Dose and time dependencies of 5-fluorouracil pharmacokinetics

Objectives: The purpose of this study was to examine the interpatient and intrapatient variability of the Michaelis-Menten plasma parameters of 5-fluorouracil administered according to a schedule combining a bolus of 400 mg/m² followed by 22-hour infusion of 600 mg/m² for 2 consecutive days.

Patients: A pharmacokinetic population approach was used to analyze the data from 21 patients with colorectal cancer.

Results: The 5-fluorouracil plasma concentrations versus time were best described by a two-compartment model with nonlinear elimination from the central compartment. The relationships between the pharmacokinetic parameters and patient characteristics were tested. On day 1 the mean values (with interindividual variability as expressed by the coefficient of variation) were 1390 mg · h⁻¹ (20%), and 5.57 mg · L⁻¹ (22%) for the maximum rate of elimination, and the half-saturating plasma concentration. The maximum rate of elimination was positively correlated to the body surface area and the percentage of liver involvement by metastatic disease determined by tomodensitometric examination. The model was successfully tested with independent data sets corresponding to other schedules. The analysis of this intrapatient variability showed that the half-saturating plasma concentration increased from day 1 to day 2, especially in the patients with low lymphocyte cell dihydropyrimidine dehydrogenase activity.

Conclusion: The pharmacokinetic parameters obtained in this study would be useful to predict the 5-fluorouracil plasma concentrations following other schedules of administration of 5-fluorouracil and to study the possible pharmacokinetic interactions between 5-fluorouracil and other drugs. (Clin Pharmacol Ther 2000;68:270-9.)

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Fluorouracil is widely used for many types of cancers. In particular, 5-fluorouracil remains the first line of therapy for patients with advanced colorectal cancer. However, the optimal infusion regimen for this drug is not known, and it is presently administered with a wide range of treatment schedules.[1-4] Both the pharmacodynamics and pharmacokinetics of 5-fluorouracil are schedule dependent. The dose-limiting toxicity is mucositis for continuous infusion and myelosuppression for the bolus injection. Regarding the mechanisms of cytotoxicity, prolonged exposure to 5-fluorouracil is required for thymidylate synthase inhibition, then high concentrations (such as those reached after short-term administration) are required for fluoridine triphosphate incorporation into ribonucleic acid.[5] The pharmacokinetics of 5-fluorouracil have been extensively studied. There are two major factors of pharmacokinetic variability. First, the drug displays nonlinear pharmacokinetics as a result of saturable metabolism located mainly in the liver.[6,7] Then the reported plasma clearance of 5-fluorouracil depends on the schedule of administration of 5-fluorouracil that varied largely according to the clinical protocols. During continuous infusion (infusion rates ranged between 300 and 1000 mg/m²/d are used), clearance of 5-fluorouracil ranged

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Table I, A. Characteristics of the 21 patients studied

Characteristics	Median	Range
Age (year)	65	40-79
Body surface area (m^2)	1.71	1.46-2.00
Weight (kg)	68	48-97
Serum creatinine (μmol $\cdot$ L^{-1})	81	53-177
Proteinemia (g $\cdot$ L^{-1})	71	55-81
Albuminemia (g $\cdot$ L^{-1})	38	27-44
Bilirubinemia (μmol $\cdot$ L^{-1})	9.4	6.2-33.4
PMNC DPD (pmol $\cdot$ min^{-1} $\cdot$ mg^{-1} protein)	140	44-392

PMNC DPD, Peripheral mononuclear cell dihydropyrimidine dehydrogenase activity.

Table I, B. Characteristics of the 21 patients studied

Characteristics	No.
Sex	
Male	14
Female	7
Performance status score*	
0	1
1	12
2	8
Liver metastatic involvement†	
No metastasis	5
≤25%	9
>25% and ≤50%	5
>50% and ≤75%	2

*From the World Health Organization.
†Percentage of liver replaced by tumor determined semiquantitatively by tomodensitometric examination.

between 100 and 350 L/h. After intravenous bolus injection of doses varying between 300 and 600 mg/m^2, clearance ranged between 30 and 120 L/h.[7] Second, there is a high interindividual variability of 5-fluorouracil pharmacokinetics. The individual clearance of 5-fluorouracil has been correlated with several covariables. Clearance of 5-fluorouracil was significantly reduced by increased age[8] and was lower in women compared with men.[9,10] Moreover, the dihydropyrimidine dehydrogenase enzyme plays a major role in liver metabolism of 5-fluorouracil, and it has been shown that dihydropyrimidine dehydrogenase activity measured in circulating lymphocytes is positively correlated to clearance of 5-fluorouracil.[11] Dihydropyrimidine dehydrogenase inhibitors are currently developed in combination with 5-fluorouracil both to prolong exposure to 5-fluorouracil and to decrease the interindividual variability of metabolism. Intraindividual pharmacokinetic variabilities were also noted. During a 5-day continuous infusion, several groups reported higher plasma concentrations during the second part of a 5-day infusion.[8,12] Last, circadian variations in the plasma concentration of 5-fluorouracil probably caused by circadian pattern for dihydropyrimidine dehydrogenase activity were shown.[13,14]

