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ORIGINAL ARTICLE

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Irinotecan in combination with thalidomide in patients with advanced solid tumors: a clinical study with pharmacodynamic and pharmacokinetic evaluation

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Abstract *Purpose*: Recent clinical studies have demonstrated a reduction of irinotecan (CPT-11) gastrointestinal toxicities when the CPT-11 is administered in combination with thalidomide in patients with diagnosis of colorectal cancer. The main purpose of this study was to investigate possible interactions between CPT-11 pharmacokinetics and thalidomide to explain the previously described gastrointestinal toxicity reduction. *Methods*: In our clinical trial, advanced cancer patients were treated with CPT-11 on a dose of 350 mg/m^2 at day 1 every 3 weeks. Only at the first cycle, CPT-11 was administered in association with thalidomide on a dose of 400 mg/day given from day 1 to day 14. From the second cycle, the treatment was continued with irinotecan alone at the same dose. Pharmacokinetics analysis of irinotecan and its metabolites, SN-38 and SN-38-glucuronide, were performed at the first and second cycle. *Results*: A total of 19 patients entered the study. The pharmacokinetic analysis were performed on 16 patients. Pharmacokinetic data suggested a decreased metabolism of irinotecan into SN-38 and SN-38-glucuronide when it was administered with thalidomide. Indeed, area under the time–concentration curve (AUC) of SN-38 was significantly lower at the first cycle than the second cycle (0.99 ± 0.45 h×μg/ml vs 1.34 ± 0.65, respectively, $P = 0.027$) whereas AUC of irinotecan and SN-38-glucuronide were higher at first cycle than second cycle (34.53 ± 11.38 h×μg/ml vs. 28.42 ± 12.23 h×μg/ml, $P = 0.064$ and 2.39 ± 1.21 h(μg/ml vs. 1.86 ± 1.11 h×μg/ml, $P = 0.018$, respectively). *Conclusions*: Our study demonstrates a significant decreased metabolism of CPT-11 into the active metabolite SN-38 when CPT-11 is administered in association with thalidomide. These observations strongly suggest an interaction of thalidomide with CPT-11 metabolism and, at least in part, it might explain the previously described improvement in tolerability.

Keywords Metastatic disease · Diarrhea · Pharmacokinetics · Irinotecan · Thalidomide

Abbreviations CPT-11: Irinotecan · CRC: Colorectal cancer · 5-FU: 5-Fluorouracil · LV: Leucovorin · NCI: National Cancer Institute · ECOG: Eastern Cooperative Oncology Group · SN-38: 7-Ethyl-10-hydroxycamptothecin · SN-38 glucuronide: 7-ethyl-10-[3,4,5-trihydroxy-pyran-2-carboxylic acid]-camptothecin (the β-glucuronide conjugate of SN-38) · AUC: Area under the time versus plasma concentration curve · $t_{1/2}\beta$: Elimination half-life · CL: Total body clearance · C_{max}: Maximal plasma concentration · T_{max}: Time to reach C_{max} · REC: Relative extent of conversion · MR: Metabolic ratio · BI: Biliary index · GR: Glucuronidation ratio · RECIST: Response evaluation criteria in solid tumor · APC: 7-Ethyl-10 (4-N-5-aminopentanoic acid)-(1-piperidino)-carbonyloxycamptothecin · NPC: 7-Ethyl-10 (4-(1-piperidino)-1-amino)-carbonyloxycamptothecin · AEDs: Antiepileptic

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drugs · CYP450: Cytochrome P-450 · ABC: ATP binding cassette

Introduction

Irinotecan, a semisynthetic water-soluble anticancer agent derived from the plant alkaloid camptothecin, is widely used in the treatment of several types of solid tumors [1–7]. The role of irinotecan was first established in patients with advanced CRC, as single agent after 5-FU failure, then in combination with 5-FU and LV on first-line treatment [8–14]. Finally, it has been evaluated in the adjuvant setting in patients with colon cancer [15–16]. The dose-limiting toxicities of CPT-11 are represented by myelosuppression and delayed diarrhea. A reversible, not cumulative and not prolonged neutropenia, of grade 3 or 4 occurs in 20–40% of patients [17–22]. Instead, delayed diarrhea has emerged as one of the most important side effects and it is observed in up to 80% of treated patients when CPT-11 is used as single agent at doses of 350 mg/m^2. With this schedule, grade 3 or 4 diarrhea occurs in 30–35% of patients, and it can be life-threatening if it is not immediately treated with high dose loperamide, oral and/or i.v. fluids or hospitalization. The risk of infectious complications is increased when diarrhea occurs and this can lead to sepsis in patients with irinotecan-induced neutropenia [23]. Furthermore, delayed grade 3–4 diarrhea is still observed in about 15–30% of patients when CPT-11 is used in combination with 5-FU [13–14]. Therefore, strategies that reduce this toxicity should be of considerable value in the clinical setting. Thalidomide, a central nervous system sedative with potent teratogenic properties, has been demonstrated to possess antineoplastic activity in several tumors models [24–26]. Many mechanisms have been put forth to explain the antineoplastic activity of thalidomide [25–27]. It has been demonstrated that thalidomide targets both the cancer cell and its microenvironment; recent studies, using in vivo models of angiogenesis, have shown that the drug is a potent antiangiogenic agent, since thalidomide treatment can decrease vascular density in granulation tissue [27]. Thalidomide has been investigated in several solid and haematological tumors, alone and in combination with cytotoxic drugs, with variable degrees of success [28–32]. In particular, clinical studies have shown that thalidomide has significant antitumour activity in the treatment of multiple myeloma which become the most common indication for its use in clinical practice.

