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ORIGINAL ARTICLE

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Phase I dose-finding study and a pharmacokinetic/pharmacodynamic analysis of the neutropenic response of intravenous diflomotecan in patients with advanced malignant tumours

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Abstract *Purpose*: To determine the maximum tolerated dose (MTD) of intravenous (iv) diflomotecan administered once every 3 weeks, and to characterize the relationship between pharmacokinetics and neutropenic effect, using a semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model. *Experimental design*: Twenty-four patients received a total of 75 cycles of iv diflomotecan that was administered as 20-min infusion, once every 3 weeks at escalating doses of 2, 4, 5, and 6 mg/m^2. Haematological and non-haematological toxicities were evaluated. Plasma concentrations of diflomotecan were measured after the first drug administration. *Results*: Dose limiting toxicity (DLT) following the first cycle occurred in 12 patients and a total of 16 patients experienced DLT at some point in the trial. During the first cycle of treatment the number of patients in the 5 and 6 mg/m^2 dose groups that experienced DLT was 3 of 4, and 3 of 3, respectively. Therefore, the dose of 5 mg/m^2 was considered the MTD and the dose of 4 mg/m^2 the recommended dose (RD). During the first cycle, 12 patients experienced DLT, six had either infection of haematological toxicity and eight complained of fatigue. The best response was a partial response in one patient treated at the 6 mg/m^2 dose level. Disease stabilization was observed in seven patients (four patients treated at 4 mg/m^2 and one patient at each dose level of 2, 5, and 6 mg/m^2). The remaining patients had all progressive disease. The median time to progression for all patients was 5.9 weeks. Pharmacokinetics of diflomotecan was described with a three-compartmental model. Mean population parameter estimates of the apparent volume of distribution of the central compartment (V_c) increased linearly with body surface area (BSA) as: V_c (L) = 41.5 × (BSA/1.85), and the mean population estimate of the apparent volume of distribution of the shallow compartment was lower in females (29.5 vs 48.8 L). Computer simulations showed the lack of clinical significance of these covariates. The time course of the neutropenic response was adequately described by a semi-mechanistic model that includes cellular processes and drug effects. *Conclusions*: The MTD and RD after a 20-min iv infusion of diflomotecan every 3 weeks are 4 and 5 mg/m^2, respectively. Diflomotecan showed linear pharmacokinetic behaviour and the selected PK/PD model described adequately the time course of neutropenia. The mean model predicted values of nadir and time to nadir after a 20-min iv infusion of 4 mg/m^2 of diflomotecan was 0.86×10^9 /L neutrophil cell counts and 11 days, respectively.

Keywords Diflomotecan · Phase I · Population Pharmacokinetic-Pharmacodynamic · Semimechanistic model · Neutropenia · NONMEM

Part of this work was presented as a poster in the 12th Population Approach Group in Europe (PAGE) meeting [12–13, June, 2003, Verona (Italy)]and in the Annual meeting of American Society of Clinical Oncology (ASCO) in 2004.

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Introduction

Diflomotecan (BN80915; 9,10-difluoro-homocamptothecin) is a new member of the "tecan" family [1]. The cytotoxicity of these compounds is due to the inhibition of topoisomerase type I, a nuclear enzyme involved in the cell replication process. The replacement of the six-membered α-hydroxy lactone ring of camptothecins (CPTs) with a seven-membered β-lactone ring of homocamptothecins (hCPTs) enhances the plasma stability of the lactone ring included in their chemical structure.

This lactone form is responsible for the antitumour activity. However, in the conventional CPTs, this active form is rapidly and reversibly hydrolysed in plasma to reach an equilibrium with its open carboxylate form, which is biologically inactive [2–4].

The antitumour activity of diflomotecan has been demonstrated in both, in vitro and in vivo preclinical studies. In in vitro studies carried out with human cancer cells, HT29, diflomotecan produced more DNA strand breaks and stimulated DNA cleavage at more sites than some CPT analogues. In addition, the antiproliferative activity of diflomotecan tested on a panel of human cancer cell lines and measured as IC_{50} parameter, was higher than for SN-38; this effect was also demonstrated in their related drug-resistant lines [5]. On the other hand, in HL-60 promyelocytic leukaemia cells diflomotecan was a potent inducer of apoptosis. This mechanism was associated with changes in the cell cycle and in other biochemical events [6].

In in vivo studies, diflomotecan displayed a potent activity in inhibiting tumour growth. This effect, demonstrated in several human tumour types transplanted in nude mice, was significantly higher than other hCPT (BN80927) and several benchmark products, irinotecan and topotecan [7].

