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Original Paper

Plasma and Salivary Pharmacokinetics of 5-Fluorouracil (5-FU) in Patients with Metastatic Colorectal Cancer Receiving 5-FU Bolus Plus Continuous Infusion with High-dose Folinic Acid

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The comparative saliva/plasma pharmacokinetics of 5-fluorouracil (5-FU) were investigated in 21 patients with metastatic colorectal cancer receiving high-dose folinic acid (LV (leucovorin) 200 mg/m^2) followed by 5-FU bolus (400 mg/m^2) and continuous infusion (600, 750, 900 or 1200 mg/m^2) on days 1 and 2. Quantitation of unchanged drug was assessed by a highly specific high-performance liquid chromatographic method. Large patient-to-patient variations in plasma and saliva 5-FU concentrations were observed. Saliva pharmacokinetics could be described using a bi-exponential pattern. The half-life of the rapid phase averaged 8.0 min, and was of the same order of magnitude as the 5-FU elimination half-life determined from plasma data. The half-life of the terminal part of the curve averaged 8 h; such decrease in salivary concentrations could be due to changes in salivary gland function caused by 5-FU, which results in reduced salivary flow rate. Between individual 5-FU concentrations in parotid saliva and plasma a statistically significant straight line could be fitted with a coefficient of correlation of 0.675. Moreover, the risk of developing 5-FU-related mucositis was significantly linked to 5-FU salivary exposure. Diarrhoea was the most frequent toxicity encountered during the trial. **© 1999 Elsevier Science Ltd. All rights reserved.**

Key words: 5-fluorouracil, plasma, parotid saliva, pharmacokinetics, metastatic colorectal cancer
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INTRODUCTION

5-FLUOROURACIL (5-FU) IS widely used alone or in polychemotherapy in the treatment of solid tumours including breast, gastrointestinal, head and neck, and ovarian cancers [1]. Colorectal cancer has traditionally been considered resistant to chemotherapy. In recent decades, 5-FU has been considered the standard single-agent treatment for colorectal cancer. In this type of pathology, many studies on the antitumour activity of this drug in various regimens, including combination and biological modulation, have been investigated. Of these regimens, continuous intravenous infusions of 5-FU may be superior to bolus injection [2, 3]. Moreover, modulation of 5-FU by folinic acid has been shown to be more effective than 5-FU alone [4–7].

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5-FU pharmacokinetics has been widely studied [8–22]. Certain studies have reported high individual variability of 5-FU pharmacokinetics and a close link between its toxicity and its individual pharmacokinetic parameters [11, 17, 22]. Moreover, a close link between acute toxicity (mainly diarrhoea and hand–foot syndrome) and 5-FU plasma levels greater than 3000 ng/ml has been reported [16]. A circadian rhythm following continuous infusion has been also described [9, 10]. Renal clearance accounts for less than 15% of the total 5-FU clearance. Total body clearance is high with 594 ± 198 ml/min/m^2. The elimination half-life of this drug ranged from 4.5 to 13 min [15].

Digestive intolerance and, particularly, the occurrence of oral mucositis remain the major limiting toxicities during prolonged 5-FU infusions. It is frequently painful and can interfere with nutrition and quality of life [23]. An effective prophylactic measure for decreasing the incidence of this

distressing toxicity can enhance patient comfort and compliance with therapy. Recently, oral cooling was proposed to prevent 5-FU-induced mucositis [24].

In the present study, the pharmacokinetic profile of 5-FU in plasma and its excretion in parotid saliva, a route of elimination heretofore given little attention, if any, were investigated. Moreover, the relationship between 5-FU salivary exposure and the occurrence of side-effects were investigated.

