Phase I Pharmacological and Bioavailability Study of Oral Diflomotecan (BN80915), a Novel E-Ring-modified Camptothecin Analogue in Adults with Solid Tumors

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ABSTRACT

Purpose: Diflomotecan (BN80915) is an E-ring modified camptothecin analogue that possesses greater lactone stability in plasma compared with other topoisomerase I inhibitors, a potential advantage for antitumor activity. As with other camptothecins, oral administration has pharmacological and clinical advantages. This Phase I study was performed to assess the feasibility of the administration of oral diflomotecan, to determine the maximum-tolerated, dose its bioavailability, and to explore the pharmacokinetics.

Experimental Design: An initial i.v. bolus was administered to assess the bioavailability of diflomotecan. Fourteen days later, diflomotecan was administered p.o. once daily for 5 days to adult patients with solid malignant tumors and repeated every 3 weeks. BN80915 and its open lactone form BN80942 were measured.

Results: Twenty-two patients entered the study and received a total of 57 cycles of oral diflomotecan at flat dose levels of 0.10, 0.20, 0.27, and 0.35 mg. The main toxicity was hematological, but some patients experienced alopecia, mild gastrointestinal toxicity, and fatigue. At the 0.35-mg dose level, 2 of 4 patients experienced dose-limiting toxicity comprising grade 3 thrombocytopenia with epistaxis and febrile neutropenia in 1 patient and uncomplicated grade 4 neutropenia lasting for >7 days in another. Toxicity was accepta-ble at the 0.27-mg dose level at which dose-limiting toxicities were observed in 3 of 12 patients (grade 4 neutropenia > 7 days, complicated by fever in 1 patient but without other signs of infection). After two cycles of diflomotecan, 6 patients had disease stabilization, which was maintained in 2 patients for 9 months and >1 year, respectively.

Diflomotecan pharmacokinetics were linear over the dose range studied. Systemic exposure correlated with the fall in WBC counts. The mean oral bioavailability (±SD) was 72.24 ± 59.2% across all dose levels. Urinary excretion of BN80915 was very low.

Conclusions: The recommended oral diflomotecan dose for Phase II studies is 0.27 mg/day × 5 every 3 weeks. This regimen is convenient and generally well tolerated with a favorable pharmacokinetic profile and high but variable bioavailability.

INTRODUCTION

The fluorinated homocamptothecin diflomotecan BN80915[2] is a recently developed water-insoluble topoisomerase I inhibitor (1, 2). Homocamptothecins are camptothecin analogues bearing a seven-membered β-hydroxylactone ring, with enhanced lactone stability, instead of the naturally occurring six-membered α-hydroxylactone. Because a one-carbon ring expansion is chemically termed a homoligation, these new lactone- or E-ring-modified compounds were named homocamptothecins. Because of the enhanced stability of the active form, homocamptothecins were expected to exert greater topoisomerase I inhibition and antitumor efficacy than the currently used analogues such as irinotecan and topotecan. This was confirmed *in vivo*, with i.p. and oral administration, in murine leukemia, human colon carcinoma, melanoma, pancreatic and ovarian cancer, brain glioblastoma, prostate and (non-)small cell lung tumor mice models (3–6), and *ex vivo* in human colon cancers (7).

Topoisomerase I inhibitors *in vitro* show more pronounced antitumor activity with protracted exposure. This can be

achieved with oral therapy, and because this is a more convenient and less expensive means of treatment (8), oral administration of diflomotecan was chosen for this study. In preclinical studies, absolute oral bioavailability of the compound ranged from 30% in rats (9) to 60% in dogs (10). Radioactivity studies in rats showed that BN80915, its lactone open ring analogue BN80942 and metabolites, were mainly recovered in the feces (69–74%), with ~16% in urine. After oral administration in mice, toxicity consisted mainly of alopecia and gastrointestinal side effects (11). The LD_{10} in these studies was 2.7 mg/m^2.

We conducted a Phase I pharmacological and bioavailability study of diflomotecan administered p.o. once daily for 5 days every 3 weeks to adult patients with solid malignant tumors. For other topoisomerase I inhibitors, fixed or flat dosing is as effective as dosing/square meter (12). Given the interpatient pharmacokinetic and pharmacodynamic variability after oral administration, we decided to use the more convenient flat dosing approach in this study. The aims of this Phase I study were to assess the feasibility of oral administration of diflomotecan to humans, to determine its maximum-tolerated dosage and bioavailability, and to explore pharmacokinetics and also pharmacodynamic correlations.