Diflomotecan is currently at the early stages of clinical development [1, 2, 8, 9], where myelosuppresion has been identified as the principal dose-limiting toxicity. To our knowledge there have been no attempts to develop a semi-mechanistic pharmacokinetic/pharmacodynamic (PK/PD) model for myelosuppresion for diflomotecan. Such models can be used, for example, to compare the haematotoxicodynamic properties of diflomotecan with other anticancer drugs for which the time course of neutropenia has been already described semi-mechanistically [10, 11].

The current study has two main objectives: to determine the maximum tolerated dose (MTD) of diflomotecan administered as a short (iv) intravenous infusion, and to establish the appropriate PK and concentration versus neutropenic effect relationships.

Patients and methods

This study was conducted in two centres in France (Hôpital Saint-Louis in Paris and Centre Oscar Lambret in Lille), and in accordance with the ethical principles stated in the Declaration of Helsinki and with the laws and regulations of France. All the clinical information used in this article was obtained from the IND approved by FDA on 30 October 2003.

Patient selection

Twenty-four patients with malignant solid tumour(s) participated in the study. Subjects were adequately informed (both verbally and by receipt of written information) of the aims, methods, potential benefits, hazards, and discomforts, and their right to abstain from participating and to withdraw their consent at any time, and all of them gave written informed consent. Patients of at least 18-years-old, with histological proof of cancer and failure of standard therapy or no option of active standard therapy, with a life expectancy greater than 12 weeks and 0–2 WHO performance status were eligible for the study. Another inclusion criteria involved adequate bone marrow reserve (absolute neutrophil count $>1.5 \times 10^9$ /L and platelet count of $>100 \times 10^9$ /L), renal function (creatinine clearance > 60 mL/min), and liver biology (serum bilirubin levels $<1.25 \times$ ULN [upper limit of normal for the reference laboratory], and serum AST and ALT levels $<2.5 \times$ ULN [AST and ALT $<5 \times$ ULN in presence of liver metastases]). Exclusion criteria were pregnancy or lactation or childbearing potential with no adequate contraception, active infection, treatment with other chaemotherapy or radiotherapy within 4 weeks of protocol entry or chaemotherapy with nitrosoureas or mitomycin within 6 weeks of protocol entry, clinical evidence of major organ failure or brain metastases, and participation in another clinical trial within 30 days prior to study screening. Table 1 shows the characteristics of the studied patient population.

Study design and dose escalation

A phase I, open-label, non-randomized, multicentre, escalating dose study of diflomotecan was administered

Table 1 Summary of patient's ($N = 24$) characteristics

Characteristic	Value
Demographics	
Age (years)	55 (34 – 71)
Weight (kg)	73 (42 – 90)
Height (cm)	169.5 (152 – 190)
BSA (m^2)	1.85 (1.36 – 2.17)
Sex (female/male)	N = 11/13
Clinical chemistry	
Serum creatinine (μmol/L)	84 (52 1–154)
Creatinine clearance (mL/min)	81.4 (19.5 – 187.8)
Alkaline phosphatase (U/L)	170.3 (59–378)
Aspartate aminotransferase (U/L)	26.8 (4–52)
Alanine aminotransferase (U/L)	29.3 (12–87)
Lactate dehydrogenase (U/L)	341.8 (114–981)
γ- glutamyl transaminase (U/L)	91.2 (10–476)
Total bilirrubin (μmol/L)	8.1 (3.9–17.1)
Total serum protein (g/L)	71.7 (57.9 – 82.5)
Albumin (g/L)	41 (28–49)
Tumor involvement	
Liver metastasis (presence/ absence)	N = 9/15
WHO perfomance status (0/1/2)	N = 10/11/3
Tumor types	
Breast cancer	N = 7
Colorectal cancer	N = 7
Non-small-cell lung cancer	N = 5
Head and neck cancer	N = 2
Soft tissue sarcoma	N = 1
Melanoma	N = 1
Parotid cancer	N = 1

Values are presented as mean value with range in parenthesis, unless N, which indicates the number of patients

as 20 min iv infusion for two cycles, 21 days apart (unless unacceptable toxicity or patient refusal occur). In case of efficacy, lack of toxicity and absence of PK contra-indication, the investigator could offer to the patient for ethical reasons, the possibility to continue the treatment cycles beyond the originally planned two iv treatment cycles. In this case, medical and follow-up procedures were the same as for the first two iv administrations.

Initially, three patients were to be enrolled per cohort. The starting dose (2 mg/m^2) was based upon the acute toxicological results in rodent species (less than one-eighth of the maximum non-lethal dose in the most sensitive species; mice and rats). The decision to escalate the dose was based on the safety findings of all patients in the cohort and enrollment in the next cohort began only after the safety and tolerance data of the last patient entered in the preceding cohort were known. If one patient in the cohort experienced a dose-limiting toxicity (DLT), three additional patients were to be added at the same dose level. DLT was defined as any toxicity of grade 3 or 4 according to NCI-CTC. If an additional patient experienced grade 3 or 4 toxicity then that dose level was considered above the MTD and the previous dose level was considered the MTD.

