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Original Paper

Co-variables Influencing 5-Fluorouracil Clearance During Continuous Venous Infusion. A NONMEM Analysis

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The objective of this study was to attempt to identify patient co-variables which could influence interpatient variability in 5-fluorouracil (5-FU) clearance during a 5-day continuous venous infusion (CVI, cisplatin 100 mg/m^2 day 1 then 5-FU 1 g/m^2/day days 2–6). The analysis was performed using a NONMEM program according to a linear one-compartment model. A total of 186 cycles (2 samples per day, 8 a.m. and 5 p.m.) were analysed from 104 patients with various cancers (the majority of head and neck and oesophagus). The study co-variables were age, sex, body surface area, hepatic metastases, peripheral mononuclear cell dihydropyrimidine dehydrogenase activity (PMNC-DPD), liver enzymes, clock-time (8 a.m. versus 5 p.m.), elapsed time during CVI. The data showed that 5-FU clearance was significantly reduced by increased age, low PMNC-DPD, high serum alkaline phosphatase and elapsed time during infusion. These data may be useful for improving knowledge of predictive factors which can influence 5-FU exposure and thus predispose to toxic manifestations. © **1998 Elsevier Science Ltd.**

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INTRODUCTION

DESPITE BEING one of the oldest anticancer drugs, 5-fluorouracil (5-FU) is still increasingly used in cancer chemotherapy. 5-FU is not only considered the standard drug for the treatment of advanced colorectal cancer, but is also one of the major drugs in the treatment of carcinoma of the oral cavity and breast. Most of the current clinical protocols which incorporate 5-FU include one or more of the so-called 5-FU biomodulators, of which folinic acid is the most frequently used [1].

Bearing in mind that 5-FU is a pro-drug which needs intracellular activation to exert its effects, there is an appreciable gap between 5-FU blood concentrations and drug effects on the target cell (tumoral or normal host cells). This, theoretically, makes it difficult to associate directly blood drug concentrations with cell toxicity. However, data from the literature [2–5] including our own experience [6, 7] have proven the existence of such relationships. The link between 5-FU pharmacokinetics and treatment response has been less explored [8], but we have recently shown the association between 5-FU systemic exposure and tumour response and overall survival in head and neck cancer patients [9]. Based on previous pharmacokinetic–pharmacodynamic relationships for 5-FU given during 5-day continuous infusion, we proposed and clinically validated [10] a pharmacokinetic-based dose adaptation strategy whose clinical applicability has been recently confirmed by others [11]. Thus, the variability in 5-FU clearance (CL) is a determinant factor which controls both the toxicity and clinical efficacy of this drug. It follows that it would be interesting to identify the individual determinants which can predict for interpatient variability in 5-FU CL.

Since renal elimination of unchanged 5-FU accounts for only 10% of the injected dose [12], renal abnormalities have, a priori, a low incidence on 5-FU pharmacokinetics. The bulk of 5-FU elimination occurs by metabolism through the liver. At this level, the enzyme dihydropyrimidine dehydrogenase (DPD) plays a major role [13], and it has been shown that DPD activity measured in circulating lymphocytes is positively correlated to 5-FU CL [14, 15]. The physiological relevance of this observation has been recently strengthened by a clinical study undertaken by our group showing the strong correlation, at the individual level, between the

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expression of DPD in liver tissue and in circulating lymphocytes [16]. In addition, several authors have followed 5-FU pharmacokinetics in patients with liver dysfunction, but it is unclear whether 5-FU pharmacokinetics were altered or not [17, 18]. Finally, age and sex are among the factors that can be involved in the pharmacokinetic variability of drugs [19]. We recently performed a retrospective analysis in patients receiving continuous venous infusion of 5-FU in order to test the possible influence of age and sex on 5-FU CL [20]. It was shown that women had a significantly lower 5-FU CL than men.

