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ORIGINAL ARTICLE

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Pharmacokinetics and pharmacodynamics of irinotecan and its metabolites from plasma and saliva data in patients with metastatic digestive cancer receiving Folfiri regimen

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Abstract *Purpose*: Irinotecan is extensively metabolized into at least four compounds and previous pharmacokinetic–pharmacodynamic studies have given varying results. We hypothesized that saliva, a noninvasive, safe and painless biological sampling process, could be a good predictor of the behavior of irinotecan and its metabolites. *Methods*: Thirty-five patients with metastatic digestive cancer were treated with a Folfiri regimen every 2 weeks. The irinotecan-administered dose was 180 mg/m^2; 17 patients participated in a dose-escalating study. Irinotecan and its metabolites (SN-38, SN-38G, APC, NPC) were quantified in plasma and saliva by high-performance liquid chromatography with fluorescence detection. *Results*: The mean irinotecan systemic clearance and steady-state volume of distribution values were 14.3 l/h/m^2 and 211 l/m^2, respectively. The intrapatient variability (22–28%) was far lower than the interindividual variability (33–88%). Age and weight were the two physiological parameters that influenced drug disposition. For irinotecan, SN-38, APC and NPC, similar pharmacokinetic profiles were observed from plasma and saliva data. The saliva/plasma AUC ratios averaged 1 for irinotecan, 0.3 for SN-38, 0.17 for APC and 0.27 for NPC. Neutropenia, diarrhea and nausea were the main toxicities encountered. From both plasma and saliva data, the percentage decrease in neutrophil count appeared to be related to irinotecan and SN-38 AUCs. *Conclusions*: All these findings provide a rationale for an individual adaptation of irinotecan dosing. In case of difficult venous access, the titration of irinotecan and of its active metabolite SN-38 in saliva may prove relevant.

Keywords Irinotecan · Plasma · Saliva · Pharmacokinetics · Pharmacodynamics

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Introduction

Colorectal cancer is the third most common cancer in both men and women and the third most prevalent cause of cancer-related death [14]. Phase III studies have demonstrated the clinical benefit of irinotecan in terms of survival, time to progression and quality of life when used as a first- or second-line chemotherapy in this disease [6, 10, 22].

The antineoplastic agent, irinotecan hydrochloride, acts as an inhibitor of DNA topoisomerase I [16]. This drug is extensively metabolized in the liver into various metabolites (Fig. 1) [16]. Irinotecan is cleaved by carboxylesterases to form SN-38, a 100- to 1,000-fold greater cytotoxic compound than irinotecan [16]. It is likely that SN-38 is responsible for the gastrointestinal toxicity attributed to irinotecan administration as a result of extensive biliary excretion of the metabolite and its accumulation in the intestine [13]. SN-38 is further conjugated in human liver by uridine diphosphate glucuronosyltransferases (and particularly UGT-1A1) to an inactive β-glucuronide derivative (SN-38G). The other metabolic pathway of irinotecan is mediated by the cytochrome P-450 3A4 enzymes. The APC is formed by oxidation of the distal piperidine group of irinotecan, and the NPC is formed by cleavage of this group. Both APC and NPC have a weak inhibitory activity on cell growth in vivo [16].

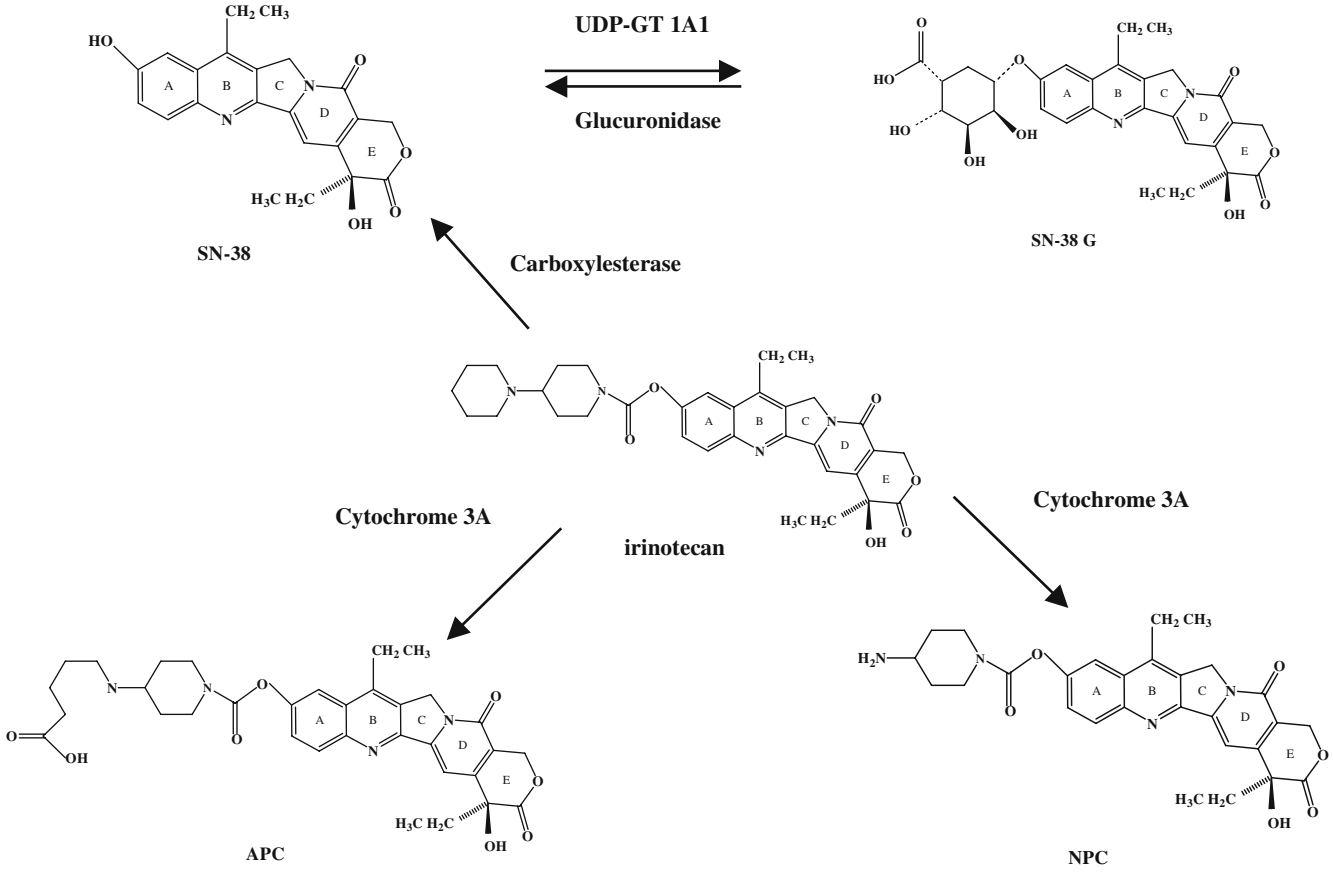

Fig. 1 Metabolic pathways of irinotecan. irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]-carbonyloxy-camptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; APC, 7-ethyl-10-[4-*N*-(5-amino-pentanoic acid)-1-piperidino]-carbonyloxycamptothecin; NPC, 7-ethyl-10-[4-amino-1-piperidino]-carbonyloxycamptothecin

Irinotecan and its metabolites are found under two forms: an active but chemically unstable α-hydroxy-delta-lactone ring form and an inactive hydroxyl–carboxylate form. Rivory et al. [21] have found low variability of this interconvertion, reporting that the carboxylate and the lactone forms became, for irinotecan and SN-38, respectively, the predominant forms in plasma soon after the end of infusion. These results suggest that a simple assay of total forms is as informative as the assay of their lactone forms.

