Relationship between 5-Fluorouracil (5-FU) Dose Intensity and Therapeutic Response in Patients with Advanced Colorectal Cancer Receiving Infusional Therapy Containing 5-FU

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BACKGROUND. A phase II prospective trial was carried out to study the concept of 5-fluorouracil (5-FU) dose-intensity in patients with advanced colorectal cancer. Forty patients were treated with 5-FU plus leucovorin (LV), with individually increasing doses of 5-FU. A 5-FU pharmacokinetic follow up was performed and a relationship was sought between its metabolism and its response to treatment, and between 5-FU's toxicity and patient survival.

METHODS. 5-FU was administered weekly by 8 hour continuous infusion. The initial dose of 1000 mg/m^2 was individually increased every 3 weeks by 250 mg/m^2 steps, potentiated by 400 mg/m^2 LV. 5-FU plasma concentrations were determined weekly by liquid chromatography.

RESULTS. Eighteen overall objective responses and 22 minor responses, stabilizations, or progressions (NR) were observed. 5-FU plasma levels were significantly higher in cases of complete or partial response, whatever the dose. They reached about 2000 μg/l as early as the second dose level (1250 mg/m^2). Only seven patients who experienced NR reached equivalent levels after the fourth step (1750 mg/m^2). High 5-FU plasma levels were predictive of an objective response and better survival (difference not significant). The acute toxicity, whatever the type, was correlated with 5-FU levels >3000 μg/l and not with the dose.

CONCLUSIONS. This study shows the wide variability of 5-FU metabolism, whatever the dose, the clear relationship between 5-FU plasma levels, toxicity, and efficacy. This relationship points out the problem of the polymorphism of 5-FU metabolism, the usefulness of the therapeutic range determination and the usefulness of the individual 5-FU dose adaptation. ***Cancer* 1996; 77:441–51.**
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Despite the fact that 30 years of clinical trials have proven the therapeutic benefit of 5-fluorouracil (5-FU) and leucovorin (LV) in treating metastatic colorectal carcinomas, the debate continues about the optimal ways to administer these drugs.[2,4] For example, although LV potentiates 5-FU efficiency, it also affects its toxicity profile. Consequently, the simultaneous administration of the folate cofactor generally leads to a reduction of the maximum tolerated dose.[4] Thus, the monthly 5 day loading regimen continues to be a standard one, with a smaller daily dose of 5-FU.[4]

Certain retrospective analyses have strongly suggested a relationship between 5-FU dose and response.[1,2,16,32] Until now, however, a dose lim-

iting hematologic and mucosal toxicity hindered both the application and the development of intensive dose strategies. In view of the short half life of the drug, different schedules of long 5-FU continuous infusion have been attempted in order to increase the time of exposure of tumor cells. They also allowed a 5-FU dose intensity of 5000 mg/m^2/week and 8400 mg/m^2/week with monthly 5 day continuous infusion and 28 day continuous infusion, respectively, versus 2000 mg/m^2/week with monthly 5 day IV bolus. In 3 randomized trials, 5 day and prolonged multiweek infusions improved the response rates when compared to conventional bolus regimens. They did not, however, improve survival.[18,27,29] These improved rates of response were obtained with the additional benefit of significantly lower rates of severe hematological and gastrointestinal toxicity. Additionally, the prolonged infusion produced hand-foot syndrome necessitating dose reductions for about a quarter of the patients.

The dose and the mean of LV administration are still debated. Contradictory results have been reported in studies comparing low (20 mg/m^2) and high doses (200 mg/m^2) of LV when 5-FU is administered by IV bolus. In vitro cytotoxic tests stressed the importance of both LV concentration in culture medium (1 to 10 μmol/l) and time of exposure (>4 hours) to optimally potentiate 5-FU cytotoxicity.[6,8,15,17] Certain other studies have reported a high individual variability of 5-FU metabolism and a close link between its toxicity and its individual pharmacokinetic parameters.[22,28] Two authors found a relationship between 5-FU plasma levels and the response to the treatment.[14,30]

Due to these contradictions, it was decided to further study the dose intensity of 5-FU and LV in a prospective clinical trial of metastic colorectal cancer. The patients were treated by increasing weekly doses of 5-FU coupled with constant high doses of LV administered at the beginning and in the middle of 5-FU infusion. A pharmacokinetic follow up looked for relationships between the individual pharmacokinetic parameters of 5-FU and the efficacy and toxicity of the treatment.

PATIENTS AND METHODS

Patients were required to have measurable metastasis of an adenocarcinoma of the colon or the rectum with a pathologically confirmed diagnosis. A local recurrence could be associated. The disease had to be measurable in two dimensions, first, by a computed tomographic (CT) scan or an ultrasound of a liver lesion, and then by a CT scan or a roentgenogram of a pulmonary lesion or a CT scan of a lesion elsewhere. Patients were also required to have a performance status (PS) of two or less, according to the World Health Organization (WHO) classification, and to have adequate hematopoietic function. Patients with any prior chemotherapy, cerebral metastasis, history of any other malignancy, or more than age 70 years were excluded. Those with previous pelvic radiotherapy on the tumor bed or a prior adjuvant chemotherapy, if it had been finished more than 6 months before the diagnosis of the metastatic recurrence, were permitted to participate. Before being carried out, the trial was submitted to the Regional Ethical Committee. Informed consent was obtained from all patients.