MATERIALS AND METHODS

Patient Selection. Eligible patients had histological or cytological proof of a solid malignant tumor that was resistant to standard forms of therapy or for whom no active standard therapy was available. Other inclusion criteria included the following: age at least 18 years; WHO performance status ≤ 2; no recent previous chemotherapy and/or radiotherapy; adequate hematopoietic function (absolute neutrophil count ≥ 1.5 × 10^9/liter and platelet count ≥ 100 × 10^9/liter), renal function (creatinine clearance > 60 ml/min or serum creatinine < 1.5 the ULN), and liver biochemistry [total serum bilirubin level ≤ 1.25 × ULN and serum AST and ALT levels ≤2.5 × ULN in the presence of liver metastasis AST and ALT were allowed ≤5 × ULN]. An additional exclusion criterion was any condition precluding oral intake or gastrointestinal absorption. The local medical ethical committees of the participating institutions in Rotterdam and Glasgow approved the study protocol and all patients gave written informed consent before entering the study.

Dosage and Dose Escalation. Two weeks after a single 20-min i.v. infusion of diflomotecan, escalating doses of oral diflomotecan were administered daily for 5 days every 3 weeks. The starting i.v. and oral dose was 0.1 mg/day, giving a total oral dose of 0.5 mg/oral cycle; this is ~10% of the estimated LD_{10} in mice. Dose escalation depended on toxicities at the prior dose level. Except for the initial dose level, the i.v. dose level was equal to the next lower oral dose level to avoid toxicity from the i.v. drug because otherwise systemic exposure from the i.v. administration would have been higher than after oral administration at the same dose level. At least 3 patients were treated at each dose level. If 1 of 3 patients experienced DLT, 3 additional patients were entered at that dose level. The RD was defined as the dose level below that which induced DLT in at least one-third of the patients (*i.e.,* ≥2 of 3 or 6 patients). Additional patients were included at the RD up to a total of 12 patients to additionally assess safety, pharmacokinetics, and pharmacodynamics. DLT was defined as grade 4 neutro-

penia (NCI-CTC version 2.0) lasting ≥8 days, grade 3 or 4 neutropenia complicated by fever, grade 4 thrombocytopenia or grade 3 complicated with hemorrhage, and/or grade ≥ 3 nonhematological toxicity, excluding inadequately treated nausea and vomiting. If a patient experienced DLT, the dose of diflomotecan was decreased by one dose level at the time of re-treatment if the patient remained on therapy. Treatment was delayed until the absolute neutrophil count had recovered to ≥1.5 × 10^9/liter and platelet count to ≥100 × 10^9/liter and continued so long as there was no disease progression and toxicity was acceptable unless this was considered not in the best interest of the patient.

Drug Administration. Diflomotecan was supplied by Ipsen Biotech (Paris, France) in brown-glass vials containing 20 mg of the compound, with 3.3 ml of 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 reconstituting the product, the 3.3-ml vial of diflomotecan was diluted with the solvent, protected from light and stored at 2–8°C. In aqueous solution, the stability of diflomotecan at room temperature is adequate for the expected duration of the i.v. infusion and oral administrations. The i.v. administration consisted of a 20-min infusion. The appropriate volume of reconstituted drug was prepared in syringes and administered p.o. by the patient daily for 5 days every 3 weeks. The solution was taken on an empty stomach in the morning. The complete dose was directly swallowed, followed immediately by up to 150 ml of water. If the patient complained about the taste of the diflomotecan, he/she was allowed to drink Coca-Cola instead of water (after a protocol amendment, initiated after the second dose level), diflomotecan being stable in this beverage in drug stability studies. Routine premedication was not prescribed. In case of nausea or vomiting, prophylactic metoclopramide or domperidone was prescribed for subsequent cycles.