The pharmacokinetics of irinotecan and the relationships between pharmacokinetic parameters and side effects have been widely studied [3–5, 8, 15, 16, 23, 24, 28, 29, 32]. The majority of these studies have used noncompartmental approaches; only Klein et al. [15] have developed a population pharmacokinetic model. Moreover, the findings of pharmacokinetic/pharmacodynamic relationships are highly variable [16].

In clinical practice, a major advantage in using saliva samples instead of blood samples for drug monitoring is that they can be obtained noninvasively, safely and painlessly. In a study performed in nine Japanese patients, Takahashi et al. [30] reported that saliva could be a good predictor of the behavior of either irinotecan or SN-38 in the body.

Thus, the purpose of the present study was (1) to describe the pharmacokinetic profiles of irinotecan and of its metabolites (lactone plus carboxylate form) in plasma and in saliva, a route of elimination heretofore given little attention, if any; (2) to estimate both inter- and intra-individual variability in pharmacokinetic parameters; (3) to examine which of the patient physiopathological parameters could have influenced drug disposition; and (4) to investigate pharmacokinetic/pharmacodynamic relationships that may prove useful in the future clinical management of this drug.

Patients and methods

Selection of patients

Thirty-five patients with advanced, inoperable, histologically proven digestive cancer were included in this trial and were admitted to the Medical Oncology Service of Anticancer Centre (Montpellier, France) between October 2001 and August 2004. Seventeen of them were a part of an escalating-dose phase II trial including 54 patients which will be published subsequently. Criteria for eligibility included the following: (1) age younger

than 81 years; (2) a performance status (WHO classification) of ≤ 3; (3) a life expectancy ≥ 3 months; and (4) adequate hematological function (absolute neutrophil count $\geq 2{,}000/mm^3$, platelet count $\geq 100{,}000/mm^3$ and haemoglobin ≥ 10 g/dl), hepatic function (bilirubin level < 1.5 times normal, prothrombin time $\geq 50\%$ and alkaline phosphatase level < 5 times normal) and renal function (creatinine level ≤ 135 μM).

The study protocols were reviewed and approved by the institutional review board. They were performed in accordance with the Declaration of Helsinki, and with current European Community and U.S. Food and Drug Administration guidelines for good clinical practice. The patients were fully informed about the procedure and the purpose of the experiment, and gave written consent.

Treatment regimen and dose-escalation schedule

All of the patients received a Folfiri regimen (irinotecan-LV5FU simplified). The LV5FU simplified regimen was administered at a fixed dose [leucovorin (200 mg/m^2) by intravenous infusion over 2 h followed by a 5-FU loading dose (400 mg/m^2), and then by 5-FU (2,400 mg/m^2) in a continuous 46-h infusion]. Irinotecan was administered during the infusion of leucovorin in 250 ml of 5% dextrose over a 90-min intravenous infusion. The administered dose of irinotecan was 180 mg/m^2 at the first course. This whole regimen was given on a 15-day cycle, exception made for patients with insufficient hematological recovery for whom the following treatment ought to be delayed by a maximum of 1 week. For the 17 patients entering the escalating dose trial, an increase in the dose of irinotecan could be made after considering intercourse toxicities (Table 1). The 18 other patients always received 180 mg/m^2 of irinotecan per course.

During the first cycle of treatment, each patient underwent a pharmacokinetic evaluation. The 17 patients with irinotecan escalating doses were studied during the first three cycles. The use of granulocyte-colony stimulating factor (G-CSF) was authorized in secondary prophylaxis after a grade 3, 4 neutropenia or after a delay for neutropenia. Systematic antiemetic premedications including methylprednisolone (120 mg per patient) and ondansetron (8 mg per patient) were administered intravenously, 30 min before the beginning of the treatment. To avoid transient acute cholinergic syndrome (early diarrhea, abdominal pain, conjunctivitis, hypotension), 0.25 mg of atropine was administered subcutaneously to all patients. Special recommendations for the management of delayed diarrhea (i.e., several days after treatment) were given to the patients. Early loperamide administration after the first liquid stool was recommended.

Blood and saliva collection

Samples [blood collected from a peripheral vein in heparinized glass tubes (5 ml)] and unstimulated saliva (3 ml) were drawn before drug administration (time 0), and then at 0.5, 1, 1.5 (end of infusion), 4, 8, 24 and 42 h after the start of infusion. For 14 patients, both blood and saliva samples have not been obtained. For these patients, the number of collected samples (plasma or saliva) was four (two patients: 0, 0.5, 1.5, 42 h), five (two patients: 0, 0.5, 1.5, 4, 8 h), and seven (six patients: 0, 0.5, 1, 1.5, 4, 8, 42 h; four patients: 0, 0.5, 1, 1.5, 4, 8, 24 h). Immediately after collection, blood samples were centrifuged (1,500*g* for 10 min) at 4°C, and then plasma and saliva samples were immediately frozen (−80°C) until assay.

Toxicity evaluation

Adverse experiences recorded from patient interrogation and physical examination; serum chemistries and complete blood cell count were determined before each treatment. Physical examination, vital signs and performance status were reevaluated every 2 weeks. Toxicity was defined according to the WHO common toxicity criteria and graded 1–4.

Analytical method

Irinotecan and its four metabolites, as total of lactone and carboxylate forms, were simultaneously assayed in human plasma and saliva by high-performance liquid chromatography with fluorescence detection [19]. For the five analytes, the limits of quantitation were 0.5 ng/ml in both matrices. The interassay precision varied from 2.6 to 10.8%. The interbatch accuracy ranged from 92.8 to 111%.

Table 1 Schedule of irinotecan escalating doses (17 patients)

	Inter-course toxicity	Administered dose (mg/m^2)
Course 1	–	180
Course 2	Hematological toxicity ≤ 2; nonhematological toxicity ≤ 3; treatment not postponed	220
	Hematological toxicity > 2; nonhematological toxicity > 3; treatment postponed	180
Course 3	Hematological toxicity ≤ 2; nonhematological toxicity ≤ 3; treatment not postponed	220 or 260
	Hematological toxicity > 2; nonhematological toxicity > 3; treatment postponed	180 or 220

Population pharmacokinetic analysis

Both from plasma and salivary data, individual pharmacokinetic parameters were estimated using an empirical Bayesian methodology. Analyses were performed using the nonlinear mixed-effect modeling approach as implemented in the NONMEM computer program (version 5.0) [2] through the Visual–NM graphical interface [31]. The compartmental analysis was performed by treating the parent drug and its metabolites independently. The population characteristics of the pharmacokinetic parameters (fixed and random effects) were estimated using the first order conditional estimation (FOCE) method.

For the parent drug and its metabolites, the pharmacokinetic model (1-, 2- or 3-compartment) was chosen on the basis of changes in the −2 log-likelihood function and qualitative assessment of diagnostic plots. Because −2 log-likelihood is approximately χ^2 distributed and the addition of 1-compartment increases the degrees of freedom by a factor of two, a change of 5.99 in −2 log-likelihood was required at the 5% significance level to select the more complex model. Tested structural models for metabolite formation included zero-order and first-order processes. Interindividual variability (IIV) was assessed according to a proportional error model associated to each fixed effect parameter. Various error models were also tested (additive, proportional or combined additive and proportional). The smallest −2 log-likelihood function value was associated to the better model. At each step of the model building, diagnostic plots were analyzed for closeness to and randomness along the line of identity on the observed versus predicted concentration plot, as well as randomness along the residual and weighted residual zero line on the predicted concentrations versus residuals or weighted residuals plot. Moreover, for each compound, the goodness of fit was also assessed by comparing the observed concentrations (DV) to the ones estimated using the Bayesian feedback (IPRED) using bias and precision [26, 27]. As for 17 patients of this study a pharmacokinetic evaluation was performed at the first three cycles, an interoccasion variability (IOV) was taken into account in the models.

