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Pharmacokinetics of 5-Fluorouracil Assessed with a Sensitive Mass Spectrometric Method in Patients on a Dose Escalation Schedule[1]

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ABSTRACT

The pharmacokinetics of 5-fluorouracil (5-FUra) was investigated in 21 patients with advanced cancer (mainly colorectal cancer). 5-FUra was administered as weekly i.v. bolus injections usually at a starting dose of 500 mg/m^2. Every 4 weeks the dose was escalated by 20% until dose-limiting toxicity was observed. Whenever possible, pharmacokinetic studies were performed at dose escalation. 5-FUra plasma concentrations were measured by high pressure liquid chromatography and a sensitive gas chromatography-mass spectrometry assay with a sensitivity as low as 3×10^{-9} M. According to the 42 plasma concentration *versus* time curves, in all but one of the patients total body clearance decreased with increasing 5-FUra doses, consistent with the nonlinear pharmacokinetics of 5-FUra. The ultrasensitive assay revealed an almost horizontal phase in the plasma concentration *versus* time curves at plasma concentrations of 10^{-8} to 10^{-9} M. This plateau did not show correlation with the area under those curves. The use of a logistic regression method showed that clinical toxicity was correlated with the area under the plasma concentration *versus* time curve of 5-FUra.

INTRODUCTION

5-FUra[3] is an antineoplastic agent that is widely used alone or in combination chemotherapy regimens for the treatment of advanced gastrointestinal cancer, breast cancer, and several other types of cancer (1). For the treatment of advanced colorectal cancer, a weekly i.v. bolus injection at a dose of about 500 mg/m^2 is a frequently used treatment schedule. Pharmacokinetic studies of 5-FUra have been reviewed by Collins *et al.* (2). A rapid initial plasma disappearance with a half-life of 10–20 min and a total body clearance of 1–1.5 liters/min were reported for most of the studies reviewed.

Collins *et al.* proposed a physiological kinetic model to describe 5-FUra pharmacokinetics with saturable elimination. However, these authors did not follow plasma concentrations below 5×10^{-6} M, allowing measurements for a time period of 90 min. We prepared stable 5-FUra derivatives and used gas chromatography-negative ion mass spectrometry (3) to measure plasma concentrations over an 8-h period. Studies yielding pharmacokinetic data for the same patient under different conditions have rarely been performed for antineoplastic agents. They are, however, very useful for understanding pharmacokinetic behavior when fewer variables must be taken into account than is the case for patient comparisons. The need for sensitive methods to study low plasma concentrations to obtain information about 5-FUra concentrations possibly reflecting release of 5-FUra from tissues has been underlined (4). We used a sensitive determination method for the comparison of model-independent parameters (5) in and between patients for subsequent dose escalations.

Most antineoplastic agents show a steep dose-response relationship, which means that plasma concentrations should be followed on an individual basis. A relationship between plasma concentrations and toxicity has been reported for methotrexate (6), and a similar relationship was found for 5-FUra given by prolonged infusion in small groups of patients (7, 8). In the present study we found correlation between the area under the plasma concentration *versus* time curve of 5-FUra after i.v. bolus injections and the risk of toxicity.

MATERIALS AND METHODS

Patients

Twenty-one patients, the majority of whom suffered from advanced colorectal cancer, were studied. Patient characteristics are given in Table 1. 5-FUra was administered weekly as an i.v. bolus injection at a starting dose of 500 mg/m^2 in 15 patients and at 600 mg/m^2 in 6 patients. Whenever possible the dose was escalated by 20% every 4 weeks until dose-limiting toxicity developed. Toxicity was evaluated according to WHO criteria (9). All patients gave their informed consent p.o. before entry into the study. After the starting dose and after the first injection of each dose escalation, venous blood samples were drawn into heparinized tubes before and immediately after the 5-FUra injection and at 1, 2, 3, 4, 5, 10, 15, 20, 30, 60, 90, 120, and 150 min and 3, 4, 5, 6, 7, and 8 h after the injection. In selected patients blood was also sampled 24 and 48 h after the injection. Freshly drawn blood samples were immediately centrifuged at 2000 rpm, and plasma was stored at −20°C until analysis.

Drugs and Chemicals

The 5-FUra and 5-ClUra used for the determination method were purchased from Sigma (St. Louis, MO); pentafluorobenzylbromide was obtained from Pierce Chemicals (Rockford, IL), and tetrabutylhydrogen sulfate was purchased from Fluka (Buchs, Switzerland) and recrystallized before use. All other chemicals were of analytical grade. Water was purified by a Millipore Reagent Q system (Millipore, Bedford, MA).

Analytical Methods

5-FUra Measured by HPLC. In principle, HPLC was performed according to the method of Au *et al.* (10). Plasma (0.18 ml) with the highest 5-FUra concentrations (obtained within 1 h after injection) was mixed with 100 μl 2 M Tris-HCl buffer (pH 6.0) and 20 μl of a 90 μM solution of 5-ClUra as the internal standard. Extraction was done with 1 ml ethyl acetate. After centrifugation the organic layer was evaporated and the residue was dissolved in 100 μl HPLC eluent which consisted of 0.01 M acetate buffer (pH 5.3); 50 μl of the eluent were injected onto the column. HPLC was performed with a C_{18}-μBondapak reverse phase column (Waters, Milford, MA; length, 30 cm; internal diameter, 4.6 mm). For the HPLC apparatus a Perkin-Elmer pump, model series 2 (Norwalk, CT), a fixed wavelength monitor, type 740 LC (Kontron, Basel, Switzerland) with detection at a wavelength of 215 nm, and a WISP autosampler, model 710B (Waters), were used. With the HPLC method used, the lower limit of detection in plasma was 10^{-6} M.

