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ORIGINAL ARTICLE

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Sequential administration of irinotecan and cytarabine in the treatment of relapsed and refractory acute myeloid leukemia

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Abstract *Purpose*: Based on reported synergy of the topoisomerase-I (topo-I) inhibitor irinotecan with antimetabolites, irinotecan and cytarabine (Ara-C) were administered sequentially to patients with acute myeloid leukemia (AML) refractory to or relapsed following high-dose Ara-C and anthracycline therapy. Pharmacokinetic and pharmacodynamic studies were performed with the first irinotecan dose. *Experimental Design*: In vitro synergy of irinotecan followed by Ara-C was confirmed in a human AML cell line as a basis for the clinical trial. Irinotecan was administered daily for 5 days, with Ara-C 1 g/m^2 12 h after each irinotecan dose. Irinotecan was initiated at 5 mg/m^2, and the dose was escalated by 5 mg/m^2 increments in cohorts of three patients and in individual patients. Pre-treatment samples were studied for topo-I activity and serial samples after the first irinotecan dose were analyzed for pharmacokinetics and for pharmacodynamic effects, including DNA damage and DNA synthesis rate. *Results*: The irinotecan dose reached 15 mg/m^2 in three-patient cohorts without reaching the maximum tolerated dose, and reached 30 mg/m^2 in individual patients. The AUC and C_{max} of both irinotecan and its active metabolite SN38 increased linearly in proportion to dose, and the mean half-lives of irinotecan conversion to SN38 and SN38 elimination were 6.2 h (CV 171%) and 7.2 h (CV 48%). Irinotecan rapidly induced DNA damage, and DNA synthesis inhibition varied among patients and treatment cycles. All courses resulted in rapid cytoreduction, and two patients achieved complete remission. Topo-I activity did not predict response. *Conclusion*: Irinotecan can be safely administered with Ara-C. This combination is active in refractory AML and warrants further study.

Keywords Irinotecan · Relapsed and refractory acute myeloid leukemia · Cytarabine · Topoisomerase-I inhibitors

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Introduction

Treatment outcome for relapsed and refractory acute myeloid leukemia (AML) is generally poor (21), and lack of effective therapy for these patients as well as for patients with untreated AML with adverse prognostic features mandates development of new approaches.

The camptothecin analogue irinotecan is metabolized by carboxylesterases to its active metabolite 7-ethyl-10-hydroxycamptothecin (SN38), which acts as a classic topoisomerase-I (topo-I) inhibitor by stabilizing the topo-I/DNA cleavable complex, resulting in obstruction of DNA replication and formation of DNA strand breaks. Irinotecan is active as a single agent in diverse malignancies [14, 18, 19, 21, 29–32, 36, 39, 41], but its major contribution appears to be in combination regimens. Irinotecan combined with 5-fluorouracil (5-FU) by 24-h infusion with leucovorin (LV) modulation (5-FU24 h/LV) produces a synergistic interaction that results in response rates as high as 58% in patients with metastatic colorectal cancer, including those previously treated with 5-FU24 h/LV without irinotecan [41].

Presence of increased topo-I levels in AML cells [15] suggests the likelihood of sensitivity to topo-I inhibitors, and limited studies have shown activity of topotecan [4, 9, 12, 13, 22, 33–35] and irinotecan [29, 31, 39] in this disease. The first report on camptothecins actually described their activity in leukemia [42]. Ohno et al. [29] subsequently found that while a single monthly dose of irinotecan was ineffective in acute leukemia, divided doses given more frequently produced response rates of 7–18%, consistent with in vivo preclinical data suggesting that repeated intermittent scheduling is superior to single injections of irinotecan in the same total doses [20, 28]. Irinotecan combined with the deoxycytidine analog gemcitabine by prolonged infusion produced an 18% response rate in refractory acute leukemia, and this rate was 28% at maximum tolerated dose (MTD) [39].