TREATMENT

All patients had long term veinous access established either by means of a catheter or by means of an implantable disc device. 5-FU was administered via a battery operated pump in weekly 8 hour continuous infusion in a mixture of 1 litre of serum and 0.9% saline. The dose was initiated at 1000 mg/m^2 and increased every 3 weeks to 250 mg/m^2 up to 2000 mg/m^2 or up to the first signs of toxicity. Two hundred mg/m^2 IV bolus LV was given just before and at the fourth hour of 5-FU infusion (H0–H4), up to a weekly total dose of 400 mg/m^2. LV doses remained constant during the whole treatment.

FOLLOW UP

Historical and physical examinations took place every week with particular attention to toxicity. Treatment efficacy was evaluated by comparing tumor measurements before and after 12 courses. Treatment was then prolonged up to 6 months, except in cases of progression. It could be prolonged further in cases of high efficacy or used again in cases of a secondary relapse after an objective response.

RESPONSE CRITERIA

Patients were evaluated for response after 3 months using standard response criteria. A complete response (CR) required the disappearance of all lesions. A partial response (PR) required at least a 50% reduction in the cross-sectional area of the indicator lesion (no individual lesion could grow and no new lesion could appear). Minor responses (MR) were characterized by a reduction of less than 50% but more than 25%. Stabilization (ST) required modification of less than 25%. In a progression (NR), the lesion had to increase more than 25% in the cross-sectional area or a new lesion had to appear. All radiographic documentation was reviewed by the coordinator.

EVALUATION OF THE TOXICITY

Toxicity was evaluated weekly with particular attention to diarrhea, mucositis, hand-foot syndrome and leucopenia. These toxic events were prospectively noted and evaluated according to WHO graduation. An electrocardiogram was performed just before and at the end of each weekly course. Hemogram, urea and creatinine dosages were taken every 15 days. In the event of a significant grade

TABLE 1
Clinical Characteristics of the 40 Patients

No. of patients	40
Sex	
Male	22
Female	18
Age	
Mean	59.3
Median	60
Minimum	35
Maximum	70
Sites of lesion	
Liver	25
Lung	9
Lombo-aortic nodes	8
Adrenal gland	1
Other	6
Local recurrence	15
Previous radiotherapy	3
Performance score	
0	16
1	14
2	10
Delay between primary tumor and metastasis	
Mean	6
Minimum	0
Maximum	24

II toxicity, principally diarrhea, hand-foot syndrome, or mucositis, the dose was reduced to 250 mg/m^2. In case of grade III toxicity, treatment was interrupted until resolution of the toxic manifestations and then readministered with a 250 mg/m^2 decrease in the dose. Treatment was stopped in cases of grade IV toxicity.

PHARMACOKINETIC STUDY

Five ml blood samples were collected in heparinized evacuated tubes on the fourth and the eighth hour of every 5-FU infusion. All of the blood samples were immediately centrifuged and stored at −20°C until analysis. Concentrations of 5-FU in the plasma were determined by liquid chromatography as described previously.[9] 5-FU was extracted from the plasma with isopropanol-ethyl acetate (85/15 v/v) in the presence of 200 mg ammonium sulfate to precipitate proteins. The organic phase was dried at 56°C under nitrogen dioxyde. The mobile phase was potassium phosphate (KH2PO4 10%). UV detection was performed at 260 nm. Chromatograms were treated with PC integrator. The limit of sensitivity was 10 ng/ml.

From two plasma concentrations, C × t was calculated by the trapezoidal rule: C being the average of 5-FU levels at each course and t, the duration of infusion. The method of calculation was the same for all of the patients and the comparisons were performed inside this population. The individual 5-FU approximated clearance (L/h/m^2) was calculated as the ratio dose/C × t in which the dose corresponded to the total dose administered during the course.

TABLE 2
Pattern of Toxicities for the First Three Months of Treatment

	WHO grade			
Toxicity	0	1	2	3
Diarrhea	26	1	12	1
Stomatitis	39	0	0	1
Nausea	38	1	1	0
Hand-foot syndrome	30	5	5	0
Leucopenia	39	0	1	0

RESULTS

Patients' Characteristics

Out of 43 patients treated, 40 were fully evaluable for toxicity, response to treatment and pharmacokinetic parameters. In those 40 cases, metastases were measurable on either a CT scan or by echography. The three remaining patients could not be studied because of a peritoneum involvement, which was not evaluable.

The first patient entered the study in March 1990 and the last in May 1991. Patients' characteristics are displayed in Table 1. Thirty two patients had been treated for colon cancer and eight for rectal cancer. All of them had undergone surgery for their primary tumor. The mean delay between the surgery for the primary tumor and the metastasis was 6 months (0 to 24 months). Of the 8 patients treated for rectal cancer, 3 had received a previous pelvic radiotherapy before surgery at a total dose of 45 grays. Of the patients treated for colon cancer, 10 had received adjuvant chemotherapy for their primary tumor, Astler-Coller stage C1 and C2. Eight of them had received one year of treatment, 5-FU plus Levamisole, according to Moertel's regimen,[23] and 2 had received 6 months, 5-FU plus LV, according to Machover's schedule.[20] The delay between prior radiotherapy or adjuvant chemotherapy and the diagnosis of the metastasis was always more than 6 months.