Numerous studies described the nonlinear pharmacokinetics of 5-fluorouracil. But only a few of them[15-21] stated values for the in vivo pharmacokinetic parameters in human beings corresponding to the saturable metabolism of 5-fluorouracil: the maximum rate of elimination (V_{max}) and the half-saturating plasma concentration (K_m) for the maximal rate of elimination and the concentration at which the elimination rate was half of V_{max}, respectively. Moreover, contradictory mean values have been proposed, with K_m ranging between 1.42 mg $\cdot$ L^{-1}, as reported by Wagner et al,[21] and 27 mg $\cdot$ L^{-1}, as reported by Sandstrom et al,[20] and with V_{max}

ranging between 944 and 3471 mg $\cdot$ h^{-1}, as reported by Port et al.[18,19]

In this study we have investigated the pharmacokinetics of 5-fluorouracil administered according to a schedule (usually called *LV5FU2*) combining 2 hours infusion of folinic acid followed by 10 minutes infusion of 400 mg/m^2 5-fluorouracil and 22 hours infusion of 600 mg/m^2 5-fluorouracil for 2 consecutive days every 2 weeks; this schedule was recently described by de Gramont et al[22] as more effective and less toxic than other monthly regimens. The objectives of our study were to determine the Michaelis-Menten parameters (ie, V_{max} and K_m) of 5-fluorouracil by a pharmacokinetic population approach from plasma concentrations versus time data and to test relationships between these parameters and patient characteristics.

PATIENTS AND METHODS

Patients and treatment schedule. Twenty-one patients with an advanced, histologically proven colorectal carcinoma were studied. Written informed consent was obtained from all patients. The primary tumor was within the colon and the rectum for 11 and 10 patients, respectively. Seven of the 21 patients had undergone prior chemotherapy. Thirteen, five, and three patients had, respectively, one, two, and three or more different metastatic sites. Demographic data on the patients are given in Table I. All patients received folinic acid followed by 5-fluorouracil administered daily for 2 consecutive days with a controlled flow pump. The treatment was given as folinic acid (200 mg/m^2 in 5% dextrose) by intravenous infusion for 2 hours followed by 5-fluorouracil by intravenous infusion for 10 minutes (400 mg/m^2 in 5% dextrose) and then continuous intravenous infusion (600 mg/m^2 in 5% dextrose) for 22

hours. This whole regimen was repeated on day 2 and was given on a 2-week cycle until disease progression. Physical examination and full blood cell count were performed every cycle. Hemogram was recorded twice weekly after the two first cycles.

Pharmacokinetic and biologic investigations. The pharmacokinetic study was performed during the first cycle of the 5-fluorouracil protocol. Blood samples (4 mL each into tubes containing lithium heparin) were obtained from a small heparinized catheter introduced into a peripheral vein in the arm opposite of the infusion arm. Blood sampling times were as follows: before administration of 5-fluorouracil, at 0, 0.25, 0.5, 1, 2, 6, 12, and 21 hours after the end of the first 10 minutes of infusion, and at 0, 1, and 21 hours after the end of the second 10 minutes of infusion. The plasma was immediately separated by centrifugation at 4°C and stored at –20°C until analysis. Plasma concentrations of 5-fluorouracil were measured by reverse-phase HPLC with the previously described method,[23] with some modifications regarding the extraction part of the analysis. Briefly, the compounds, with 5-fluorouracil and 5-fluorocytosine used as an internal standard, were extracted from plasma by isopropanol-ethyl acetate (85:15 vol/vol) in the presence of ammonium sulfate to precipitate the proteins according to the previously described method.[24] The organic phase was dried at 56°C under nitrogen. Liquid chromatography was carried out by use of a Nucleosil C18 (7 µm, 250 × 4.6 mm) column (Bischoff, Leonberg, Germany) eluted by a mobil phase consisting of 50 mmol/L potassium phosphate buffer (pH 3.0) (1.0 mL/min) and an ultraviolet detection at 265 nm. The limit of quantification was 50 ng/mL plasma. The accuracy and precision of the method was assessed by seeding two plasma at 5-fluorouracil at nominal values of 500 and 2500 ng/mL: the interassay (n = 6) coefficients of variation for precision were, respectively, 9.2% and 8.3%. The concentrations for the seeded control samples were found to be within –11% and +15% of the nominal values.