Drug administration

Diflomotecan was supplied by Ipsen Biotech (Paris, France) in the form of vials containing 20 mg of the compound, with 3.3 mL dimethyl acetamide and a glass bottle of solvent, containing 3.5 g of Montanox VGDF80, and 0.4 g of sodium chloride and water for injection to make up 200 mL of aqueous, sterile isotonic solution. For reconstitution, the 3.3 mL vial of diflomotecan was diluted with the solvent, protected from light and stored at 2–8°C. Diflomotecan was infused during 20 min using a peristaltic pump. Nausea and vomiting was prevented at the second administration with a single iv administration of ondansetron (8 mg) or granisetron (3 mg) before treatment was administered over 15 min.

Treatment assessment

One week before treatment a complete screening evaluation including clinical status, haematology, and biochemistry was performed. Radiological evaluation was carried out within 3 weeks before treatment. During treatment, clinical status, biochemistry, and toxicity symptoms were evaluated weekly. Complete blood cell count, including WBC differential, platelet counts, and haemoglobin was performed twice a week. In case of febrile neutropenia, blood analyses were performed daily. Tumour evaluation, carried out, according to WHO response criteria, was performed at baseline and after the second course of therapy and for patients who remained in the study, at the end of every other cycle.

Sample collection for pharmacokinetic analysis

Blood collection to obtain plasma samples for the analysis of BN80915 (the active form) and BN80942 (code name of the corresponding inactive open lactone form) was done only during the first course of treatment at 0, 10, 20 (end of infusion), 30, and 45 min and 1.5, 3, 4.5, 6, 9, 12, 24, and 48 h after the start of the administration. Blood samples (8 mL) were collected in glass tubes containing sodium heparinate as anticoagulant, and placed immediately on ice and centrifuged at 2000 *g* for 15 min at 4°C. Three aliquots of 1.2 mL of plasma were frozen at −80°C until analysis.

Analytical determination

Plasma concentrations of BN80915 and BN80942 were determined by LC-MS/MS using ^{13}C-labelled analogues of both forms (BN81011 and BN81012, respectively). Analytes were extracted with diethyl ether and were separated by a Kromasil (100 C-18, 5 × 0.46 cm, 5 μm) column, with a mobile phase composed of acetonitrile/water (38:62 v/v) containing 0.1% acetic acid at a flow rate of 1 mL/min and isocratic conditions. The lower limit of quantification was 0.25 and 0.5 ng/mL for BN80915 and BN80942, respectively. The linear concentration range for the technique was established between 0.1 and 500 ng/mL for BN80915 and between 0.25 and 500 ng/mL for BN80942. The precision of the analytical technique corresponding to BN80915 was between 0.7 and 16.9% (coefficient of variation), and accuracy was between −13.0 and 7.3% (percentage of relative error).

Data analysis

All the analyses were performed using the non-linear mixed effects modelling approach with the software NONMEM version V [12], using the FOCE estimation method with INTERACTION. Interpatient variability was modelled exponentially, and residual errors were described with a combined error model. Selection between models was based on the precision of parameter estimates, goodness-of-fit plots, as well as on the minimum value of the NONMEM objective function [−2 log(likelihood), −2LL].

The population PK model was first developed, and subsequently, the time course of neutropenia was described using the individual predicted PK profiles. Only the active lactone form (BN80915) was considered for the analysis. The covariates initially selected using the generalized additive model (GAM) approach [13], implemented in the software Xpose version 3 [14], were then tested in NONMEM, and the potential clinical significance of the final selected covariate models were evaluated by simulations. The PK model was validated computing for each of the model parameters, the median

(MPE (%)) and median absolute (MAPE (%)) performance error obtained from 1,000 model-based simulated data sets [15]. The PK/PD was validated using the posterior predictive check [16]. One thousand model-based data sets were simulated. For each of the simulated data sets, the percentage of individuals treated with 4 mg/m^2 dose exhibiting different degrees of neutropenia was computed, transforming the simulated neutrophil count at nadir into a 5 (0–IV)-categories scale [17]. The same procedure was followed to calculate the toxicity percentages corresponding to the raw data. Distributions of percentages of neutropenia of grades 3 and 4 and time to nadir obtained from the simulations were represented graphically together with their mean value.

PK models

Plasma drug disposition was characterized by compartment models. Distribution and elimination were modelled as first-order processes, however models including plasma concentration-dependent elimination and distribution were also evaluated.