In the present study irinotecan was combined with another deoxycytidine analogue, cytarabine (Ara-C), which is a mainstay of AML therapy. The combination of topotecan with Ara-C has produced encouraging responses in previous studies [4, 9, 12, 13, 22, 34], and irinotecan may be preferable to topotecan in AML therapy because AML cells express multidrug resistance proteins including P-glycoprotein (Pgp) [23, 26] and (BCRP) [37] and, while both drugs are substrates for BCRP and Pgp [17, 40, 43], Pgp affects cytotoxicity of irinotecan less than that of topotecan [27]. Indeed, irinotecan was highly effective as a single agent against multidrug resistant myeloid leukemias in in vivo preclinical studies [28].

Previous studies on the sequence of administration of irinotecan in combination regimens demonstrated that administration of irinotecan first was superior to the reverse sequence or to simultaneous administration [6, 38]. Since Ara-C is mechanistically different from 5-FU and gemcitibine and differences may exist between AML and colon carcinoma and neuroblastoma with regard to baseline and drug-perturbed growth kinetics that may result in altered drug interactions, the drug interaction between SN38 and Ara-C was first studied in vitro using a human AML cell line model. In the present study, after in vitro synergy between sequentially administered SN38 and Ara-C was demonstrated in a human AML cell line model, we undertook a clinical trial of this combination regimen in this sequence.

Materials and methods

In vitro assessment of synergistic interaction between SN38 and Ara-C in human acute myeloid leukemia cells

To test for a synergistic interaction between SN38 followed by Ara-C in human AML cells, the effect of SN38 pre-treatment on the in vitro IC_{50} of Ara-C was determined in HL60 cells. Cells were seeded at 10,000 cells/well in 96-well tissue culture plates in RPMI 1640 medium (Life Technologies, Inc., Grand island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (Life Technologies), 2 mM L-glutamine, 20 U/ml penicillin and 20 μg/ml streptomycin (Life Technologies). SN38 (Pharmacia Corporation, Kalamazoo, MI, USA) was prepared from 5 mM frozen stock in 100% dimethylsulfoxide and Ara-C from frozen clinical preparation stock of 358 mM (Cytarabine Injection, Preservative free, Faulding Pharmaceutical Co., Paramus, Australia). Drug dilutions were made in supplemented RPMI 1640 medium. In one set of experiments, HL60 cells were exposed to SN38 for 2 h, washed twice in phosphate-buffered saline (PBS), resuspended in drug-free medium and returned to the incubator and Ara-C was added to these cultures 24 h later. In a second set of experiments, HL60 cells were cultured with SN38 for 24 h, and Ara-C was added 24 h later. Cells were then cultured for 96 h, drug effects were assessed by the wst-1 colorimetric assay and IC_{50}'s were estimated as previously described [5]. To assess the effects of SN38 pre-treatment on the IC_{50} of Ara-C, the combined drug effects were evaluated relative to control cultures exposed to the corresponding SN38 concentration alone (0, 0.001 and 0.003 μM SN38, 2 h or continuous exposure). All cytotoxicity experiments were performed in quadruplicate.

Patient eligibility

Eligibility criteria included AML diagnosed by French–American–British (FAB) criteria [3], refractory to or in relapse following HiDAC and anthracycline chemotherapy. Prior chemo- or radiation therapy for an antecedent malignancy or other medical condition did not disqualify. Other criteria included age ≥15 years, performance status 0–3, serum creatinine $< 1.5\times$ normal, and total bilirubin and SGOT $< 2\times$ normal. All patients signed informed consent.