In addition, 20 mL blood was collected in Vacutainer cell preparation tubes (Becton Dickinson & Co, Rutherford, NJ) before administration of 5-fluorouracil for peripheral mononuclear cell dihydropyrimidine dehydrogenase determination. Contaminating red blood cells were hypotonically lysed. Peripheral mononuclear cells were suspended in 35 mmol/L sodium phosphate buffer containing 10% glycerol and stored at –80°C until analysis (within 2 months). The cytosolic dihydropyrimidine dehydrogenase activity was measured with the previously reported method[14] after selective ultracentrifugation of peripheral mononuclear cell. The

assay consisted of the incubation of 50 µL of peripheral mononuclear cell cytosol (0.05 to 0.10 mg of cytosolic protein) with ^{14}C-5FUH$_2$ (20 µmol/L final), β–reduced nicotinamide adenine dinucleotide phosphate (250 µmol/L final), and magnesium chloride (2.5 mmol/L final). Total volume was 125 µL (in 35 mmol sodium phosphate buffer pH 7.5 containing sodium azide 0.001 mol/L). The duration of incubation was 30 minutes at 37°C. The reaction was stopped by addition of 125 µL ice-cold ethanol followed by 30 minutes storage at –20°C. After centrifugation, the ^{14}C-5FUH$_2$ in the supernatant was analyzed by use of the previously reported reverse-phase HPLC method. Mobil phase at a flow rate of 0.8 mL/min was eluted in fractions of 0.8 mL. The radioactivity of the fractions eluted between 7 and 10 minutes corresponded to the formed 5FUH$_2$. Cytosolic protein concentrations were determined by the dye-binding method (Bio-Rad SA, Ivry sur Seine, France) with bovine γ-globulin used as standard. Thus enzyme activity was expressed as nmoles of 5FUH$_2$ formed per minute and per milligram of protein.

Pharmacokinetic analysis. Plasma 5-fluorouracil levels were analyzed with the nonlinear mixed-effects modeling program (NONMEM,[25] version V, level 1.1) with the first-order method and the PREDPP package (University of California, San Francisco, Calif).[26] A proportional error model was used for the interpatient variables. A combination model (ie, additive plus proportional) was used for residual variability.

In the first phase of analysis, the data collected within the first 24-hour period of treatment (day 1) were used to test the different pharmacokinetic models: linear elimination (according to a rate constant [k_{10}]) and nonlinear elimination (according to the Michaelis-Menten parameters). For each of these models, one- (corresponding parameter: central volume [Vc]) and two-compartment (corresponding parameters: Vc, rate constant from central to peripheral volume [k_{12}], and rate constant from peripheral to central volume [k_{21}]) models were tested. Then the influence of 13 patient covariables on the pharmacokinetic parameters was tested. These covariables were weight, height, body surface area calculated according to the formula of Dubois and Dubois,[27] sex, age, proteinemia, albuminemia, bilirubinemia, presence of hepatic metastasis (a score ranged between 0 and 3 was alocated to hepatic metastasis covariable in function of the percentage of liver replaced by tumor determined semiquantitatively by tomodensitometric examination: score of 0 if no liver metastasis, 1 for ≤25% of liver replaced by tumor, 2 for >25% and ≤50%, 3 for >50% and ≤ 75%), performance status score from the World Health Organization,
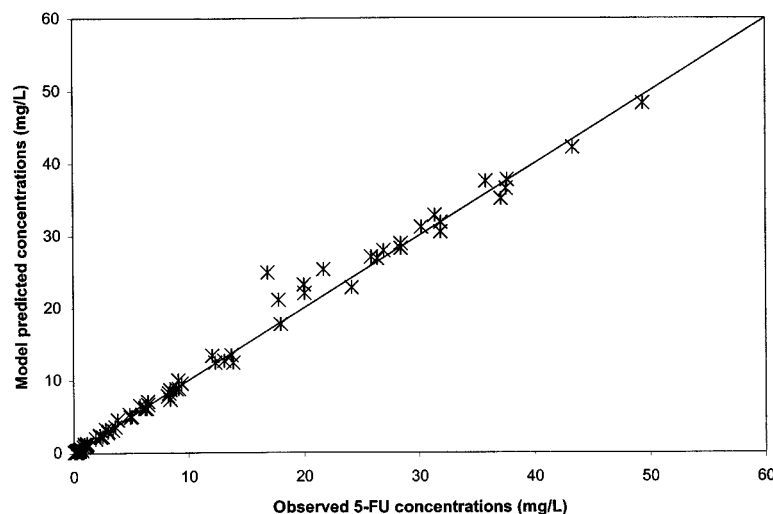
Fig 1. Analysis of 5-fluorouracil plasma concentrations according to two-compartment model with nonlinear elimination from central compartment: individual model-predicted concentrations versus observed concentrations (n = 21 patients). *Continuous line* is the line of identity.