In order to evaluate the antitumoral activity of a combination including thalidomide and CPT-11, recently a phase II clinical study has been performed in patients with metastatic CRC [33–34]. Patients were treated with thalidomide 400 mg/day per os administered at bedtime continuously plus CPT-11 325–350 mg/m^2 i.v. repeated every 21 days. An interim analysis was done after enrolment of the first nine patients and it showed a striking absence of gastrointestinal toxicity; of interest, only one patient experienced a grade 2 (NCI scale) diarrhea. As described above, a grade 3 diarrhea is expected in more than 30% of patients when CPT-11 is administered every 3 weeks while in this study the incidence of diarrhea was extremely low. Of the assessable patients, one achieved a complete and two patients a partial remission. A following phase II clinical study was conducted on 18 patients with diagnosis of metastatic CRC confirming previous results in terms of antineoplastic activity and toxicity, with a grade 3 diarrhea (NCI scale) observed in only one (2%) patient [34–35]. Because of these findings and for the relevance that CPT-11 has in clinical practice, we designed the present study to provide an explanation for this effect. We performed a pharmacokinetic analysis of CPT-11 in order to evaluate the possible influence of thalidomide on irinotecan disposition in cancer patients.

Patients and methods

Patient selection

The main eligibility criteria included a histologically confirmed diagnosis of malignant tumour with metastatic disease or brain tumour; previous palliative chemotherapy; age $\leq$ 70 years; ECOG performance status of $\leq$ 2; measurable disease; leukocyte count $\geq$3,500 per mm^3; platelet count $\geq$100,000 per mm^3; serum creatinine $\leq$ 1.3 mg/dl; serum bilirubin $\leq$ 1.5 mg/dl; aspartate aminotransferase and alanine aminotransferase $\leq$ 2.5 times normal values; life expectancy more than 3 months. Exclusion criteria were: symptomatic cardiac disease, recent history of myocardial infarction, active infections, and inflammatory bowel disease. The study was approved by the local ethics committee, and patients were informed of the investigational nature of the study and their written informed consent was obtained

Study design and treatment

Planned treatment consisted of CPT-11 infused over a 90 min period in 250 ml of NaCl 0.9% at the dose of 350 mg/m^2 i.v. administered at day 1 every 3 weeks for a maximum of 12 cycles. Thalidomide was administered at dose of 400 mg/day per os at bedtime from day 1 to day 14 only at the first cycle. Because of the aim of the study, thalidomide was not administered continuously but only at the first cycle to see possible interactions of thalidomide on CPT-11 metabolism, and following the same CPT-11 treatment proposed by Govindarajan et al. [33]. From the second cycle treatment was continued with CPT-11 alone and stopped in case of progressive disease, unacceptable toxicity or patient's refusal. At the first and

second cycle blood samplings were performed for pharmacokinetic analyses of CPT-11 and its main metabolites SN-38 and SN-38-glucuronide.

To prevent nausea and vomiting, ondansetron 8 mg i.v. and dexamethasone 16 mg i.v. were administered before CPT-11. Atropine 0.25 mg was given subcutaneously in case of cholinergic syndrome and given prophylactically in the following cycles. Loperamide 2 mg orally every 2 h and oral rehydration were prescribed in case of delayed diarrhea. No prophylactic treatment with cytokines was used. Granulocyte colony-stimulating factor in combination with antibiotics was used in case of grade 4 neutropenia and fever ≥38°C. Treatment was delayed if, on the planned day of treatment, neutrophils were $< 1{,}000$ per mm^3, platelets were $< 100{,}000$ per mm^3, persistent diarrhea, stomatitis or other toxicities at grade higher than 1 were present. CPT-11 dose modifications were based on the worst preceding toxicity observed in each patient.

Pharmacokinetics

Pharmacokinetic analysis of CPT-11 and its main metabolites, SN-38 and SN-38-glucuronide, was performed in 16 patients at the first and the second cycle of chemotherapy. Blood was withdrawn from a peripheral catheter placed in a vein of the forearm at baseline, at the end of CPT-11 infusion, and then 5 and 30 min and 2, 3, 4, 6, 24 and 48 h after the end of infusion. After blood samples were immediately centrifuged, plasma was stored at –20°C until the assay for concentrations of CPT-11 and its catabolites was performed following a high performance liquid chromatographic method previously reported [36]. Briefly, plasma concentrations of total CPT-11 and SN-38 were evaluated following extraction of plasma with methanol containing 0.1% HCl 10 N; samples were then centrifuged and the clear supernatant was evaporated to dryness in a SpeedVac concentrator, equipped with a SPD 111V centrifuge and a RVT 4104 refrigerated vapor trap (Savant, Holbrook, NY). The resulting pellet was reconstituted in methanol acidified with 0.1% HCl 10 N and eluted through a μBondapack C_{18} stationary phase (300×3.9 mm, 10 μm; Waters) by KH_2PO_4 0.1 M/acetonitrile (65:35, v/v) pH 4.0, containing sodium heptansulfonate 3 mM [36]. The chromatographic apparatus was an Alliance system (Waters) equipped with a model 474 scanning fluorescence detector (excitation and emission wavelengths, 375 and 525 nm, respectively). Data analysis was performed by the Empower software (Waters) SN-38-glucuronide plasma concentrations were determined by digestion of samples with β-glucuronidase at 37°C for 2 h before extraction, and the difference between peak height corresponding to SN-38 from β-glucuronidase-treated versus untreated samples corresponded to the plasma levels of SN-38-glucuronide.