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[3] The abbreviations used are: 5-FUra, 5-fluorouracil; HPLC, high pressure liquid chromatography; 5-ClUra, 5-chlorouracil; AUC, area under the plasma concentration *versus* time curve; MRT, mean residence time; GC-MS, gas chromatography-negative ion mass spectrometry.

Table 1 *Patient characteristics*

No. of patients	21
Male/female	14/7
Performance status (WHO)	
Median (range)	2 (0–2)
Age (yr)	
Median (range)	62 (32–78)
Tumor types	
Colorectal	18
Lung	1
Stomach	1
Breast	1

5-FUra Measured by GC-MS. Before extraction, samples were diluted to obtain a 5-FUra concentration in the range of 10^{-9} to 10^{-6} M in connection with linearity of the mass spectrometer. Plasma samples of 0.9 ml with the lowest 5-FUra concentrations (obtained more than 1 h after injection) were mixed with 0.1 ml 2 M Tris-HCl buffer (pH 6.0) and 30 μl 5-ClUra as the internal standard (final concentration, 10^{-8} M in plasma). These mixtures were extracted twice with 4 ml isopropyl alcohol:ether (22:78, v/v). 5-FUra and the internal standard were extracted from the combined organic layers into 1.0 ml of 0.2 M phosphate buffer (pH 10.5). After addition of 1.0 ml of 0.5 M tetrabutylammonium hydrogen sulfate and 0.2 M phosphate buffer (pH 10.5), 4 ml of dichloromethane containing 10 μl pentafluorobenzylbromide were added and the vial was shaken vigorously at room temperature for 1 h. After centrifugation the water layer was removed. The organic layer was evaporated with nitrogen at 65°C, the residue was dissolved in 1.25 ml hexane:acetone (3:1) and washed with 100 μl 0.1 N HCl, and the organic layer was again evaporated and redissolved, now in 100 μl hexane. A 2-μl aliquot of the hexane layer was injected into a capillary gas chromatographic system (OV 17) connected with a Kratos MS 80 mass spectrometer, and the pentafluorobenzylbromide derivatives of the halogenated uracils were detected by electron capture negative ion mass spectrometry with ammonia as the moderating gas (3). The mass spectrometer was operated at a resolution of 3000 (10% valley) for increased selectivity. The signals of 5-ClUra and 5-FUra were recorded by selected ion recording with a sweep of zero parts per million at m/z 319. and m/z 334. respectively, using a lock mass at m/z 311. The lower limit of detection of the GC-MS method was 3×10^{-9} M.

Possible changes in 5-FUra concentration during storage were checked in 37 samples over the concentration range 10^{-8}–10^{-5} M after an average storage time of 28 months and the difference of the means appeared to be insignificant at a significance level of 5% (Student's t test). For samples spiked with known concentrations of 5-FUra the intraday variabilities of the assay were <3.5 and <3% (expressed as standard deviation, $n = 7$) for 5-FUra plasma concentrations of 3×10^{-6} M and 10^{-4} M, respectively. The between-day variabilities were 4.8 and 4.8% (expressed as standard deviation for the mean values of 7 measurements made on 3 days) for 5-FUra plasma concentrations of 3×10^{-6} and 10^{-4} M, respectively.

In 60 specimens in which 5-FUra concentration could be determined by both GC-MS and HPLC the average difference did not amount to more than 5%, which is not significantly different from zero at a significance level of 5% (Student's t test).

Pharmacokinetic Calculations

The AUC was calculated for the interval 0–90 min. The half-life of plasma disappearance ($t_{½}$) was calculated according to the method of Gibaldi and Perrier (11) and the MRT, the volume of distribution (V_d), and the total body clearance (Cl) were calculated according to the method of van Rossum and van Ginneken (5).