Pharmacokinetic analysis from plasma data

Because the metabolized (F_m) fractions of the irinotecan dose into SN-38, APC or NPC were unknown in this patient population, the volume of distribution and the total clearance divided by F_m had to be estimated for each metabolite. Although enterohepatic recycling of SN-38G to SN-38 has been described in the literature, it was not identifiable in this analysis.

Following selection of the basic structural and statistical models, the influence of covariates (weight, age, body surface area, sex, aspartate aminotransferase, alanine aminotransferase, bilirubin, albumin and total proteins) was assessed. Firstly, the individual empirical pharmacokinetic estimates were plotted against all preselected potential covariates and secondly, selected covariates were added to the model and tested for statistical significance (χ^2 test).

Several secondary pharmacokinetic parameters were calculated from the individual (Bayesian estimates) primary pharmacokinetic parameters: the volume of distribution at steady state (Vss, only for irinotecan), the half-life ($t_{1/2}$) of the terminal part of the curves and the area under plasma concentration–time curves from time zero to infinity (AUC_P).

Pharmacokinetic analysis from salivary data

The main pharmacokinetic parameters to estimate were the half-life ($t_{1/2}$) of the terminal part of the curves and the area under saliva concentration–time curves from time zero to infinity (AUC_S). These two parameters were computed from the individual (Bayesian estimates) primary pharmacokinetic parameters.

The extent of diffusion into saliva of the parent drug and its metabolites was calculated from the following ratio: AUC_S/AUC_P.

Pharmacokinetic–pharmacodynamic analysis

The pharmacodynamics, especially the dose-limiting toxicities, were explored by plotting the percentage decrease in absolute neutrophil count, platelet count and hemoglobin at nadir (first course only) against both irinotecan and SN-38 AUCs computed from plasma or saliva data. Only the first courses were used because of the possible influence of previous irinotecan administrations on the pharmacodynamics of either the parent drug or metabolite SN-38. The percentage decrease was defined as follows: % decrease = $100(BV_0-BV_{nadir})/BV_0$, where BV_{nadir} was the value of the biochemical variable at the nadir and BV_0 is the pretreatment value. The data were modeled using linear, log-linear, exponential and sigmoidal maximum models. The performance of the model was evaluated by using the log-likelihood value. As previously reported [1, 3, 7, 18, 23, 25], the sigmoidal maximum effect model, as described by a modified Hill equation, produced superior fits as compared with the other models and was used to demonstrate the relationship between the percentage decrease in the above parameters and the systemic or salivary exposure (AUC_P or AUC_S, respectively) according to the following equation:

$$\text{Decrease } (\%) = \frac{(\text{ME} \times \text{AUC}^{\gamma})}{(\text{AUC}_{50}^{\gamma} + \text{AUC}^{\gamma})}.$$

ME denotes the asymptotic maximum effect, AUC_{50} is the AUC value that results in 50% decrease of the ME and γ is the sigmoidicity factor which describes the shape of the curve. The computed Pk-fit program was used for this analysis [12].

Statistical analysis

For the 17 patients receiving three different doses of irinotecan, an analysis of variance (ANOVA) was performed to compare irinotecan and SN-38 AUCs, computed from plasma data, between doses. Before the statistical analysis, AUCs were normalized to the same administered dose (180 mg/m^2) and were previously transformed into their logarithms so as to achieve a nearly normal distribution of parameter values. The same ANOVA analysis was performed on normalized irinotecan AUC computed from salivary data. The computed program Pk-fit was used for this analysis [12].

Results

Patient characteristics

A total of 35 patients were entered onto this pharmacokinetic study (19 men and 16 women; median age, 62 years). The demographic characteristics of the patients are illustrated in Table 2. Except for one patient (WHO = 3), all of them had an excellent performance status. These patients were typical of the entire study population described elsewhere [3, 11]. Among the 17 patients receiving dose escalation of irinotecan, two patients received G-CSF after the first course and for one patient the course was delayed (1 week). After the second course, eight patients received G-CSF and the treatments of four patients were delayed (1 week). With the exception of methylprednisolone (known to induce cytochrome P-450 activity) which was given to all patients, only one of them received sodium valproate (an antiepileptic drug known to inhibit the UGT-1A1 enzyme).

Table 2 Patient characteristics

Characteristics	Mean (CV, %)	Number of patients
Patients		35
Male		19
Female		16
Age (years)	62 (14.3)	
Weight (kg)	68 (17.1)	
Body surface area (m^2)	1.76 (10.7)	
WHO performance status		
0		24
1		10
3		1
Tumor type		
Colo-rectal		33
Liver		1
Cardia		1
Metastasis		
None		1
Liver alone		24
Peritoneum		1
Lymph nodes		1
Lung		3
Peritoneum/Retroperitoneum		4
Bones		1
Line of chemotherapy		
First line		33
Second line		2
WBC count (10^9/l)	8.6 (18.7)	
Neutrophil count (10^9/l)	5.9 (30.1)	
RBC count (10^{12}/l)	4.2 (18.7)	
Haemoglobin (g/dl)	12.2 (11.9)	
Platelet count (10^9/l)	355 (36.0)	
Bilirubin (μM)	11.6 (74.5)	
AST (U/l)	26 (33.6)	
ALT (U/l)	30 (28.8)	
Total proteins (g/l)	75 (11.4)	
Serum albumin (g/l)	38 (23.1)	

WBC white blood cells; *RBC* red blood cells; *AST* aspartate transferase; *ALT* alanine transferase

Pharmacokinetics from plasma data

Mean (± SD) plasma concentration–time profiles in the patient population (first course of chemotherapy) are presented in Fig. 2a for irinotecan, SN-38 and SN-38G, and in Fig. 2b for APC and NPC.

Dispositions of irinotecan was best described with a linear 3-compartment open model (Fig. 3). The six-dimensional vector θ of kinetic parameters considered in the population analysis consists of CL, initial volume of distribution (V_1), transfer rate constants (k_{21} and k_{31}), distribution rate (α) and elimination rate (β). In the last step of the population analysis, a relationship between CL and patient's age (CL decreased with age: $r = -0.42$, $P = 0.0095$) was taken into account in the model. The inclusion of this second stage model significantly improved the fit of the basic model (the objective function decreased from 5,115 to 5,010) and provided a substantial decrease in unexplained clearance IIV (from 49.7 to 41.7%). Population pharmacokinetic parameters are presented in Table 3. The V_{ss} was 377 l and the half-life of the terminal log-linear part of the curve averaged 11.7 h.

Disposition of SN-38 was best described with a 2-compartment open model with first-order formation from irinotecan (Fig. 3). The five-dimensional vector θ of kinetic parameters considered in the population analysis consists of CL/F_{msn}, transfer rate constants (k_{12} and k_{21}), formation rate constant (k_f) and initial volume of distribution (V_1/F_{msn}), where F_{msn} is the metabolized fraction of the irinotecan dose into SN-38. A relationship between V_1 and patient's weight (V_1 increased with weight: $r = 0.44$, $P = 0.0081$) was taken into account in the final model. The inclusion of this covariate significantly improved the goodness of fit (the objective function decreased from 1,784 to 1,723) and decreased the IIV on V_1 (from 43.2 to 38.2%). Population pharmacokinetic parameters are presented in Table 3. Mean half-life of the terminal log-linear part of the curve was estimated to be 28.1 h.