PK/PD models

The structure of the model used to describe the time course of the neutropenic effects of diflomotecan was previously described by Friberg et al. [10]. The model incorporates some of the key and known processes of the cellular cycle: proliferation, maturation of precursor cells represented by the first-order rate constant (k_{Prol}) and the mean transition time (MTT), respectively. MTT is reflected as a chain of three transit compartments connected by the first-order rate constant (k_{tr}), which is equal to $(n+1)$/MTT, n being the number of transit compartments. The elimination of circulating (observed) cells and the homaeostatic regulation are represented by k_{Circ} and γ, respectively. Drug effects are modelled as a decrease in the k_{Prol} as: $k_{Prol} \times (1 - \text{Slope} \times C_p)$, where Slope is a parameter to be estimated by the model and C_p represents the predicted plasma concentrations of diflomotecan. During model development, different number of transit compartments, different estimates of k_{tr} and k_{Circ}, a zero-order rate of proliferation, reversible E_{MAX} or sigmoidal E_{MAX} and irreversible models accounting for drug effects were also explored.

Results

The results that are shown in this study are derived from a total of 24 patients with characteristics listed in Table 1. All patients had received prior chaemotherapy with a median number of regimens of 3.5 (ranging from 1–14), 17 patients received also radiotherapy with a median dose of 50 Gy. Patient dose levels were 2, 4, 5, and 6 mg/m^2, although during the first administration one patient received 1 mg/m^2 instead of 2 mg/m^2 and another patient was mistakenly overdosed at 11.4 mg/m^2 . Thus, the number of patients who have received, in the first course of treatment, 1, 2, 4, 5, 6, and 11.4 mg/m^2 of diflomotecan was 1, 2, 13, 4, 3, and 1, respectively. The maximum number of cycles was seven, and the number of patients contributing with 1, 2, 3, 4, 6, and 7 cycles of chaemotherapy was 5, 9, 1, 4, 3, and 2, respectively, resulting in a total of 75 cycles. Dose administration was delayed in seven patients (six patients and one patient in the 4 and 5 mg/m^2 dose groups, respectively) in nine treatment cycles (eight cycles and one cycle in the 4 and 5 mg/m^2 dose groups, respectively). Dose reduction occurred in five patients (three patients and two patients in the 4 and 6 mg/m^2 dose level, respectively) in six treatment cycles (three cycles both for the 4 and 6 mg/m^2 dose level).

Dose-limiting toxicity

DLT following the first cycle occurred in 12 patients and a total of 16 patients experienced DLT at some point in the trial. During the first cycle of treatment the number of patients in the 5 and 6 mg/m^2 dose groups who experienced DLT was 3 of 4, and 3 of 3, respectively. Therefore, the dose of 5 mg/m^2 was considered the MTD and the dose of 4 mg/m^2 the recommended dose (RD). During the first cycle, 12 patients experienced DLT, six had infection of haematological toxicity and eight complained of fatigue. One patient in the 5 mg/m^2 dose group died in the setting of febrile neutropenia.

Haematological and non-haematological toxicity (Table 2)

The haematological toxicity increased with the dose level. Following the first cycle, 9 patients experienced grade 4 neutropenia and 13 patients experienced grade 4 neutropenia overall during the course of the trial. In addition, 3 patients experienced grade 4 thrombocytopenia at doses higher than the RD. The patient who received the 11.2 mg/m^2 dose experienced grade 4 (0.17×10^9 /L) neutropenia on day 12 after the infusion. Fifteen days after infusion the cell counts corresponded to a neutropenia of grade 0. On the other hand, the gastrointestinal toxicity represented by the nausea, vomiting, and diarrhoea events was in general mild. The incidence of the fatigue appeared to be dose-related although could be associated to disease progression or anaemia.

Anti-tumour activity

Two patients were not evaluated for response. The best response was a partial response in one patient treated at the 6 mg/m^2 dose level. Disease stabilization was observed in seven patients (four patients treated at 4 mg/

Table 2 NCI-CTC grade worst haematological and non-haematological toxicity and after iv diflomotecan (all cycles)

Dose (mg/m^2)	Neutropenia		Thrombocytopenia		Anemia		Nausea		Vomiting		Diarrhea		Fatigue	
	3	4	3	4	3	4	2	3	2	3	2	3	2	3
2	0	0	0	0	0	0	0	1/3	0	1/3	0	0	3/3	0
4	3/14	7/14	5/14	0	2/14	1/14	3/14	0	6/14	1/14	0	2/14	7/14	4/14
5	0	3/4	1/4	1/4	1/4	0	0	0	0	0	0	0	1/4	3/4
6	0	3/3	1/3	2/3	2/3	0	0	0	1/3	1/3	2/3	0	0	3/3
Total	3/24	13/24	7/24	3/24	5/24	1/24	3/24	1/24	7/24	3/24	2/24	2/24	11/24	10/24

n/*N*, number of patients who presented a specific grade of toxicity (3 or 4 of haematological toxicity and 2 or 3 of different gastrointestinal disorders and fatigue) per total number of patients in each group of dose, 2, 4, 5, and 6 mg/m^2

m^2 and one patient at each dose level of 2, 5, and 6 mg/m^2). The remaining patients had all progressive disease. The median time to progression for all patients was 5.9 weeks.