RESULTS

Mean 5-FUra plasma concentration *versus* time curves for 5-FUra doses of 500, 600, and 720 mg/m^2 are shown in Fig. 1. The lowest plateau was established with help of the low quantitation limit of negative ion mass spectrometry (3); the curves were systematically determined during a period of 7–8 h after injection of the agent. In 3 patients, additional analysis of 24- and 48-h samples gave values lying significantly above the quantitation limit (data not shown). The pharmacokinetic findings are summarized in Table 2. The parameters have been separated into highest and lowest concentrations because of the wide range of plasma concentrations over the period 0–8 h. The AUC was calculated for the interval 0–90 min and did not differ by more than 5% from the AUC calculated for 0–8 h. The MRT of the lowest dose (500 mg/m^2) is comparable to the previously reported MRT (7). After dose escalation the MRT increased in all patients but one, which is consistent with nonlinear pharmacokinetics. The average of all MRT values for a dose of 500 mg/m^2 does not differ significantly (t test) from the average of MRT values for a dose of 600 mg/m^2. The same holds for comparison of the MRT values of doses of 600 and 720 mg/m^2. However, when intrapatient differences are compared (with a paired t test) the differences between MRT values for both 500 and 600 mg/m^2 and between those for 600 and 720 mg/m^2 are significant ($P < 0.05$). The plasma concentration during the interval between 4 and 8 h after an injection was described by the ordinate coefficient of the concentration (C) and the $t_{½}$, as shown in Table 2. The AUC was calculated for this phase too (not shown). For this parameter as well as for the value of C, no statistically significant differences were observed between the average values for the different doses (t test). For $t_{½}$, averaging was not done because of the special nature of this data set, which included very large values for $t_{½}$. No significant changes in V_d were found when the doses were compared (t test). Fig. 2 shows the total body clearance (Cl) of 5-FUra, which was calculated by dividing the dose by the AUC, for different doses per patient. The average total body clearance per m^2 was 558, 404, and 349 ml/min/m^2 for doses of 500, 600, and 720 mg/m^2, respectively. The decrease was found to be highly significant (paired t test) when the decrease was compared for the individual patient between doses of 500 and 600 mg/m^2 ($P < 0.005$) and between 600 and 720 mg/m^2 ($P < 0.01$). For comparable 5-FUra doses, the values are in the same range as those previously reported (2). When the dose was increased, the clearance decreased, which corresponds with nonlinear pharmacokinetics. It must be kept in mind here that clearance is determined mainly by the highest plasma concentrations (time less than 90 min).

Besides the assessment of the pharmacokinetics of 5-FUra in this group of patients, we were able, because of the availability of toxicity data, to make a preliminary attempt to detect relationships between the pharmacokinetic findings and the toxicity of 5-FUra. In this respect, AUC seemed likely to be the most reliable pharmacokinetic parameter of drug exposure. Toxicity was designated as + when WHO toxicity (hematological and/or nonhematological) ≧grade 1 was observed, and as − for WHO toxicity grade 0. Toxicity was evaluable in 37 of the 42 courses. Grades of toxicity were distributed as follows: grade 0 in 16 courses; grade I in 10 courses; grade II in 6 courses; grade III in 3 courses; and grade IV in 2 courses. The data of the first 5-FUra courses were analyzed apart from those of all courses. The fact that not all of the patients received the same number of courses at the same dosages may have led to some degree of patient selection and influenced the outcome of the analysis.

According to the logistic regression model (12), the relationship between AUC and the risk of toxicity that fit our data was described by the equation

$$\%\ \text{of risk of toxicity} = \frac{1}{1 + e^{\alpha - \beta \log \text{AUC}}}$$