Dispositions of SN-38G, APC and NPC were best described with a 1-compartment open model with first-order formations (Fig. 3). The three-dimensional vector θ of kinetic parameters considered in the population

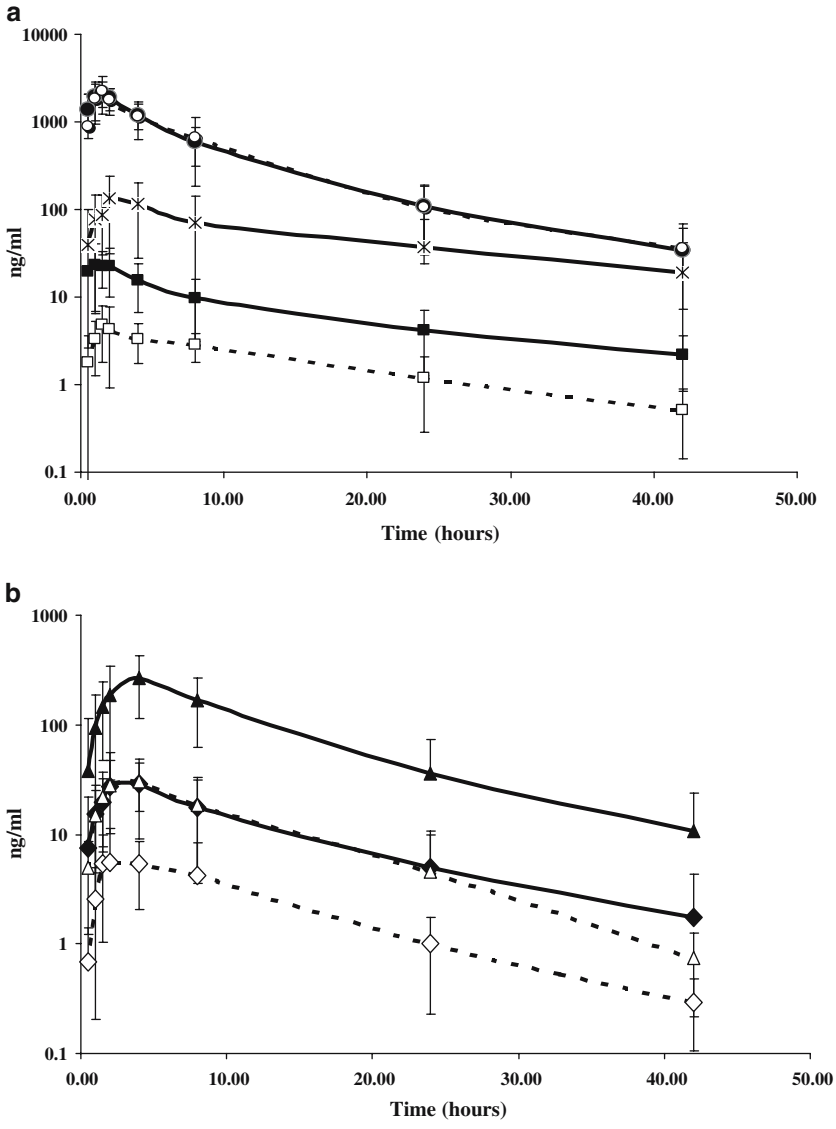

Fig. 2 Mean (± SD) plasma and saliva concentration–time profiles of irinotecan (*filled circle*, *open circle*), SN-38 (*filled square*, *open square*) and SN-38G (*multiplication sign*) (**a**); APC (*filled triangle*, *open triangle*) and NPC (*filled diamond*, *open diamond*) (**b**), in 35 patients after the first administration of irinotecan. *Filled symbols* plasma; *open symbols* saliva. *Lines* are obtained from mean concentrations connected point by point: *solid line* (plasma), *dotted line* (saliva)

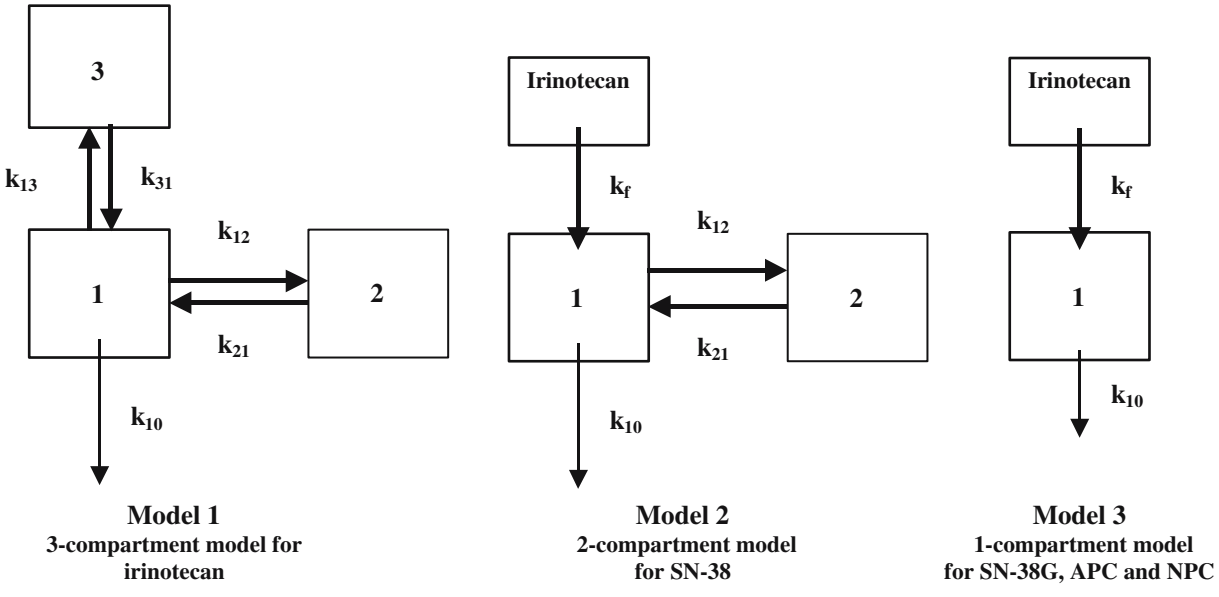

Fig. 3 Different models used

analysis consists of CL/F_m, formation rate constant (k_f) and volume of distribution (V/F_m). $F_m = F_{msng}$ corresponds to the amount of SN-38 metabolized to SN-38G and $F_m = F_{mapc}$ or F_{mnpc} corresponds to the metabolized fraction of the irinotecan dose into APC or NPC, respectively. Population pharmacokinetic parameters are presented in Table 3. Mean half-lives of the terminal log-linear part of the curves were 18.1 h for SN-38G, 9.6 h for APC and 9.23 h for NPC.

Examples of model performances are presented in Fig. 4 for the two main compounds, irinotecan and SN-38. The vast majority of the weighted residuals lays within two units of perfect agreement and was symmetrically distributed around the zero ordinate.

The interindividual distribution of irinotecan, SN-38 and SN-38G AUCs are illustrated in Fig. 5. The distribution of irinotecan AUC was close to normal. Concerning SN-38 and SN-38G AUCs, no clear evidence of bimodal distribution characteristic of a genetic polymorphism was shown. One patient of 74 years had very high AUC values compared to the other patients. This result can be explained by a partial impaired liver function due to macronodular metastasis and an occlusive syndrome avoiding a good fecal elimination of the drug and its metabolites.