Individual plasma concentrations of drugs and their catabolites were fitted according to a two-compartment open model using nonlinear least-squares regression analysis by means of a computer software (MWP-HARM, MediWare, Groeningen, The Netherlands). The calculation of pharmacokinetic parameters included: AUC, $t_{1/2}\beta$, Vd and CL, while C_{max} and T_{max} were obtained from visual inspection of the plasma profiles.

REC of irinotecan was calculated by the following equation $REC = AUC_{SN\text{-}38}/AUC_{CPT\text{-}11}$. Moreover, MR, defined as $(AUC_{SN\text{-}38} + AUC_{SN\text{-}38\text{-}glucuronide})/AUC_{CPT\text{-}11}$, BI, calculated as $(AUC_{CPT\text{-}11}) \times (AUC_{SN\text{-}38}/AUC_{SN\text{-}38\text{-}glucuronide})$ and GR, evaluated as $AUC_{SN\text{-}38\text{-}glucuronide}/AUC_{SN\text{-}38}$, were also determined for each patient.

Assessability, toxicity, and response criteria

The pretreatment evaluation included history and physical examination, performance status assessment, complete blood cell with differential and platelet counts, complete blood profile, tumoral markers when indicated, urinalysis, ECG, chest X-ray or computed tomography scan, abdominal computed tomography scan and/or sonogram, and any other appropriate diagnostic procedure to evaluate metastatic sites. During treatment, a physical examination, a complete blood cell count, blood profile, urinalysis and toxicity evaluation was performed every 3 weeks, immediately before drug administration. Sites of metastatic disease were radiologically re-evaluated every three cycles according to RECIST criteria [37]. A chest X-ray and/or an abdominal sonogram were repeated at least every 6 months if there was no evidence of lung or abdominal disease, respectively. Toxicities were scored according to standard NCI criteria. Duration of responses was calculated from the first day of treatment to the date of first observation of progressive disease or last examination.

Statistical analysis

The main objective of the study was to evaluate possible pharmacokinetic interactions between thalidomide and CPT-11 and its metabolites; secondary objective of the study was to compare toxicities of CPT-11 + thalidomide (administered only at the first cycle) with CPT-11 alone (second and following cycles). The method described by Friedman [38] was used to determine the number of patients to be included. In particular because grade 2–4 diarrhea incidence is observed in approximately 60% of the patients when CPT-11 is administered at 350 mg/m^2 every 3 weeks whereas previous reports with CPT-11 + thalidomide have reported an incidence of grade 2–4 diarrhea of less than 10%, our objective was to demonstrate a difference in grade 2–4 diarrhea of 50% between the first and second cycle (10 vs. 60%, respectively). Therefore, for the Friedman's method with a two-side α of 0.05 and a power of 0.8 a minimum sample size of 17 patients receiving both

CPT-11 + thalidomide and CPT-11 alone were needed. Pharmacokinetic data from cycle 1 and cycle 2 were pooled to calculated mean and standard deviation values, while differences between cycles 1 and 2 were analysed by paired Student's *t* test and checking for the pairing with the Spearman correlation coefficient. All calculations were performed by the GraphPad Prism 4.0 software (GraphPad, San Diego, CA) and all values are represented as mean ± standard deviation. A *P* value of <0.05 was considered to be significant.

Results

Patients and toxicity

As outlined in Table 1, 19 patients with advanced solid tumors entered the study. Median age was 55 years (range 28–70 years), ECOG performance status was 0–1 in ten patients and 2 in nine and most patients were heavily pretreated. All patients with recurrent or progressive brain tumor and two with metastatic lesions to the brain, nine patients in total, were on treatment with steroids and AEDs. Overall, 72 cycles of CPT-11 were administered with a median of 3 cycles per patient (range 2–12 cycles). Fourteen patients (74%) received no more than 3 cycles of chemotherapy because of rapidly progressive disease. Two of seven patients with diagnosis of brain tumors continued thalidomide from the third cycle up to the end of treatment with CPT-11. After first cycle (with CPT-11 + thalidomide) overall toxicities included mainly nausea and vomiting (grade 2–3, 16% of patients), diarrhea (grade 2, 5%; grade 3, 16%) and somnolence (grade 2, 5%). After the second cycle (CPT-11 alone), toxicities observed were mainly nausea and vomiting (grade 2–3, 21%, grade 4, 5%) and diarrhea (grade 2, 26.5%) (Table 2). Hematological toxicity was rare; one episode of grade 3 neutropenia occurred at the first and second cycle, no episodes of thrombocytopenia were observed. Side effects associated more directly with thalidomide as somnolence, vertigo and xerostomia were infrequent and mild in intensity. Nine patients were receiving enzyme-inducing AEDs (phenytoin and phenobarbital) in combination with chronic dexamethasone and the incidence of toxicities seemed to be the same in comparison to patients not receiving AEDs. Five patients received more than three cycles of chemotherapy, but toxicities observed on following cycles were mild (data not reported). With respect to the evaluation of antitumor activity, all patients were assessable for response, although tumour response was not a primary end point of this study. We observed a minor response in a patient with diagnosis of glioblastoma (defined as 25–50% reduction in disease), four patients (21%) with a disease stabilizations and 14 (75%) progressions. Minor response and disease stabilization lasted a median of 5 months (range 2–8 months).