Table II. Testing of the pharmacokinetics and the covariable model from data of day 1

Model investigated		Change in objective function*	P value
Pharmacokinetic model	Two-compartment and non-linear elimination†	—	—
	Two-compartment and linear elimination‡	+228	<.0005
	One-compartment and non-linear elimination‡	+92	<.0005
Covariable model	$V_{max} = \theta 1 \cdot BSA \cdot (1 + \theta 2 \cdot META\S)$ with mean value ($\pm CI95\%$): $\theta 1 = 751$ (± 105) mg $\cdot$ h^{-1} $\cdot$ m^{-2}, $\theta 2 = 0.068$ (± 0.052)†	—	—
	V_{max} independent of BSA‡	+10	<.01
	V_{max} independent of META‡	+5	<.05

BSA, Body surface area in m^2.
*By comparison with the final model.
†Final model.
‡Alternative model tested.
§Hepatic metastases status: META = 0 if no liver metastasis, 1 if ≤25% of liver replaced by tumor, 2 if >25% and ≤50%, and 3 if >50% and ≤75%.

peripheral mononuclear cell dihydropyrimidine dehydrogenase activity, creatinine clearance calculated according to the Cockcroft-Gault equation,[28] and clock-time (0 if sample obtained between 7 AM and 7 PM, 1 in other cases). In a second phase of analysis, the data collected during day 2 were analyzed simultaneously with those of day 1 according to the best pharmacokinetic model. In fitting the data, NONMEM computed the value of a statistical function, the minimal value of the objective function, which is equal to minus twice the log likelihood. Both structural model selection and the testing of the relationships between covariables and pharmacokinetic parameters were based on the objective function value. The structural model that was

selected gave the lowest value of the objective function. If two models gave the same or similar values, then the most parsimonious model was chosen. For testing of the covariables, the different models were compared by use of the approximation to the χ^2 distribution of the objective function value of the reduced model (eg, model without covariable) minus that of the full model (eg, model with covariable); the number of degrees of freedom is equal to the difference in the number of parameters between two nested models. For example, a difference in the objective function larger than 3.8 (associated with a *P* value of < .05 and degree of freedom of 1) was required to consider the model with nonlinear elimination (corresponding parameters: V_{max} and K_m) more
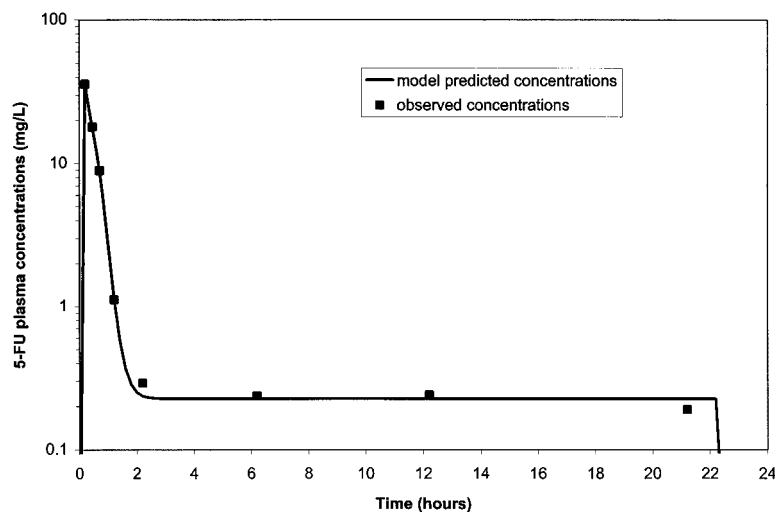
Fig 2. Plasma concentrations of 5-fluorouracil given by 10-minute infusion of 400 mg/m² followed by 22-hour infusion of 600 mg/m² in one patient. *Curve* corresponding to the two-compartment model with nonlinear elimination from the central compartment is shown.

appropriate than the model with linear elimination (corresponding parameter for elimination was clearance).