PK modelling

A total of 258 plasma samples were used to establish the PK model. Plasma concentration versus time profiles of diflomotecan were best described using a three-compartment model with first order elimination. Before testing the available covariates for significance, the mean population estimates of the apparent volume of the central (V_c), shallow (V_{P1}), and deep (V_{P2}) compartments were 43.8, 36.1, 12.8 L, respectively. The corresponding estimates for the total elimination clearance (CL), and the distribution clearances between the central and shallow (CL_{D1}), and between the central and deep compartments (CL_{D2}) were 21.8, 65.8, and 1.4 L/h, respectively. Inclusion of interpatient variability was significant ($P < 0.01$) for V_c, V_{P1}, and CL, with estimates of 53, 34, and 57%, respectively. Covariates body surface area (BSA) for V_c, and SEX for V_{P1}, were statistically significant. Table 3 lists the population model parameter estimates obtained from the selected model. Figure 1 shows typical and individual model predictions. The values of MPE and MAPE for all the parameters included in the final model were less than 15 and 22%, respectively, indicating absence of bias and a good precision in the parameter estimates.

Figure 2 shows the distribution of the plasma concentrations of diflomotecan obtained from 1000 males and females receiving 4 mg/m^2 of the drug as a 20-min iv infusion with a BSA value of 1.85 m^2 (left panel) and 1000 female patients with BSA of 1.4 and 1.98 m^2 (right panel). In both cases the observed differences in the distribution profiles are unlikely to have the SEX and BSA covariates linked to clinical implications.

PK/PD modelling

A total of 505 neutrophil determinations were used to develop the PK/PD model. The model proposed by Friberg et al. [10] was also selected in the present work, where no significant ($P > 0.05$) covariate effects were detected. Table 3 lists the parameter estimates corresponding to the final model. The rates of proliferation, transition, and elimination of circulating (observed) cells are the same and equal to $(n+1)$/MTT. Figure 3 shows the typical model-predicted neutrophil time profiles that describe very well the mean tendency of the data for each dose group. The results from the model validation procedure using the posterior predictive check showed that the selected model was supported by the data. For each of the data descriptors the mean values obtained

Table 3 Population PK and PD parameter estimates of diflomotecan

	Estimate	IPV
Pharmacokinetics		
V_c (L)	41.5 (0.08)	52 (0.54)
V_{P1}_male(L)	48.8 (0.11)	Ne
V_{P1}_female(L)	29.5 (0.08)	
CL (L/h)	21.6 (0.11)	51 (0.30)
CL_{D1} (L/h)	80.4 (0.30)	Ne
V_{P2} (L)	12.8 (0.21)	Ne
CL_{D2} (L/h)	1.5 (0.33)	Ne
Proportional error (%)	21.2 (0.08)	Na
Additive error (ng/mL)	0.33 (0.18)	Na
Pharmacodynamics		
$Circ_0$ (cells × 10^9 /L)	4.58 (0.08)	39 (0.28)
MTT (days)	5.38 (0.13)	21 (0.44)
Slope (mL/ng)	0.144 (0.19)	61 (0.69)
γ	0.159 (0.06)	Ne
Additive error (cell × 10^9 /L)	0.85 (0.79)	Na
Proportional error (%)	38 (0.61)	Na

Estimates are listed with the relative standard error in parenthesis. Relative standard errors are computed as the ratio between the standard error and the parameter estimate. Interpatient variability (IPV) is expressed as coefficient of variation (%). V_c, apparent volume of distribution of the central compartment modelled as $V_c \times (BSA/1.85)$; V_{P1_male}, $V_{P1\_females}$, apparent volumes of distribution of the shallow compartment in males and females, respectively; *CL*, clearance of the central compartment; $CL_{D1}$and CL_{D2}, distribution clearances between the central and the shallow, and between the central and deep compartments, respectively; V_{P2}, apparent volume of distribution of the deep compartment. $Circ_0$, absolute neutrophil cell counts at baseline; *MTT*, mean transition time; *Slope*, the inhibitory effects of diflomotecan on the rate of proliferation of precursor cells; γ, parameter controlling the rebound effect; *Ne*, not estimated; *Na*, not applicable