Toxicity

During the first 3 months of treatment, 14 patients developed diarrhea and 10 suffered hand-foot syndrome. One episode of stomatitis and one episode of leucopenia were observed. The toxicity was mild, except for two patients who developed grade III diarrhea and stomatitis, respectively (Table 2). The toxicity was never life threatening. As recommended in the protocol, the dose of 5-FU was reduced to 250 mg/m^2 in case of grade II toxicity. The 2 cases of grade III toxicity required a 15-day interruption of treatment and then a 250 mg/m^2 decrease of the 5-FU

TABLE 3
Incidence of Toxic Manifestations According to the Dose of 5-FU (2082 Courses, One Year Mean Duration of Treatment)

Fu dose (mg/m^2)	No. of courses	Diarrhea (%)	Hand-foot syndrome (%)	Mucositis (%)	Leucopenia (%)
1000	503	22 (4.3)	14 (2.7)	1 (0.2)	2 (0.4)
1250	498	22 (4.4)	14 (2.8)	2 (0.4)	2 (0.4)
1500	495	21 (4.2)	14 (2.8)	2 (0.4)	2 (0.4)
1750	345	12 (3.4)	13 (3.8)	1 (0.3)	0 (0)
2000	157	7 (4.4)	11 (7)	0 (0)	0 (0)
≥2250	44	1 (2.2)	0 (0)	0 (0)	0 (0)

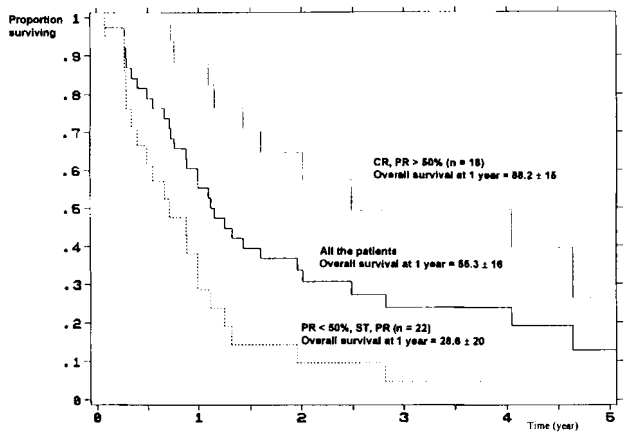

FIGURE 1. Overall survival rates of the 40 patients. The patients who experienced CR, PR > 50% had a better survival ($P < 0.01$). Five patients were alive at 5 years.

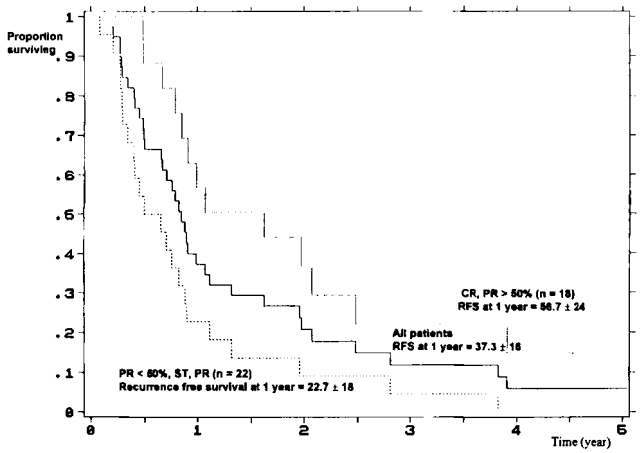

FIGURE 2. Recurrence free survival (RFS) rates of the 40 patients. The patients who experienced CR, PR > 50% had a better survival ($P = 0.02$).

dose. During the duration of treatment for every patient, 2082 weekly courses (mean duration of treatment was 1 year), 86 episodes of diarrhea (83 grade 1 and 2, 3 grade 3), 66 hand-foot syndrome (60 grade 1 and 2, 6 grade 3), 6 mucositis (grade 1 and 2) and 6 leucopenia (grade 1 and 2) were observed. The low incidence of acute toxicity can be explained by the fact that the dose of 5-FU was secondary adjusted according to its metabolism in order to avoid toxicity.

The number of each type of toxicity was classified according to the dose of 5-FU for the first 3 months of treatment and for the whole treatment. No relationship was found between the step of dose and the incidence of toxicity (Table 3).

Response to Therapy

Treatment efficiency was evaluated after 3 months. Six CR and 12 PR were observed. Therefore, the overall response rate was 45%. There were nine MR, inferior to 50%, nine ST, and four P. There were 3 PR in the group of 10 patients previously treated by adjuvant chemotherapy.

The results of the survival analysis are presented in Figures 1 and 2. Median survival was 14 months. Overall and recurrence-free survivals after 1 year were 55 ± 16 and 51%, respectively (Fig. 1). The recurrence-free survival curves differed significantly according to the quality of the response (56% ± 24 for CR and PR versus 22.7% ± 18 for MR, ST and P) ($P = 0.02$). Overall survival also differed between the 2 groups (at one year 88% ± 15 versus 28.6% ± 20), depending on the time of evaluation, ($P < 0.01$). After 3 years, the overall survival remained at 50% in cases of initial objective response, whereas there was only one survivor among patients with MR, ST and P.