In summary, published data indicate that biological variables, such as DPD or gender, may influence 5-FU CL. However, these data come from separate clinical analyses performed on different groups of patients. The objective of the present study was to perform a multivariate analysis including lymphocyte DPD, liver function tests, age and sex in order to assess their link with 5-FU CL. This was done on a group of 104 cancer patients, all receiving the same 5-FU-based chemotherapy protocol. The analysis was performed using the NONMEM method [21, 22]. Unlike traditional pharmacokinetic analysis in which the data from each individual are analysed separately, this method deals with all patients simultaneously. It is therefore possible to determine the influence of co-variables on pharmacokinetic parameters while dispensing with individual pharmacokinetic parameters.

PATIENTS AND METHODS

Patients and treatment

The characteristics of the patients are summarised in Table 1. 104 patients (91 men and 13 women; mean age 59 years; range 31–84) with squamous cell carcinoma of the head and neck, all treated in a single institution (Centre Antoine Lacassagne), were included in the present study. Tumour localisations were head and neck, 60; oesophagus, 20; pancreas, 7; rectum, 6; colon, 4; lung, 2; unknown, 5. Performance status was ≤ 2 in all patients. Patients had not been pretreated with chemotherapy and all were treated with curative intent with neoadjuvant chemotherapy consisting of cisplatin plus 5-FU (three cycles planned at 3-week intervals). For each cycle, the treatment on day 1 consisted of 6-h hydration with 5% dextrose (2 l) sodium chloride (NaCl 6 g/l) and potassium chloride (KCl 3 g/l), followed by cisplatin (100 mg/m^2) 1 mg/min intravenously (i.v.) in NaCl 9 g/l (0.5 l) with 1.6% mannitol (0.25 l) then 5% dextrose (1 l), NaCl (6 g/l), and KCl (3 g/l). Then, on days 2–6, a 5-day continuous venous infusion of 5-FU was administered (controlled flow pump; initial dose, 1 g/m^2/d). A total of 186 chemotherapy cycles were analysed. After completion of induction chemotherapy, patients were given locoregional treatment with curative locoregional treatment, with curative radiotherapy alone (65–70 Gy) or unilateral/bilateral surgical resection complemented, when necessary, by irradiation of the tumour bed and nodal regions (55 Gy).

Table 1. *Patients' characteristics*

Characteristics	No. of patients (104)
Sex	
Male	91
Female	13
Mean age, years (range)	59 (31–84)
Mean body surface area, m^2 (range)	1.7 (1.25–2.17)
Hepatic metastasis (yes/no)	7/97
Mean DPD, nmol/min/mg (range)	0.235 (0.065–0.410)
Mean AST, U/l (range)	32 (8–157)
Mean ALT, U/l (range)	33 (7–232)
Mean γGT, U/l (range)	88 (14–348)
Mean ALP, U/l (range)	89 (43–267)

AST, aspartate transaminase; ALT, alanine transaminase; γGT, gamma glutamyltranspeptidase; ALP, alkaline phosphatase.

Pharmacokinetic and biological investigations

5-FU pharmacokinetic investigation was performed for each cycle. The 5-day infusion of 5-FU started at 8 a.m. on day 2. Blood sampling times were as follows: 5 p.m. on day 2, 8 a.m. and 5 p.m. on days 3 and 4, and 5 p.m. on days 5 and 6. This specific sampling was dictated by our previous experience in pharmacokinetics and pharmacodynamics of 5-FU given as a 5-day infusion [10]. Venous blood (5 ml) was drawn on edathamil (EDTA) tubes and samples were immediately centrifuged (10 min at 2500 rpm) in the hospital ward. Plasma was sent to the laboratory and stored at -20°C until analysed. 5-FU concentrations in plasma were analysed by high-performance liquid chromatography [6]. The intra- and interday coefficients of variation for 5-FU measurement were $< 10\%$.

Hepatic function tests, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transpeptidase (γGT), were performed prior to therapy (typically the day before). In addition, 20 ml of blood were collected for peripheral mononuclear cell DPD (PMNC-DPD) determination. Because of the previously reported circadian variability of DPD activity in PMNC [14], blood samples were collected between 8 a.m. and 11 a.m. to minimise the influence of circadian variability. PMNC-DPD activity was assayed as previously reported [14, 15]. This method for determining DPD activity is both sensitive (limit of sensitivity, 0.010 nmol of product) and reproducible (intra- and interday coefficient of variation $< 10\%$).