Testing of the pharmacokinetic model with independent data. The final pharmacokinetic model was tested with four independent data sets corresponding to other schedules of 5-fluorouracil administration by comparing graphically the observed concentrations and the model predicted values. Plasma data of 5-fluorouracil from the Department of Medicine and Therapeutics of the University of Aberdeen were obtained in 10 patients with colorectal cancer after 10 minutes intravenous infusion of 370 mg/m².[29] Data from the Centre Paul-Papin (Angers) corresponded to 4- and 8-hour intravenous infusion of 1000 and 1250 mg/m² (20 patients for each group, randomly chosen from a larger database[30]), respectively. Finally, data from the Centre René-Gauducheau (Nantes) were obtained during 96-hour continuous intravenous infusion of 1 g/m²/d.[12]

RESULTS

Pharmacokinetic model. Pharmacokinetic data of 5-fluorouracil were available in 21 patients. The data of 5-fluorouracil at day 1 were best described by a two-compartment model with a nonlinear elimination from the central compartment (Table II). No interindividual variability on k_{12} was used because the elimination of this intersubject variability term improved the precision on estimates of the remaining parameters. There was good agreement between model-predicted and observed concentrations (Figs 1 and 2). Mean (± SD) maximum plasma concentrations (end of 10-minute infusion) and

concentration before the end of the 22-hour infusion were, respectively, 31.3 ± 8.9 and 0.178 ± 0.046 mg/L. The estimated parameters for residual variability were 10.4% and 0.062 mg · L⁻¹ for, respectively, the proportional and the additional part of the combination model.

Relationships between covariables and day 1 pharmacokinetic parameters. The mean pharmacokinetic parameters and their interindividual variables are summarized in Table III. No covariable was found correlated with K_m or k_{21}. During the individual testing, four covariables were significantly and positively correlated with V_{max}: body surface area (BSA), peripheral mononuclear cell dihydropyrimidine dehydrogenase activity (DPD), percentage of liver involvement by metastatic disease (META), and proteinemia (PROT). After independent deletion of each of these covariables from an intermediate model, such as follows:

$$V_{max} = \theta 1 \cdot BSA \cdot (1 + \theta 2 \cdot DPD) \cdot (1 + \theta 3 \cdot META) \cdot (1 + \theta 4 \cdot PROT)$$

the final model was as follows (Table II):

$$V_{max} = \theta 1 \cdot BSA \cdot (1 + \theta 2 \cdot META)$$

Testing of the pharmacokinetic model with independent data. Fig 3 shows the mean observed 5-fluorouracil plasma concentrations corresponding to the four independent data sets and concentrations predicted according to the two-compartment model with nonlinear elimination, the mean pharmacokinetic parameters (day 1) shown in Table III (for central volume of dis-
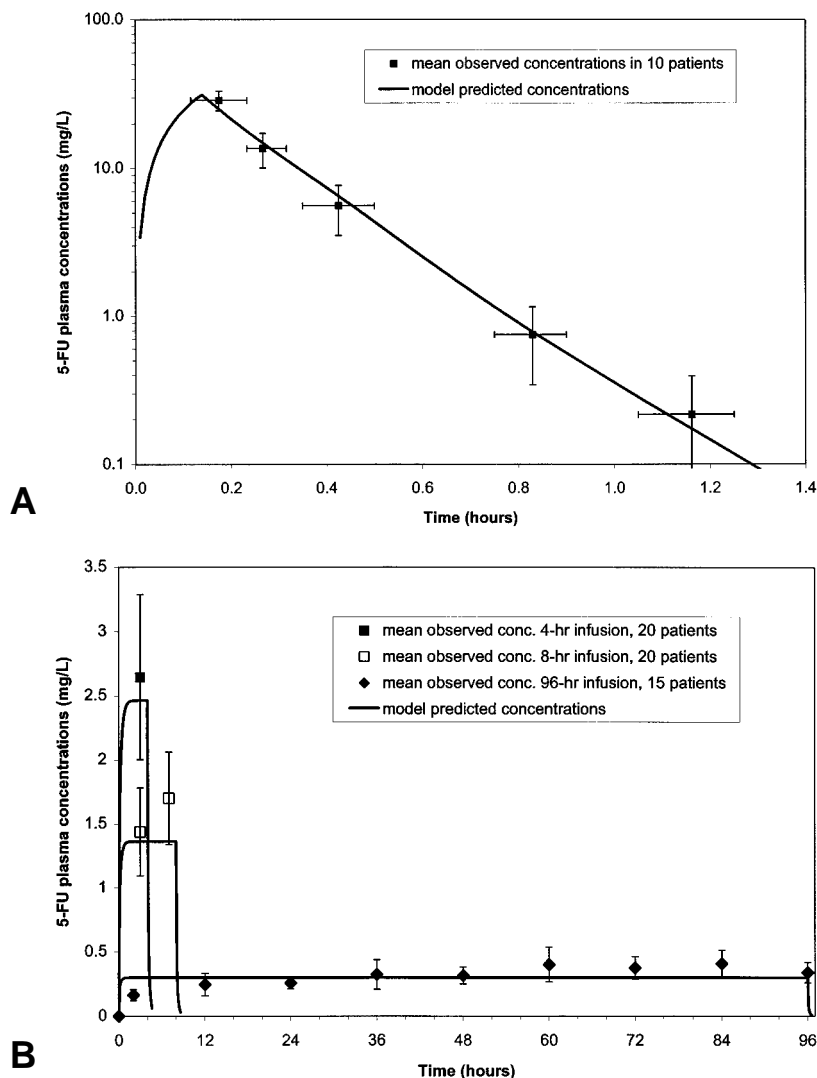
Fig 3. Testing of two-compartment model and nonlinear elimination with independent data sets. Model predicted according to mean pharmacokinetic parameters and mean (±95% confidence intervals) observed 5-fluorouracil concentrations after 10-minute intravenous infusion of 370 mg/m² **(A),** horizontal bars correspond to the range of sample time) or 4-hour (1000 mg/m²), 8-hour (1250 mg/m²), and 96-hour (4000 mg/m²) intravenous infusion **(B)**.