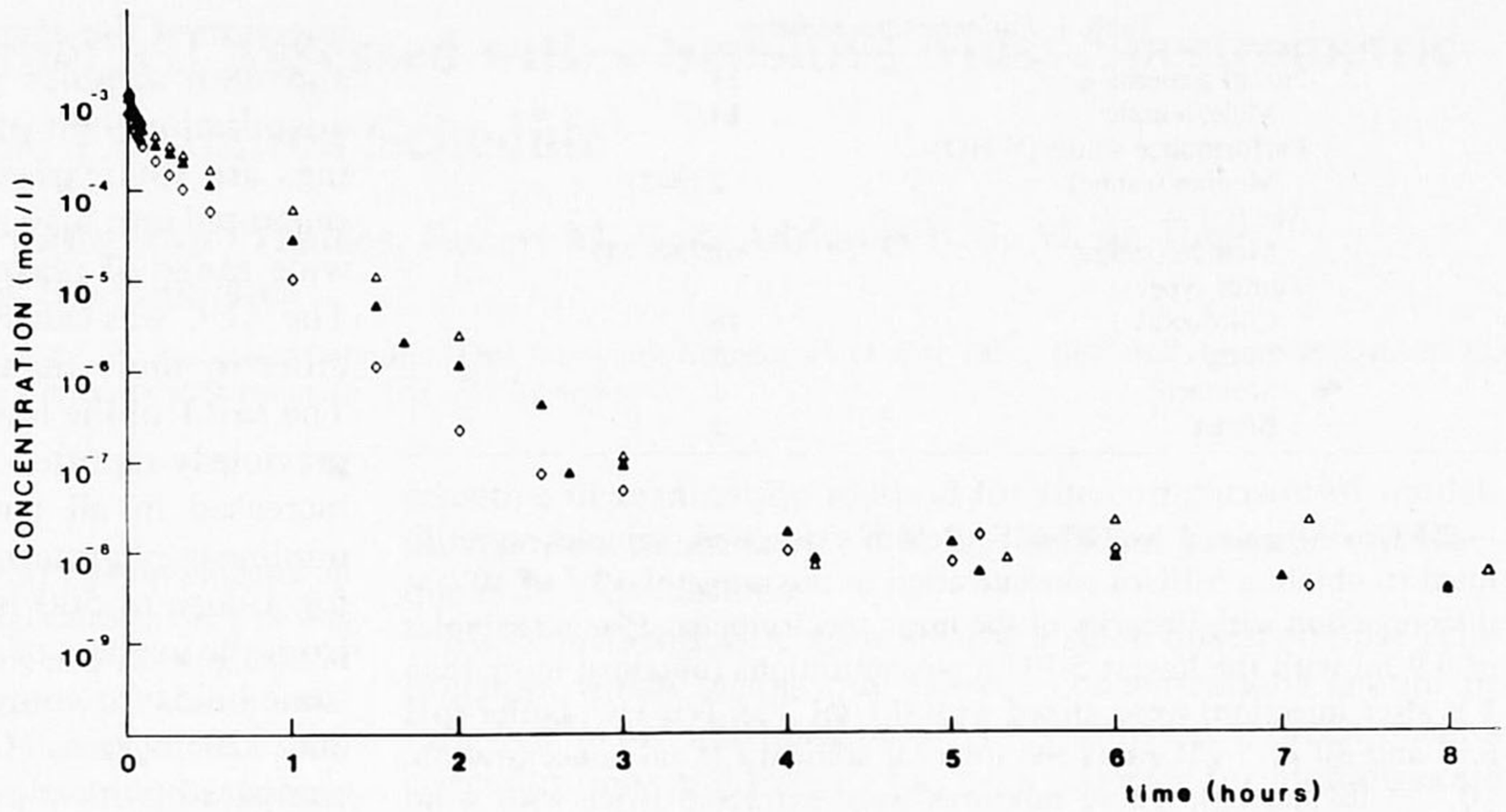


Fig. 1. Mean 5-FUra plasma concentration *versus* time curves of patients treated with different dosages of 5-FUra. ◇, 500 mg/m²; ▲, 600 mg/m²; △, 720 mg/m². For averaging measured concentrations, data were selected for a minimum of five measurements per time point for dosages of 500 and 600 mg/m² and for a minimum of three measurements per time point for dosages of 720 mg/m².

The constants α and β in this equation were derived from our set of data and were 18.1 and 14.1 for the first courses and 11.3 and 8.9 for all courses, respectively (Figs. 3 and 4). Slight differences between the curve based on the data of the first courses and that based on the data of all courses are evident. At an AUC of approximately 18 mg.h/liter the risk of toxicity is about 50% in both the first courses and all courses. However, for AUCs >18 mg.h/liter the risk of toxicity is slightly higher in the first courses as compared with all courses, and the opposite is the case for AUCs <18 mg/h/liter.

DISCUSSION

The present study has two interesting aspects: (*a*) the results show that the use of a sensitive assay permits measurements of 5-FUra plasma concentrations over a much longer period than has been reported previously; and (*b*) correlation was found between a 5-FUra pharmacokinetic parameter (AUC) and the observed signs of clinical toxicity.

It is clear from the course of the 5-FUra plasma concentrations that in this dose range the level may rise much more steeply than would be expected under linear pharmacokinetics after a 20% increase of the dose. The nonlinearity concerned the changes in the MRT and the total body clearance values at the different dose levels. This might be explained by saturation of the rate-limiting enzyme in the elimination pathway. In general, only a few physiological processes determine the shape of a pharmacokinetic curve (13). Data in the literature indicate that 5-FUra-catabolizing enzymes in the liver and lung play an important role in the rapid elimination phase (2). Collins *et al.* (2) applied a numerical method to fit 5-FUra plasma concentrations into a physiological pharmacokinetic two-compartment model for concentrations down to approximately 5 μM. In this model, elimination according to Michaelis-Menten kinetics was assumed to occur in both the first and the tissue compartment (K_m = 15 μM). We used the same physiological parameters for numerical calculation of the plasma concentration *versus* time curves for different starting concentrations, as can be seen in Fig. 5. The lower concentrations were calculated according to this model. Comparison with the measured concentrations shows considerable deviation from this two-compartment model at the lower concentrations. We propose the addition of an extra compartment possibly reflecting the 5-FUra released from tissues (4). This third compartment could have various locations in relation to the other compartments, as discussed by Wagner for three-compartment models (14). It is clear, however, that ultimately the slowest release determines the slope of the curve for the last plasma disappearance phase wherever the "deep compartment" is situated. Generally, caution must be exercised in designating physiological processes to pharmacokinetic observations. However, in the present study the range of measured 5-FUra concentrations was very large compared with those mentioned in reports on other pharmacokinetic studies. Such extreme concentration differences promote such designations. We showed that the higher concentrations were induced by only a small dose increment. In most of the patients, however, the lowest concentrations, which were measured between 4 and 8 h after administration of the drug, were not systematically dependent on the administered dose. This phenomenon might be explained by saturation kinetics of 5-FUra tissue uptake, only the duration of exposure above a certain critical level being of importance, not the concentration. Plasma concentrations in samples taken 24 and 48 h after the injection were of the order of the quantitation limit but still significantly higher than the background signal. The significance of the persistently long-lasting low 5-FUra plasma concentrations is not clear. They are unequivocally below the level for which cytotoxicity is known from *in vitro* studies in the colony-forming unit-cell system (15) and experiments with colon cancer cells (16). They are also below known levels for continuous i.v. infusion of 5-FUra (1100 mg/m²/day) which leads to plasma concentrations of 0.5 to 2.5 μM, levels associated with mucositis and intestinal symptoms (17). The height of the lowest phase relative to the applied dose schedule requires more investigation.