For 17 of the 35 patients participating in a dose-escalating phase II study, it was possible to estimate the IOV (22–28% depending on the compound). This was far lower than the IIV (Table 3). For these patients, the ANOVA analysis performed on SN-38 AUC, normalized to an administered dose of 180 mg/m^2, indicated no statistically significant difference between the three courses (mean values: 0.31 ± 0.055 mg h/l at the first cycle, 0.32 ± 0.85 mg h/l at the second cycle and

Table 3 Population parameters from plasma data

	Population parameters			Derived parameters		
	Parameters	Mean	IIV, %	Parameters	Mean	IIV, %
Irinotecan[a]						
IOV, 26.7%	V_1 (l)	14.9 (8.85)	43.6	V_{ss} (l)	377.1	28.1
	CL (l/h)	22.2 (7.46)	41.6	AUC (mg h/l)	13.1	37.6
	α (h^{-1})	0.28 (4.64)	11.6	$t_{1/2}$ β (h)	11.7	20.5
	β (h^{-1})	0.059 (22.0)	20.5			
	k_{21} (h^{-1})	2.64 (13.3)	69.4			
	k_{31} (h^{-1})	0.095 (5.76)	14.4			
Bias: −21.8 ng/ml (95% confidence interval, −52.5, 8.86); Precision: 321.7						
SN-38G[b]						
IOV, 22.8%	V/F_{mSNG} (l)	4316 (9.7)	67.7	AUC (mg h/l)	1.86	76.3
	CL/F_{mSNG} (l/h)	190 (19.3)	33.0	$t_{1/2}$ (h)	18.1	46.3
	k_f (h^{-1})	2.66 (14.5)	13.9			
Bias: 0.35 ng/ml (confidence interval, −1.66, 2.35); Precision: 20.9						
APC[a]						
IOV, 22.2%	V/F_{mAPC} (l)	1671 (5.45)	43.8	AUC (mg h/l)	2.77	66.6
	CL/F_{mAPC} (l/h)	126 (11.2)	79.1	$t_{1/2}$ (h)	9.60	21.2
	k_f (h^{-1})	1.12 (9.3)	93.8			
Bias: −0.26 ng/ml (95% confidence interval, −2.6, 3.1); Precision : 29.9						
SN38[a]						
IOV, 23.7%	V_1/F_{mSN} (l)	1675 (0.25)	35.4	AUC (mg h/l)	0.319	43.5
	CL/F_{mSN} (l/h)	901 (14.8)	33.3	$t_{1/2}$ (h)	28.1	26.6
	k_{12} (h^{-1})	1.24 (15.2)	26.4			
	k_{21} (h^{-1})	0.068 (32.1)	38.2			
	k_f (h^{-1})	1.54 (17.0)	31.9			
Bias: −0.26 ng/ml (95% confidence interval, −0.61, 0.10); Precision : 3.7						
NPC[a]						
IOV, 28.2%	V/F_{mNPC} (l)	11430 (1.6)	70.0	AUC (mg h/l)	0.36	88.3
	CL/F_{mNPC} (l/h)	860 (17.8)	76.5	$t_{1/2}$ (h)	9.23	25.4
	k_f (h^{-1})	2.18 (6.9)	93.5			
Bias: −0.16 ng/ml (95% confidence interval, −0.65, 0.32); Precision: 5.15						

Values in parentheses are the error of estimate expressed as coefficient of variation; bias and precision are calculated by comparing observed concentrations (DV) to the ones estimated using the Bayesian feedback (IPRED)

IIV interindividual variability expressed as coefficient of variation; *IOV* interoccasion variability; *CL* total body clearance, V_1 initial volume of distribution, *Vss* steady-state volume of distribution; k_{12}, k_{21} and k_{31} transfer rate constants; α distribution rate; β rate of the terminal part of the curve; F_{mSN}, fraction of the irinotecan dose metabolized to SN38; k_f formation rate constant; *AUC* total area under the plasma concentration-time curve (normalized to a 180 mg/m^2 administered dose); $t_{1/2}$, half-life of the terminal part of the curve; F_{mAPC} or F_{mNPC}, fraction of the irinotecan dose metabolized to APC or NPC; F_{mSNG} fraction of the amount of SN-38 metabolized to SN38G

[a]combined additive and proportional error model

[b]proportional error model

0.33 ± 0.15 mg h/l at the third cycle; $P = 0.943$). For the parent drug a weak statistical significant difference occurred, either from plasma and salivary data (mean dose normalized AUC computed from plasma data: 12.4 ± 3.22 mg h/l at the first cycle, 13.2 ± 3.18 mg h/l at the second cycle and 14.3 ± 4.33 mg h/l at the third cycle, $P = 0.0283$; mean dose normalized AUC computed from salivary data: 12.8 ± 2.27 mg h/l at the first cycle, 13.0 ± 2.47 mg h/l at the second cycle and 14.2 ± 3.37 mg h/l at the third cycle, $P = 0.0393$).

Pharmacokinetics from saliva data

Mean (± SD) saliva concentration–time profiles in the patient population (first course of chemotherapy) are presented in Fig. 2a for irinotecan and SN-38, and in Fig. 2b for APC and NPC. For the parent drug and its metabolites, similar pharmacokinetic profiles were observed from plasma and salivary data. Concentrations of the SN-38G in saliva were lower than the lower limit of quantitation of the used method.

An empirical Bayes methodology using NONMEM was used to compute individual pharmacokinetic parameters. The same models as described earlier for plasma data were selected (data not shown). Derived population parameters are presented in Table 4. Only the terminal half-life and the AUC will be reported. For the parent drug and its metabolites, similar pharmacokinetic profiles were observed from plasma and salivary data. Irinotecan concentrations were of the same order of magnitude in saliva and plasma, the saliva/plasma AUC ratio averaged 1.05. For the active metabolite SN-38, concentrations in saliva were about three times lower than in plasma; the saliva/plasma AUC ratio was 0.30. For APC and NPC, the saliva/plasma AUC ratios were 0.17 and 0.27, respectively. Results are presented in Table 4. The IOV was very near to that reported from the plasma data. The mean half-lives of the terminal log-linear part of the curves: 11 h for irinotecan, 22.3 h for SN-38, 8.44 h for APC and 8.45 h for NPC were similar to those computed from the plasma data (11.7, 28.1, 9.6 and 9.23 h, respectively). Examples of model performances are presented in Fig. 6 for the two main compounds, irinotecan and SN-38. Significant relationships were found between AUCs computed from saliva and plasma data, with coefficients of correlation of 0.931 (irinotecan, $P < 0.0001$), 0.852 (SN-38, $P < 0.0001$), 0.730 (APC, $P < 0.0001$) and 0.675 (NPC, $P < 0.0001$).

Hematological and nonhematological toxicities

The total number of hematological and nonhematological toxicities encountered during this study is reported in Table 5. Mucositis is the major toxicity of 5-FU and was observed in 11–12% of patients. This drug has a weak hematological toxicity in opposite to irinotecan that demonstrated significant hematological and digestive (nausea, diarrhea) toxicities. Neutropenia, diarrhea, and nausea were the main toxicities encountered. Although severe neutropenia was not clearly irinotecan dose-related, there was a trend toward a higher incidence at the highest dose levels. The nadir of neutropenia was between day 8 and day 15, and was of brief duration. None of the patients experienced episode of febrile neutropenia or grade 3–4 diarrhea.

Pharmacokinetic–pharmacodynamic analysis

Both from salivary and plasma data, the percentage decrease in neutrophil count appeared to be related to the irinotecan or SN-38 AUC. Scatter plots of AUC versus the percentage change in neutrophil count for all of the patients at the first course are presented in Fig. 7. The goodness of fit to these data was verified by plotting weighted residuals versus model-predicted decrease in neutrophil count. The vast majority of weighted residuals was symmetrically distributed around the zero line. Using this analysis, the irinotecan AUC values producing a 50% decrease in neutrophil count were 13 mg h/l from plasma data and 12.4 mg h/l from salivary data. The high values of the sigmoidicity factor, 2.8–3 reflect the very steep AUC–toxicity relationship observed. By excluding one patient with very high AUC, similar results were obtained. AUC_{50} was 12.3 mg h/l and the sigmoidicity factor was 2.8. For SN-38, AUCs producing a 50% decrease in neutrophil count were 0.28 mg h/l from plasma data and 0.083 mg h/l from salivary data. No relationships were found between the decrease (%) in platelet count and hemoglobin level, and the irinotecan or SN-38 AUC.

Discussion

Irinotecan is clearly one of the most important new anticancer drugs developed in the last few decades for digestive cancer treatment. Irinotecan pharmacokinetic has been extensively studied but pharmacokinetic–pharmacodynamic relationships have given variable results. Here, we described, for the first time, the pharmacokinetic behavior of irinotecan and its four metabolites both in plasma and saliva. In addition, relationships between irinotecan and SN-38 exposures (AUCs) and hematological toxicities were studied. Unstimulated saliva sampling was used to avoid a modification of the equilibrium between plasma and saliva drug concentrations due to an increase of the saliva flow. All patients of this study receiving atropine, saliva secretion are influenced by the anticholinergic effects of this compound as well as by the cholinergic effects of irinotecan. Thus, our results cannot be extrapolated to a population that do not receive atropine without additional studies.