Treatment Assessment. Before therapy a complete medical history was taken and physical examination performed. A complete blood cell count, including WBC differential and serum biochemistry, comprising sodium, potassium, calcium, chloride, urea, creatinine, total protein, albumin, total bilirubin, alkaline phosphatase, AST, ALT, γ-glutamyl transferase, lactate dehydrogenase, and glucose, were performed, as was measurement (24-h urine collection) of creatinine clearance and urine analysis. Weekly evaluations included history, physical examination, toxicity assessment according to the NCI-CTC version 2.0, and serum chemistry. A complete blood cell count was determined twice weekly. Tumor evaluation according to the WHO response criteria was performed at baseline and after every two oral courses of therapy.

Sample Collection for Pharmacokinetic Analysis. Blood samples for pharmacokinetic analysis, collected in 4.5-ml glass tubes containing lithium heparinate as anticoagulant, were obtained on the first day after i.v. administration and on the first and fourth or fifth day of the first oral treatment course for all patients. Samples were taken at the following time points: before dosing and at 10, 20, 30, and 45 min then 1.5, 3, 4.5, 6, 8, 10, and 24 h after the start of the i.v. administration and after the first and either the fourth or fifth administration of oral diflomotecan. Additional samples were taken 48 h after the i.v. administration and before oral administration on days 3 and 4. Immediately after sampling, tubes were placed on ice and centrifuged at 2000 × *g* for 15 min at 4°C. For each sample, two aliquots of plasma of ~1 ml were placed in
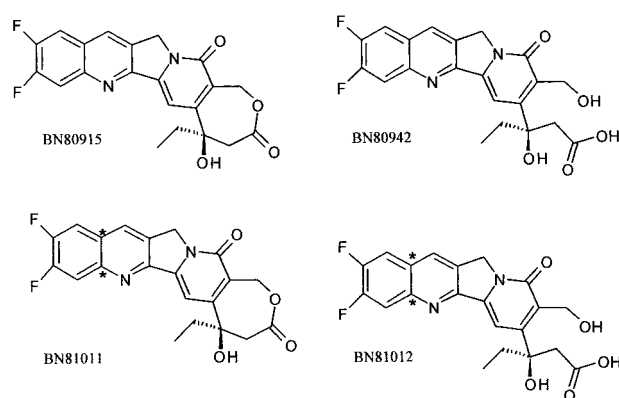
Fig. 1 Chemical structure of diflomotecan BN80915 (active lactone form), BN80942 (inactive open ring form), and its analogues BN81011 and BN81012, respectively.

polypropylene tubes and frozen at −80°C until analysis. Urine was collected for 24 h on day 1 after i.v. difomotecan and after oral diflomotecan on days 1 and 5.

Pharmacokinetic Assays. The plasma concentrations of BN80915 and BN80942 (code name of the corresponding inactive open lactone form) were determined by LC-MS/MS using ^{13}C-labeled analogues of BN80915 (BN81011) and BN80942 (BN81012). All structure formulas are presented in Fig. 1. The lower limit of quantification was 0.05 ng/ml plasma for both BN80915 and BN80942. For the determination of BN80915 and BN80942 in human urine, the analytical method involved extracting the analytes with diethyl ether followed by the same LC-MS/MS method; the limits of quantification were 0.25 and 0.5 ng/ml for BN80915 and BN80942, respectively. The precision of the analytical technique in plasma for BN80915 was between 7.4 and 10.9% (CV) accuracy was between 2.6 and 0.42% (percentage of relative error); for BN80942, the precision and accuracy were 8.8–15.0 and 0.05–5.0%, respectively. In urine, the precision and accuracy of the analytical technique were between 5.3–18.9% (CV), with relative error (percentage) 2.5 and 5.5% for BN80915. For BN80942, the CV percentage ranged from 10.4 to 17.4%, whereas relative error ranged from 10.4 to 3.7%.

Pharmacokinetic Data Analysis. The pharmacokinetic analyses of BN80915 and BN80942 plasma concentration-time data set after i.v. and oral administration were performed by a noncompartmental approach using a WinNonLin pharmacokinetic program (version 2.1, Scientific Consulting, Inc., 1998). The following pharmacokinetic parameters were determined: empirical time of peak plasma level (T_{max}); the empirical peak plasma level (C_{max}); and the apparent elimination rate constant (λ_z), estimated by linear regression of the terminal phase of the semilogarithmic plasma levels curve when this was clearly defined. The elimination half-life ($t_{1/2}$) was defined as $ln2/\lambda_z$. The AUC was estimated by the linear-log trapezoidal rule. The AUC ratio of BN80915/total was calculated where total = BN80915+BN80942. The absolute oral bioavailability of BN80915 (F), expressed in percentages, was calculated by dividing the $AUC_{oral}/dose_{oral}$ ratio by the $AUC_{i.v.}/dose_{i.v.}$ ratio of BN80915.