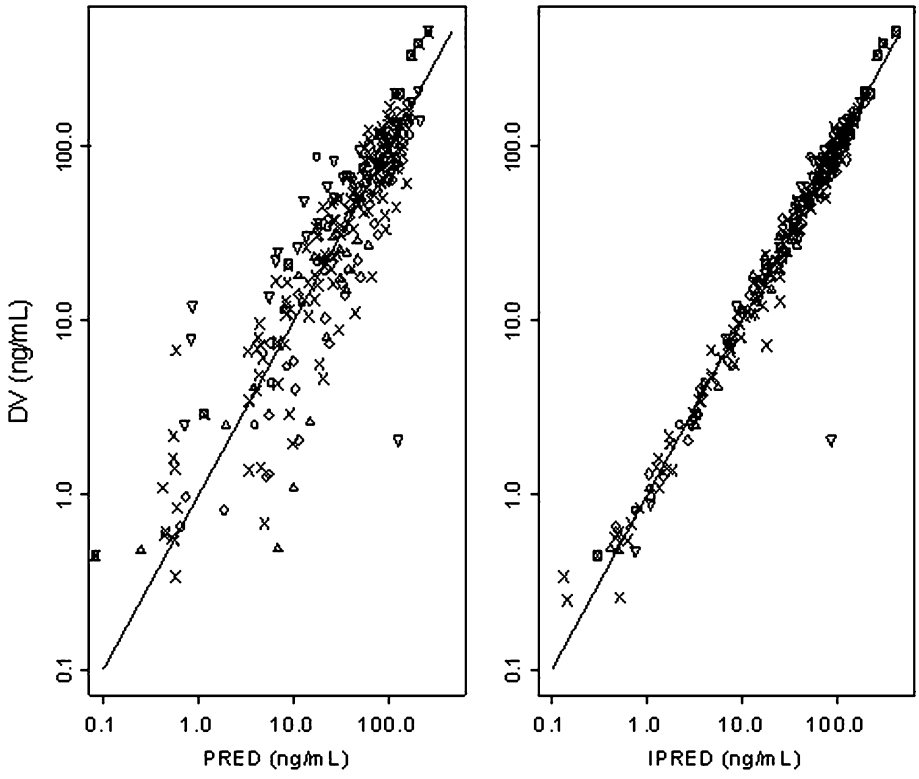

Fig. 1 Goodness-of-fit plots. *DV*, observed values; *PRED*, typical model predictions; *IPRED*, individual model predictions. *Different symbols* represent different doses of diflomotecan. *Solid lines* correspond to the line of identity. The *log scale* has been used for clarity in the plot

from the simulations were very similar to those extracted from the raw data. Percentages of grade 3 neutropenia were 22 versus 18, percentages of grade 4 neutropenia were 22.5 versus 21, and the mean times to nadir were 12.5 versus 11 days for raw and simulated data, respectively.

Discussion

The current report describes the diflomotecan PK behaviour and the PK/PD analysis of the neutropenic effect induced by diflomotecan administered iv, once ev-