Treatment schedule

Patients received irinotecan intravenously over 90 min daily for 5 days, with Ara-C 1 gram/m^2 intravenously over 60 min 12 h after the start of each irinotecan dose. The regimen was administered as inpatient therapy because of the 12-h schedule, but, when possible, patients were discharged at completion of chemotherapy. The Ara-C dose was decreased to 400 mg/m^2 if cerebellar toxicity developed. The initial irinotecan dose was 5 mg/m^2, and irinotecan doses were escalated by 5 mg/m^2 dose increments in cohorts of three patients based on regimen-related dose-limiting toxicity (DLT), in a standard phase I design. DLT was defined as grade 3 or higher non-hematologic or non-infectious toxicity using the National Cancer Institute Common Toxicity Criteria, or lack of hematologic recovery within 30 days of initiation of therapy, attributable to drug effect rather than to refractory leukemia. If none of three patients

exhibited a DLT, the irinotecan dose level was escalated for the next three patients. If one patient exhibited a DLT, the dose level was repeated for three more patients. If two or three patients exhibited a DLT, the previous dose level would be considered the MTD, and nine additional patients would be treated at this dose level. Patients completing therapy without therapy-related DLT, continuing to meet protocol eligibility criteria and wishing to continue therapy were treated with irinotecan at the next dose level. Dose escalations for individual patients were not be used in establishing MTD.

Response criteria

Criteria for response and relapse were those of the 1990 National Cancer Institute-sponsored workshop on definitions of diagnosis and response in AML [8].

Pharmacokinetic studies

Blood samples were collected for determination of irinotecan and SN38 concentrations on day 1 at 0 (pre-infusion), 0.5, 1, 1.5 (end of infusion), 2, 3, 4, 6, 8, and 10 h, on days 2, 3, and 4 at 0 h, and on day 5 at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 10 h, and were also collected in secondary dose escalations within individual patients on days 1 and 5 at 0, 0.5, 1.5, 2, 4, and 6 h and on days 2, 3 and 4 at 0 h. Blood was collected in heparinized tubes and centrifuged immediately at 4°C. Plasma was stored at −20°C until analysis.

To precipitate proteins and extract drugs and internal standards, 800 µl of acidified methanol were added to plasma samples. After thorough mixing, the protein was pelleted by centrifugation, the supernatant was transferred to another glass tube and the methanol was evaporated. The resulting residue was reconstituted in 200 µl of a 1:1 solution of acidified methanol and 3% triethylamine, transferred to a microcentrifuge tube, and centrifuged at 10,000 g for 5 min. Eighty microliter of the supernatant was injected into the high performance liquid chromatograpy (HPLC) system for analysis.

A validated HPLC method with fluorescence detection was used to quantify both total irinotecan and SN38, using campothecin as an internal standard. The HPLC system consisted of a Waters M600 pump with autosampler and a M470 fluorescence detector operated by a PC with Waters Millenium software. The separation was carried out on a Waters Nova-Pak C18 column equipped with a μBondapak C18 guard column, with the mobile phase consisting of 20% acetonitrile and 80% triethylamine buffer. The detection was by fluorescence, with excitation at 370 nm and emission at 510 nm. The ratio of the peak areas for irinotecan, SN38 and campothecin was used for quantitation. The assay has a lower limit of quantitation of 2.5 ng/ml for both irinotecan and SN38. Quality assurance was maintained by simultaneously assaying the quality control samples prepared in bulk.

Pharmacokinetic modeling

The plasma pharmacokinetics of irinotecan and SN38 were characterized by fitting the data to candidate pharmacokinetic models, initially by maximum likelihood non-linear regression, as implemented in ADAPT II [10]. Discrimination between candidate models was by Akaike's information criterion [1] and the Rule of Parsimony, and data were weighted by the inverse of the observation variance. The maximum likelihood parameter estimates were then used as initial Bayesian priors (parameter likelihood distributions), and each patient profile refitted by maximum a posteriori (MAP) Bayesian estimation (ADAPT II). Bayesian prior parameter estimates were updated twice during the course of the analysis for the final fitting procedure. The plasma concentrations from the entire 5-day study period for both irinotecan and SN38, as well as the conversion of irinotecan to SN38, were co-modeled (fit simultaneously) in each patient.