After i.v. and oral administrations, the amount of un-

Table 1 Patient characteristics

		No. of patients
Patients entered		22
Patients assessable for toxicity		22
Age, (yr)		
Median	55	
Range	34–71	
Sex		
Female		9
Male		13
Performance status		
Median	1	
Range	0–2	
Tumor type		
Colorectal		5
Sarcoma		4
Adenocarcinoma of unknown primary origin		3
Melanoma[a]		2
Ovarian cancer		2
Other[b]		6
Previous therapy		
Chemotherapy		20
No. of chemotherapy regimens		
Median	2	
Range	0–3	
Radiotherapy and chemotherapy		10

[a] One patient also had renal cell carcinoma.
[b] Mesothelioma, head and neck, cervical, breast, anal, and prostate cancer.

changed drug excreted in the urine was determined and expressed as 9% of the administered dose. Pharmacokinetic data are reported as mean ± SD.

RESULTS

A total of 22 patients entered this study and received the oral formulation of diflomotecan between June 1999 and December 2000. Patient characteristics are listed in Table 1. All patients were eligible and assessable for both toxicity and response.

The majority of patients had mild disease-related symptoms at baseline. Nine patients were female and 13 male. Twenty patients had received prior chemotherapy, the median number of regimens being 2, but 1 patient had previously received stem cell transplantation after myeloablative chemotherapy. This patient was treated at the first dose level and had no myelotoxicity. Ten patients had received prior radiotherapy. The most common tumor types were colorectal cancer, adenocarcinoma of unknown primary origin, and sarcoma. The flat oral dose levels of diflomotecan studied were 0.10, 0.20, 0.27, and 0.35 mg/day. The total number of assessable courses was 57, and the median number of courses/patient was 2 (range, 1–8).

Myelosuppression was the principal DLT. Five patients required dose reductions after experiencing DLT. Once dose reduction had taken place, toxicity of subsequent courses administered to the patients was incorporated in the evaluation of that lower dose level.

Table 2 NCI-CTC grade worst hematological toxicity and DLTs of oral diflomotecan (all cycles)

Dose mg/day	No. of patients/ no. of cycles	Leukocytopenia (Grade)		Neutropenia (Grade)		Thrombocytopenia (Grade)		Anemia (grade)	
		3	4	3	4	3	4	3[a]	4
0.10	3/6	0	0	0	0	0	0	0	0
0.20	3/5	1	0	1	0	0	0	1	0
0.20[b]	3/5	2	0	1	1	1	0	0	0
0.27	12/29	3	3	1	5[c]	1	0	1	0
0.27[b]	2/2	1	1	1	1	1	0	1	0
0.35	4/10	2	2	0	2[d]	2[e]	0	2	0

[a] All, except for 1, patients experiencing grade 3 anemia had grade 1 or 2 at baseline.
[b] Dose level after dose reduction.
[c] Three DLTs: 3 patients had grade 4 neutropenia > 7 days, complicated by fever in 1 patient.
[d] One DLT: 1 patient had febrile neutropenia (also grade 4 neutropenia > 7 days).
[e] One DLT: 1 patient had grade 3 thrombocytopenia with bleeding, requiring platelet transfusion (with also grade 4 neutropenia > 7 days).

Table 3 Worst NCI-CTC grade nonhematological toxicity of oral diflomotecan (all cycles)

Dose mg/day	No. of patients/ no. of cycles	Nausea (grade)		Vomiting (grade)		Diarrhea (grade)		Fatigue (grade)	
		2	3	2	3	2	3	2	3
0.10	3/6	0	0	0	0	0	0	1[a]	0
0.20	6[b]/10	1	0	0	0	0	1	1	0
0.27	14[c]/31	5	0	2	0	2	0	8[d]	0
0.35	4/10	0	0	0	0	1	0	2	0

[a] Patient had grade 1 at baseline.
[b] Three patients after dose reduction.
[c] Two patients after dose reduction.
[d] Four patients with grade 1 at baseline.