Table 1 Patients' characteristics

	n
Patients	19
Male	8
Female	11
Age (years)	55
Median (range)	(28–70)
ECOG[a] performance status	
0	5
1	5
2	9
Primary	
Brain	7
Colon	4
Oesophagus	1
Stomach	1
Lung	1
Larynx	1
Uterus	2
Kidney	2
Number of metastatic sites	
Single	9
Multiple	10
Sites of disease	
Liver	9
Lung	8
Abdomen	3
Brain	9
Others	7
Previous chemotherapy treatments	
1	2
2	9
3	8
Antiepileptic treatments	
Phenobarbital	2
Phenytoin	7

[a] *ECOG* Eastern Cooperative Oncology Group

Pharmacokinetics

Sixteen of 19 patients were enrolled in the pharmacokinetic part of the study. In fact, because of grade 3 delayed diarrhea at first cycle, three patients at second cycle received CPT-11 at a reduced dosage and blood withdrawal for pharmacokinetic analysis was not performed. Pharmacokinetic analysis of CPT-11, showed a decreased metabolism of the parent drug into the active metabolite SN-38 when followed by thalidomide (Fig. 1). Indeed, CPT-11 AUC value was higher at cycle 1 with respect to cycle 2 ($P=0.064$) even though it was not statistically significant, whereas mean AUC value of SN-38-glucuronide was significantly lower at first cycle than at second cycle ($P=0.027$) (Table 3). Moreover, at first cycle, AUC value of SN-38-glucuronide was significantly increased with respect to second cycle ($P=0.018$). The comparison of pharmacokinetics of CPT-11 and its metabolites with or without thalidomide did not reveal any difference among C_{max}, T_{max} and $t_{1/2}\beta$ values (Table 3). Further analysis demonstrated that thalidomide led to a decrease in BI, REC and MR values at first cycle with respect to second cycle, when patients were treated with CPT-11 alone even if only the decrease of BI and REC was statistically significant ($P=0.011$ and

Table 2 Overall maximum toxicity/patient (19 evaluable patients)

Adverse event	First cycle (CPT-11 + thalidomide)				Second cycle (CPT-11)			
	National Cancer Institute—Common Toxicity Criteria grade (%)							
	1	2	3	4	1	2	3	4
Nausea/vomiting	8 (42.4)	2 (10.6)	1 (5.3)	0	6 (31.8)	3 (15.9)	1 (5.3)	1(5.3)
Diarrhea	8 (42.4)	1 (5.3)	3 (15.9)	0	4 (21.2)	5 (26.5)	0	0
Stomatitis	1(5.3)	1 (5.3)	0	0	5 (26.5)	0	0	0
Neutropenia	1 (5.3)	1 (5.3)	1 (5.3)	0	1 (5.3)	2 (10.6)	1 (5.3)	0
Anemia	1 (5.3)	3 (15.9)	1 (5.3)	1 (5.3)	1 (5.3)	2 (10.6)	0	0
Somnolence	5 (26.5)	1 (5.3)	0	0	0	2 (10.6)	0	0
Insomnia	2 (10.6)	0	0	0	0	0	0	0
Vertigo	1 (5.3)	2 (10.6)	0	0	2 (10.6)	0	0	0
Peripheral neurotoxicity	1 (5.3)	0	0	0	0	0	0	0

Cholinergic syndrome: 7 patients (37.1%), Febrile neutropenia: 0 patients
Values in parentheses are in percentage

$P = 0.014$, respectively). Instead, GR value was significantly increased at first cycle during the treatment with CPT-11 + thalidomide (2.56 ± 1.00) with respect to the second cycle (1.44 ± 0.76; $P = 0.0003$) (Table 3).

Discussion

Of late, CPT-11 has become one of the most important anticancer drugs in the management of patients with metastatic CRC and it has shown a clinical activity against several types of tumors [2–7]. However, CPT-11 clinical advantages have been tempered by significant adverse effects, the most important of which is an unpredictable delayed diarrhea, sometimes associated with myelosuppression, irrespective of the schedule used [17–23]. Therefore, efforts to reduce the incidence of severe diarrhea should be of considerable value.

Several trials have evaluated the antineoplastic activity of thalidomide alone or in combination with anticancer drugs with encouraging results in hematologic malignancies and solid tumors [28–32]. Two small phase II clinical studies have investigated the clinical activity of thalidomide administered in association with CPT-11 in patients with metastatic CRC. The data have showed a response rate of about 30% [33–35] and a striking decrease in incidence of gastrointestinal toxicity, in particular severe diarrhea has been observed in only 1–2% of patients. These data have encouraged us to investigate the possible interaction of thalidomide on irinotecan disposition in cancer patients in an attempt to provide an explanation of this clinical effect.

Clinical studies have demonstrated that there is a significant relationship between high value of BI and the severity of diarrhea induced by CPT-11 [40–42]. Noteworthy, the present pharmacokinetic results provided a possible explanation of decreased toxicities, previously observed when CPT-11 was administered in combination with thalidomide, because it was found that the combined treatment was significantly associated with reduced BI and REC values and augmented GR value. In particular, a significant decreased SN-38 AUC value and increased CPT-11 and SN-38-glucuronide AUC values were calculated at first cycle with respect those obtained at the second cycle. These data suggest an influence of thalidomide on CPT-11 metabolism despite there being only few hypotheses about the sites of interaction that could explain the gastrointestinal toxicities observed. First of all, thalidomide could affect the main metabolic pathway of CPT-11 that yields SN-38 but, up to date, little is known concerning drugs that could interact with carboxyl esterases, except for ciprofloxacin and loperamide [43, 44].

Second, microsomial CYP 450 enzymes, in particular the isoform CYP3A4, represent a parallel pathway of CPT-11 activation [45]. In fact, irinotecan is metabolised to yield APC, a second major inactive metabolite of CPT-11, and NPC, which is produced in a negligible percentage with respect to APC and may be further converted into SN-38 [39]. However, several studies suggest that a metabolic interaction between CPT-11 and thalidomide should be unlikely. Thalidomide undergoes spontaneous hydrolysis and only a reduced fraction is metabolized to hydroxylated compounds by CYP2C19 [46]. Furthermore, in multiple mieloma patients, only three hydrolisys breakdown products were detected in urine but no hydroxylated metabolites [47]. Finally, in vitro studies demonstrated that human hepatic cytochromes did not metabolize thalidomide to yield hydroxylated products at detectable levels [48,49], while thalidomide did not affect liver metabolism of several drugs which are substrate of CYPs, including CYP3A4 [48].