tribution, k_{12}, k_{21}, and K_m), and $V_{max} = \theta 1 \cdot BSA$ (with $\theta 1 = 831$ mg $\cdot$ h^{-1} $\cdot$ m^{-2} corresponding to the final model when the covariable "percentage of liver involvement by metastatic disease" is not available).

Comparison of pharmacokinetic parameters between day 1 and day 2. Graphical examination of the mean plasma concentrations versus time showed that elimination of 5-fluorouracil decreased from day 1 to day 2 of treatment (Fig 4). To investigate the modification of pharmacokinetic parameters between the two days, the parameters K_m and V_{max} were allowed to vary from one day to

the other for each patient. In first time, no covariable was taken into account. The results were compared with those when K_m and V_{max} were constant within the 2 days. With the objective function used as criterion, best fit was obtained for K_m as parameter varying from day 1 to day 2 (Table IV). Then relationships between K_m at day 2 and covariables were tested. K_m at day 2 was found to be significantly correlated with dihydropyrimidine dehydrogenase according to the equation:
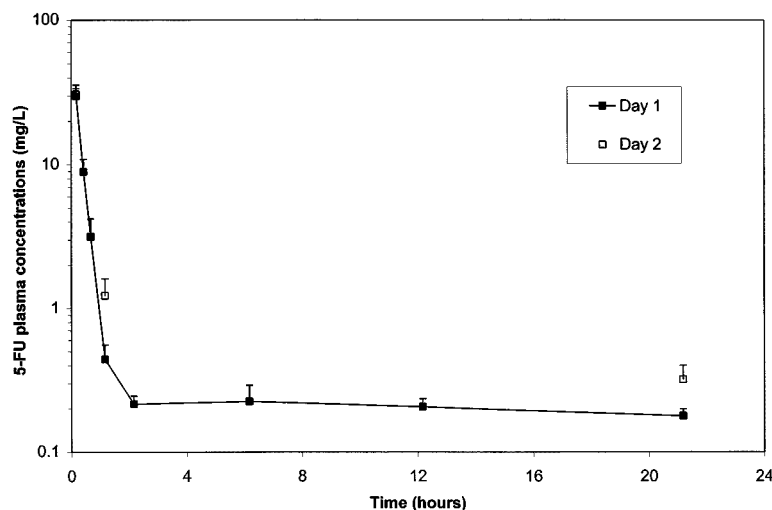
$$K_{mDay\,2} = \theta 1 \cdot (1 - \theta 2 \cdot DPD)$$

Fig 4. Mean (±95% confidence interval) plasma concentrations of 5-fluorouracil after intravenous administration according to 10-minute infusion 400 mg/m² 5-fluorouracil immediately followed by 22-hour infusion 600 mg/m² 5-fluorouracil for 2 consecutive days (n = 21 patients).

