was found that

**Table 4** Effect of gender, body surface area, and creatinine clearance on the pharmacokinetic parameter $AUC_{0-\infty}$ and $C_{max}$ of capecitabine and its metabolites

| | Pharmacokinetic parameter | Gender | | Body surface area | | Creatinine clearance | |
|---|---|---|---|---|---|---|---|
| | | *P*-value | Magnitude (%)[a] | *P*-value | Magnitude (%)[b] | *P*-value | Magnitude (%)[c] |
| Capecitabine | AUC | 0.0001 | 87 | 0.03 | 64 | 0.29 | 26 |
| | $C_{max}$ | 0.005 | 108 | 0.53 | 26 | 0.95 | −2 |
| 5′-DFCR | AUC | 0.03 | 67 | 0.32 | 37 | 0.66 | 14 |
| | $C_{max}$ | 0.04 | 73 | 0.69 | 16 | 0.94 | 3 |
| 5′-DFUR | AUC | 0.04 | 21 | 0.06 | 25 | 0.14 | 19 |
| | $C_{max}$ | 0.20 | 19 | 0.87 | 3 | 0.93 | 2 |
| 5-FU | AUC | 0.03 | 31 | 0.04 | 41 | 0.89 | 2 |
| | $C_{max}$ | 0.08 | 33 | 0.67 | 11 | 0.40 | −17 |
| FBAL | AUC | 0.02 | 37 | 0.09 | 36 | 0.06 | 40 |
| | $C_{max}$ | 0.0006 | 46 | 0.01 | 45 | 0.12 | 26 |

[a] Percentage greater in female patients than in male patients
[b] Percentage change for a 30% decrease in body surface area
[c] Percentage change for a 50% decrease in creatinine clearance

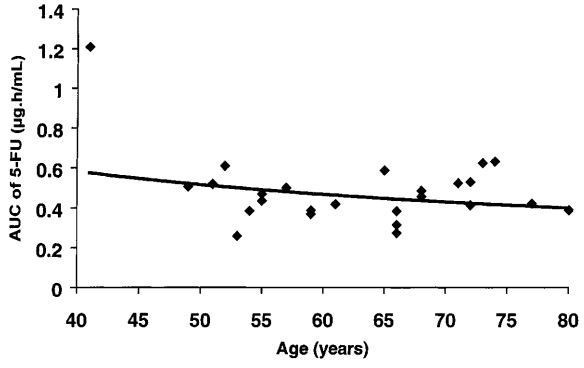


**Fig. 4** AUC of 5-FU (µg · h/ml) as a function of age ($R^2$ = 0.0810)

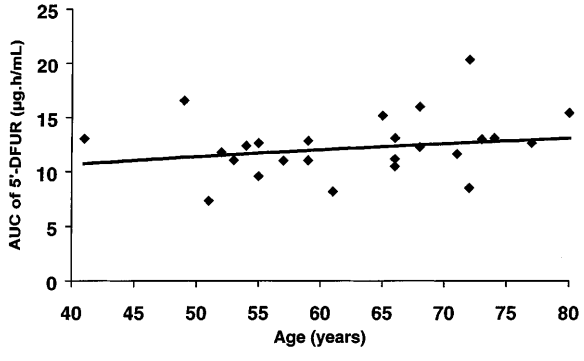


**Fig. 5** AUC of 5′-DFUR (µg · h/ml) as a function of age ($R^2$ = 0.0473)

$AUC_{0-\infty}$ and $C_{max}$ of intact drug and $C_{max}$ of FBAL were greater in females than in males.

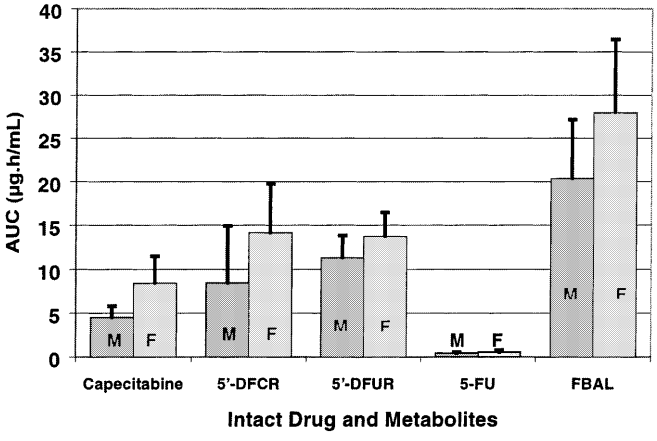


**Fig. 6** AUC of capecitabine and its metabolites as a function of gender (geometric means and geometric SD)

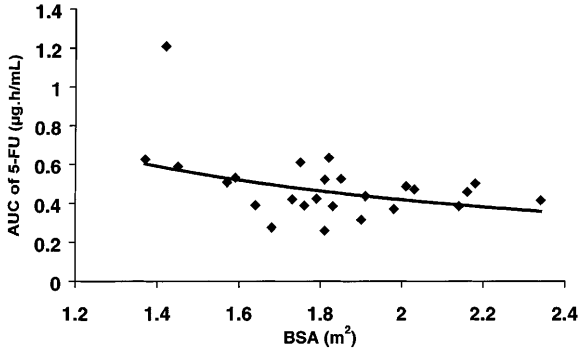


**Fig. 7** AUC of 5-FU (µg · h/ml) as a function of BSA ($R^2$ = 0.1784)

## Discussion

The primary objective of this study was to compare the bioavailability of 5′-DFUR following administration of two tablet formulations of capecitabine. Since the composition and dissolution profiles of the two tablets were very similar, bioequivalence between the two formulations was the expected outcome of this study.