The previously described marked reduction in gastrointestinal toxicity when CPT-11 was administered in association with thalidomide was not observed in the present study. A reason to explain the low incidence of severe gastrointestinal toxicity observed could be that almost half of the present patients received steroids and AEDs. Clinical and preclinical studies have demonstrated that AEDs and dexamethasone are potent inducers of hepatic cytochrome P450 enzymes, thus

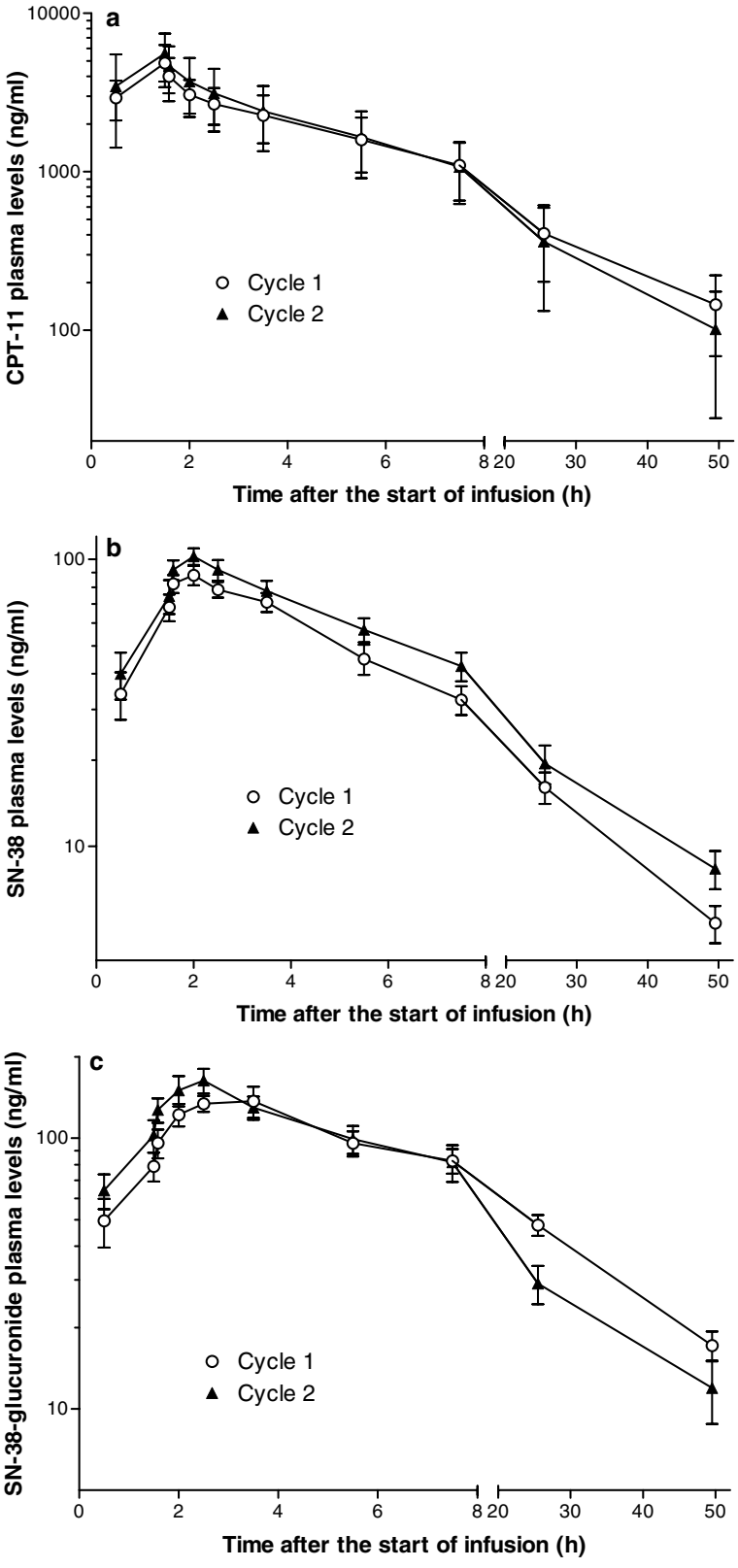

Fig. 1 Plasma profiles of **a** CPT-11, **b** SN-38 and **c** SN-38-glucuronide at first and second cycle of chemotherapy. Plasma concentrations of drug and metabolites were available from 16 out of 19 patients enrolled in the present study. *Symbols* mean values; *bars* standard deviation

increasing CPT-11 clearance [38] and improving treatment tolerability despite higher CPT-11 doses [39]. For these reasons, we cannot exclude that thalidomide can offer a protection against diarrhea induced by CPT-11 because of the low number of patients (9 out of 19) who were not taking steroids and AEDs. Moreover, there are not data about a possible effect of thalidomide on glucuronidation process or on excretion of CPT-11

Table 3 Pharmacokinetic parameters of CPT-11, SN-38 and SN-38-glucuronide at first and second cycle in 16 patients