The difference between recurrence-free survival and overall survival in the group that experienced an objective response can be explained by the fact that, after 6 months of treatment, in the case of a stabilized CR or PR, the treatment was generally stopped or, in one case, was performed every 15 days. As early as the diagnosis of recurrence was done the same schedule was used again, this time with an individual 5-FU dose adjustment, calculated according to the results of the study in order to reach

TABLE 4
Incidence of Toxic Manifestations According to 5-FU Plasma Levels of the Previous Course (24 Cases of Toxicity for 3 Months of Treatment)

5FU levels (μg/l)	<1600	1600	2800	3000	3400	3800	4300	4600	4800	5800	6800	7200	7800
No. of toxicities	0	1	1	2	2	6	3	3	1	1	1	1	1

Only two patients who underwent previous pelvic irradiation experienced toxicity after 5FU plasma levels under 3000 μg/l.

and to keep the optimal plasma levels (see below). Therefore, a second objective response or a long stabilization could be obtained. In the population alive after 3 years, 3 patients remained in persistant CR, 1 patient was still treated in a persistant PR every 15 days, 9 patients had relapsed, 7 underwent the same treatment schedule with 5-FU dose adjustment, and 2 were treated by 4 day continuous infusions of 5-FU plus cisplatin every 3 weeks and died 5 months later. Five patients remain alive after 5 years, 3 of whom are under the same treatment. The others are being reevaluated after a new interruption of treatment.

For 10 out of the 22 patients in MR, ST or P at the evaluation, the same weekly schedule was continued with individual 5-FU dose adjustments in order to intensify the treatment (see below). A partial response was then recorded for two patients. For five other patients, a second line chemotherapy scheme was attempted with 5-FU plus cisplatin or interferon, without any efficacy. Only six patients were alive after one year.

Pharmacokinetic Study

The pharmacokinetic study was carried out for all 40 patients. Two thousand eighty two courses were studied. Large variations of 5-FU plasma concentrations were found among the patients whereas the concentrations were stable for each patient during the 3 courses of each step. In 70% of the courses, a 10% to 15% increase of 5-FU levels was observed during the infusion, from the 4th to the 8th hour.

Twenty four cases of acute toxicity were noted during the first three months of treatment. They appeared in the week following the administration of 5-FU concentrations greater than 3000 μg/l (Table 4) or C $\times$ t over 24 mg.h.l-1. For 2 patients, diarrhea was observed with 5-FU levels below 3000 μg/l. These 2 patients had been previously treated for rectal carcinoma with 40 Gy pelvic radiotherapy before surgery. Furthermore, the higher the 5-FU levels were, the more severe the toxicities were. Diarrhea and hand-foot syndrome were associated when 5-FU levels rose to more than 4000 μg/l. The toxicity disappeared as soon as 5-FU doses were reduced to the inferior step (−250 mg/m^2). Rather than the dose, the decrease of 5-FU levels to less than 3000 μg/l led to the disappearance of the toxicity when 2 patients who had toxic levels and an acute toxicity experienced a spontaneous decrease of 5-FU levels without any 5-FU dose change and simultaneously a resolution of their toxic manifestations.

A relationship between 5-FU plasma levels and the response to the treatment and the survival was studied according to two complementary approaches. It was first regarded during the first 3 months of treatment when 5-FU plasma levels were predictive for the quality of the response. The patients were divided into two groups according to their mean plasma levels for each step dose. They were considered in the group "high levels" if more than half of their mean levels for the first three steps were superior to the overall population mean. 5-FU plasma levels for each dose step were: 1300 μg/l for 1000 mg/m^2, 1600 μg/l for 1250 mg/m^2, and 2000 μg/l for 1500 mg/m^2. In the group "low levels," their mean levels were below. Seventeen patients were in the group "high levels," 21 in the group "low levels," and 2 patients could not be situated because they were just on the midline. In the group "high levels," 14 out of the 17 patients experienced a CR or a PR and 3 patients experienced a MR, a ST, or a P, whereas in the group "low levels," only 3 out of the 21 patients had a PR and 18 a MR, a ST, or a P. The correlation between high 5-FU levels and low 5-FU levels and the quality of the response (CR + PR vs MR + ST + P) ($\kappa^2 = 8.8$, $P < 0.01$) was significant for the 2 groups.

A similar relationship may exist between 5-FU plasma levels during the first eight courses and the survival. At 1 year, the overall survival was 70.6% $\pm$ 22 for the group "high levels" and 45 $\pm$ 22 for the group "low levels" (Fig. 3). At 2 years, it was 45 $\pm$ 22 and 23.5 $\pm$ 20, respectively. The difference was not significant ($P = 0.2$). The recurrence-free survivals showed the same difference (at 1 year 44.3 $\pm$ 25 versus 33.3 $\pm$ 20) (Fig. 4), probably because of the small number of patients and the fact that 8 patients in the first group and 10 in the second group had been treated after recurrence or in progression by the same schedule, with an individual 5-FU dose adjustment in order to reach and to keep the optimal plasma levels (see below).