Hematological Toxicity. The severity of hematological toxicity was clearly dependent on the dose level (Table 2). At the 0.27-mg oral dose, 1 of the initial 6 patients treated had DLT in the form of grade 4 neutropenia for 12 days, permitting escalation to the next dose level. However, at the 0.35-mg dose level, 2 of 4 patients had DLT. One of these patients had febrile neutropenia and grade 4 neutropenia for 11 days, the other had grade 3 thrombocytopenia with recurrent epistaxis requiring platelet transfusion and associated with grade 4 neutropenia lasting 13 days. Because 2 of 4 patients had experienced DLT, no additional dose escalation was attempted. Six additional patients were included at the dose level immediately below that which was unacceptably toxic, to further characterize the safety profile, pharmacokinetic parameters, and pharmacodynamic relationships at the dose recommended for additional studies. Of these additional 6 patients treated with 0.27 mg of oral diflomotecan, 2 experienced DLT consisting of grade 4 neutropenia for >7 days; 1 of these patients had febrile neutropenia without other signs of infection. Both patients continued treatment at the lower dose level of 0.2 mg for one and three cycles, respectively, until disease progression. Hence, a total of 3 of 12 patients experienced DLT at the 0.27-mg dose level, which was defined as the RD according to the protocol. All 3 patients experiencing DLT at the RD were heavily pretreated with more than or equal to two lines of prior chemotherapy. Moreover, only 2 of 31 courses at the RD were delayed because of prolonged neutropenia. All DLTs were hematological, and toxicity at the RD was easily manageable.

The median time to neutrophil nadir was 8 days (range,

7–13 days). Blood transfusions were given with 17 of 57 cycles to 45.5% of patients (10 of 22); but only one platelet transfusion was required.

Nonhematologic Toxicity. Gastrointestinal toxicity was mild (Table 3) and mainly a result of the unpleasant taste of diflomotecan. Diarrhea was rare and never clinically serious, with grade 3 diarrhea observed only once and lasting just a few hours. Only 2 patients experienced grade 2 mucositis during a single cycle. Fatigue was frequently observed but often related to disease progression or anemia. Three patients, who had baseline grade 1 or 2 fatigue, experienced grade 3 fatigue related to disease progression. A relationship with the study drug could not be ruled out in several other cases of grade 1 or 2 fatigue. Alopecia was seen in 10 of the 22 patients, but in 4, this was present at baseline. One patient experienced grade 3 vertigo, but this was not observed on rechallenge with the study medication and was therefore not considered related. Nephrotoxicity grade 1 was observed in 1 patient but resolved with continued treatment. There was no grade 4 nonhematological toxicity.

Antitumor Activity. No objective responses were observed. However, 6 patients had disease stabilization after two cycles of diflomotecan. One of these patients with pulmonary metastases from an anal carcinoma withdrew consent to additional treatment after two cycles but remained free of disease progression for 9 months. Another patient with colon carcinoma completed eight cycles and was still without signs of disease progression 1 year after start of the treatment. The other 4 patients, with desmoplastic round cell sarcoma, cervical cancer, cancer of unknown primary and clear cell sarcoma, developed
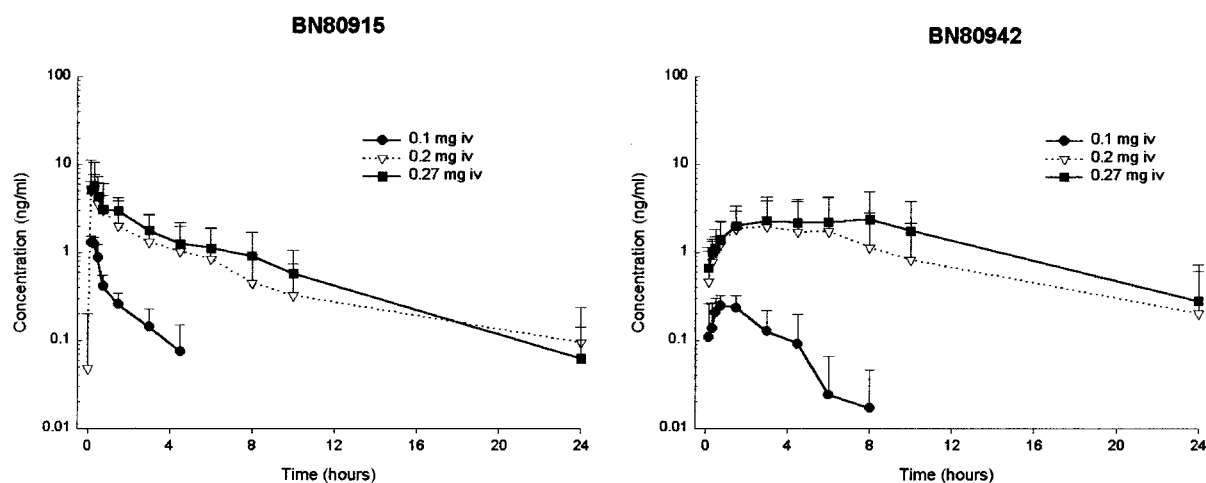
BN80915