	Mean ± SD		
	First cycle	Second cycle	p[a]
CPT-11			
AUC (h×μg/ml)	34.53 ± 11.38	28.42 ± 12.23	0.064
Clearance (l/h/m^2)	11.33 ± 3.98	14.53 ± 6.02	0.038[a]
$t_{1/2}\beta$ (h)	19.05 ± 6.44	15.72 ± 7.62	0.195
V_d (l/m^2)	321.27 ± 181.62	335.46 ± 233.70	0.798
C_{max} (ng/ml)	4,883.1 ± 1,368.7	5,864.9 ± 1,757.1	0.046[a]
T_{max} (h)	1.53 ± 0.04	1.52 ± 0.04	0.718
SN-38			
AUC (h×μg/ml)	0.99 ± 0.45	1.34 ± 0.65	0.027[a]
$t_{1/2}\beta$ (h)	20.71 ± 20.02	19.17 ± 10.81	0.798
C_{max} (ng/ml)	99.1 ± 22.7	111.6 ± 39.5	0.377
T_{max} (h)	2.08 ± 0.53	1.93 ± 0.39	0.188
SN-38-glucuronide			
AUC (h×μg/ml)	2.39 ± 1.21	1.86 ± 1.11	0.018[a]
$t_{1/2}\beta$ (h)	24.44 ± 19.19	22.53 ± 18.00	0.778
C_{max} (ng/ml)	136.3 ± 37.7	181.2 ± 76.0	0.223
T_{max} (h)	2.79 ± 0.98	2.69 ± 0.93	0.727
BI	15,627.8 ± 7,767.5	22,964.7 ± 10,896.2	0.011[a]
REC	0.031 ± 0.019	0.052 ± 0.026	0.014[a]
MR	0.105 ± 0.052	0.122 ± 0.061	0.232
GR	2.559 ± 1.001	1.444 ± 0.764	0.0003[a]

[a]Comparison between the first and the second cycle
AUC area under the time/concentration curve, $t_{1/2}\beta$ terminal half-life, V_d volume of distribution, C_{max} maximal plasma concentration, T_{max}, time to peak, *BI* biliary index, *REC* relative extent of conversion, *MR* metabolic ratio, *GR* glucuronidation ratio

and its metabolites through ABC members, as well as P glycoprotein (ABCB1) and ABCC2 as observed for other drugs [50–52].

Overall, present results do not show a significant reduction of gastrointestinal toxicities induced by CPT-11 when the latter is combined with thalidomide. However, a significant pharmacokinetic interaction between thalidomide and CPT-11 has been demonstrated, emphasizing the important influence of drug interactions on treatment tolerability of CPT-11-based chemotherapy. Due to the complex metabolic pathways that yield the active metabolite SN-38 from CPT-11, via both carboxyl esterases and cytochrome P450 enzymes, several drugs could significantly affect the disposition of CPT-11 and its metabolites, and further researches will be warranted.

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References

1. Kunimoto T, Nitta K, Kanaka T, Uehara N, Baba H, Takeuchi M, Yokokura T, Sawada S, Miyasaka T, Mutai M (1987) Antitumor activity of 7-ethyl-10 (4-(1-piperidino)-1 piperidino)-1-carboniloxi-campthotecin, a novel water soluble derivative of campthotecin, against murine tumor. Cancer Res 47:5944–5947
2. Canal P, Gay C, Dazeuse A, Douillard JY, Bugat R, Brunet R, Adenis A, Herait P, Lokiec F, Mathieu-Boue A (1996) Pharmacokinetics and pharmacodynamics of irinotecan during a phase II clinical trial in colorectal cancer. J Clin Oncol 14:2688–2695
3. Fuchs CS, Moore MR, Harker G, Villa L, Rinaldi D, Hecht JR (2003) Phase III comparison of two irinotecan dosing regimens in second-line therapy of metastatic colorectal cancer. J Clin Oncol 21:807–814
4. Negoro S, Masuda N, Takada Y, Sugiura T, Kudoh S, Katakami N, Ariyoshi Y, Ohashi Y, Niitani H, Fukuoka M; CPT-11 Lung Cancer Study Group West (2003) Randomized phase III trial of irinotecan combined with cisplatin for advanced non-small-cell lung cancer. Br J Cancer 88:335–341
5. Friedman HS, Petros WP, Friedman AH, Schaaf LJ, Kerby T, Lawyer J, Parry M, Houghton PJ, Lovell S, Rasheed K, Cloughsey T, Stewart ES, Colvin OM, Provenzale JM, McLendon RE, Bigner DD, Cokgor I, Haglund M, Rich J, Ashley D, Malczyn J, Elfring GL, Miller LL (1999) Irinotecan therapy in adults with recurrent or progressiv malignant glioma. J Clin Oncol 17:1516–1525
6. Perez EA, Hillman DW, Mailliard JA, Ingle JN, Ryan JM, Fitch TR, Rowland KM, Kardinal CG, Krook JE, Kugler JW, Dakhil SR (2004) Randomized phase II study of two irinotecan schedules for patients with metastatic breast cancer refractory to an anthracycline, a taxane, or both. J Clin Oncol 22:2849–2855
7. Kohene CH, Catane R, Klein B, Ducreux M, Thuss-Patience P, Niederle N, Gips M, Preusser P, Knuth A, Clemens M, Bugat R, Figer I, Shani A, Fages B, Di Betta D, Jacques C, Wilke HJ (2003) Irinotecan is active in chemonaive patients with metastatic gastric cancer: a phase II multicenter trial. Br J Cancer 89:997–1001
8. Pitot HC, Wender DB, O'Connell MJ, Schroeder G, Goldberg RM, Rubin J,Mailliard JA, Knost JA, Ghosh C, Kirschling RJ,Levitt R, Windschitl HE (1997) Phase II trial of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 15:2910–2919
9. Rougier P, Bugat R, Douillard JY, Culine S, Suc E, Brunet P, Becouarn Y, Ychou M, Marty M, Extra JM, Bonneterre J, Adenis A, Seitz JF, Ganem G, Namer M, Conroy T, Negrier S, Merrouche Y, Burki F, Mousseau M, Herait P, Mahjoubi M (1997) Phase II study of irinotecan in the treatment of advanced colorectal cancer in chemotherapy-naïve patients and patients pretreated with fluorouracil based chemotherapy. J Clin Oncol 15:251–260
10. Cunningham D, Glimelius B (1999) A phase III study of irinotecan (CPT-11) versus best supportive care in patients with metastatic colorectal cancer who have failed 5-fluorouracil therapy. Sem Oncol 26(suppl 5):6–10
11. Rougier P, Van Cutsem E, Bajetta E, Niederle N, Possinger K, Labianca R, Navarro M, Morant R, Bleiberg H, Wils J, Awad