The present study also looked for possible relationships between clinical toxicity and pharmacokinetics. For the treatment of various malignant disorders (*e.g.*, colorectal cancer and breast cancer), 5-FUra is often administered as a single agent or as part of a combination chemotherapy regimen at a dose level around 500 mg/m². For a substantial number of patients this regimen will not produce dose-limiting toxicity and may therefore be suboptimal. In *in vitro* and *in vivo* studies higher 5-FUra doses have generally led to better antitumor responses (18, 19). We found a correlation between toxicity and 5-FUra pharmacokinetics when a logistic regression model (12) was fitted to the 5-FUra AUC. These mathematical descriptions might prove to be useful planning dosages for individual patients. However, the problem of toxicity was not the primary subject of the study, and the results must be considered preliminary. The attempt to detect correlation between clinical toxicity and pharmacokinetics was made in a relatively small number of patients, which

Table 2 *Pharmacokinetic data of patients treated with 5-FUra at different doses*

Patient	Dose (mg/m²)	Body surface area (m²)	MRT[a] (min)	V_d[a] (liters)	$t_{½}$ (h)[b]	C[b] (nM)	AUC[a] (mg·h/liter)	Toxicity[c] (+/−)
J. C.	500	1.8	12.9	12.4	7.5	166.1	15.6	−
	600		15.0	9.3	4.9	23.8	28.9	−
	720		17.3	10.1	3.0	11.0	37.1	+
M. K.	500	1.9	22.6	17.1	1.9	127.8	20.9	−
	600		33.0	19.5	2.3	144.5	32.4	+
A. H.	500	1.6	13.4	9.5	[d]	10.8	19.3	+
	600		17.8	10.6	4.6	19.7	27.0	+
	720		20.4	11.3	2.7	82.2	33.2	+
S. B.	500	1.95	13.7	10.6	3.2	33.8	20.5	−
	600		21.8	15.0	1.8	21.5	28.4	−
	720		26.7	13.3	1.5	700.6	46.8	+
C. T.	500	1.65	12.8	11.1	[d]	2.7	15.3	+
	600		14.3	10.6	0.8	702.9	22.3	+
	720		20.0	14.3	2.7	37.7	28.5	+
W. A.	500	2.0	22.9	36.8	4.2	11.6	10.4	−
	600		17.0	17.5			20.2	+
J. St.	600	1.88	26.8	21.7	3.4	23.5	23.0	−
J. Zw.	500	2.25	12.9	17.7	3.8	12.9	13.6	−
	600		17.3	20.3	22.9	11.9	19.1	+
A. A.	500	2.0	12.2	15.6	[d]	0.1	13.0	−
	600		16.9	14.1	8.5	3.9	23.3	+
A. B.	500	1.8	17.1	22.5	7.7	11.9	11.4	−
	600		23.1	17.8	18.0	5.8	23.8	+
J. Zy.	600	1.8	17.8	13.4	3.0	12.1	24.3	−
	720		21.0	14.8	2.5	15.6	32.1	−
	864		27.5	23.0	1.9	78.3	32.5	+
W. F.	500	1.85	17.0	16.2	3.2	9.1	16.1	−
S. K.	500	1.95	13.3	20.3	2.1	20.3	10.9	−
	600		16.1	11.4	5.8	6.1	27.6	+
	720		23.7	15.7	2.1	57.0	34.2	+
	864		25.5	16.9	2.3	185.0	41.3	NE[e]
B. H.	500	1.83	16.3	18.4	2.2	30.5	13.7	−
	600		19.0	16.8	11.0	7.9	20.7	+
L. V.	500	1.68	20.9	14.7	3.0	21.9	19.9	+
	600		24.0	10.5	2.3	35.3	39.2	+
S. H.	600	1.7	16.7	14.5	2.2	29.0	19.6	NE
J. Sl.	600	1.55	20.9	13.8	2.3	42.8	28.3	+
	720		22.9	12.7	19.0	45.8	33.8	NE
C. H.	600	1.9	19.0	11.2	1.8	53.5	32.4	+
A. W.	600	1.5	19.5	10.6	0.9	467.0	27.5	NE
P. W.	500	1.44	22.3	17.9	18.7	13.3	18.3	−
J. B.	500	2.0	20.6	16.9	1.8	79.7	20.3	NE
Mean ± SD	500		16.7 ± 4.1	17.4 ± 6.7		36.2 ± 49.5	15.9 ± 3.7	
	600		19.8 ± 4.6	14.4 ± 3.8		94.8 ± 192.0	26.0 ± 5.3	
	720		21.7 ± 3.0	13.2 ± 2.0		135.7 ± 250.3	35.1 ± 5.8	

[a] Calculated for the period 0–90 min.