BN80942

Fig. 2   Mean plasma levels of diflomotecan (BN80915) and its open lactone form (BN80942) after i.v. short infusion of diflomotecan at 0.1, 0.2, and 0.27 mg.
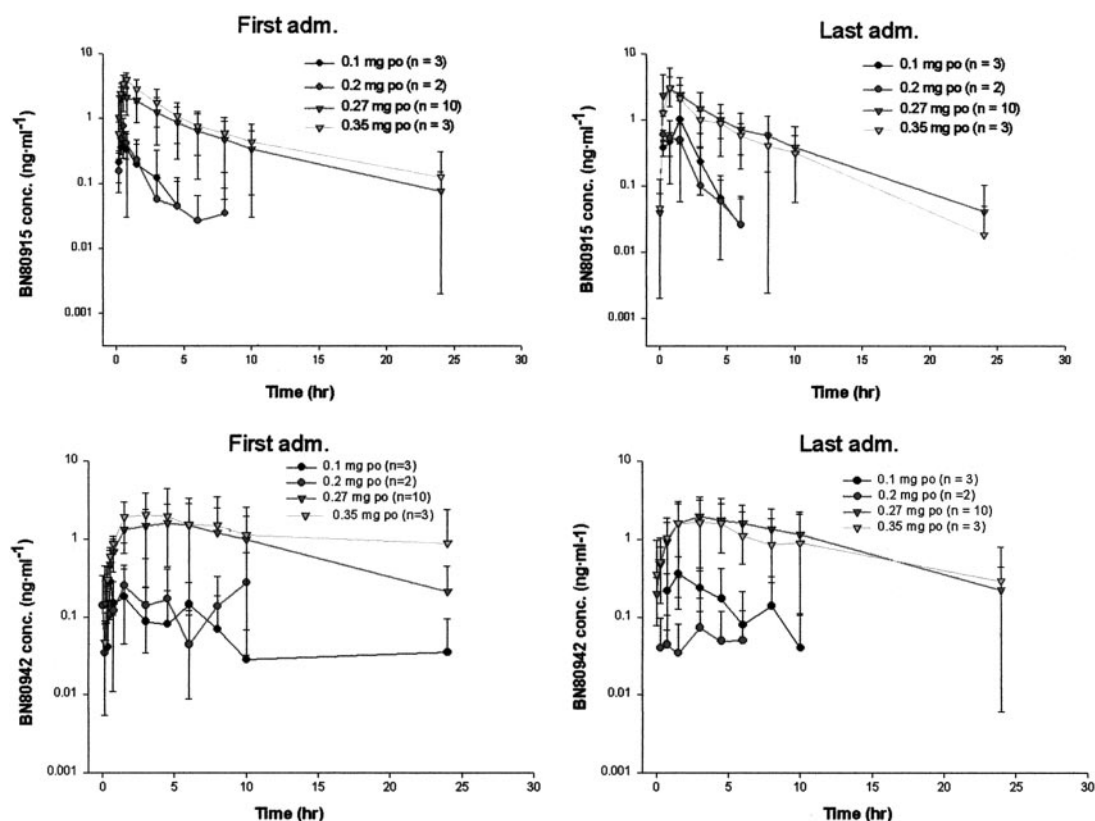

Fig. 3   Mean plasma levels of BN80915 (*top*) and BN80942 (*bottom*) after repeated oral administrations of diflomotecan at 0.1, 0.2, 0.27, and 0.35 mg.

progressive disease after two to four subsequent cycles of diflomotecan.