L, Herait P, Jacques C (1998) Randomized trial of irinotecan versus fluorouracil by continous infusion after fluorouracil failure in patients with metastatic colorectal cancer. Lancet 352:1407–1412
12. Graeven U, Ridwelski K, Manns M (2000) Irinotecan with bolus 5-fluorouracil and folinic acid in patients with previously untreated metastatic colorectal cancer: an active and safe regiment. Proc Am Soc Clin Oncol 19 abstract no. 952
13. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alakl M, Gruia G, Awad L, Rougier P (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomized trial. Lancet 355:1041–1047
14. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL, Miller LL (2000) Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 343:905–914
15. Saltz LB, Niedzwiecki D, Hollis D (2004) Irinotecan plus fluorouracil/leucovorin (IFL) versus fluorouracil/leucovorin alone (FL) in stage III colon cancer (intergroup trial CALGB C89803). Proc Am Soc Clin Oncol 22: abstract no. 3500
16. Sasaki K, Takasaka H, Kiriyama K (2004) Adjuvant postoperative chemotherapy for Dukes C colorectal cancer; weekly low-dose Irinotecan (CPT-11) plus 5 FU versus oral 5FU only. Proc Am Soc Clin Oncol 22: abstract no. 3735
17. Canal P, Gay C, Dezeuse A, Douillard JY, Bugat R, Brunet R, Adenis A, Herait P, Lokiec F, Mathieu-Boue A (1996) Pharmacokinetics and pharmacodynamics of irinotecan during a phase II clinical trial in colorectal cancer. J Clin Oncol 10:2688–2695
18. De Forni M, Bugat R, Chabot GG, Culine S, Extra JM, Gouyette A, Madelaine I, Marty ME, Mathieu-Boue A (1994) Phase I and pharmacokinetics study of the camptothecin derivative irinotecan, administered on a weekly schedule in cancer patients. Cancer Res 54:4347–4354
19. Abigerges D, Chabot GG, Armand J, Herait P, Gouyette A, Gandia D (1995) Phase I and pharmacologic studies of the camptothecin analogue irinotecan administered every 3 weeks in cancer ptients. J Clin Oncol 13:210–221
20. Catimel G, Chabot GG, Guastella JP, Dumortier A, Cote C, Engel C, Gouyette A, Mathieu-Boue A, Mahjoubi M, Clavel M (1995) Phase I and pharmacokinetics study of irinotecan (CPT-11) administered daily for three consecutive days every three weeks in patients with advances solid tumors. Ann Oncol 6:133–140
21. Bleiberg H, Cvitkovic E (1996) Characterisation and clinical management of CPT-11 induced adverse events: the european perspective. Eur J Cancer 32A(suppl 3):S18–S23
22. Conti JA, Kemeny NE, Saltz LB, Huang Y, Tong WP, Chou TC, Sun M, Pulliam S, Gonzalez C (1996) Irinotecan is an active agent in untreated patients with metastatic colorectal cancer. J Clin Oncol 14:709–715
23. Abigerges D, Armand JP, Chabot GC, Da Costa L, Fadel E, Cote C, Herait P, Gandia D (1994) Irinotecan high-dose escalation using intensive high-dose loperamide to control diarrhea. J Nat Cancer Inst 86:446–449
24. Stephens TD, Bunde CJ, Fillemore BJ (2000) Mechanism of action in thalidomide teratogenesis. Biochem Pharmacol 59:1489–1499
25. McHugh SM, Rifkin IR, Deighton J, Wilson AB, Lachmann PJ, Lockwood CM, Ewan PW ((1995) The immunosuppressive drug thalidomide induces T helper cell type 2 (Th2) and concomitantly inhibits Th1 cytokine production in mitogen- and antigen-stimulated human peripheral blood mononuclear cell cultures. Clin Exp Immunol 99:160–167
26. Kenyon BM, Browne F, D'Amato RJ (1997) Effects of thalidomide and related metabolites in a mouse corneal model of neovascularization. Exp Eye Res 64:971–978
27. Or R, Feferman R, Shoshan S (1998) Thalidomide reduces vascular density in granulation tissue of subcutaneously implanted polyvinyl alcohol sponges in guinea pigs. Exp Hematol 26:217–221
28. Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, Munshi N, Anaissie E, Wilson C, Dhodapkar M, Zeddis J, Barlogie B (1999) Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med 341:1565–1571
29. Rajkumar SV, Fonseca R, Dispenzieri A, et al (1999) Thalidomide in the treatment of relapsed and refractory myeloma. Blood 94(suppl 1):316a
30. Fine HA, Figg WD, Jaeckle K, Wen PY, Kyritsis AP, Loeffler JS, Levin VA, Black PM, Kaplan R, Pluda JM, Yung WK (2000) A phase II trial of the antiangiogenic agent thalidomide in patients with recurrent high-grade gliomas. J Clin Oncol 18:708–715
31. Figg WD, Dahut W, Duray P, Hamilton M, Tompkins A, Steinberg SM, Jones E, Premkumar A, Linehan WM, Floeter MK, Chen CC, Dixon S, Kohler DR, Kruger EA, Gubish E, Pluda JM, Reed E (2001) A Randomized phase II trial of docetaxel (taxotere) plus thalidomide, an angiogenesis inhibitor, in patients with androgen-independent prostate cancer. Clin Cancer Res 7:1888–1893
32. Motzer RJ, Berg W, Ginsberg M, Russo P, Vuky J, Yu R, Bacik J, Mazumdar M (2002) Phase II trial of Thalidomide for patients with advanced renal cell carcinoma. J Clin Oncol 20:302–306
33. Govindarajan R, Heaton KM, Broadwater R, Zeitlin A, Lang NP, Hauer-Jensen M (2000) Effect of thalidomide on gastrointestinal toxic effects of irinotecan. Lancet 356:566–567
34. Govindarajan R (2000) Irinotecan/thalidomide in metastatic colorectal cancer. Oncology 16(suppl):23–26
35. Govindarajan R, Maddox A, Gray P (2003) Irinotecan and thalidomide prolong disease free and overall survival in 5FU refractory metastatic colorectal cancer. Proc Am Soc Clin Oncol 22:249: abstract no. 997
36. Barileiro I, Gandia D, Armand JP, Mathieu-Boue A, Re M, Gouyette A, Chabot GG (1992) Simultaneus determination of the camptothecin analogue CPT-11 and its active metabolite SN-38 by high-performance liquid chromatography: application to plasma pharmacokinetics studies in cancer patients. J Chromatogr Biomed Appl 575:275–280
37. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC, Gwyther SG (2000) New guidelines to evaluate the response to treatment in solid tumors. European organization for research and treatment of cancer, National Cancer Institute of United states, National Cancer Institute Of Canada. J Natl Cancer Inst 92:205–216
38. Friedman LM, Furberg DC, De Mets DL. Fundamentals of clinical trials. Third Edition, Springer
39. Crews KR, Stewart CF, Jones-Wallace D, Thompson SJ, Houghton PJ, Heideman RL, Fouladi M, Bowers DC, Chintagumpala MM, Gajjar A (2002) Altered irinotecan pharmacokinetics in pediatric high-grade glioma patients receiving enzyme-inducing anticonvulsant therapy. Clin Cancer Res 8:2202–2209
40. Gupta E, Lestingi TM, Mick R, Ramirez J, Vokes EE, Ratain MJ (1994) Metabolic fate of irinotecan in humans: correlation of glicuronation with diarrhea. Cancer Res 54:3723–3725
41. Gupta E, Mick R, Ramirez J, Wang X, Lestingi TM, Vokes EE, Ratain MJ (1997) Pharmacokinetic and pharmacodinamic evaluation of the topoisomerase inhibitor irinotecan in cancer patients. J Clin Oncol 15:1502–1510
42. Mick R, Gupta E, Vokes EE, Ratain MJ (1996) Limited sampling models for irinotecan pharmacokinetics-pharmacodynamics: prediction of biliary index and intestinal toxicity. J Clin Oncol 14:2012–2019
43. Haaz MC, Rivory LP, Riche C, Robert J (1997) The transformation of irinotecan (CPT-11) to its active metabolite SN-38 by human liver microsomes. Differential hydrolysis for the lactone and carboxylate forms. Naunyn Schmiedebergs Arch Pharmacol 356:257–262
44. Charasson V, Haaz MC, Robert J (2002) Determination of drug interactions occurring with the metabolic pathways of irinotecan. Drug Metab Dispos 30:731–733