For some patients of this study, due to venous, clinical or technical problems, only sparse samples were available (4–7 blood samples); thus classical compart-

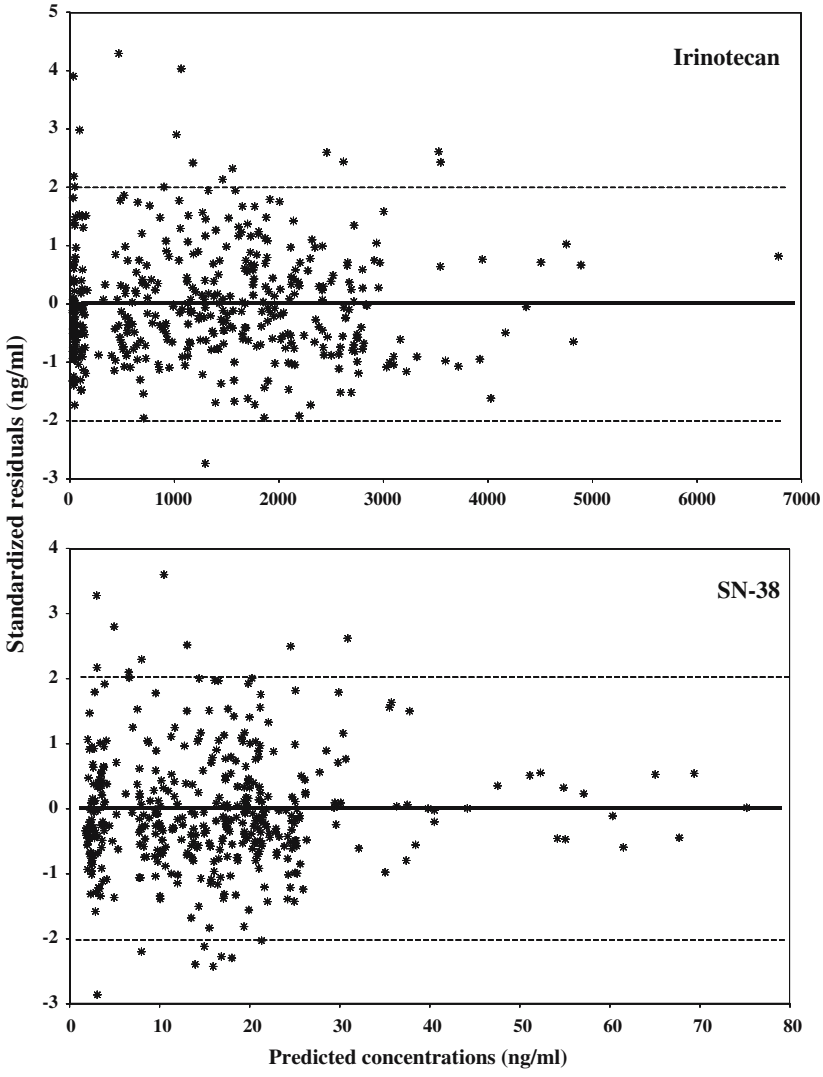

Fig. 4 Weighted residuals versus predicted concentrations (plasma data)

mental or noncompartmental analysis could not be done. Therefore, an empirical Bayes methodology was used to estimate individual pharmacokinetic parameters, either from plasma or from saliva data. In this analysis, population characteristics of the parameters to be estimated were used as prior information to estimate each individual pharmacokinetic parameters. Moreover, such an approach avoided a possible bias in the estimation of the elimination half-life (i.e., underestimation) when the last sampling time (i.e., 42 h) was not available. For 17 of the 35 patients included in this study, a pharmacokinetic evaluation was performed at the first three chemotherapy cycles, and IOV was taken into account in the models. Assessment of both IIV and IOV of pharmacokinetic parameters is of central importance to establish optimal dosage recommendations by the clinicians.

Pharmacokinetic parameters and the IIV in these parameters determined in our patients are comparable to those reported previously [3–5, 8, 15, 23, 24, 28, 29, 32]. The highest variabilities were observed in the AUCs of SN-38G, APC and NPC (76.4, 66.6 and 88.3%, respectively). This was probably related to the interpatient variations in carboxylesterases, UGT-1A1 and cytochrome P-450 3A4 activities. In contrast, in our study, the IOV was moderate (22–28%), indicating consistent irinotecan pharmacokinetics within individual patients. In this paper, formation of SN-38 from irinotecan was determined to be a first-order process. In contrast, Rivory et al. [20] and Dodds et al. [9] suggested that nonlinear process might be involved. However, frequent sampling within the first 30 min following irinotecan administration would be necessary to detect this process. In the present study, the mean half-lives of the terminal log-linear part of the plasma concentration–time curves were 11.7, 28.1, 18.1, 9.6 and 9.23 h for irinotecan, SN-38, SN-38G, APC and NPC, respectively. The terminal disposition phases of APC, NPC and of the parent drug were quite similar suggesting that a formation rate-limitation of the metabolite disposition occurred. In this case, the real metabolite half-life could not be estimated. The terminal disposition phases of SN-38 and SN-38G are delayed compared with the elimination of irinotecan and cytochrome P-450-mediated