### Rationale for choosing AUC of 5′-DFUR as primary parameter

Since 5′-DFUR is a major metabolite of capecitabine in plasma and is the direct precursor of the cytotoxic agent 5-FU, it is likely that it reflects much better the rate and extent to which 5-FU reaches the site of action (tumor) than plasma concentrations of the other chemical species. For this reason, 5′-DFUR was chosen as the primary metabolite to test bioequivalence.

AUC but not $C_{max}$ was chosen as the primary parameter for assessment of the bioequivalence before the start of the study. This choice was based on several factors. First, the maximum tolerated dose (MTD) of 3000 mg/m$^2$ per day for an intermittent dosing schedule produced a mean $C_{max}$ of 13.4 μg/ml for 5′-DFUR, which is greater than the value of 6.1 μg/ml obtained at the MTD of 1657 mg/m$^2$ per day following a continuous dosing schedule [12]. Thus, safety in humans does not correlate with $C_{max}$ values. Furthermore, preclinical experiments in mice have shown that the antitumor activity is similar when the same daily dose of capecitabine is administered once or twice daily (H. Ishitsuka, data on file, F. Hoffmann-La Roche). Assuming dose-proportional pharmacokinetics in mice, the once-daily dosing would have produced a $C_{max}$ twice as high as the twice-daily dosing regimen with the same AUC. Therefore, this suggests that AUC and not $C_{max}$ correlates with antitumor activity in mice.

In vitro studies investigating the cell killing effect of 5-FU have shown that 5-FU is an 'AUC-dependent drug', that is, short exposure requires high concentrations to obtain the cell killing effect whereas the same effect can be obtained with longer exposure to lower concentrations [13]. Finally, in the clinical use of cytotoxic agents, efficacy generally correlates better with AUC rather than with $C_{max}$ [14]. Based on these facts, the AUC of 5′-DFUR was chosen as the only primary parameter for the assessment of bioequivalence.

### Bioequivalence for 5′-DFUR

Based on this primary parameter, equivalence was concluded, since the 90% confidence interval of the estimate of the ratio of the AUC of 5′-DFUR after administration of formulation B to that after administration of formulation A was within the accepted limits of 80% to 125%. For the secondary parameter $C_{max}$ of 5′-DFUR, the 90% confidence interval of the estimate of formulation B relative to that of formulation A was within the wider acceptance region of 70% to 143%. This region for $C_{max}$ is justified in view of the high intrapatient variability (CV = 32%).

There was no difference seen between the $t_{max}$ values of the two formulations. Overall, these results suggest no clinically significant difference between the two formulations in the extent to which 5′-DFUR reaches the systemic circulation and no relevant difference in the rate at which 5′-DFUR reaches the systemic circulation. For the other metabolites 5′-DFCR, 5-FU, and FBAL, similar results were obtained.

### Bioequivalence for intact drug

For capecitabine, the extent and rate at which it reached the systemic circulation appeared to be higher following treatment with formulation B compared with formulation A and equivalence could not be concluded. Examination of individual data indicated that two patients did not consume breakfast prior to administration of formulation B as specified in the protocol. Since food intake is known to decrease AUC and $C_{max}$ of capecitabine [15], this effect might have influenced the outcome of the analysis. Indeed, after exclusion of these two patients, the $AUC_{0-\infty}$ of capecitabine following ingestion of formulation B was 109% of that following ingestion of formulation A and the confidence interval was 98% to 120%.

### Exploratory analyses for the effect of selected variables

Single and multifactor analysis revealed no clinically significant effects of the investigated covariates 'age', 'gender', 'BSA', and '$Cl_{CR}$' on $AUC_{0-\infty}$ or $C_{max}$ of capecitabine or its metabolites. A statistically significant effect of 'gender' was obtained only for $AUC_{0-\infty}$ and $C_{max}$ of intact drug and for $C_{max}$ of FBAL, which were higher in females than in males. However, this effect is not of clinical significance as both intact drug and FBAL have no intrinsic antiproliferative activity. Comparison of the safety and efficacy of capecitabine showed no differences between male and female patients with colorectal cancer (Subgroups analysis on safety and efficacy, data on file, Hoffmann La Roche), which confirms our interpretation of these pharmacokinetic results. Since these analyses for the effect of selected variables were exploratory and the number of patients was small, no firm conclusions can be drawn from these results.

The effect of adjusting dose for BSA is unclear for most cytotoxic agents [16, 17]. One small previous study has suggested that both gender and BSA have important effects on the pharmacokinetics of 5-FU given as an i.v. infusion [18]. However, in the current study, BSA had only a borderline effect on the AUC of 5-FU. As the magnitude of this effect was small, one can assume that it will be not clinically significant. In oncology, dosing is traditionally based on BSA [17, 19]. The results obtained in this study suggest that dose adjustments based on BSA should reduce interpatient variability in systemic exposure. Therefore the current practice of BSA-adjusted dosing of capecitabine might be of benefit to the patients. Based on the results of the regression analyses, no subgroup of patients with different pharmacokinetic characteristics could be identified.

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