The pharmacokinetic parameters considered for the parent and metabolite included drug clearance out of the body, distributional clearances between the compartments and volumes of distribution for each compartment. Area under the curve (AUC) was calculated by integration of the plasma-concentration time profile. Steady-state volume of distribution (Vss) was calculated as the sum of volumes for each individual compartment. Half-lives were computed as (Ln 2)/k, where k is the rate constant for the elimination phase of interest. Maximum observed concentration (Cmax), time of Cmax (Tmax), and minimum observed concentration (Cmin) were determined by visual inspection of the raw data.

DNA damage

DNA damage was assessed by the comet assay (Trevigen Inc, Gaithersburg, MD, USA), according to the manufacturer's instructions. This single-cell gel electrophoresis assay measures migration of denatured, cleaved DNA fragments out of cells under the influence of an electrical field. Undamaged DNA migrates more slowly and remains within the confines of the nucleus when a current is applied, while damaged DNA migrates out of the nucleus. Measurement of the DNA comet tail shape and migration pattern, visualized by SYBR Green staining, quantifies DNA damage. Immunofluorescence was captured using a silicone intensified target (SIT) camera (C2400-8, Hamamatsu, Japan) attached to a fluorescence microscope (Zeiss Axiophot, Goettingen, Germany). The camera signal was processed in real time by a low-light enhancement image processing system (Argus-10 image processor, Hamamatsu, Japan) and

comet tail lengths were measured using the system's operating software. At least 50 cells were evaluated on each slide and the data were expressed as the average comet tail length (in pixels) per slide.

Thymidine incorporation

DNA synthesis at serial time points was measured by incorporation of tritiated thymidine. Cells were incubated at 37°C for 10 min with 1 mCi/ml tritiated thymidine, then washed twice with ice-cold PBS. Protein was precipitated with 10% trichloric acid. Following cell lysis in 0.4 N sodium hydroxide, radioactivity was measured and quantified as CPM/10^6 cells plotted against time.

Topoisomerase-I catalytic activity

Topoisomerase-I activity was measured in nuclear cell extracts [10] using a commercial Topo-I kit (Topogen, Columbus, OH, USA) according to the manufacturer's instructions. The test determines the conversion of a fixed amount of supercoiled DNA into relaxed DNA. Since nuclear protein extract efficiency varied, different protein extract titration ranges were used for individual patients. Topo-I activity was defined as the minimal nuclear protein extract concentration required to fully relax 0.25 μg of supercoiled DNA during 30-min incubation at 37°C.

Results

In vitro synergy between SN38 and Ara-C in human acute myeloid leukemia cells

SN38 pre-treatment sensitized HL60 cells to Ara-C (Fig. 1). Specifically, 2-h pre-treatment with 0.001 μM