NONMEM analysis

The analysis was performed using the NONMEM program (double precision, version IV, level 1.1) [23] running on Vax openVMS (version 6.1). A one-compartment kinetic model with first-order elimination (PREDPP package, subroutine ADVAN1, TRANS2) [24] was used to examine the influence of a variety of co-variables on the population mean value for CL. Indeed, in a first analysis, a one-compartment kinetic model incorporating a saturable elimination, expressed as a function of V_{max}, K_m and V, was used: K_m values were larger than the maximum plasma 5-FU concentration observed, respectively, 6.58 and 2.74 mg/l. Moreover, the objective function obtained from the fit to the saturable elimination model was not significantly different to that obtained from the fit to the first-order elimination model. Interindividual variability in CL and residual variability were modelled according to a proportional error (constant CVs). The residual variability includes the measurement errors in plasma 5-FU concentration and in time statements and also intersubject variability. There were 50 patients treated for 1 cycle, 35 for 2 cycles, 15 for 3 cycles, 2 for 4 cycles, 1 for 5 cycles and 1 for 6 cycles. An identification number was assigned to each patient allowing the data collected from different cycles of the same patient to be recognised. NONMEM can obtain estimates of

individual CL by the 'posthoc' option: individual-specific true values of CL were obtained according to a Bayesian approach from the observed 5-FU concentrations and by taking into account the computed interindividual and residual variabilities. The influence of the following co-variables on 5-FU CL was tested: sex, age, PMNC-DPD activity (DPD), body surface area (BSA), presence of hepatic metastases (HM), ALP, AST, ALT and γGT. In order to evaluate change in CL during the infusion, time from the start of the infusion (TIME) and clock-time (CLO = 0 if 8 a.m. sample, and = 1 if 5 p.m. sample) were assigned to each 5-FU plasma level as co-variables.

In fitting the data, NONMEM computed the value of a statistical function, the minimum value of objective function, which is proportional to minus twice the log likelihood of the data. The objective function values were used to evaluate the increase in goodness of fit upon inclusion of each co-variable. The differences in objective function values were obtained by comparing a restricted model in which the tested co-variable is absent to a non-restricted model in which the co-variable is included. These differences were asymptotically distributed as chi-square with one degree of freedom [24]. A change in the objective function value of > 3.8, associated with a P value of < 0.05, was required to identify a co-variable as being significant. All significant co-variables were then forced into a multivariate intermediate model, and each was eliminated in a backwards stepwise approach to determine if its exclusion was statistically significant. In the evaluation of the intermediate model, the objective function values were used to evaluate the decrease in goodness of fit obtained upon independent deletion of each co-variate: a change in objective function of > 7.8, associated with a P value of < 0.005, was required to retain a co-variate. The significant remaining co-variates represented the final model.

RESULTS

For all 5-FU cycles investigated, the mean value of CL was 235 l/h with an interindividual variability of 27%. Table 2 shows the goodness of fit upon inclusion of each co-variable. BSA, clock-time (8 a.m. versus 5 p.m.), and 3 out of 4 liver enzymes had no significant influence on 5-FU CL. In a second stage, each of the significant co-variables: age, PMNC-DPD and ALP (found significant at the screening test), was tested according to two equations: $CL = \theta1 \times (1 - \theta2 \times age) - \theta3 \times TIME$, and $CL = \theta1 - \theta2 \times (1 + \theta3 \times age) \times TIME$ same equations for ALP; and $CL = \theta1 \times (1 + \theta2 \times DPD) - \theta3 \times TIME$, and $CL = \theta1 - \theta2 \times (1 - \theta3 \times DPD) \times TIME$. In each case, the second equation would mean that the decrease in CL during the infusion was dependent on the co-variable. For the three co-variables, the best fit corresponded to the first equation. Thus, at this stage, the full model included the three co-variables which were independent of time: $CL\ (l/day) = \theta1 \times (1 - \theta2 \times age) + \theta3 \times DPD - \theta4 \times ALP - \theta5 \times TIME$. The final estimates (with 95% confidence intervals) of the regression coefficients were as follows: $\theta1 = 14\,000$ (10 200–17 800); $\theta2 = 1.04 \times 10^{-2}$ (0.78×10^{-2}–1.30×10^{-2}); $\theta3 = 9040$ (2780–15 300); $\theta4 = 9.5$ (0–19.9); $\theta5 = 420$ (60–780).