PATIENTS AND METHODS

Patients and eligibility criteria

21 patients (15 males, 6 females) with advanced, inoperable, histologically proven colorectal cancer participated in this study. The study protocol was reviewed and approved by the institutional review board. It was performed in accordance with the Declaration of Helsinki, and with current European Community and US Food and Drug Administration guidelines for good clinical practice. All patients gave written informed consent. Patients with altered performance status (> 2, WHO classification), a serum creatinine level of > 130 μM, an abnormal blood-liver test (bilirubin > 3.0 mg/100 ml and aspartate aminotransferase > 2 times normal) or abnormal liver echography, and known coronary heart disease or cardiac failure were not included. In addition, all patients had adequate bone marrow function (white blood cell (WBC) $\geq 3000/mm^3$ and platelets $\geq 100\,000/mm^3$). Complete history, physical examination, blood chemistry, complete blood count (including platelet count) and evaluation of the extent of tumour (using appropriate investigations) were required prior to study entry.

The mean age of these patients was 62 years (± 8.8 years, range: 43–77). Their average height was 170 ± 10.3 cm (range: 151–191 cm) and their average weight was 71.3 ± 16.6 kg (range: 43–107 kg). A total of 33 courses were investigated. The majority of subjects showed a good WHO performance status of 0–1; only 1 patient had a WHO grade 2. Among these patients, 11 had undergone prior therapy, including 5 patients previously treated with 5-FU.

Drug administration and doses

Patients received the LV5FU2 chemotherapy regimen consisting of folinic acid and 5-FU daily for two consecutive days according to the treatment schedule reported by de Gramont and associates [6, 7]. The treatment was given as folinic acid (200 mg/m^2 in 0.9% (w/v) sodium chloride) by intravenous (i.v.) infusion over 2 h followed by a 5-FU loading dose administered in 2 min (400 mg/m^2 in 5% dextrose) then continuous infusion (600, 750, 900 or 1200 mg/m^2 in 1000 ml of 5% dextrose) for 22 h using a non-portable infusion pump (Diginfusa 5,010, Arcomed AG, Switzerland). The whole regimen was repeated on day 2. The second course of chemotherapy was given on a 14-day cycle with a dose escalation of 5-FU continuous infusion (+ 25, + 50 or + 100%) according to an individual dose adaptation schedule based on the area under plasma concentration-time curve (AUC_p) value [25]. On the first day of treatment, 2 patients received oxaliplatin (130 mg/m^2) by intravenous infusion over 2 h, just before the folinic acid infusion.

Blood and parotid saliva sampling

Samples (at least 2 ml) were taken 0.3, 1, 2, 3, 4, 18 and 22.3 h after the start of the 5-FU infusion on day 1. Salivary samples were collected over a period not exceeding 2 min. Venous blood samples were drawn into heparinised tubes. Immediately after collection, blood samples were centrifuged (2000 *g*) at + 4°C for 10 min, then the supernatant was removed. Both plasma and parotid saliva samples were stored at − 40°C until assay.

Analytical method

Plasma 5-FU determination was performed by high-performance liquid chromatography with ultraviolet absorbance detection, as previously described [26]. Precision, expressed as % coefficient of variation, ranged from 2.7 to 13% and the mean recovery from 94 to 105%. The limit of quantitation was 20 ng/ml.

In saliva, the extraction procedure was as follows: to a 0.5 ml aliquot of saliva, 100 μl of internal standard solution (chlorouracil, 5 μg/ml) was added and gently mixed. After addition of 500 μl of acetonitrile and centrifugation (10 min at 4000 *g*), the supernatant was transferred in a fresh tube and mixed with 3 ml of ethylacetate. The mixture was centrifuged at 4000 *g* for 10 min. The upper organic phase was collected in a glass tube and evaporated to dryness under a stream of nitrogen for 30 min at 40°C. The dried residue was reconstituted in 200 μl of water and then filtered through a 0.45-μm HV4 membrane (Millipore, Molsheim, France). A 20-μl volume was injected on to the column. Chromatographic analysis was performed using the same chromatographic conditions as that described for plasma. Inter-day precision and accuracy of the method were assessed by the determination of saliva quality control samples (50, 400 and 800 ng/ml). The precision was lower than 12% and the accuracy did not exceed 5%. The limit of quantification was 10 ng/ml.

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using the Pkfit software [27].