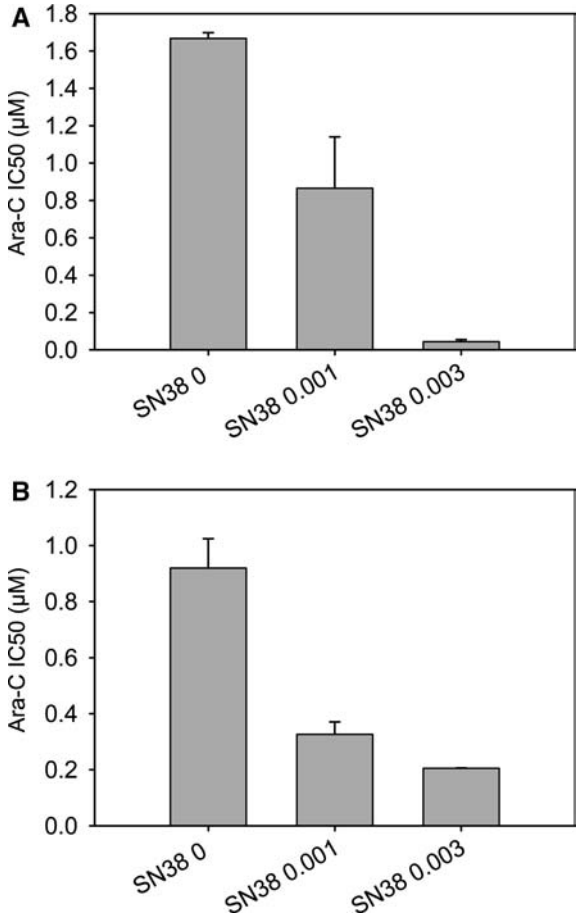

Fig. 1 In vitro synergy between SN38 and Ara-C in HL60 cells. Cells were treated with SN38 for 2 h, followed 24 h later by Ara-C for 4 days continuous exposure (**a**), or exposed continuously to SN38, with Ara-C added 24 h after initiation of SN38 exposure (**b**). *Bars* represent Ara-C IC_{50} concentrations, assessed by evaluating the combined drug effects relative to control cultures exposed to the corresponding SN38 concentration alone (0, 0.001 and 0.003 μM SN38, 2 h (**a**) or continuous exposure (**b**)). *Error bars* show standard deviations of quadruplicate experiments

Table 1 Clinical data

Cohort	Patient	Age, sex	Irinotecan doses (mg/m^2)	Treatment outcome	ANC	Grade 3–4 toxicities
1	1	52F	5, 10, 15, 20	RD, RD, RD, RD	NR, NR, NR, NR	None
	2	53M	5	RD	18	Infection
	3	50F	5	RD	14	None
2	4[a]	45F	10	RD	59	GI[b] hemorrhage, hepatic, infection, metabolic, neurologic, pain, pulmonary, renal
	5	46M	10, 15, 20	RD	27, NR, NR	None
	6	65M	10, 15	RD	15, NR	None
3	7	71M	10, 15, 20	CR	NR, 35, NR	None
	8	73M	10	RD	NR	Infection
	9	62M	10	D	NR	Cardiovascular, skin, hepatic, infection, pulmonary, renal
4	10	58M	10, 15, 20	CR	19, 29, 31	None
	11	36F	10, 15	RD	NR, NR	Infection
	12	69F	10, 15, 20	RD	NR, NR, NR	Pulmonary
5	13	57M	15	RD	33	Cardiovascular
	14[a]	75M	15, 20, 25, 30	RD	27, NR, NR, NR	Skin[b]

RD resistant disease, *CR* complete remission, *d* death, *NR* no recovery [a]DLT [b]Regimen-related toxicity