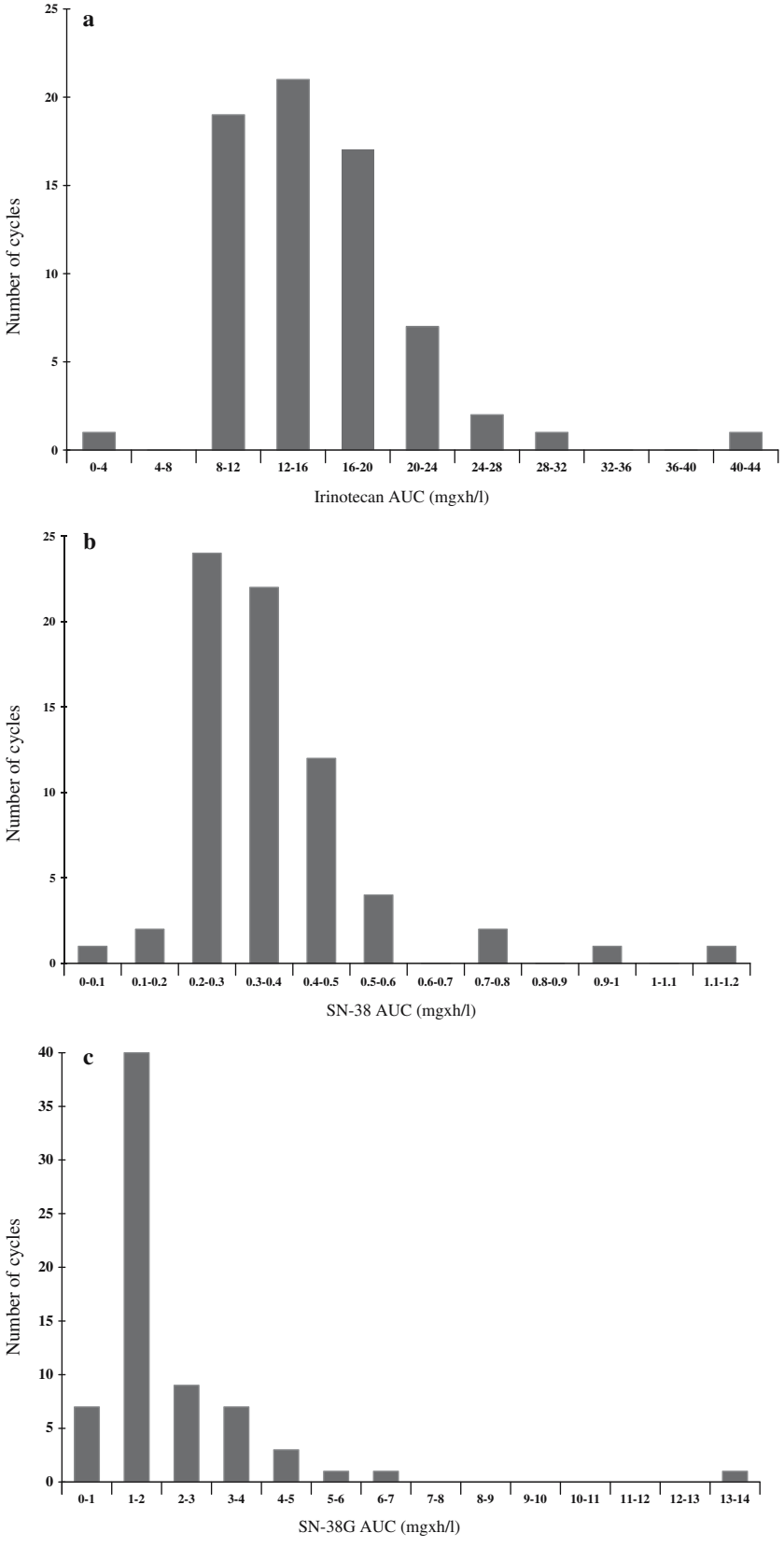

Fig. 5 Distribution of normalized AUC values (35 patients, 69 cycles) treated with an intravenous infusion of irinotecan: (**a**) irinotecan, (**b**) SN-38 and (**c**) SN-38G

metabolites, suggesting that the SN-38 disposition may involve an elimination rate-limitation process. The enterohepatic recycling of SN-38G to SN-38, although nonidentifiable in this analysis, may also be partly responsible for the prolonged terminal half-lives of SN-38 and SN-38 G. Over the dose range studied (180–

Table 4 Derived population parameters from salivary data

Parameters	Population mean	IIV, %
Irinotecan		
AUC (mg h/l)	13.2	26.9
$AUC_{saliva}/AUC_{plasma}$	1.05	18.0
$t_{1/2}$ terminal part of the curve (h)	11.0	26.4
IOV, %		24.6
Bias: −6.67 ng/ml (95% confidence interval, −27.8, 41.1); precision: 323.8		
SN 38		
AUC (mg h/l)	0.096	44.9
$AUC_{saliva}/AUC_{plasma}$	0.30	25.6
$t_{1/2}$ terminal part of the curve (h)	22.3	27.9
IOV, %		25.3
Bias: −0.068 ng/ml (95% confidence interval, −0.15, 0.02); precision: 0.78		
APC		
AUC (mg h/l))	0.431	135
$AUC_{saliva}/AUC_{plasma}$	0.17	35.3
$t_{1/2}$ terminal part of the curve (h)	8.44	31.2
IOV, %		25.6
Bias: −0.67 ng/ml (95% confidence interval, −1.3, 0.02); precision: 6.17		
NPC		
AUC (mg h/l)	0.083	79.9
$AUC_{saliva}/AUC_{plasma}$	0.27	33.3
$t_{1/2}$ terminal part of the curve (h)	8.45	27.9
IOV, %		16.3
Bias: −0.14 ng/ml (95% confidence interval, −0.24, 0.01); precision: 1.09		

IIV interindividual variability; *IOV* interoccasion variability; *AUC* area under the saliva concentration–time curve (normalized to a 180 mg/m^2 administered dose); $t_{1/2}$ half-life

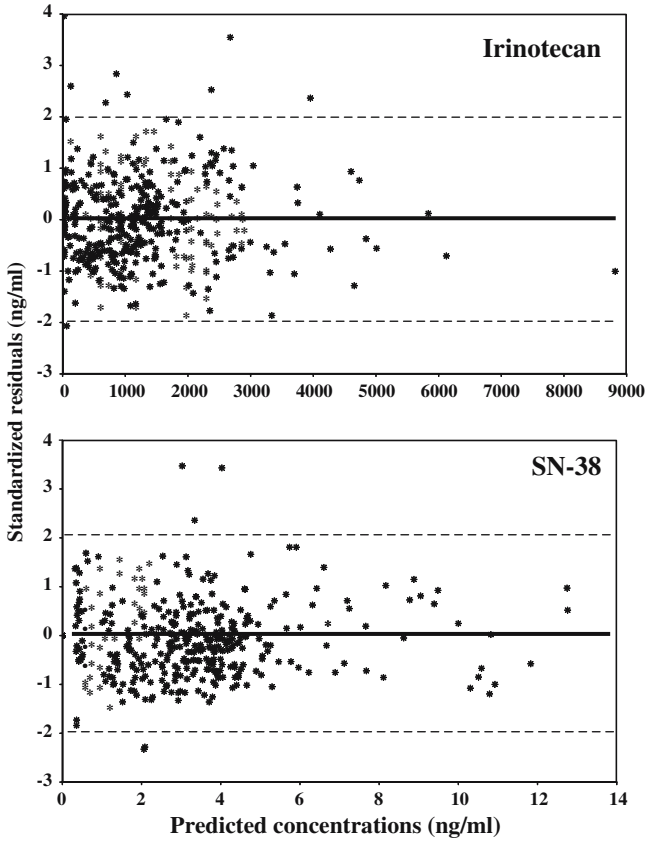

Fig. 6 Weighted residuals versus predicted concentrations (salivary data)

Table 5 Hematological and nonhematological toxicities

	Grade 1–4 Patient (%)	Grade 3 or 4 Patient (%)
Course number 1		
Mean irinotecan dose: 180 mg/m^2		
Number of patients: 35		
Neutropenia	37	9
Anemia	40	–
Diarrhea	40	–
Nausea	43	–
Vomiting	14	–
Constipation	26	–
Mucositis	11	–
Course number 2		
Mean irinotecan dose: 220 mg/m^2		
Number of patients: 17		
Neutropenia[a]	26	14
Anemia	17	–
Diarrhea	35	–
Nausea	53	–
Vomiting	18	–
Constipation	24	–
Mucositis	12	–
Course number 3		
Mean irinotecan dose: 250 mg/m^2		
Number of patients: 17		
Neutropenia[a]	24	12
Anemia	41	–
Diarrhea	59	–
Nausea	41	–
Vomiting	18	–
Constipation	18	–
Mucositis	12	–

[a]Two patients (12%) were under G-CSF treatment at the second course and 8 (47%) at the third course

