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Report

Non-linear pharmacokinetics of irinotecan in mice

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Irinotecan (CPT-11) is a topoisomerase I inhibitor used in the treatment of metastatic colorectal cancer. Its conversion by carboxyl esterases is necessary to form the active metabolite SN-38. The aims of the study were to evaluate the linearity of CPT-11 pharmacokinetics and the influence of the schedule of administration of CPT-11 in mice, using a population pharmacokinetic approach with the NON-linear Mixed Effects Model (NONMEM) program. Mice were treated using two doses and two schedules of administration [10 mg/kg/day (daily × 5) × 2 or 50 mg/kg/day on days 1 and 12]. Plasma concentrations of both CPT-11 and SN-38 were determined by HPLC. A pharmacokinetic model based on both immediate conversion of CPT-11 to SN-38 for a fraction of the administered dose and saturable process for the remaining fraction fitted the data well. Refinements of the model allowed us to evaluate both the impacts of the dose and the schedule of administration on the pharmacokinetic parameters. We conclude that the pharmacokinetics of CPT-11 is not linear in mice. Extrapolation of both pharmacokinetic and pharmacodynamic preclinical results to humans may be limited by species particularities for this drug. [© 2002 Lippincott Williams & Wilkins.]

Key words: Carboxylesterases, irinotecan, mouse, pharmacokinetic.

Introduction

Irinotecan (CPT-11) is a water-soluble derivative of camptothecin, commonly used in the treatment of metastatic colorectal cancer. It is a prodrug converted by a carboxylesterase to an active metabolite, SN-38. SN-38 is a potent topoisomerase I inhibitor which is 1000-fold more cytotoxic, *in vitro*, than CPT-11.[1] SN-38 can then be inactivated by glucuronidation (SN-38G) by UDP-glucuronosyl-transferase. While carboxylesterases are usually ubiquitous, a specific carboxylesterase for CPT-11 has been isolated from rat serum. The reaction is a two-step process: the first step is the hydrolysis of the ester bond and the transfer to the acyl group on the carboxylesterase; the second step is the deacylation of the enzyme to allow its recovery.[2]

In human, CPT-11 is administered on 3-weekly short i.v. infusions at a dose of 350 mg/m^2, although neither the schedule of administration nor the linearity of the pharmacokinetics of CPT-11 and SN-38 have been assessed by specific experiments. After CPT-11 administration, two major digestive toxicities can be observed: an immediate toxicity consisting of a cholinergic syndrome and a delayed diarrhea. The cholinergic syndrome could be attributed to high plasma concentrations of CPT-11. Neutropenia represents the other dose-limiting toxicity of CPT-11.

Pharmacokinetic studies in animals represent a necessary preclinical investigation during the development of a drug. They are also performed in order to elucidate pharmacokinetic questions arising from clinical observations of marketed compounds. In the mouse, since blood sampling usually requires the sacrifice of the animal, the pharmacokinetic analysis is performed from pooled data based on mean concentrations at the same time-point. The inter-animal pharmacokinetic variability cannot be assessed, which could lead to biased results. Moreover, most of the pharmacokinetic models reported in the literature for the analysis of mouse data after administration of CPT-11 are simple (i.e. one- or two-compartment models with first-order elimination) due to the low number of available time-points.[2–4]

To address the questions of both the linearity of CPT-11 pharmacokinetic and the influence of the schedule of administration on carboxylesterase, we performed a pharmacokinetic study of CPT-11 in mouse by a population pharmacokinetic approach using the NON-linear Mixed Effects Model (NONMEM) program.[5]

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Materials and methods

Animals, drug administration and chemical analysis

Female BALB/c mice, 4–5 weeks old, were purchased form Iffa Credo (St Germain sur l'Abresle, France), and housed in cages maintained in a controlled environment with food and water *ad libitum*. After a 2-week quarantine, they were used for experiments. CPT-11 was administered i.v. in the caudal vein in a volume of 10 μl/g body weight. We investigated two CPT-11 dose levels administered following two schedules of administration: 10 mg/kg/day daily 5 days a week for 2 consecutive weeks (from day 1 to 12), and 50 mg/kg administered on days 1 and 12. The animals received the same total dose of 100 mg/kg. The pharmacokinetic experiments were performed on days 1 and 12. Blood samples were recovered by cardiac puncture in anesthetized mice at 5 min, and 1, 3, 7 and 24 h after the administration (three mice per time-point). Each sample was immediately centrifuged and plasma stored at −20°C until analysis. CPT-11 and SN-38 plasma concentrations were determined from 50-μl plasma samples by HPLC according to Rivory *et al.*[6] Briefly, plasma samples were thawed on ice to avoid further conversion of CPT-11 to SN-38 due to plasmatic carboxylesterase. Then, 50 μl samples were added to 100 μl HCl 0.01 N and 50 μl camptothecin solution (1 μg/ml) as internal standard. After addition of acetonitrile:methanol solution (50:50, v/v), samples were centrifuged for 15 min at 1000 *g*. The supernatant was used for HPLC analysis. Calibration curves were established for each determination using 50 μl of mouse serum containing 25–5000 ng/ml for CPT-11 and 12.5–1000 ng/ml for SN-38. The limit of quantification was 5 ng/ml for both CPT-11 and SN-38.