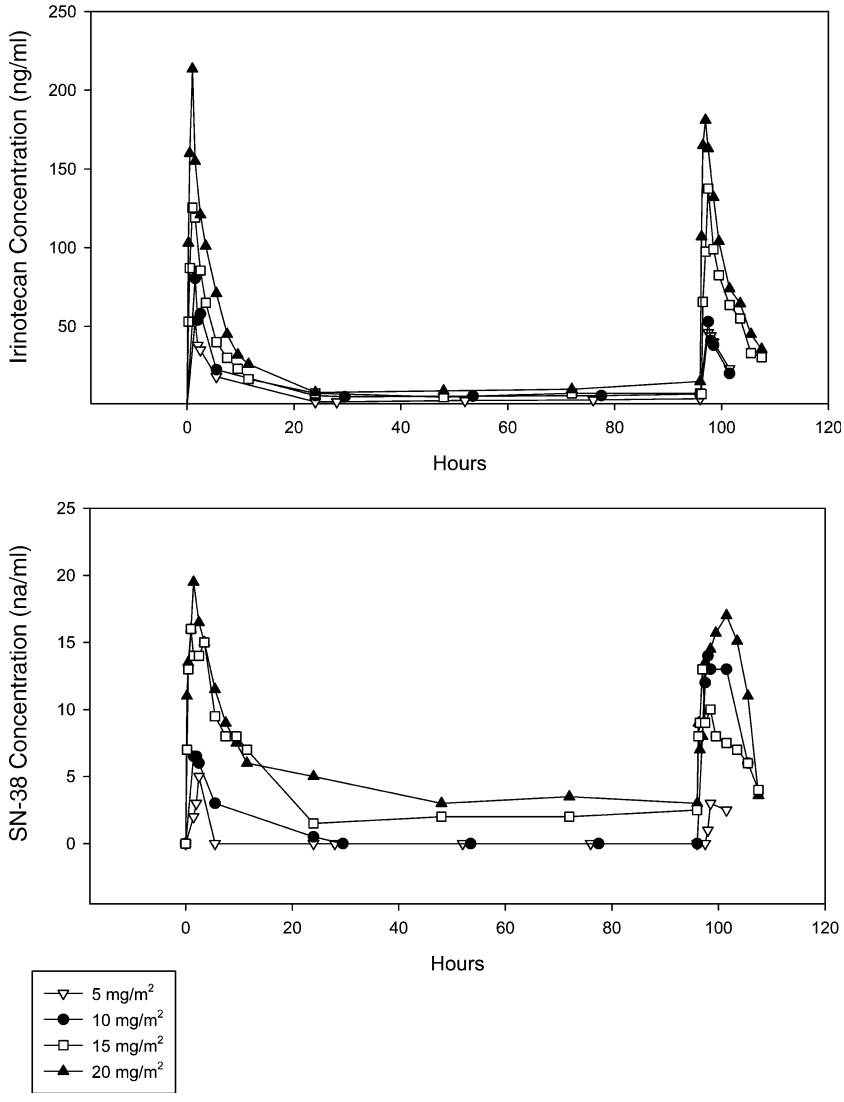

Fig. 2 Median irinotecan (*above*) and SN38 (*below*) plasma concentrations (Ng/ml) following administration of 5, 10, 15 and 20 mg/m^2

and 0.003 μM SN38 reduced the IC_{50} of Ara-C from 1.7 μM to 0.9 μM and less than 0.1 μM, respectively (Fig. 1a), and pre-treatment with and continuous exposure to 0.001 and 0.003 μM SN38 reduced the IC_{50} of Ara-C from 0.9 μM to 0.3 μM and 0.2 μM, respectively (Fig. 1b).

Clinical data

Fourteen patients (ages 36–75 years; median, 58 years) were enrolled. Irinotecan doses up to 15 mg/m^2 were administered in three-patient cohorts, without reaching MTD (Table 1). No patient in the 5 mg/m^2 cohort experienced a DLT. One patient in the first 10 mg/m^2 cohort had a DLT (gastrointestinal), so the cohort was repeated. In the second 10 mg/m^2 cohort, no patient experienced a regimen-related DLT, but one patient died of pneumonia, and was therefore considered inevaluable for DLT. A third cohort was therefore treated at 10 mg/m^2, and no DLT was seen (toxicities included infection and hypoxemia associated with pulmonary atelectasis, neither of which was regimen-related). The dose was therefore escalated to 15 mg/m^2, but dose escalation was subsequently stopped in favor of a 6-day, rather than 5-day, regimen.

In addition, with intrapatient irinotecan dose escalation, doses of up to 30 mg/m^2 were administered. The 14 patients received 30 treatment courses (Table 1). Intrapatient dose escalation was also generally well tolerated. Neutropenia was seen with all courses. The median duration of absolute neutrophil count (ANC) less than 500 was 27 days (range 14–59 days) for 11 courses in which neutrophil recovery occurred; neutrophils did not recover following 19 courses for which the outcome was refractory leukemia. Fever was the next most common toxicity (11 courses), followed by cardiovascular and pulmonary toxicities (5 courses each). Other toxicities