By removing each co-variable in turn from the multivariate intermediate model, the difference in the objective function was significant: 308, 68, 67 and 19, respectively, for age, PMNC-DPD, TIME and ALP. This means that CL was significantly ($P < 0.001$) dependent on each of these co-variables and that the co-variables were not redundant. The multivariate intermediate model therefore remained the final model. The difference in the objective function between this final model and the model without co-variable ($CL = \theta$) was 436. In the final model, the estimate of the coefficient of variation for interindividual variability in CL was 31% with a confidence interval of 26–35%. This variability was not lower than that found with the model without co-variable ($CL = \theta$): 27% with a confidence interval of 23–30%. In the final model, residual variability was estimated at 19%.

DISCUSSION

The purpose of this study was to examine the impact of different physiological variables on the interpatient variability of the clearance of 5-FU. This investigation was performed by using NONMEM, a powerful statistical tool particularly well suited for estimating population parameters [22]. This method determines the pharmacokinetic parameters from observational unbalanced and noisy data. Some of us recently applied the NONMEM approach for modelling carboplatin clearance including several patient variables [25]. The administration schedule was a 5-day continuous infusion of 5-FU which is representative of most 5-FU chemotherapy protocols presently used. Interestingly, the mean 5-FU clearance value which was found (235 l/h) is close to what we have previously reported (181 l/h) by applying classic pharmaco-

Table 2. Univariate analysis of the relationships between 5-FU CL and patient co-variables

Question addressed	Equation	Change in objective function	P
Body surface area (BSA)	$CL = \theta1 \times (1 + \theta2^{\star} \times BSA)$	0	NS**
Age	$CL = \theta1 \times (1 - \theta2 \times age)$	293	< 0.0005
Sex (0 if female, 1 if male)	$CL = \theta1 \times (1 - \theta2 \times sex)$	2	NS
Hepatic metastases	$CL = \theta1 \times (1 - \theta2 \times HM)$	0	NS
PMNC-DPD	$CL = \theta1 \times (1 + \theta2 \times DPD)$	29	< 0.001
AST	$CL = \theta1 \times (1 - \theta2 \times AST)$	0	NS
ALT	$CL = \theta1 \times (1 - \theta2 \times ALT)$	0	NS
γGT	$CL = \theta1 \times (1 - \theta2 \times \gamma GT)$	2	NS
ALP	$CL = \theta1 \times (1 - \theta2 \times ALP)$	29	< 0.001
Time	$CL = \theta1 \times (1 - \theta2 \times TIME)$	61	< 0.001
8 a.m. versus 5 p.m.	$CL = \theta1 \times (1 + \theta2 \times CLO)$	1	NS

Each equation was tested versus $CL = \theta1$. The objective function values were used to evaluate the increase in goodness of fit upon inclusion: a decrease in objective function of at least 3.8 associated with a P value of < 0.05 was required to identify a covariate as being significant.
*θ2 values throughout the table were positive; **NS, the change in objective function is not significant. HM, hepatic metastases; CL, clearance; TIME, time from the start of infusion; CLO, clock-time. For other abbreviations see legend of Table 1.

kinetic rules (CI = dose/AUC) in a series of 1092 cycles from the same category of patients receiving the same chemotherapy protocol as presently investigated [20]. Considering the present treatment schedule, the possible interaction of cisplatin on 5-FU clearance could be questioned as cisplatin was given the day before 5-FU infusion was begun. In fact, we recently analysed the impact of different so-called 5-FU modulators on cellular DPD activity [26], and cisplatin, tested at clinically compatible concentrations, had no effect on this key enzyme for 5-FU elimination. Nevertheless, experimental data strongly suggest that cisplatin acts as a modulator of 5-FU cytotoxicity as we previously established in tumour cell line experiments [27]. Explanations concerning the pharmacological mechanisms of the 5-FU–cisplatin synergy have been supplied by studies on human cancer cells [28] and laboratory animals [29]. Both studies suggested that cisplatin significantly enhances 5-FU cytotoxicity by indirectly increasing the intracellular levels of reduced folates. Thus, it is suggested that the possible impact of cisplatin on 5-FU pharmacology is situated on a pharmacodynamic rather than a pharmacokinetic level.