Model-independent parameters. C_{ss} is the observed steady-state plasma concentration. C_{max} is the maximum concentration in parotid saliva. The area under the plasma (AUC_p) and parotid saliva (AUC_s) concentration–time curve from time zero to infinity was obtained by the linear trapezoidal approximation with correction to time infinity by dividing the last observed data point by the elimination rate constant. Total plasma clearance (CL) was estimated as Dose/AUC_p and the apparent volume of distribution (V_d) as the ratio of CL to the apparent rate constant of elimination.

Model-dependent parameters. In each course, the plasma data were fitted using a one-compartment model. The parotid saliva concentrations of 5-FU decreased biexponentially with time; $t_{\frac{1}{2}}\alpha$ and $t_{\frac{1}{2}}\beta$ represent the half-lives of the two observed phases. The coefficients and exponents of the exponential terms were estimated using the weighted ($1/Y$) least-squares method.

Statistical analysis

The values for each parameter are reported as mean values ± standard deviation (S.D.). Plasma concentrations and AUC_p were plotted against parotid saliva concentrations and AUC_s, respectively. Linear regression was performed using unweighted least-squares analysis of the data. The significance of the regression was confirmed using the *F*-test. For the 12 patients receiving two different 5-FU doses at 14-day intervals, a Friedman test was performed to compare

normalised (to a 600 mg/m^2 maintenance dose) AUC_p and AUC_s, as well as normalised C_{ss} across doses.

The relationship between 5-FU salivary exposure and the occurrence of side-effects was investigated using the Chi-square test.

RESULTS

Pharmacokinetic parameters from plasma data

Figure 1(a) shows mean (± SE) 5-FU concentration–time (semi-logarithmic) curves for plasma. The data were fitted to a one-compartment model and linear elimination kinetics. This model did not produce systemic deviations in the residuals and provided the lowest Akaike criterion. Mean (± S.D.) pharmacokinetic parameters are presented in Table 1.

For patients receiving a dose escalation of 5-FU continuous infusion, C_{ss} were compared after normalisation to a 600-mg/m^2 maintenance dose; no significant differences occurred ($P = 0.7701$, first course: 0.251 ± 0.0825 mg/l versus second course: 0.262 ± 0.0661 mg/l). Likewise, the dose-normalised AUC_p did not differ between doses ($P = 1.00$); they were 8.93 ± 3.13, 11.1 ± 2.03 and 8.13 ± 1.90 mg/l×h after 1000, 1300 and 1600 mg/m^2 total administered dose, respectively. The mean elimination half-life was 10.4 ± 5.4 min. V_d and CL averaged 0.76 ± 0.49 l/kg and 1.09 ± 0.368 l/min/m^2, respectively. Large interindividual variability of the pharmacokinetic parameters was observed.

Pharmacokinetic parameters from salivary data

Figure 1(b) shows parotid saliva levels of 5-FU. The drug concentrations versus time followed a biexponential decay pattern. Mean (± S.D.) pharmacokinetic parameters are presented in Table 2. There was notable interpatient variability in pharmacokinetics.

Twelve minutes after the start of 5-FU administration, drug concentrations in parotid saliva greatly exceeded those in plasma; for 9 courses out of 33, concentrations ranged