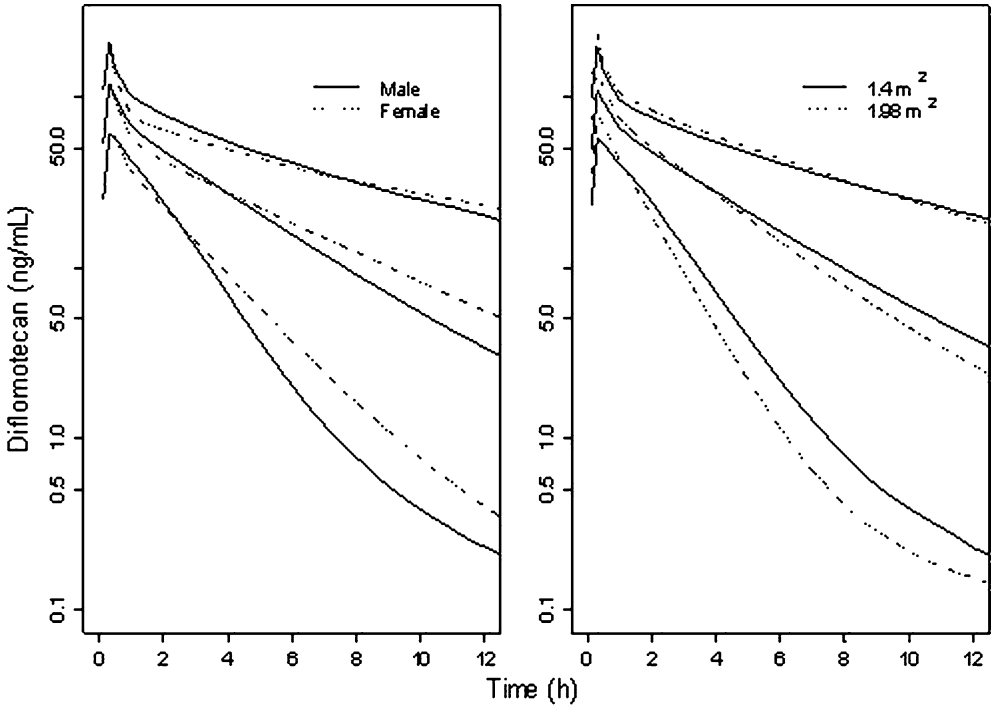

Fig. 2 The panels show the simulated PK profiles corresponding to the 2.5 (*lower*), 50 (*middle*), and 97.5th (*upper*) percentiles, respectively. The *left panel* shows the covariate effects of sex and in the *right panel* the effects of the BSA are presented. During the simulations a single 20-min iv infusion of 7.2 mg was assumed

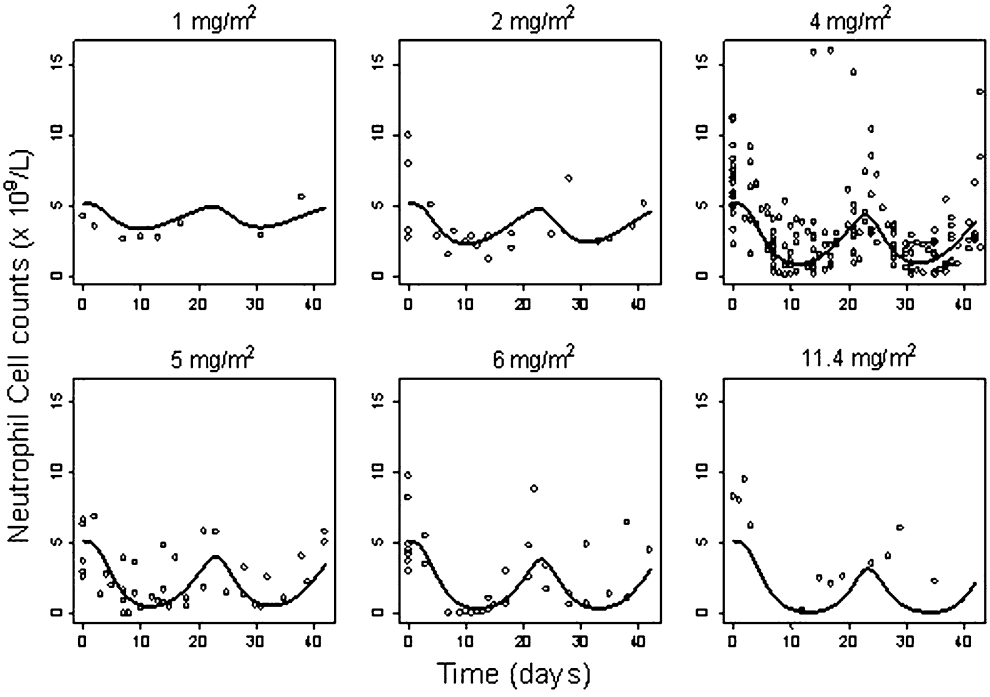

Fig. 3 Observed (*symbols*) and typical model-predicted (*solid lines*) neutrophil cell counts versus time profiles for each dose group. For clarity, the panels include only data from the first two cycles of treatment

ery 3 weeks, in a phase I dose escalation trial. This study shows that iv administration of diflomotecan is feasible in adult patients with solid tumours. As with other topoisomerase type I inhibitors, the main induced toxicological side-effects were haematological and, in particular, severe neutropenia. Similar results were also found in a phase I study with oral administration of diflomotecan [8]. Gastrointestinal toxicity was mild, especially the incidence of diarrhoea that was not related to diflomotecan dose level. Based upon the safety data, the recommended dose was established at 4 mg/m^2 administered as a 20 min infusion once every 3 weeks, corresponding to a 7.4 mg per cycle for a patient with BSA of 1.85 m^2 (the mean in the population studied). The RD of diflomotecan after oral administration is 0.27 mg/day × 5 every 3 weeks [8], a value which corresponds to a total of 1.35 mg of diflomotecan per cycle. Although antitumour activity was not a primary end point, a partial response was obtained in one patient and disease stabilization in seven patients, four of them at the RD level.