Table III. Pharmacokinetic parameters of 5-fluorouracil at day 1

Parameters	Mean (95% confidence interval)	Interindividual variability* (95% confidence interval)
Central volume of distribution (L)	12.7 (9.6-15.8)	31% (11%-42%)
k_{12} (rate constant from central to peripheral volume) (h^{-1})	5.35 (3.32-7.38)	—†
k_{21} (rate constant from peripheral to central volume) (h^{-1})	5.69 (4.00-7.38)	27% (0%-43%)
V_{max} (maximum rate of elimination) (mg · h^{-1})	1390 (1213-1567)	20% (2%-28%)
K_m (mg · L^{-1})	5.57 (4.36-6.78)	22% (0%-36%)

*Coefficient of variation.
†Interindividual variability on k_{12} was fixed to zero.

with $\theta 1 = 11.6$ mg/L (95% confidence interval: 7.5 to 15.7 mg/L) and $\theta 2 = 0.99$ $nmol^{-1}$ · min · mg (95% CI: 0.48 to 1.50 $nmol^{-1}$ · min · mg).

DISCUSSION

Simultaneous analysis of concentrations versus time data from 21 patients allowed us to use a relatively complex pharmacokinetic model: two-compartment model with nonlinear elimination. A similar model was previously proposed by Collins et al,[15] who determined, by comparison of model simulations with literature data, a K_m value lower than the mean value obtained in this study (1.95 versus 5.57 mg · L^{-1}, respectively). More generally, the discrepancies between the values of K_m and V_{max} proposed in the literature can be explained by the diversity of the methodologies used for determining 5-fluorouracil concentrations: positron emission tomography,[17] nuclear magnetic resonance

spectroscopy,[18,19] or HPLC measurements.[15,16,21] For positron emission tomography and nuclear magnetic resonance spectroscopy methods, liver concentrations were obtained. Hepatic arterial infusion and sampling were also used.[15,21] In fact, the study performed by Sandström et al[20] is the only one in which the method was similar to that of our analysis: population pharmacokinetic analysis with NONMEM of plasma 5-fluorouracil after intravenous bolus injections of 600 mg/m² in patients with breast cancer. They obtained mean K_m and V_{max} (27 mg · L^{-1} and 2528 mg · h^{-1}, respectively) that differ largely from our values (5.57 mg · L^{-1} and 1390 mg · h^{-1}, respectively). This schedule of administration combining two very different rates of administration (ie, 2400 mg/m²/h and 27 mg/m²/h) was more appropriate to determine the parameters corresponding to a saturable process than the unique bolus injection. The V_{max} and K_m can be estimated more accurately

Table IV. Mean values (95% confidence interval) of maximum rate of elimination (V_{max}) and Michaelis constant (K_m) of 5-fluorouracil at day 1 and day 2

	K_m	V_{max}	Change in objective function*	P value
Final model				
K_m varying, V_{max} constant			—	—
Day 1	6.08 (3.81-8.35)			
Day 2	9.83 (6.68-13.2)			
Constant		1400 (1093-1707)		
Alternative models tested				
Both K_m and V_{max} constant			73.8	<.0005
Constant	6.05 (4.50-7.60)	1260 (1050-1470)		
K_m constant, V_{max} varying			21.7	
Day 1		1520 (1123-1917)		
Day 2		1240 (954-1526)		
Constant	7.24 (4.38-10.1)			
Both K_m and V_{max} varying			14.9	<.001
Day 1	5.83 (4.07-7.59)	1370 (1152-1588)		
Day 2	10.4 (0-19.1)	1490 (688-2292)		

*By comparison with the final model.

when data corresponding to different levels of saturation are available.

The interest in knowing these parameters is in being able to predict the concentrations after administration of higher doses of 5-fluorouracil. For drugs with nonlinear pharmacokinetics, simulations require the knowledge of parameters such as K_m and V_{max}. Now the good tolerance of the protocol LV5FU2 has already stimulated clinical trials with higher doses of 5-fluorouracil to increase the probability of efficacy.[3] For example, an increase of 25% of 5-fluorouracil bolus dose administered in this study would lead to an increase of 100% plasma 5-fluorouracil concentrations at 30 minutes after administration. The testing of the pharmacokinetic model with independent data sets confirmed that it is possible to predict accurately the mean observed 5-fluorouracil plasma concentrations (particularly those corresponding to the 10-minute and 4-hour intravenous infusions) from the mean pharmacokinetic parameters of this study. For the 8-hour and 96-hour intravenous infusions, good agreement was observed for the first observed concentrations, but the later observed values were larger than the predicted ones; this point will be discussed below. Finally, the mean values we obtained for K_m and V_{max} are consistent with the mean 5-fluorouracil clearance previously observed during 5-day continuous venous infusion of 1 g/m²/d: 235 L · h⁻¹.[8] Indeed, the ratio $V_{max}/K_m = 250$ L · h⁻¹ approximates the clearance when plasma 5-fluorouracil concentrations are far below K_m, which is the case for this schedule of administration.