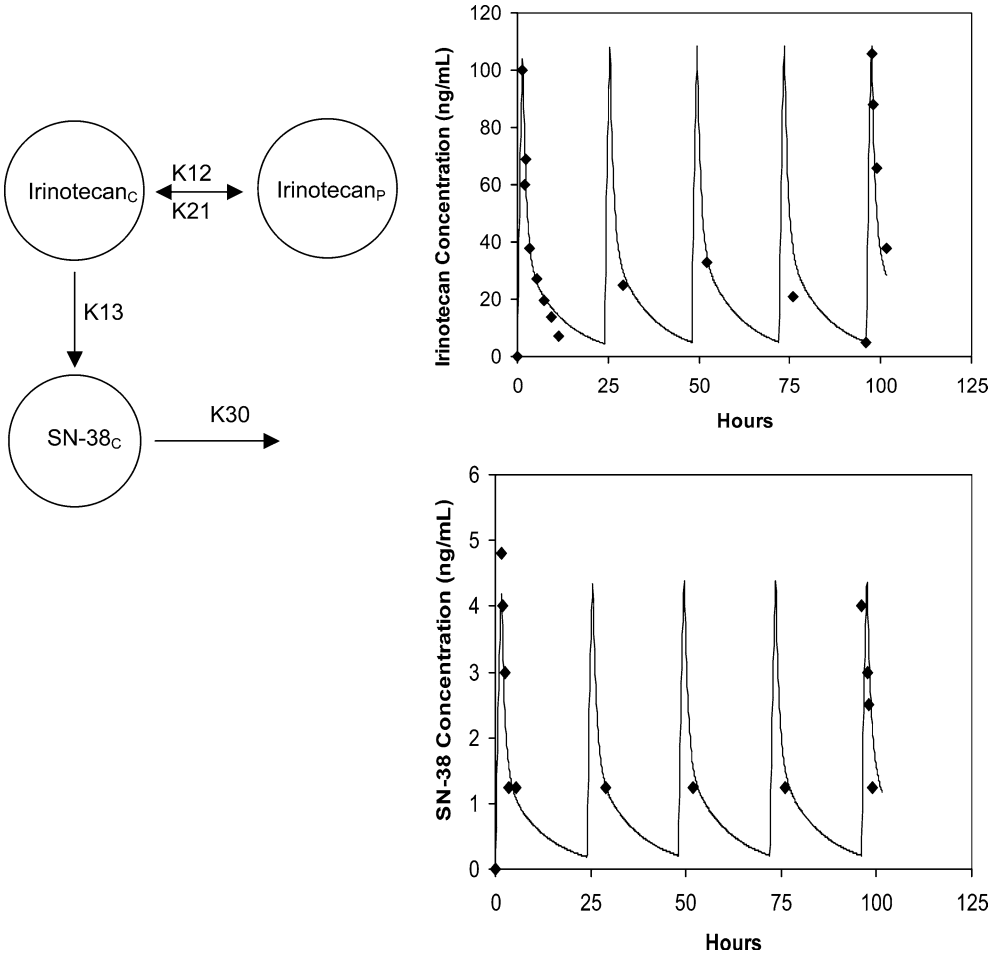

Fig. 3 Pharmacokinetic model for irinotecan and SN38 (*left*), and example of fit of the model to patient data (*right*). Irinotecan$_c$, irinotecan$_p$ and SN38$_c$ are the apparent volumes of distribution for the central and peripheral compartments of irinotecan, and the central compartment for SN38, respectively. *K*'s represent rate constants for the movement of drug to each compartment. *Dark symbols* are measured irinotecan concentrations, and *solid lines* represent fit of the model to the data

Table 2 Irinotecan and SN38 pharmacokinetic parameters. K_{12} is the rate constant describing movement of irinotecan from the central to the peripheral compartment, K_{21} is the rate constant describing movement of irinotecan from the peripheral to the central compartment, K_{13} the rate constant for conversion of irinotecan to SN38, and K_{30} is the elimination rate constant for SN38 out of the body

	K_{12} (1/h)	K_{21} (1/h)	K_{13} (1/h)	K_{30} (1/h)	Vc (l/m^2)	Vmet (l/m^2)	$CL_{Irinotecan}$ (l/h/m^2)	CL_{SN38} (l/h/m^2)
Geometric mean	0.51	0.28	0.29	1.7	63.9	20.9	21.9	73.0
CV%	87	117	161	81	49	89	37	67

included gastrointestinal, hemorrhage, hepatic, metabolic, neurologic, pain and renal, all in fewer than four courses.