5-FU plasma levels were studied according to the quality of the response to the treatment. The 40 patients

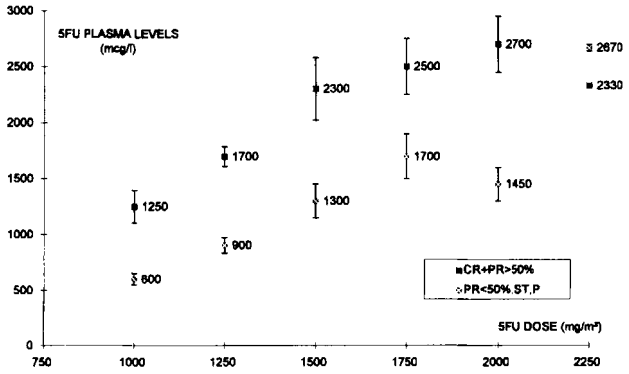

FIGURE 3. Mean 5-FU plasma levels for every dose step according to the response to the treatment (CR + PR > 50% vs PR < 50% + ST + P) (40 patients).

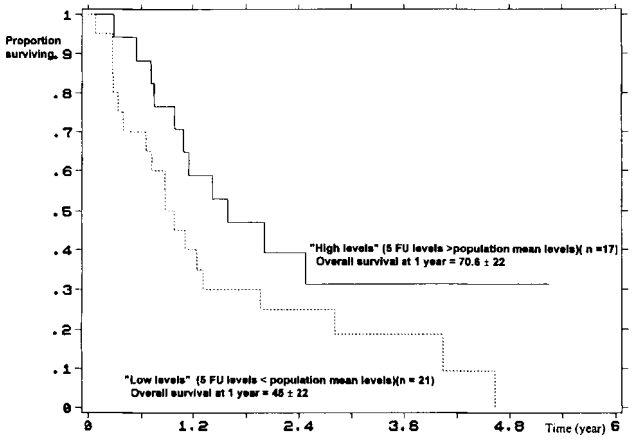

FIGURE 4. Overall survival for the 2 groups of patients: "high 5-FU plasma levels" (17 patients) and "low 5-FU plasma levels" (21 patients). The difference was not significant ($P = 0.2$).

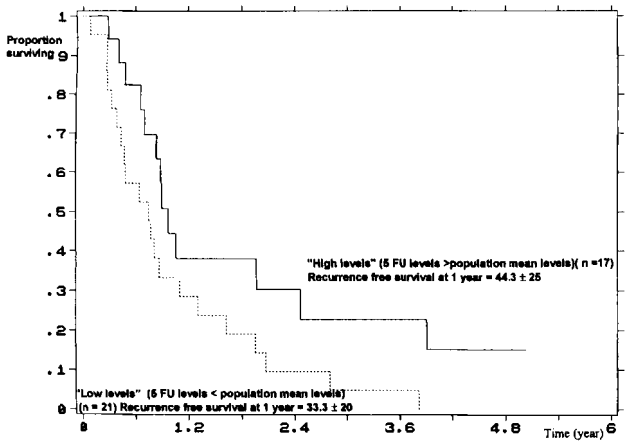

FIGURE 5. Recurrence free survival for the 2 groups of patients: "high 5-FU plasma levels" (17 patients) and "low 5-FU plasma levels" (21 patients). The difference was not significant ($P = 0.2$)

were divided into 2 groups: those in whom the responses were complete or partial (18 patients, group 1), and those in whom there were minor responses, stabilizations and progressions (22 patients, group 2). 5-FU plasma levels differed significantly between the two groups. The results are summarized in Table 5 and Figure 5. 5-FU plasma levels were higher in group 1 for each dose step. The difference was highly significant between the 2 groups for the steps, 1000, 1250 and 1500 mg/m^2 ($P < 0.001$). 5-FU concentrations of group 1 were initially high and reached 1800–2000 μg/l as early as the first or second step. Conversely, for the patients who had MR, ST, or P, 5-FU levels were initially gathered at much lower levels. They were then scattered with an increasing dose, allowing them to reach high levels, equivalent to those of patients who experienced CR or PR. At the step of 1750 mg/m^2, the difference of mean 5-FU plasma levels between the 2 groups was just significant ($P = 0.041$). Concentration of drug $\times$ and time of infusion (C $\times$ t values) were calculated for each patient. An equivalent C $\times$ t (13.6 mg.h.l-1) was obtained with 1250 mg/m^2 for patients in group 1 and 1750 mg/m^2 for patients in group 2 (Table 5).

Patients in group 1 reached 5-FU plasma levels of about 1800–2000 μg/l as early as the first or the second step, whereas patients in group 2 had very low initial 5-FU plasma levels and had to wait generally at least until the fourth dose step to reach the same levels than those of group 1. For every patient, the pharmacokinetic follow up was performed weekly for the duration of treatment. If 5-FU plasma levels remained constant for some patients during the treatment, a progressive modification of 5-FU plasma levels, especially a decrease, was often observed in spite of constant doses of 5-FU. A close follow up of tumor markers (C.E.A. and CA 19-9) showed a secondary rise of their levels as soon as 5-FU levels fell below 2000 μg/l. As a consequence of the great individual differences of 5-FU metabolism, the maximum tolerated dose (MTD) varied widely. It was 1500 mg/m^2 for patients who had a CR or a PR. One patient was in the toxic zone as early as the first course at 1000 mg/m^2 and had to receive a dose reduced to 850 mg/m^2 in order to keep the therapeutic, non toxic range. However, all of the patients in MR, ST and P tolerated 1500 mg/m^2, and 14 out of 22 reached 1750 mg/m^2. For the other eight patients, the progression of the disease obliged the researchers to stop the treatment.