Pharmacokinetic data analysis

Plasma CPT-11 and SN-38 concentrations from all mice were analyzed simultaneously using the NONMEM program (version V, level 1.1)[7] with the first-order estimation (FO) method and the PREDPP package[8] running on a PC computer. A proportional error model and a combination model (i.e. additive plus proportional) were used for the inter-animal and the residual variability, respectively. The model-building process was guided by graphical evaluation and by comparing the objective functions. The selected structural model was the one giving the lowest value of the objective function. The different models were compared using the approximation to the χ^2 distribution of the objective function value of the reduced model minus that of the full model; the number of degrees of freedom (d.o.f.) is equal to the difference in the number of parameters between two nested models. For example, a difference in the objective function greater than 3.8 (associated with $p<0.05$ and d.o.f. = 1) was required to consider the model with non-linear elimination (corresponding parameters: V_{max} and K_m) more appropriate than the model with linear elimination (the corresponding parameter for elimination was CL).

Results

The mean (±SD) maximum plasma observed for both CPT-11 and SN-38 at 5 min after i.v. injection showed a dose-dependent pharmacokinetics: 336±49 and 3695±289 ng/ml for CPT-11 after 10 and 50 mg/kg injections; 1465±216 and 1128±98 ng/ml for SN-38 after 10 and 50 mg/kg injections, respectively.

Considering the early maximum concentrations of SN-38, we chose to build a model dividing the administered dose of CPT-11 in two fractions: the first one was the fraction introduced within the central volume as CPT-11 (F_1) and the second one was the one immediately converted to SN-38 (F_2, with $F_1+F_2=1$). The significant change in the objective function was confirmed by the better adjustment of SN-38 concentrations obtained 5 min after administration. However, it did not explain the similarity of SN-38 concentrations associated with a 10-fold difference in CPT-11 concentrations between the two dose levels (i.e. 10 and 50 mg/kg).

The second step was to refine the model with a saturation process. A covariate was associated to the F_2 fraction, variable with the dose [i.e. $F_2=\theta_1\times(1-\theta_2\times\mathrm{DOSE})$, with DOSE = 0 when the dose is 10 mg/kg and DOSE = 1 when the dose is 50 mg/kg]. Moreover, the transformation of CPT-11 to SN-38 was considered as a Michaelis–Menten kinetic (V_{max} and K_m). The objective function again decreased significantly ($p<0.0005$). This model allowed a better fitting of the data and predicted a SN-38 C_{max} after 50 mg/kg of CPT-11 of 1115 ng/ml, while the observed concentration was 1128 ± 98 ng/ml. Finally, we introduced a constant rate of elimination for CPT-11 (K_{10}), characterizing the route of elimination distinct from SN-38. A further significant decrease of the objective function was

observed. The optimal structural model describing the data is shown in Figure 1. The corresponding graphical representation of SN-38 concentrations versus time is shown in Figure 2. It compares SN-38 concentrations predicted by the final model versus observed concentrations.

Table 1 summarizes the evaluation of the final model and the decrease of the objective function when each component of the model was successively omitted.