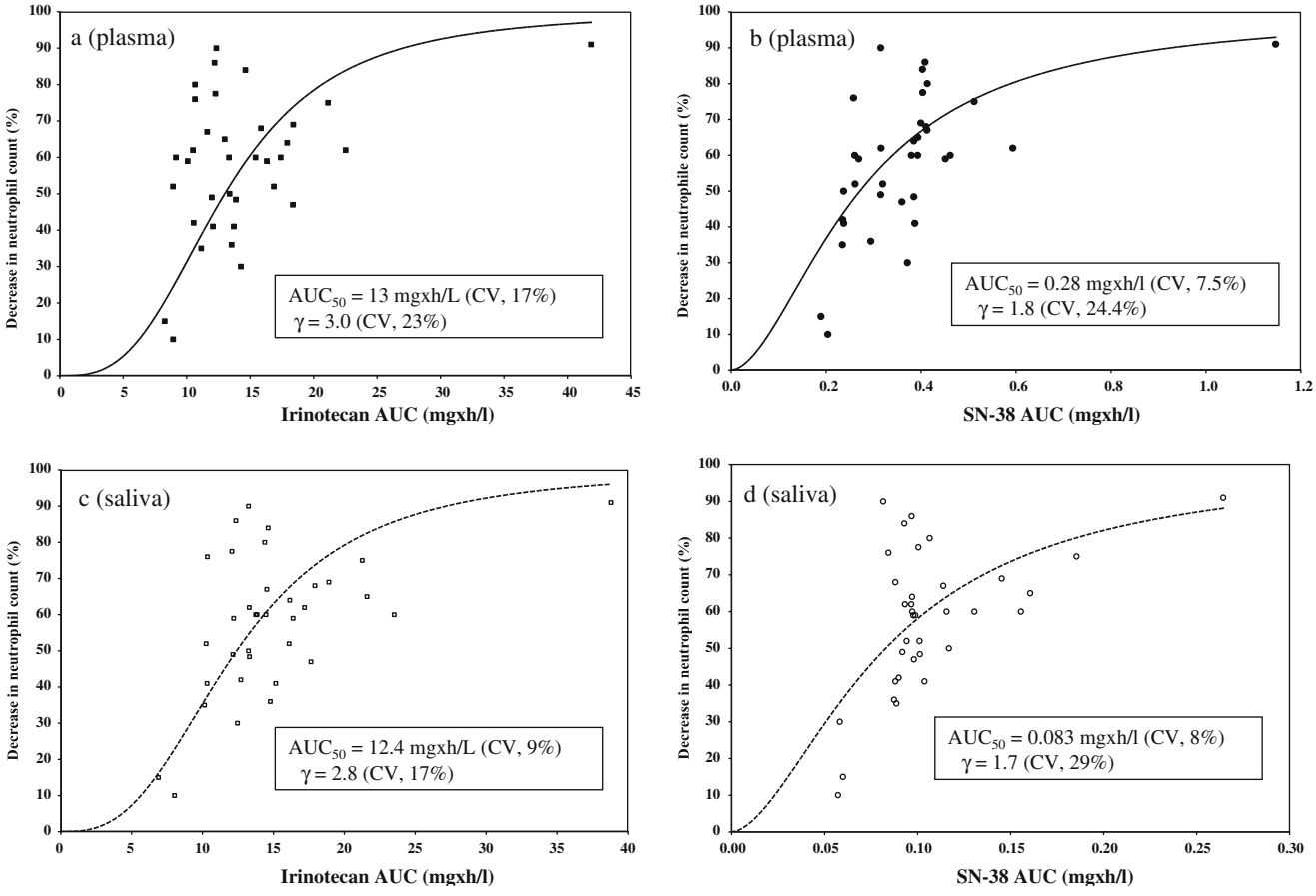

Fig. 7 Relationship between (**a, c**) irinotecan AUC and (**b, d**) SN-38 AUC and the percentage decrease in neutrophil count. *Lines* represent the fitting to the maximum effect model

260 mg/m^2), the SN-38 AUC_P increased in proportion to dose, while a weak statistical significant difference was observed for irinotecan AUCs computed either from plasma and salivary data. It has been reported that irinotecan systemic exposure increases in dose independent way at doses ranging from 33 to 180 mg/m^2 [16]. In our study highest doses have been administered to 17 patients. The observed nonlinear pharmacokinetics could be due to a saturation of a metabolic pathway. However, these results must be confirmed on a larger number of patients due to the weak statistical significance and the large interindividual variability in pharmacokinetic parameters observed.

Body surface area was not found to be significant in our model; these results were in agreement with Mathijssen et al. [17]. On the other hand, patient age was found to significantly decrease the systemic clearance of irinotecan and explained 8% of the IIV in clearance. Such a result was in agreement with Klein et al. [15]. Moreover, patient weight explained part of the variation in the initial volume of distribution of SN-38 (5%). In opposite to the results published by Chabot et al. [5], hepatic function did not appear to influence irinotecan systemic clearance. These discrepancies could be explained by the differences in the characteristics and/or size of the patients between the two populations. Only one patient of this study had elevated hepatic enzymes and presented high AUC values compared to the other patients. This result can be explained by a partial impaired liver function due to macronodular metastasis and an occlusive syndrome avoiding a good fecal elimination of the drug and its metabolites.

Similar pharmacokinetic profiles were observed from plasma and salivary data. As previously reported from plasma data, IOV in pharmacokinetic parameters was relatively low but large IIV was observed. Rapid diffusion of irinotecan and SN-38 into saliva occurred, while concentrations of SN-38G in saliva were lower than the limit of quantitation of our analytical method. For irinotecan, high relationship was found between AUC_P and AUC_S ($r = 0.931$); the saliva/plasma AUC ratio averaged 1.05. For the active metabolite SN-38, the relationship between AUC_P and AUC_S was slightly lower than that for irinotecan ($r = 0.852$); the saliva/plasma AUC ratio was 0.30. These results were in agreement with those published by Takahashi et al. [30] in Japanese patients. Considering the high binding rate of irinotecan and SN-38 to plasma proteins, about 80 and 99%, respectively [16], the secretion of these two compounds in saliva remains important. The carboxyl-

ate form becomes the predominant irinotecan form in plasma soon after the end of infusion (lactone to total irinotecan AUC = 0.37), while SN-38 was present predominantly as the lactone form at all times (lactone to total SN-38 AUC = 0.64) [21]. The difference in the saliva/plasma ratios found between irinotecan and SN-38 could be due to the differential protein binding, lipophilic properties and lactone/carboxylate form ratio in plasma of the two compounds. The carboxylate forms being unlikely to cross the biological barriers, the secretory mechanisms of the lactone forms of irinotecan and SN-38 in saliva are not due to simple passive diffusions but rather due to active processes. For the two metabolites, APC and NPC, low diffusion into saliva occurred. The higher polarity of these two compounds compared to that of irinotecan and SN-38 could explain this low diffusion.

Neutropenia, diarrhea and nausea were the main toxicities observed during this study. In spite of the known toxicity of irinotecan on the gastrointestinal tract, none of the patients experienced grade 3 or 4 diarrhea. The better tolerance of the once every 2 weeks schedule, the good management of delayed diarrhea and the small number of grade 3, 4 neutropenia could explain the absence of severe diarrhea.

Both the irinotecan and SN-38 AUCs computed from plasma and salivary data were significantly correlated with the percentage decrease in neutrophil count. These results were in agreement with previously published works [1, 3, 7, 23, 25]. The SN-38 AUC_P necessary to produce a 50% decrease in the neutrophil count found in this study was 0.28 mg h/l compared with 0.195 mg h/l [3], 0.25 mg h/l [3, 18], 0.33 mg h/l [23] and 0.65 mg h/l [1]. In this study, the irinotecan AUC_P necessary to produce a 50% decrease in the neutrophil count was 13.0 mg h/l compared with 25.7 mg h/l [3], and 25 mg h/l [1, 5]. The differences observed between the present study and the other studies could be explained by association of another drug with weak hematological toxicity (5-FU) and differences in the characteristics and/or size of the patient populations evaluated. From plasma and saliva data, the high value of the Hill constant, 2.8–3.0 found by plotting irinotecan AUC against the percent decrease in neutrophil count, reflects the very steep AUC–toxicity relationship observed.

In conclusion, there is a large IIV in the distribution, metabolism and elimination of the irinotecan. Considering the risk of weak efficacy or high toxicity of the treatment, there is a need for a more individual dosing. Pharmacogenetic studies can only evaluate the SN-38 elimination pathway via research for some mutations on the UGT-1A1 promoter. However, large IIV is also expected from the cytochrome P-450 pathway. Without any method quantifying the cytochrome phenotype, pharmacokinetic analysis will be the most effective process to estimate IIV. The undeniable problem generally caused by this analysis is the blood sampling. Saliva presents the advantage of being noninvasive, safe and painless. We have demonstrated that saliva could be a good predictor of the behavior of irinotecan in the body. In many clinical situations, including patients with difficult venous access, the titration of irinotecan and of its active metabolite SN-38 in saliva may prove relevant. All these findings provide a rationale for the conduct of new trials in which individual adaptation of the treatment could be proposed.

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