The relationship between 5-FU plasma concentrations and 5-FU dose in the two groups was studied. The hypothesis was first made that the correlation was represented by a straight line of linear regression. For the two groups, this hypothesis has been confirmed by a statistical test ($P < 0.001$). The equation was of ($y = ax + b$) type, where y was 5-FU plasma levels and x was 5-FU

TABLE 5
Mean Pharmacokinetic Parameters of the 40 Patients According to their Response to the Treatment

5-FU dose mg/m²/w		1000	1250	1500	1750	2000	2250
	No. of patients	18	18	15	8	3	1
5-FU plasma levels (μg/l)	OR	1250 ± 145 (260 ⇒ 6500)	1700 ± 90 (170 ⇒ 3500)	2300 ± 280 (160 ⇒ 5200)	2500 ± 250 (950 ⇒ 4350)	2700 ± 250 (1000 ⇒ 3850)	2330
	No. of patients	22	22	22	14	4	2
	NR	600 ± 50 (150 ⇒ 1800)	900 ± 70 (130 ⇒ 2000)	1300 ± 150 (200 ⇒ 4500)	1700 ± 200 (350 ⇒ 3700)	1450 ± 150 (800 ⇒ 1950)	2670 ± 30 (2640 ⇒ 2700)
	p	0,001	0,001	0,001	0,041		
C × t (mg.lh.l⁻¹)	OR	8,8 ± 0,15	13,6 ± 1,2	18,4 ± 2,3	20 ± 2	21,6 ± 6	
	NR	4,8 ± 0,06	7,2 ± 1	11,2 ± 1,5	13,6 ± 1,6	13 ± 2	
$\frac{\text{5-FU Dose}}{C \times t}$ (l/mm)	OR	1,9	1,53	1,36	1,46	1,54	
	NR	3,47	2,9	2,23	1,92	2,56	

OR: objective response; NR: no response; p: progression.

dose. For the first group: $y = 1.61x - 405$, and for the second group: $y = 1.85x - 1367$. The coefficients a of the two equations (i.e. the slopes) have been compared and were equivalent whereas the coefficients b were significantly different ($p = 0.02$). Therefore the two groups appeared to have equivalent slopes with an increasing dose but the mean initial level was much lower for the second group. An approximated clearance could be calculated with the dose of 5-FU normalized to C × t. Although the values decreased with the dose, 5-FU kinetics being non linear, the group 2 values remained significantly higher for each dose step probably because of metabolic capacities saturable at a higher level.

The practical consequence of these findings was the predictability of 5-FU concentrations. A simple linear relation between plasma levels and 5-FU dose and the coefficient a was common to all patients. However, the coefficient b differed according to the metabolic capacities evaluable as early as the initial doses. For this reason, a simple table was established for adjusting 5-FU dose according to these coefficients, adaptable to all situations. The purpose of this dose adjustment was to reach the optimal therapeutic and nontoxic range, between 2000 and 3000 μg/l in 3 to 4 weekly courses. It was decided to progressively obtain this range of value, in order to avoid eventual levels that would be too high. The dose modifications were valuable whatever the type of 5-FU metabolism, they depend only on 5-FU concentrations and previous doses (Table 6). This table was then used for these patients for long term adjustment of their treatment.

DISCUSSION

A relationship between 5-FU dose and 5-FU response in metastatic colorectal cancer has been strongly suggested by retrospective analyses, but, until now, a dose limiting hematologic and mucosal toxicity hindered both the application and the development of intensive dose strategies.[2,16,32] Therefore, there remains much confusion regarding the optimal dose scheduling of 5-FU.[1,4,26] A weekly administration was adopted for this study since Shah found a real advantage in the weekly administration of a 48 hour 5-FU infusion in comparing 3 schedules of 5-FU.[31] 5-FU continuous infusion schedules improved the response rates in 3 randomized trials, maybe because they allow a dose intensification with a different pattern of toxicity, usually less than IV bolus.[18,27,29] An 8 hour continuous infusion appeared to be a good compromise between 2 constraints, a continuous infusion for obtaining a concentration-steady state, and a day hospitalization. It also allowed pharmacokinetic plasma assays in strict conditions of infusion with a constant flow rate. At the steady state, it has been previously reported that there was a linear correlation between 5-FU dose and plasma concentration.[7,32] During continuous infusion it has been shown that 5-FU blood levels proportionally followed modifications in the 5-FU dose rate. The predictability of 5-FU concentrations and, moreover, the 5-FU dose adjustment to obtain the wanted 5-FU plasma levels, was possible[7,32] whereas with a 5-FU bolus, dose increments were followed by disproportionate and variable elevations of 5-FU blood concentrations.[10,17,35] The practical consequence was that with bolus injection, it was impossible to predict what the blood concentration would be for a given dose.[10,17,35]

The initial dose of 1000 mg/m² was chosen according to literature[5,31] and from past experience (data not shown). High dose folinic acid was administered twice, at 4 hour intervals, to provide better conditions to maintain isomer/LV plasma concentrations ≥1 to 10. 10-6 Mol/l for the duration of 5-FU infusion. Certain in vitro cytotoxicity