[b] Calculated for the period 4–8 h; this part of the plasma concentration *versus* time curve was described by

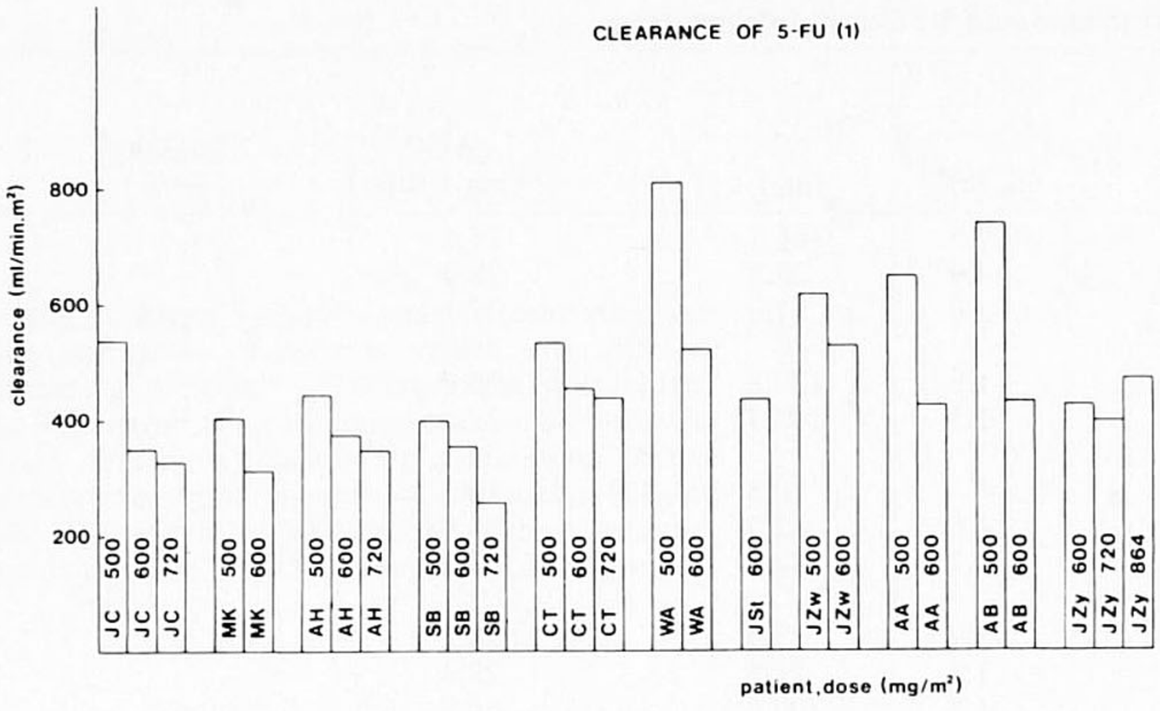
$$Cp = C.e^{\frac{-0.693t}{t_{½}}}$$

in which *Cp* is the plasma concentration and *C* is the ordinate of this phase at $t = 0$ h.

[c] Toxicity (WHO): +, ≧ grade 1; −, grade 0.

[d] Not determined due to increasing concentrations *versus* time.

[e] NE, not evaluable.



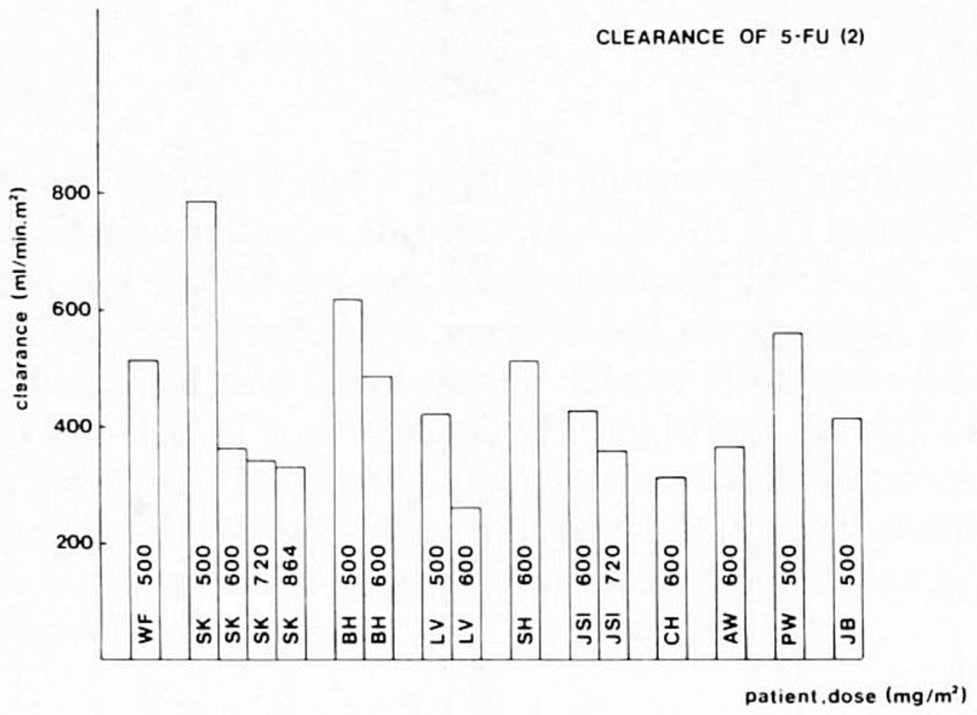


Fig. 2. Total body clearance of 5-FUra (*5-FU*) for different doses per patient.