The mean parameters (with the 95% confidence interval as estimator of the precision) corresponding to the final model were: two-compartment model for CPT-11 with $V_c = 12.6 \pm 1.8$ ml/g body weight and $V_p = 27.6 \pm 9.0$ ml/g body weight; one-compartment model for SN-38 with $V = 1.85 \pm 0.45$ ml/g body weight; Michaelis–Menten parameters for biotransformation of CPT-11 to SN-38 were $V_{max} = 2.81 \pm 0.71$ μmol/h/g body weight and $K_m = 0.10 \pm 0.04$ μM; F_2 fraction was dependent on the dose administered according to the equation: $F_2 = 0.44 \ (\pm \ 0.10) \times [1 - 0.83 \ (\pm 0.043) \times D]$ with $D = 0$ if the dose was 10 mg/kg, $D = 1$ if the dose was 50 mg/kg; linear elimination for CPT-11 with $K_{10} = 0.38 \ (\pm \ 0.06)\ h^{-1}$ and for SN-38 with $K_{10} = 2.9 \ (\pm \ 0.4)\ h^{-1}$. The final model attributed inter-animal variabilities of 22, 38, 37 and 14% for V_c, V_{max}, F_2 and K_{10}, respectively. Inter-animal variability was fixed to zero for V, K_m, K_{10}, K_{12} and K_{21}. These latter parameters were chosen as those for which smallest inter-animal variabilities were obtained when run without constraint (i.e. no value fixed to zero) was performed.

The modification of the parameters V_{max} and F_2 due to the repetition of the administrations was also explored by assigning the value 1 at the covariate 'day' for data after 10 mg/kg at day 12 (following repeated administrations) and the value 0 in other cases. By taking into account this covariate, no significant change of the objective function was observed.

Lastly, no significant difference was observed between the pharmacokinetic parameters corresponding to the injection of 50 mg/kg at days 12 and 1.

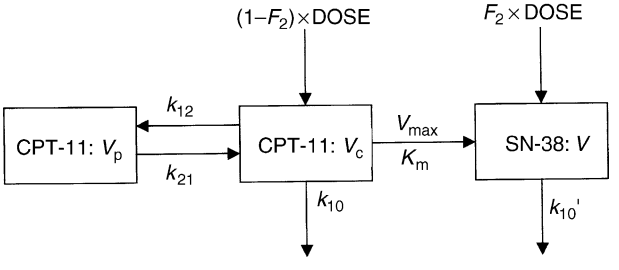

Figure 1. Optimal structural model: two compartments for CPT-11 (V_p for the peripheral compartment and V_c for the central compartment) and one compartment for SN-38; K_m and V_{max} characterize the Michaelis–Menten process; $F_2 \times$ DOSE for the amount of administered CPT-11 immediately converted to SN-38.

Discussion

The purpose of this study was to describe CPT-11 metabolism and its particular pharmacokinetics in mouse with a population pharmacokinetic approach, using the NONMEM program. This type of methodology is a powerful tool for pre-clinical experiments allowing the simultaneous analysis of both CPT-11 and SN-38 data from different animals. This pharmacokinetic approach has been necessary to assess the complexity of the metabolism of this molecule and particularly the conversion of CPT-11 to SN-38. The naive pooling approach would require a larger number of samples and, thus, of animals.

CPT-11 conversion to SN-38 by carboxylesterase is a two-step reaction. The hydrolysis reaction is faster than the deacylation step, which limits the reaction rate.[2] We built a model considering that a fraction of the CPT-11 dose was immediately converted to SN-38 (due to the systemic localization of the enzyme) and the other fraction of the dose ('non-immediate' conversion) was converted according to a Michaelis–Menten kinetic. This is consistent with the data from Kaneda *et al.*[4] and Stewart *et al.*[9] describing a saturation process for CPT-11 conversion to SN-38 in mice. Indeed, the two fractions were highly dependent on the administered dose; the model shows an approximately 5-fold decrease of the 'immediate' conversion when the dose is 5-fold increased and the 'non-immediate' conversion is better described with a Michaelis–Menten kinetic, characterizing a saturation process.

The final model led us to describe routes of elimination for both CPT-11 and SN-38. It was obtained with a limited number of samples and only two doses. Further studies (i.e. a comparison between simulated and observed concentrations following injections of different doses) would be necessary to confirm the usefulness of this model.

As it has already been suggested in a clinical study in children,[10] we wanted to address the question of the schedule dependency of CPT-11. The influence of the schedule of administration did not appear to be significant regarding to the conversion of CPT-11.