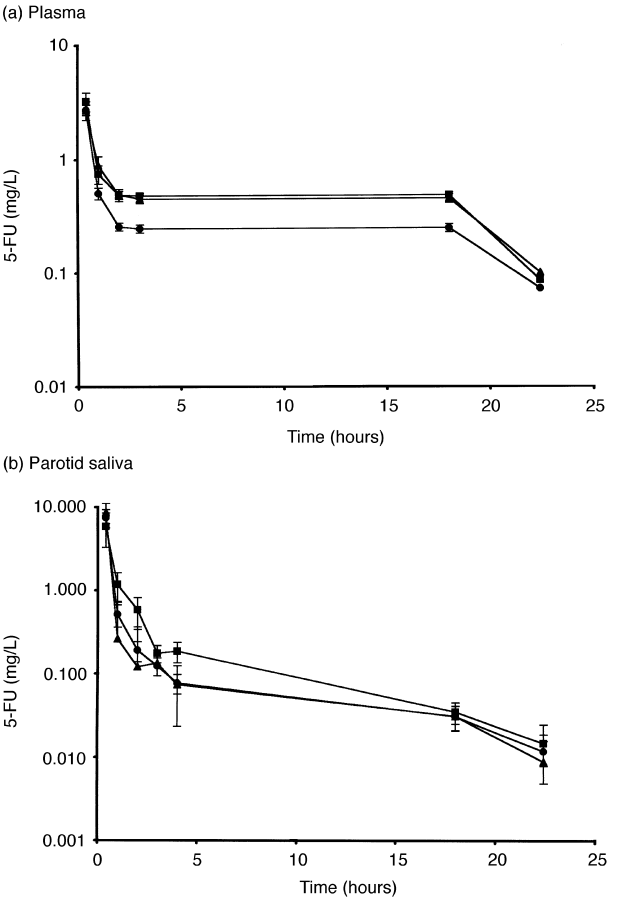

Figure 1. Mean (± SEM) 5-FU plasma (a) and parotid saliva (b) concentrations versus time curve following intravenous administration of 5-FU to patients with metastatic colorectal cancer. Maintenance doses: (•) 600 mg/m^2; (■) 900 mg/m^2; (▲) 1200 mg/m^2.

Table 1. Mean ± S.D. pharmacokinetic parameters determined from plasma

Dose (mg/m^2)						
Loading dose	Maintenance dose	C_{ss} (mg/l)	$t_{\frac{1}{2}elim}$ (min)	$AUC_{0-\infty}$ (mg×h/l)	V_d (l/kg)	CL (l/min/m^2)
Course 1						
400	600 ($n = 18$)	0.244 ± 0.088	9.67 ± 4.26	8.33 ± 2.74	0.71 ± 0.44	1.17 ± 0.40
Course 2						
400	750 ($n = 1$)	0.437	6.13	16.0	1.41	0.77
400	900 ($n = 6$)	0.478 ± 0.085	9.98 ± 6.72	14.5 ± 2.63	0.49 ± 0.25	0.81 ± 0.19
400	1200 ($n = 8$)	0.447 ± 0.104	12.8 ± 6.94	13.0 ± 9.05	1.01 ± 0.62	1.19 ± 0.32

n = number of patients. C_{ss}, steady-state plasma concentration; $t_{\frac{1}{2}elim}$, half-life of elimination; $AUC_{0-\infty}$, area under concentration–time curve from time 0 to infinity; V_d, apparent volume of distribution; CL, total plasma clearance.

Table 2. Mean ± S.D. pharmacokinetic parameters from salivary data

Doses (mg/m^2)					
Loading dose	Maintenance dose	C_{max} (mg/l)	$t_{\frac{1}{2}}\alpha$ (min)	$t_{\frac{1}{2}}\beta$ (h)	$AUC_{0-\infty}$ (mg × h/l)
Course 1					
400	600 ($n = 18$)	7.47 ± 7.44	7.29 ± 4.16	7.34 ± 5.40	6.25 ± 5.52
Course 2					
400	750 ($n = 1$)	28.1	5.90	1.64	18.5
400	900 ($n = 6$)	5.89 ± 6.40	9.00 ± 9.82	7.07 ± 5.92	7.11 ± 4.13
400	1200 ($n = 8$)	8.70 ± 6.25	9.02 ± 6.52	10.5 ± 6.59	6.69 ± 4.03

n = number of patients. C_{max}, maximum concentration in saliva; $t_{\frac{1}{2}}\alpha$, $t_{\frac{1}{2}}\beta$, half-lives of the two observed phases; $AUC_{0-\infty}$, area under the concentration–time curve from time zero to infinity.

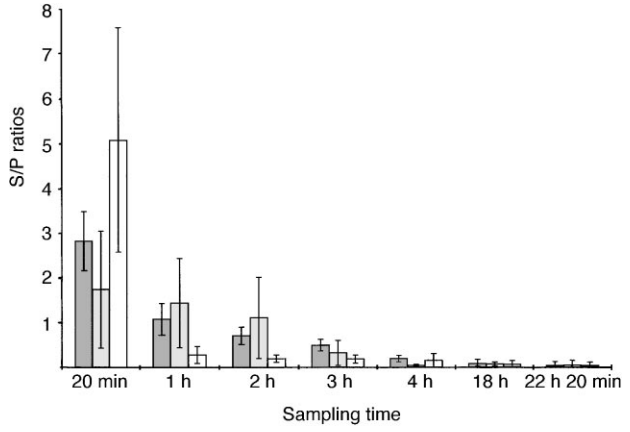

Figure 2. S/P ratios versus time. Error bars represent SEM. Maintenance doses: (■) 600 mg/m^2; (▨) 900 mg/m^2; (□) 1200 mg/m^2.