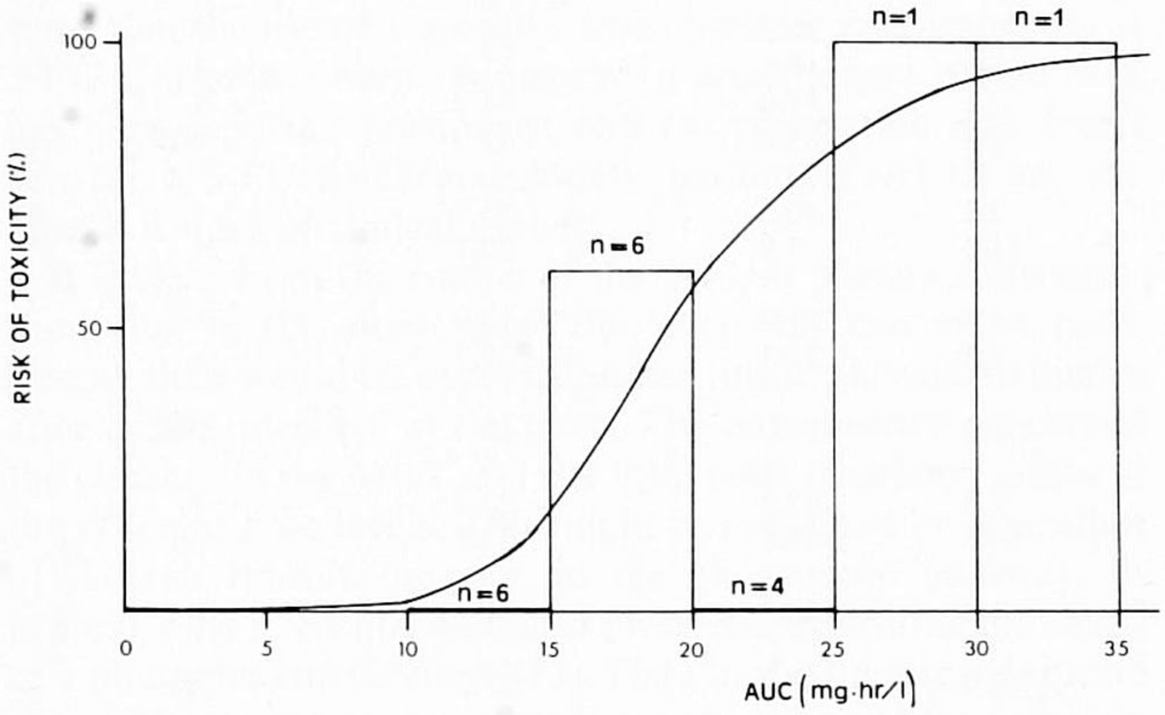


Fig. 3. Relationship of area under the 5-FUra plasma concentration *versus* time curve and the percentage risk of toxicity in the first courses. The curve displayed was modeled as described in "Results." *n*, number of courses with AUC values as indicated on the *abscissa*. *Bars* on the *abscissa*, percentage of courses which were toxic in the given AUC interval.

also meant that toxicity could only be analyzed qualitatively, *i.e.*, as present or not present. Useful further subdivision into grades of severity was not possible with the mathematical model we used. This differs from the phase I study done by Egorin *et al.* (20) on menogaril, where the use of a comparable mathematical model showed a relationship between the AUC of menogaril and the percentage of decrease in WBC and absolute neutrophil counts. This approach has the advantage of giving quantitative results but for several reasons could not be used for the present study: (*a*) at the lower dose levels an initial rise of the WBC occurred during the first course in several patients; (*b*) the method cannot be applied concurrently to nonhemato-