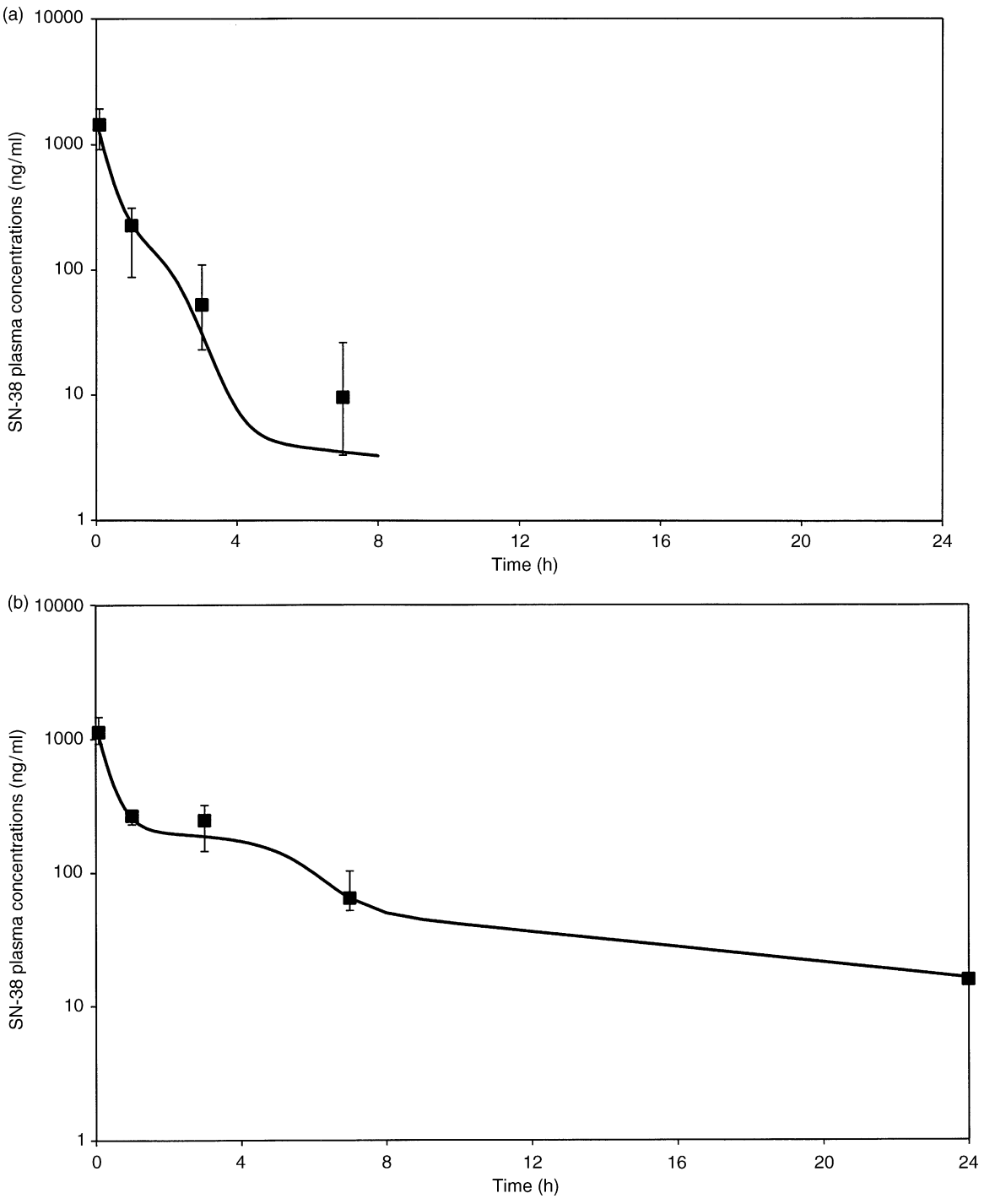

Figure 2. SN-38 concentration profile after a dose of (a) 10 and (b) 50 mg/kg of CPT-11 predicted by the final model compared with the mean concentrations observed (interval corresponding to the extreme observed values). Solid line: profile predicted by the final model. Squares: mean SN-38 observed values.

Indeed, including the covariate 'day' as we exposed it above, we did not observe a significant change in one of the parameters studied. However, when considering the saturation process, the schedule of administration is an important parameter. Fractionation of the dose (i.e. delivering the same total dose in several administrations of lower doses) allows the maintenance of active SN-38 plasma levels without reaching high plasma levels of CPT-11 associated with toxicity.[11]

Table 1. Evaluation of the final model of CPT-11 in mice

Alternative models tested	Increase of objective function	$p <$
One compartment for CPT-11	15	0.001
Only transformation of CPT-11 into SN-38 ($K_{10} = 0$)	18	0.001
Fraction of the dose immediately converted in SN-38		
independent of the dose (F_2 constant)	40	0.0005
null ($F_2 = 0$)	113	0.0005
Fraction F_1 converted into SN-38 according to a first-order process (K_a in place of K_m and V_{max})	51	0.0005

CPT-11 showed a wide inter- and intrapatient variability in clinical trials.[1,12–14] Using the NONMEM program to analyze patient data would offer the possibility to identify parameters related to those variabilities. The relevance of fractionating administrations could be evaluated both in terms of toxicity and efficacy.