Pharmacokinetics and Pharmacodynamics. Pharmacokinetics could be evaluated in 18 patients, 1 each at the 0.2 and 0.35 mg/day dose levels and 2 at the 0.27 mg/day dose level

being inevaluable. Mean plasma levels (±SD) for BN80915 (closed ring) and BN80942 (open ring) at each i.v. and oral dose level are shown in Figs. 2 and 3, respectively.

The pharmacokinetic parameters of BN80915 and the *AUC* ratio for BN80942 after the first and fifth oral administration are

Table 4 BN80915 pharmacokinetics and AUC ratio with total (BN80915 + BN80942) after first and fifth day oral administration of diflomotecan

NOTE: Data are expressed as mean $\pm$ SD, T_{max}*, median value.

| Dose (mg/day)	No. of patients	AUC (ng·h/ml)	C_{max} (ng/ml)	Ratio BN80915/total	T_{max}* (h)	$t_{1/2}$ (h)	F (%)
0.1							
Day 1	3	0.89 ± 0.99	0.50 ± 0.21	0.44[(n = 1)]	0.50	0.94 ± 0.73	16.8[(n = 1)]
Day 5	3	1.62 ± 0.58	1.07 ± 0.86	0.34[(n = 1)]	1.50	1.55 ± 0.35	
0.2							
Day 1	2	0.96 ± 0.83	0.76 ± 0.24	0.44[(n = 1)]	0.51	1.14 ± 1.17	37.3–18.1[(n = 2)]
Day 5	2	1.36 ± 1.29	0.70 ± 0.25	ND	0.88	1.07 ± 0.74	54.5–21.3[(n = 2)]
0.27							
Day 1	10	11.79 ± 7.21	2.64 ± 1.20	0.40 ± 0.10	0.51	3.89 ± 2.06	64.7 ± 36.1[(n = 10)]
Day 5	10	14.47 ± 10.46	3.27 ± 3.13	0.40 ± 0.09	0.75	3.72 ± 1.43	111.7 ± 127.6[(n = 8)]
0.35							
Day 1	3	18.03 ± 11.88	4.22 ± 0.77	0.45 ± 0.24	0.75	5.70 ± 2.67	97.1 ± 20.60[(n = 3)]
Day 5	3	11.26 ± 6.15	3.02 ± 1.42	0.45 ± 0.20	0.82	3.29 ± 1.00	63.1 ± 12.8[(n = 3)]
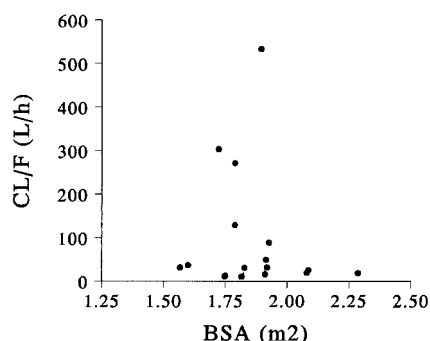
Fig. 4 Relationship between BSA/m^2 and absolute CL/F (flat dose divided by the AUC of BN80915), expressed in liter/h.

presented in Table 4. The *AUC* was underestimated at the lowest dose level as results from the later time points were below the limit of detection. The mean oral bioavailability ($\pm$SD) was 72.24 $\pm$ 59.2% across all dose levels. No significant changes in pharmacokinetic parameters were observed between the first and fifth administration at the RD level. The AUC and C_{max} were proportional to dose, and the T_{max} was independent of dose, indicating a linear and dose-independent kinetics. Urine analysis revealed that at the RD level the mean percentage of dose excreted in the urine was 5.44% in the 24 h after first administration and 4.33% in the 24 h after final dose. The mean ratios of BN80942/BN80915 in urine after i.v. and p.o. were 2.8 and 2.9, respectively (data not shown). Renal elimination accounts, therefore, for only a small proportion of total drug clearance.

Pharmacodynamic relationships between BN80915 *AUC* or C_{max} and percentage of fall of WBCs, neutrophils, and platelets, using E_{max} model, were established at the three highest dose levels (data not shown).