TABLE 6
5-FU Dose Adjustment According to 5-FU Plasma Levels at the Previous Course

In absence of toxicity		
5-FU plasma levels (μg/l)	5-FU dose adaptation (% of previous dose)	In case of toxicity
<500	+50%	
500 to 1000	+40%	Grade 2 toxicity: 200 mg dose decrease
1000 to 1300	+30%	
1300 to 1500	+20%	
1500 to 1800	+10%	
1800 to 2000	+5%	
2000 to 3000	No modification	Grade 3 toxicity: 1 week break then 300 mg dose decrease
3000 to 3200	−5%	
3200 to 3500	−10%	
>3500	−30%	

studies stressed this level to be necessary for 5-FU optimal potentiation.[15] Forty five percent of objective responses were observed. These results were equivalent to those previously reported with weekly high doses of 5-FU.[2,4,5] Doses reached after 3 months of treatment were higher than those usually used but a little lower than those reported by Ardalan.[2] However, a high incidence of toxicity occurred which required an interruption of treatment every 6 weeks. The incidence of toxicity in this study was very low compared with the usual regimens and allowed the researchers to treat the patients every week, without interruption. Except for a grade III mucositis, the usual profile of toxicity observed was represented by mild diarrhea and hand-foot syndrome ($\leq$ grade II). Both quickly disappeared with a 250 mg/m^2 dose reduction.

This pharmacokinetic study showed great variations of 5-FU plasma concentrations in the 40 patients. The study also showed that the same dose administered after adjustment for body surface or weight led to varying therapeutic intensities. 5-FU plasma concentrations showed a wide range for each dose step. As previously described in the relevant literature, the plasma kinetics of 5-FU were not linear.[21,30] 5-FU plasma clearance decreased with dose increment, probably because of a saturable metabolic process.

With this regimen, a close link was found between the acute toxicity and the 5-FU plasma levels. Concentrations of more than 3000 μg/l were followed by diarrhea or hand-foot syndrome and appeared to be more closely related to the toxicity than to the dose of 5-FU. This level corresponded to a value of AUC over 24 mg.h.l-1 which was very close to previous results reported with different schedules.[33] Thyss et al. have demonstrated a relationship between an elevated AUC of 5-FU over 30 mg.h.l-1 and the frequency of cycles with leucopenia, mucositis and diarrhea for patients with head and neck cancer treated by chemotherapy combining cisplatin and 5 day continuous infusion of 5-FU. Milano et al. found the same AUC threshold value for patients with metastatic colorectal cancer treated by 5 day continuous infusion of 5-FU without cisplatin.[22] Thus, cisplatin did not influence the maximum tolerated AUC of 5-FU. The lower value of AUC found in the regimen used in this study[24,30] could be explained by the addition of LV, which is known to potentiate 5-FU toxicity. It is important to note that with this regimen, patients could tolerate about the same value of AUC every week and so the same treatment intensity was tolerated monthly with 5 day continuous infusion. Yoshida et al. found that for patients with colorectal cancer treated by 5-FU continuous infusion, the 5-FU concentrations at the steady state and AUC administered more than 72 hours were higher in the group with toxicity than in the group with no toxicity.[38] In addition, Trump et al. reported equivalent results for patients treated by 3 day continuous infusion of 5-FU, showing a close relationship between steady state 5-FU plasma concentration and the risk of leucopenia and mucositis.[34] Van Groeningen et al. described this relationship mathematically with a 5-FU bolus schedule.[35]

In an attempt to decrease the incidence of toxicity,[28] researchers controlled AUC at the middle of a 5 day infusion by adjusting the dose of 5-FU with a nomogram to maintain the total AUC under the toxic value. This approach permitted a significant decrease in toxicity when compared with a constant dose of 5-FU. A close link between 5-FU concentrations in plasma and therapeutic outcome was also found. Two approaches were used for studying this relationship. One showed that the patients who had plasma levels higher than the mean levels of the overall population had better chances of experiencing an objective response than patients who had lower levels

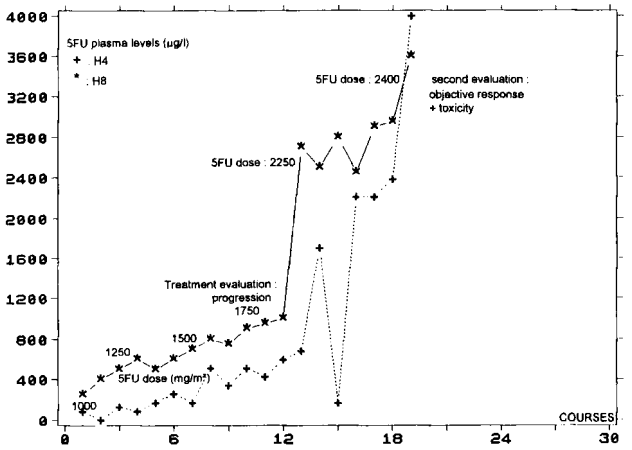

FIGURE 6. Example of a patient's pharmacokinetic follow up. He first experienced a progression at 3 months and then an objective response at 5 months after a great and quick increase of 5-FU dose (from 1750 mg/m^2 to 2250 and 2400 mg/m^2). The 5-FU plasma levels were >2000 µg/l but they were also superior to 3000 µg/l and a diarrhea occurred.