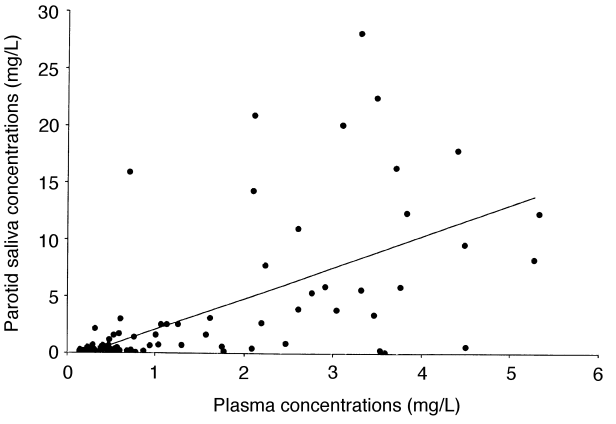

Figure 3. Relationship between salivary and plasma 5-FU concentrations. Coefficient of correlation: $r = 0.675$ (165 df, $P < 0.001$); slope = 2.73, intercept = − 0.65.

from 15 to 28 mg/l, they were 5–10-fold higher than that in plasma. At this time, the mean (± S.D.) S/P ratios (concentrations in saliva over those in plasma) averaged 2.8 ± 2.7, 1.7 ± 1.3 and 5.1 ± 2.6 for maintenance doses of 600, 900 and 1200 mg/m^2, respectively; afterwards, these ratios decreased with time (Figure 2). Three hours after the beginning of administration, all concentrations in parotid saliva were lower than in plasma (S/P ratios of 0.49 ± 0.57, 0.32 ± 0.28 and 0.28 ± 0.27 for maintenance doses of 600, 900 and 1200 mg/m^2, respectively); 5-FU was no longer detectable 12 min after the end of infusion ($t = 22.3$ h; mean S/P ratios of 0.03–0.04). The dose-normalised AUCs after total administered doses of 1000, 1300 and 1600 mg/m^2 were 6.25 ± 5.52, 5.49 ± 3.20 and 4.10 ± 2.63 mg/l×h, respectively; in spite of a 34% decrease at the highest dose, these values were not statistically different ($P = 0.247$). The ratio AUC_s/AUC_p averaged 0.66 ± 0.49.

The half-life of the rapid phase averaged 8.0 ± 5.9 min, the same order of magnitude as the 5-FU elimination half-life determined from plasma data. The half-life of the terminal part of the curve was 7.9 ± 5.8 h.

When the 5-FU concentrations in parotid saliva were plotted against 5-FU plasma concentrations (Figure 3) a statistically significant straight line could be fitted with a coefficient of correlation of 0.675 (165 df, $P < 0.001$) and a slope of 2.73. For the relationship between AUC_p and AUC_s, the coefficient of correlation (31 df, $R = 0.3269$, $P = 0.0604$) was relatively low.

Toxicity

Table 3 summarises the toxic effects observed throughout treatment. No toxicity greater than WHO grade 4 was observed. The toxicity was never life-threatening. Diarrhoea was the most frequently toxicity encountered during the trial (12/33 cycles). Leucopenia was the only haematological toxicity encountered with 1 and 2 patients suffering grade 1 and 3 leucopenia, respectively, and 1 patient presenting grade 4 leucopenia associated with fever that required hospitalisation with parenteral antibiotics.

At the first course, 50% of patients experienced one or more episodes of non-haematological toxicity. Although grades 2 and ≥ 3 were more pronounced during the second course of chemotherapy with increased 5-FU doses, these data suggested that dose escalation of 5-FU results in a weak increase in toxicity.