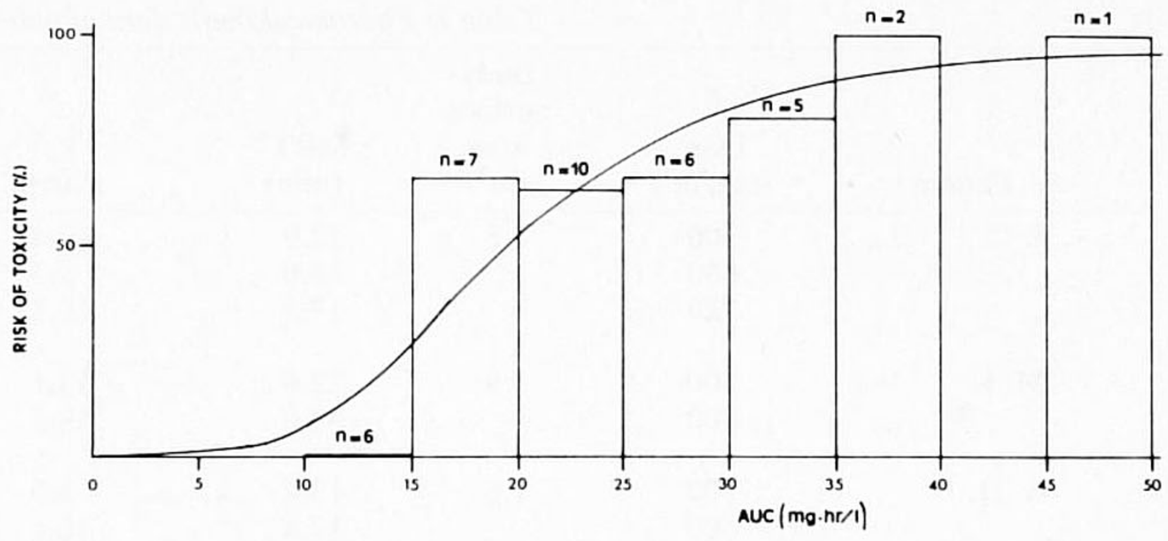


Fig. 4. Relationship of area under the 5-FUra (*5-FU*) plasma concentration *versus* time curve and the percentage risk of toxicity in all courses. The curve displayed was modeled as described in "Results." *n*, number of courses with AUC values as indicated on the *abscissa*. *Bars*, on the *abscissa*, percentage of courses which were toxic in the given AUC interval.

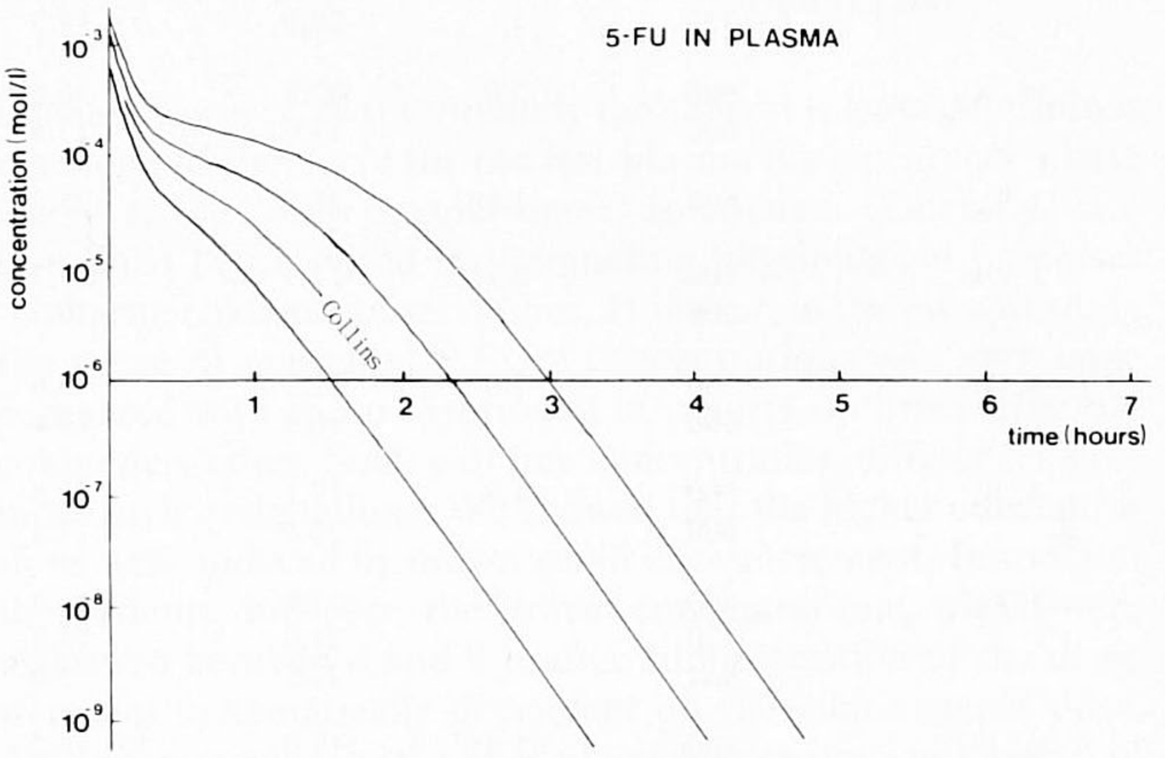


Fig. 5. Numerical computer fit according to parameters used by Collins *et al.* (2) for three starting concentrations, 0.5, 1.0, and 1.5 mM. Extrapolations to lower concentrations and comparing the data with those of Fig. 1 indicate that after measuring in this range an extra compartment should be added. *5-FU*, 5-FUra.

logical toxicity. It must also be taken into account that with this model a substantial proportion of the patients treated with 5-FUra and this dose schedule will not show toxicity. If the intention is to treat these patients up to the level of maximum tolerable doses, dose escalation will have to be guided by standard clinical signs of toxicity. Although relatively few data are available, for antineoplastic agents in general, the literature on correlations between toxicity and plasma concentrations has been reviewed by Powis (7). Thus far, routine monitoring of plasma concentrations to avoid the toxicity of antineoplastic agents has been defined only for methotrexate (6). On the basis of the present data it might prove possible to develop similar predictive rules for 5-FUra.

Finally, initial measurements have shown that the assay method described here is sufficiently sensitive for reliable determination of tissue concentrations, and the investigation has given rise to ongoing studies on the relationship between 5-FUra plasma pharmacokinetic data and cellular pharmacodynamics.

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