To test the appropriateness of flat dosing strategy in this study, the BSA was plotted *versus* the absolute apparent CL/F (calculated by dividing the absolute administered oral dose of diflomotecan by the *AUC* of BN80915) in Fig. 4. As expected, no relationship was found between the apparent CL/F and BSA.

DISCUSSION

This study shows that oral administration of diflomotecan as a solution is feasible in adult patients with solid tumors. Diflomotecan was well tolerated, with gastrointestinal toxicity and fatigue limited and few patients experiencing alopecia. Only 1 patient experienced short-lasting grade 3 diarrhea and 4 patients grade 2 diarrhea This is in marked contrast to irinotecan where diarrhea is severe (grade 3–4) in ~25% of patients (13). As with other oral topoisomerase I inhibitors, bone marrow depression was the main toxicity. At the RD level, 3 of 12 patients had neutropenia lasting >7 days and 1 experienced febrile neutropenia but without other signs of infection or sepsis; all 3 of these patients were heavily pretreated. Dose delays because of prolonged hematological toxicity were rare. Many patients entered the study with grade 1 or 2 anemia, and although no more than a two grade increase in anemia was observed, erythrocyte transfusion policy was liberal. The percentage of patients requiring blood transfusions (45.5%) wasn't, therefore, excessive. Indeed this is in the range reported for other oral topoisomerase I inhibitors in Phase I studies using the same 5-day schedule (48–55%; Refs. 14, 15).

We conclude that treatment toxicity was acceptable at the RD level of 0.27 mg once daily × 5, every 3 weeks.

Antitumor activity was not a primary end point, and the patient population was relatively heavily pretreated with few patients having tumors such as colon cancer that might be expected to be sensitive to diflomotecan. Nevertheless, one of the patients with stabilization of their disease for a prolonged period of time on oral diflomotecan had metastatic colon cancer. Taken with the preclinical data, this suggests that Phase II testing of oral diflomotecan should include patients with colorectal cancer.

Oral diflomotecan has a linear dose-independent pharmacokinetic profile over the higher part of the dose range studied with significant inter- and intrapatient variability. Accumulation of BN80915 was not observed after multiple administrations of diflomotecan. The urinary excretion of BN80915 was very low, indicating that elimination is mainly through extrarenal mechanisms. As for many other chemotherapeutic agents, it was interesting to note that there was no correlation between BSA and absolute CL/F, vindicating the flat-dosing regimen used in this study. Population pharmacokinetic models might identify means of reducing the

inter- and intrapatient variability in the future. Until then, the more convenient flat dosing of diflomotecan is as accurate as the more complex dosing by body surface area. Pharmacodynamic relationships of oral diflomotecan AUC and E_{max} with percentage decrease in WBCs, neutrophils, and platelets were demonstrated that should render toxicities predictable. These pharmacodynamic relationships also raise the prospect of developing pharmacokinetic models to facilitate individualized dosing, thus avoiding excessive toxicity or subtherapeutic drug levels.

Compared with other oral topoisomerase I inhibitors that are administered in similar schedules (14–19), oral diflomotecan has a range of (pre)clinical advantages. In addition to the superior antitumor activity *in vivo*, this study indicates that nonhematological toxicity was less common and less severe than with most other oral topoisomerase I inhibitors (20), whereas hemorrhagic cystitis was never encountered. As expected, the E-ring modification leading to enhanced preclinical stability resulted in a high BN80915/total ratio, an additional potential advantage in terms of antitumor activity. Also of note interest is the exceptionally high oral bioavailability of diflomotecan (72.2%). This compares favorably with other oral topoisomerase I inhibitors: 11.3% for lurtotecan; 32–44% for topotecan; and 48.6% for 9-AC (20). One of the reasons for the high variability in oral bioavailability, also observed in other oral topoisomerase I inhibitors, might be related to individual differences in intestinal drug efflux transporters such as Breast Cancer Resistance Protein.

In conclusion, the recommended flat dose of oral diflomotecan for Phase II studies in adults, is 0.27 mg once daily × 5, every 3 weeks. Oral diflomotecan is well tolerated and has a favorable, although variable, pharmacokinetic profile with high lactone stability and acceptable bioavailability. Given the broad and superior preclinical activity along with enhanced stability, compared with other topoisomerase I inhibitors and hints of activity in this study, Phase II studies in a range of solid tumor types are warranted.

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