Among the variables which were tested by the NONMEM approach, it was found that the full predictive model included four independent variables which were, by order of decreasing importance, age, PMNC-DPD, time and ALP. It should be noted that age had a greater impact on 5-FU clearance as compared to the other co-variables. This point is illustrated by the more narrow confidence intervals for age attested by the regression coefficient θ_2 in comparison to the other regression coefficients. A previous study of ours indicated that 5-FU clearance was significantly lower in women in comparison with men [20]. In the present population analysis, gender did not appear as an independent parameter having significant weight in predicting 5-FU clearance. It should be noted that the number of women in the present study was low. Moreover, this apparent discrepancy is not surprising when considering differences in both the statistical approaches and the way of determining 5-FU clearance. In addition, it must be emphasised that the previously found difference in 5-FU clearance between men and women was rather small since average values were 172 and 155 l/h, respectively. Another discrepancy with previous published studies [20] involved the importance of patient age which is presently demonstrated by NONMEM analysis. It must be stressed that in the previous work the influence of patient age was tested by rank analysis comparing three groups of patients (< 50 versus 51–70 versus > 70). In the present study, by testing patient age as an independent and continuous variable, the NONMEM approach revealed that this parameter had a significant impact on 5-FU clearance (Figure 1), although the interindividual variability overrode the effect of age. Age-related physiological changes such as decrease in liver mass, hepatic enzyme activity and hepatic blood flow may account for the reduced elimination of certain metabolised drugs in the elderly population [30]. In a recent study, DPD activity was determined in 138 donor liver samples [31] and age was found to have no significant influence on liver DPD activity. In an analysis of the pharmacokinetics of 5-FU given by 5-day continuous intrahepatic infusion [32], we previously showed that the drug is particularly highly extracted by the liver (93%). The clearance value of drugs with high hepatic extraction is strongly dependent on the hepatic blood flow. Thus, the deleterious effect of age on 5-FU clearance could be explained, at least in part, by age-related decreases in liver mass or hepatic blood flow.

There is no doubt that DPD plays a central role in 5-FU elimination. Positive correlations have already been reported between PMNC-DPD and 5-FU clearance [14, 15]. The validity of PMNC-DPD as a surrogate for liver DPD activity has recently been shown [16]. Thus, it is not surprising that a population analysis by NONMEM identified PMNC-DPD as an independent variable having a significant impact on 5-FU clearance. Further information concerning the clinical importance of PMNC-DPD determination with regard to 5-FU clearance is now necessary. PMNC-DPD is expressed within a wide range of interpatient variability [33, 34]. Attempts to correlate PMNC-DPD and 5-FU clearance from population studies have led to significant associations but with weak coefficients of correlation [15, 34]. As a corollary, other authors [35] and ourselves [34] have failed to demonstrate a significant link between pretreatment PMNC-DPD and 5-FU pharmacodynamic outcome. It must be considered that only very low PMNC-DPD activity is of clinical importance because it is linked to depressed 5-FU clearance followed by severe 5-FU-related side-effects [33, 36]. Based on reported cases from the literature [37], a PMNC-DPD threshold for 5-FU related toxicity risk was suggested as located at 0.100 nmol/min/mg protein. The application of this parameter value to a large population showed that approximately 3% of unselected patients exhibited PMNC-DPD activity below the threshold [34]. It is clear that for this low percentage of cases, PMNC-DPD activity becomes the predominant factor governing 5-FU clearance and, in this situation, the presently developed predictive model reaches its limit of applicability. Alternatively, it is possible that in certain cases PMNC-DPD can be disconnected from DPD activity expressed in hepatic parenchyma. We thus recently reported on a young woman with hepatic metastases from colorectal cancer who had developed severe toxic manifestations including a neurological disorder following the first cycle of 5-FU treatment [38]. The toxic event was associated with high concentrations of 5-FU in blood and cerebrospinal fluid. PMNC-DPD values were in the normal range. In contrast, liver DPD decreased in conjunction with a marked elevation in liver enzymes. It is thus interesting to emphasise the role played by liver function in 5-FU clearance as presently