Diflomotecan in plasma undergoes multi-compartmental kinetics. None of the covariates tested showed statistically significant effects on the PK behaviour of diflomotecan, however it should be noted that eligibililty criteria excluded patients with severe renal or hepatic dysfunction. The dose level was not selected as a covariate in the model, however, as the patient number is small, a dose effect on the PK cannot be excluded. It should be noted that in a different phase I study, a linear and dose-independent diflomotecan PK were observed [8]. Diflomotecan total plasma clearance is associated with a degree of inter-patient variability of 51%, considered as a moderate to high value and mostly responsible for the amplitude in the 95% prediction intervals presented in Fig. 2. Since the renal excretion of diflomotecan was found to be low [8], this result suggests that the degree of inter-patient variability is highly contributed by the liver metabolism. Similar values of inter-patient variability are reported for other anticancer drugs: lurtotecan, 32% [18], topotecan, 33% (after inclusion of creatinine clearance, body weight, and ECOG performance status as covariates with a 22% of inter-occasion variability) [17], irinotecan 32% [19], and SN-38, 63% [20]. On the other hand, and in contrast with irinotecan, the metabolites of diflomotecan are biologically inactive and therefore do not contribute to the uncertainty in the response.

In vitro experiments showed that CYP3A4 accounted for 78% of the diflomotecan metabolism, suggesting a major role for CYP3A4 isoforms on the overall CYP3A-mediated metabolism [21]. In a phase I clinical study with 22 patients [9], who were genotyped for ABCG2 gene (responsible for resistance to various anticancer drugs) it was found that patients carrying a variant of ABCG2421C > A allele appeared to have altered plasma concentrations of diflomotecan. On the other hand, the variants in CYP3A4 and CYP3A5 genes seemed to play a minor role in the inter-patient variability observed for diflomotecan [9].

The finding that BSA does not affect drug exposure suggests that there is no need to adjust the dose per m^2 . This result is in accordance with several reports in the literature indicating that, for a great variety of anticancer drugs including diflomotecan, and contrary to clinical oncology practice, there is no direct correlation between clearance and BSA [8, 22].

The haematological effects are quite often the leading cause for dose-limitation in the use of anti-neoplasic cytotoxic agents. In this context, not only the extent of toxicity but also its duration is important, since for a same value at nadir, a higher risk of infection is associated with a prolonged duration of the toxicity [23]. Therefore, it is highly desirable to have models capable of describing simultaneously the degree of the induced side-effect and its duration [10].

The data reported for diflomotecan in this paper were adequately described using a recently developed PK/PD model [10]. Predictions were excellent (see Fig. 3) and the model validation, using a posterior predictive check, showed that the model was indeed supported by the patient data. This model allows the discrimination between system and drug-related parameters. Interestingly, the estimates of the system-related parameters obtained during this analysis were very similar to those that have been reported previously for $Circ_0$, MTT, and γ [10, 11]: 4.72–5.51 ($x10^9$ /L), 3.65–5.62 days, and 0.119–0.23, respectively. The corresponding estimates in the present study are 4.58 ($\times 10^9$ /L), 5.38 days, and 0.159. For comparison purposes, the slope parameter estimate based on total plasma concentrations of diflomotecan (see Table 3) was corrected by the unbound fraction in plasma (0.09) and expressed in units of μM^{-1} (molecular weight = 398.36). The estimated value was equal to 637.4 μM^{-1}. This value is higher than the ones reported for other anticancer drugs evaluated with the same model [10] indicating that diflomotecan is more potent. This result is in accordance with previous studies where the antiproliferative effects toward HT-29 colon carcinoma cells of diflomotecan ($IC_{50} = 0.3$ nM) were stronger compared with SN-38 ($IC_{50} = 20$ nM) and topotecan ($IC_{50} = 40$ nM) [24]. Diflomotecan has also shown higher antiproliferative activity in ex vivo colon tumor tissue than SN-38, CPT, topotecan, adriamycin, and etoposide [25]. It is important to take into consideration that the model used in the current study was developed using data obtained after a single drug administration with a given schedule (20-min iv infusion, once every 3 weeks). It is very likely that diflomotecan, in the course of future clinical development, would be administered under different dosing schedules. Therefore, the ability of the current model to describe the neutropenic effect and duration using different drug regimens has to be confirmed yet.

In conclusion, the RD of diflomotecan for phase II studies administered in adults as a 20-min iv infusion once every 3 weeks is 4 mg/m^2. The PK of diflomotecan were described properly with a three open compartment PK model, along with a moderate value of inter-patient variability in total plasma clearance. There was no apparent effect of dose on diflomotecan PK behaviour within the dose range tested. The time course of the haematological toxicity induced by diflomotecan and measured by the neutrophil cell counts was adequately described using a semi-mechanistic model developed and published recently [10].

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