Relationship between 5-FU salivary exposure and the occurrence of side-effects

Only the relationship between the occurrence of mucositis and AUCs was statistically significant ($P < 0.001$). During this study, mucositis was observed in 24% of patients, in those exhibiting the highest salivary concentrations.

DISCUSSION

The present pharmacokinetic study was conducted in 21 patients during two successive courses of chemotherapy. The

Table 3. Toxicity observed during two courses of chemotherapy

Toxicity	No. of cycles (≥ Grade 2)		Number of patients WHO grade			
	Course 1 ($n = 18$)	Course 2 ($n = 15$)	1	2	3	4
Mucositis	0	1	4	1	–	–
Diarrhoea	4	5	3	9	–	–
Nausea	0	1	5	1	–	–
Alopecia	0	0	1	–	–	–
Cutaneous	0	1	1	–	1	–
Anorexia	0	0	1	–	–	–
Anaemia	0	1	–	1	–	–
Leucopenia	1	2	1	1	–	2
Fever	1	1	–	2	–	–

second course was given on a 14-day cycle with a dose escalation of 5-FU continuous infusion (+25, +50 or +100%) according to an individual dose adaptation schedule [25]. The interpatient variability in 5-FU pharmacokinetic parameters determined from plasma data are in agreement with the data previously published [8–19]. To date, few references have been made to the excretion of 5-FU in parotid saliva. As this drug is not protein-bound to a large extent in plasma [28], it is theoretically an appropriate candidate for salivary investigations. Only Milano and associates [29] have studied the salivary passage of 5-FU during continuous infusion in 10 patients. We confirmed their results, with a significant correlation between individual concentrations of 5-FU in saliva and plasma; a similar observation has been made by others in the Beagle dog [30]. However, the values were scattered, so the concentration of this drug in parotid saliva does not appear to be useful in indirect, non-invasive estimations of levels of unbound 5-FU in plasma.

Of particular interest was the rapid diffusion of 5-FU in parotid saliva; the concentrations reached 20 min after the start of 5-FU administration were, on average, 3-times higher than those in plasma, and the S/P ratio decreased with time. A similar observation has been made by Hayashi and associates in rats [31]. The parotid saliva concentrations of 5-FU decreased biexponentially with time; the half-life of the rapid phase averaged 8.0 min, it was of the same order of magnitude as the 5-FU elimination half-life determined from plasma data. Between 3 and 22 h (end of continuous infusion), plasma 5-FU concentrations were relatively stable whilst 5-FU salivary concentrations decreased with a half-life averaging 8 h. Such a phenomenon could be due to changes in salivary gland function caused by 5-FU, which results in reduced salivary flow rate [32]. These results were in accordance with the findings of Hayashi and Watanabe [33] in the perfused rat mandibular gland; the authors concluded that 5-FU itself influenced salivary excretion of 5-FU by decreasing the salivary flow rate. The secretory mechanisms of 5-FU in saliva are not due to simple passive diffusions but rather to active processes.

Diarrhoea was the most frequent toxicity encountered during this study. Nausea and vomiting were moderate; leucopenia was the only haematological toxicity encountered. No toxicity greater than WHO grade 4 was observed; the toxicity was never life-threatening. Although grades 2 and ≥ 3 were more pronounced during the second course, when 5-FU doses were increased, these data suggested that dose escalation of 5-FU results in a weak increase in toxicity. The risk of developing FU-related mucositis was significantly linked to 5-FU salivary exposure ($P < 0.001$). Indeed, mucositis was observed in patients presenting with the highest 5-FU salivary concentrations 20 min after the start of 5-FU administration. From these observations, the individual follow-up of 5-FU salivary concentrations just after the initiation of treatment (approximately 20 min) and during the subsequent 3 h, should be of interest to detect patients with a risk of developing mucositis in order to begin, as soon as possible, a treatment to prevent toxicity. Moreover, such measurements are non-invasive. Recently, a randomised study demonstrated the utility of oral cooling in the prevention of 5-FU-induced stomatitis [24].

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