Conclusion

Pre-clinical studies are still a major step in the development of new drugs, as already shown by Burtin *et al.*[15] Unfortunately, it is difficult to extrapolate these results to humans since carboxylesterase is greater both qualitatively and quantitatively in rodents, particularly at a systemic level, and since localization of the enzyme is different in humans and mice (i.e. plasmatic activity in mice is very important compared to humans). The present model would not fit human pharmacokinetic data, but could help evaluate a possible saturation process that could influence the pharmacokinetics of both CPT-11 and SN-38. This study shows that population pharmacokinetic analysis of sparse data with NONMEM provided additional information about drug disposition and the influence of the covariates.

References

1. Chabot GG, Abigerges D, Catimel G, *et al*. Population pharmacokinetics and pharmacodynamics of irinotecan (CPT-11) and active metabolite SN-38 during phase I trials. *Ann Oncol* 1995; **6**: 141–51.
2. Tsuji T, Kaneda N, Kado K, Yokokura T, Yoshimoto T, Tsuru D. CPT-11 converting enzyme from rat serum: purification and some properties. *J Pharmacobiodyn* 1991; **14**: 341–9.
3. Bissery MC, Vrignaud P, Lavelle F, Chabot GG. Experimental antitumor activity and pharmacokinetics of the camptothecin analog irinotecan (CPT-11) in mice. *Anti-Cancer Drugs* 1996; **7**: 437–60.
4. Kaneda N, Nagata H, Furuta T, Yokokura T. Metabolism and pharmacokinetics of the camptothecin analog CPT-11 in the mouse. *Cancer Res* 1990; **50**: 1715–20.
5. Beal SL, Sheiner LB. Methodology of population pharmacokinetics. In: Garrett ER, Hirtz J, eds. *Drug fate and metabolism*. New York: Marcel Decker 1985; **5**: 135–83.
6. Rivory LP, Robert J. Reversed-phase high-performance liquid chromatographic method for the simultaneous quantitation of the carboxylate and lactone forms of the camptothecin derivative irinotecan, CPT-11, and its metabolite SN-38 in plasma. *J Chromatogr B* 1994; **661**: 133–41.
7. Boeckmann AJ, Sheiner LB, Beal SL. *NONMEM users guides, part V: introductory guide*. Technical Report of the Division of Clinical Pharmacology, University of California, San Francisco 1994.
8. Beal SL, Boeckmann AJ, Sheiner LB. *NONMEM users guides, part VI: PREDPP guide*. Technical Report of the Division of Clinical Pharmacology, University of California, San Francisco 1994.
9. Stewart CF, Zamboni WC, Crom WR, Houghton PJ. Disposition of irinotecan and SN-38 following oral and intravenous irinotecan dosing in mice. *Cancer Chemother Pharmacol* 1997; **40**: 259–65.
10. Furman WL, Stewart CF, Poquette CA, *et al.* Direct translation of a protracted Irinotecan schedule from a xenograft model to a phase I trial in children. *J Clin Oncol* 1999; **17**: 1–10.
11. Kuhn JG. Pharmacology of irinotecan. *Oncology* 1998; **12**(suppl 6): 39–42.
12. Drengler RL, Khun JG, Schaaf LJ, *et al.* Phase I and pharmacokinetic trial of oral irinotecan administered daily for 5 days every 3 weeks in patients with solid tumors. *J Clin Oncol* 1999; **17**: 685–96.
13. Negoro S, Fukuoka M, Masuda N, *et al.* Phase I study of weekly intravenous infusions of CPT-11, a new derivative of camptothecin, in the treatment of advanced non-small-cell lung cancer. *J Natl Cancer Inst* 1991; **83**: 1164–8.
14. Rowinsky EK, Grochow LB, Ettinger DS, *et al.* Phase I and pharmacological study of the novel topoisomerase I inhibitor 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin (CPT-11) administered as a ninety-minute infusion every 3 weeks. *Cancer Res* 1994; **54**: 427–36.
15. Burtin P, Mentre F, Van Bree J, Steimer JL. Sparse sampling for assessment of drug exposure in toxicological studies. *Eur J Drug Metab Pharmacokinet* 1996; **21**: 105–11.

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