The second advantage of having an adequate pharmacokinetic model for 5-fluorouracil over the model-independent analysis is the possibility to test relationships between the corresponding pharmacokinetic parameters (ie, V_{max} and K_m) and patients covariables. The final model for covariables indicates that V_{max} tends to increase with body surface area and the liver metastatic volume of involvement. If a relationship between body surface area and V_{max} is not surprising, an opposite relationship would be more expected with liver involvement: lower is the volume of normal hepatic tissue, lower are the hepatic capacities of 5-fluorouracil elimination. But Boisdron et al[31] have previously observed a positive correlation between 5-fluorouracil clearance and the volume of hepatic metastases in advanced colorectal cancer, suggesting an increase of the 5-fluorouracil uptake by the tumor that is known to be substantial.[32]

Peripheral mononuclear cell dihydropyrimidine dehydrogenase activity proposed as a predictive marker of 5-fluorouracil catabolism[11,14] was poorly correlated with the V_{max} when tested individually and did not persist in the final model. This emphasized the limits of this mononuclear cell determination that is not necessarily representative of the dihydropyrimidine dehydrogenase activity in the liver,[33] particularly in the case of liver metastases. This study confirmed the influence of some covariables (ie, body surface area and liver metastatic volume of involvement) on 5-fluorouracil pharmacokinetics but without allowing to control the interindividual variability of this drug.

Bressolle et al[10] recently performed a NONMEM analysis of 5-fluorouracil plasma concentrations after the same schedule of 5-fluorouracil administration. The main

covariable for pharmacokinetic variability was the time showing a circadian rhythm defined by the sum of two cyclic components (peak times for 5-fluorouracil concentrations were 4.2 and 0.41 hours). We failed to observe any circadian rhythm because only 27 from the 176 blood samples were performed during the 12 PM to 9 AM interval. Moreover, it was also shown recently that no uniform time of peak or trough concentration was observed between individuals.[34] This finding complemented an earlier study on the high interindividual variability in the circadian pattern of dihydropyrimidine dehydrogenase enzyme activity.[35] Then the time would not be expected as a significant covariable for our analysis.

In terms of time-dependency, we confirmed in this schedule of administration that was previously shown in continuous 5-fluorouracil infusion: 5-fluorouracil clearance decreases during the 5-day infusion.[8,12] Later observation of the 5-fluorouracil concentrations corresponding to the data sets of intravenous infusion for 8 and 96 hours that were used for the independent evaluation of this pharmacokinetic model confirms this tendency (Fig 3, *B*). The simultaneous analysis of our 5-fluorouracil plasma concentrations at day 1 and day 2 showed that the rate of elimination was lower during the second day. The best fit corresponded to the model where K_m was free to vary from one day to another: the mean K_m increased from 6.08 to 9.83 mg · L^{-1}, suggesting a lower affinity of 5-fluorouracil for the dihydropyrimidine dehydrogenase enzyme. McLeod et al[36] have shown an autoregulation of 5-fluorouracil metabolism: administration of 5-fluorouracil induced a decrease of mononuclear cell dihydropyrimidine dehydrogenase activity in human beings and of liver dihydropyrimidine dehydrogenase activity in rats. This inhibition appeared to be specific for dihydropyrimidine dehydrogenase, but the mechanism was not clear. Repression of the transcription or messenger RNA translation would be associated with a decrease of V_{max} rather than an increase of K_m. A possible mechanism for increased K_m could be the accumulation of endogenous substrates of dihydropyrimidine dehydrogenase, such as uracil, as a result of the first day of treatment. When the 5-fluorouracil is administered during the second day of the schedule, a competitive interaction would occur between these substrates and 5-fluorouracil, leading to an apparent Michaelis constant, $K_m´$, with

$$K_m´ = K_m · (1 + [I]/Ki)$$

where [I] and Ki are, respectively, the concentration of inhibitor and the inhibition constant. Because K_m at day 2 is negatively correlated with dihydropyrimidine dehy-

drogenase activity, we could make the hypothesis that the amount of accumulated substrate is dependent on this activity.

Finally, this model will allow one to study the possible pharmacokinetic interactions between 5-fluorouracil and other drugs such as oxaliplatin or irinotecan that are planed to be combined into the LV5FU2 protocol. Indeed, it would be possible to evaluate whether a drug combined to 5-fluorouracil had an impact on V_{max} or K_m by simultaneous analysis of 5-fluorouracil plasma concentrations obtained in absence and in combination with this drug. The model could also be useful to analyze the pharmacokinetic data after administration of 5-fluorouracil prodrugs such as capecitabine when both unchanged drug and metabolites were quantified.

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