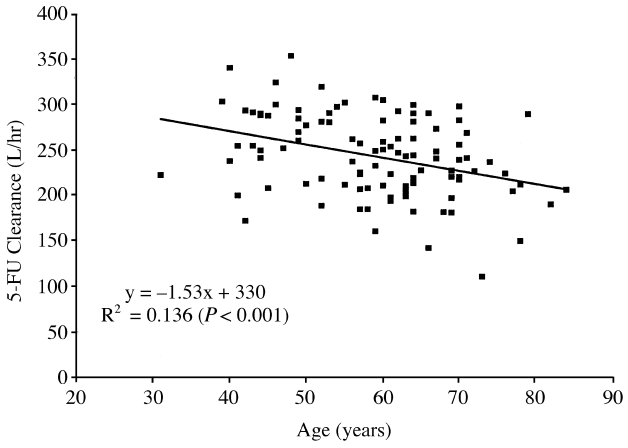

Figure 1. Relationship between age and individual CL (obtained by *post hoc* option during the NONMEM analysis where no co-variable was taken into account). The linear regression is shown.

highlighted by the population study using NONMEM; plasma ALP was found to be significantly and inversely related to the drug elimination capacity (Table 2). This finding corroborates a previous observation by us where, by simple linear regression analysis, a weak but significant correlation was established between plasma ALP and 5-FU clearance [39]. The present data emphasise the need to evaluate prospectively the benefit of 5-FU dose reduction in patients with hepatic disease.

The present study involved testing the impact of perfusion time on 5-FU clearance during the course of drug infusion (5 days). Interestingly, the analysis reveals that globally, during infusion, 5-FU clearance progressively decreases since the factor time was negatively correlated to 5-FU clearance value (Table 2). In general, non-linear pharmacokinetics of 5-FU have been reported for bolus injections where plasma concentrations can exceed K_m values for liver DPD [3]. We previously reported on 5-FU clearance during 5-day continuous infusion and noted that this pharmacokinetic parameter was globally unchanged when the 5-FU dose was increased within a 2-fold range ($550–1100\,mg\,m^{-2}\,day^{-1} \times 5$ [38]. However it must be stressed that in this latter study 5-FU clearance was determined as the total dose per cycle divided by the total cycle AUC and thus the change in 5-FU concentration profile during cycle was not taken into account. Abnormally high 5-FU concentrations during the second part of a 5-day continuous infusion were recently reported by Fety and associates [40]. This observation is in agreement with the present study and thus the risk of 5-FU accumulation during the second part of 5-day cycles must be borne in mind as regards consequences for drug toxicity.

We have shown that by using an appropriate statistical approach based on NONMEM analysis, it is possible to identify several independent patient characteristics which have a significant influence on 5-FU clearance during a 5-day continuous infusion. Previous reports by others [35] and ourselves [9] have identified target 5-FU plasma concentrations at the steady state or 5-FU area under curves which were related to an optimal 5-FU therapeutic index during 5-day continuous infusion. Given these findings, there is a potential clinical interest for 5-FU dose tailoring as it has been performed and validated for carboplatin [21, 41]. However, although the four co-variables are significantly correlated to the 5-FU CL, they did not decrease the interindividual variability. We consider that at this stage the relatively high error we found in the estimate between observed and predicted 5-FU clearance does not allow faithful 5-FU dose adaptation prior to treatment.

We consider the NONMEM approach herein described as representing a significant step forward because it identifies independent variables for predicting 5-FU clearance which are linked to patient status and treatment characteristics. At this stage, it is important to note that in our study body surface area was found to have no significant influence on 5-FU clearance. This strengthens the conclusions of a recent review article by Gurney [42] indicating that the routine use of body surface area for dose calculation of anticancer drugs should be re-evaluated. Additive co-variables other than the ones presently investigated can be sought by exploring other patient groups and schedules with modulated 5-FU. This would be particularly useful in colorectal cancer, for instance, which is the best target for 5-FU-based chemotherapy. It is the hope of the present study to stimulate such future investigations.

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