(14/17 versus 3/21) ($p < 0.01$). Additionally, patients who experienced an objective response had significantly higher levels, whatever the dose, than patients who failed to respond. Notably, these patients reached levels of 1800–2000 µg/l as early as the second step dose, so that they were quickly in a narrow range, close to the toxic levels. On the contrary, for patients who experienced a treatment failure, the initial concentrations were very low and they became equivalent much later. For patients whose 5-FU clearance was elevated, the dose of 5-FU was initially too low and therefore patients had insufficient medication during the major part of the treatment. This could explain some primary failures due to an insufficient dose of 5-FU rather than a real tumor cell resistance. As an illustration of this hypothesis, 2 patients who failed to respond after 3 months of treatment received an increased last dose of 1750 mg/m^2 up to 2250 mg/m^2 and then to 2400 mg/m^2. In these two cases, objective responses were obtained. It is important to note that 5-FU plasma levels were more than 2000 µg/l in the seven courses which preceeded the objective response (Fig. 6). The quick dose increment and the obtainment of the optimal plasma range appear much more likely to be the cause of the response than a prolonged treatment (5 months). The relationship between 5-FU pharmacokinetics and treatment response has been less extensively explored than toxicity and remains less clear. Hillcoat was the first to show that for patients with digestive tract cancer, treated by 5 day continuous infusion of 5-FU, AUC values were significantly higher when objective response or stabilization were observed.[14] Some authors have reported similar results lately.[30,38]

This study covered survival according to 5-FU plasma levels. The overall survival at one year and later was better for patients who had plasma levels higher than the mean concentrations, but the difference was not significant ($P < 0.2$). The lack of statistical difference may be due in part to the small number of patients and also to the fact that for 8 patients who experienced an objective response and 10 patients who failed to respond, the treatment was continued with an individual dose adjustment. This result may have lessened the differences between the two groups. Milano has shown, for patients with head and neck cancer, treated by 5 day continuous infusion 5-FU and cisplatin, a better overall survival with increased AUC.[22]

This wide variability in 5-FU metabolism has some consequences on the 5-FU maximum tolerated dose. For the majority of patients (22), 1750 mg/m^2 was either just sufficient or just insufficient to reach 5-FU plasma concentrations higher than 2000 µg/l. On the contrary, for 10 patients, 1500 mg/m^2 5-FU led to concentrations ≥ 3000 µg/l and to an acute toxicity. One patient was in the toxic area as early as 1000 mg/m^2. This required a reduction of the dose, down to 850 mg/m^2 for obtaining the therapeutic range. These results point out the problem of the genetic polymorphism of the 5-FU metabolism.[8,12,13,19] A few cases of patients with complete deficiency in dihydropyrimidine dehydrogenase (DPD), the key enzyme of 5-FU catabolism, have been reported.[12,13] Extremely high and prolonged levels of 5-FU were measured after a low dose of 5-FU and the subsequent toxicity was severe, sometimes fatal. Consequences are multiple. First, there is a wide polymorphism of 5-FU metabolism and a large panel of degrees of DPD activity with a gaussian distribution among a large population of patients.[8,19] Moreover, a relationship between the DPD activity in lymphocytes and 5-FU plasma levels has been reported in certain studies.[9,12,19,24] This could partly explain primary treatment failures and severe toxicities. It must be kept in mind that DPD is largely widespread in tissues and that the degree of DPD activity in tumor cell lines plays a major role in resistance to 5-FU, as well as thymidylate synthase activity.[3] Second, the polymorphism of 5-FU metabolism and the link between 5-FU plasma levels and the response to the treatment begs the question of the individual adjustment of the 5-FU dose. The method of the test dose appears to be difficult to implement since the plasma kinetics of 5-FU are complex. The pretreatment DPD activity determination would be a fine solution except that this method, which seems to be of great interest for the detection of DPD deficiency, remains insufficient for the predictability of 5-FU plasma concentrations in practice and cannot be a useful indicator for improving 5-FU dose adaptation strategy.[8,19] Moreover, the potential modifications of 5-FU metabolism

during prolonged treatment will also affect the outcome. So, the individual dose adjustment with a pharmacokinetic follow up appears to be actually more interesting and practicable. The predictability of 5-FU concentrations seems possible with infusions. Spicer et al. found a linear correlation between the dose of 5-FU and the plasma concentration at the steady state for patients treated by very prolonged continuous infusion of 300 to 500 mg/m^2 5-FU.[32] Erlichman et al., Milano et al., and Thyss et al. made similar observations with 5 day continuous infusion schedules and concluded that under these conditions, 5-FU concentrations proportionally followed 5-FU dose modifications.[7,22,33] Equivalent results were found in this study with weekly 8 hour infusion, (i.e. a simple linear relation between plasma levels and 5-FU dose). This study established a dose adjustment table to reach the optimal therapeutic range determined. The table was simple and practical, but usable only for this schedule. The originality of this dose adjustment was its aim, the dose intensification. Since it was found that a large proportion of patients had insufficient doses of 5-FU and that the dose could be responsible for a failure, researchers looked for an intensification with a control of the risk of toxicity. A large prospective multicentric study was started to prove the interest of this approach in term of efficacy, tolerance, and survival.

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