45. Haaz MC, Riche C, Rivory LP, Robert J (1998) Biosynthesis of an aminopiperidino metabolite of irinotecan [7–ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecine] by human hepatic microsomes. Drug Metab Dispos 26:769–774
46. Ando Y, Fuse E, Figg WD (2002) Thalidomide metabolism by the CYP2C subfamily. Clin Cancer Res 8:1964–1973
47. Lu J, Palmer BD, Kestell P, Browett P, Baguley BC, Muller G, Ching LM (2003) Thalidomide metabolites in mice and patients with multiple myeloma. Clin Cancer Res 9:1680–1688
48. Teo SK, Sabourin PJ, O'Brien K, Kook KA, Thomas SD (2000) Metabolism of thalidomide in human microsomes, cloned human Cytochrome P-450 isoenzymes, and Hansen's disease patients. J Biochem Mol Toxicol 14:140–147
49. Lu J, Helsby N, Palmer BD, Tingle M, Baguley BC, Kestell P, Ching LM (2004) Metabolism of thalidomide in liver microsomes of mice, rabbits, and humans. J Pharmacol Exp Ther 310:571–577
50. Patel NJ, Zamek-Gliszczynski MJ, Zhang P, Han YH, Jansen PL, Meier PJ, Stieger B, Brouwer KL (2003) Phenobarbital alters hepatic Mrp2 function by direct and indirect interactions. Mol Pharmacol 64:154–159
51. Weiss J, Kerpen CJ, Lindenmaier H, Dormann SM, Haefeli WE (2003) Interaction of antiepileptic drugs with human P-glycoprotein in vitro. J Pharmacol Exp Ther 307:262–267
52. Xiong H, Suzuki H, Sugiyama Y, Meier PJ, Pollack GM, Brouwer KL (2002) Mechanisms of impaired biliary excretion of acetaminophen glucuronide after acute phenobarbital treatment or phenobarbital pretreatment. Drug Metab Dispos 30:962–969