Two patients (Patients 7 and 10) achieved complete remissions. Patient 7 was a 71-year-old man with primary refractory AML, FAB M1, with monosomy 7 whose leukemia had previously been refractory to standard-dose Ara-C, daunorubicin and etoposide induction, and to HiDAC and mitoxantrone salvage therapy. Patient 10 was a 58-year-old man with AML, FAB M2, with a normal karyotype, in second relapse. Previous therapy had included Ara-C, daunorubicin and etoposide induction, HiDAC and etoposide intensification and autologous hematopoietic stem cell transplantation in first remission, and HiDAC and mitoxantrone reinduction for first relapse.

Given the appreciable response rate and the low toxicity rate, the study was terminated in favor of a subsequent study of a 6-day, rather than a 5-day, regimen.

Pharmacokinetics

Median pharmacokinetic profiles for irinotecan and SN38, by dose, are shown in Fig. 2. The very low doses of 5 mg/m^2 resulted in irinotecan and SN38 concentrations that approached or were below the assay limit of quantification, precluding a complete pharmacokinetic analysis. Thus, only irinotecan doses of 10 mg/m^2 and greater were included in the pharmacokinetic analysis. The mean peak plasma concen-

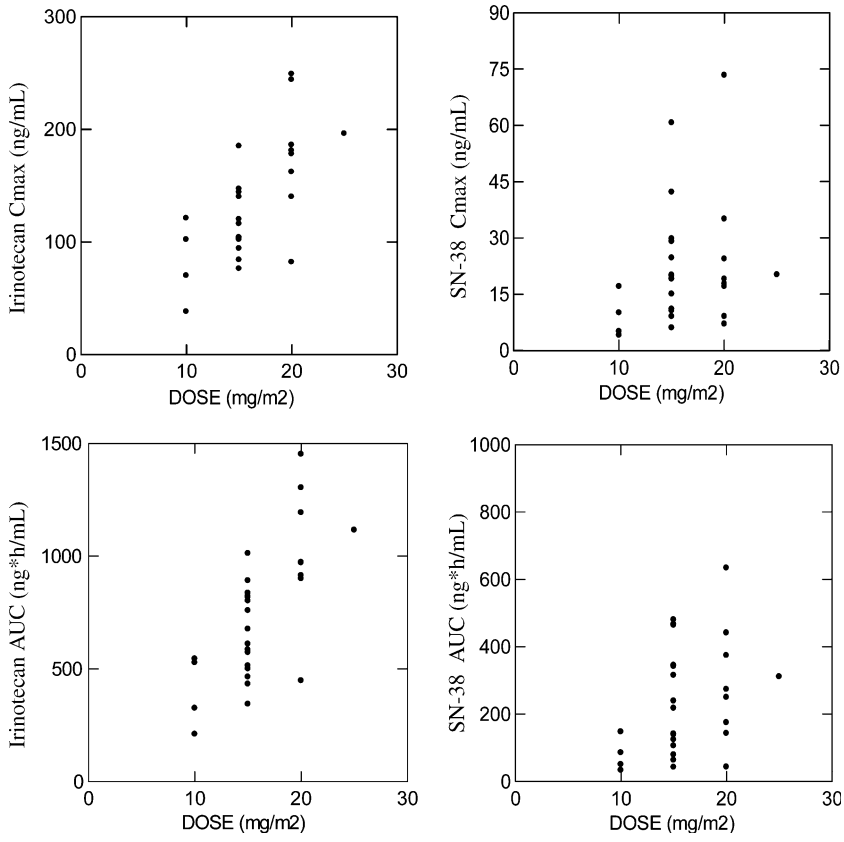

Fig. 4 Irinotecan (*left*) and SN38 (*right*) Cmax (*top*, Ng/ml) and AUC (*bottom*, Ng.h/ml) values as a function of irinotecan dose administered

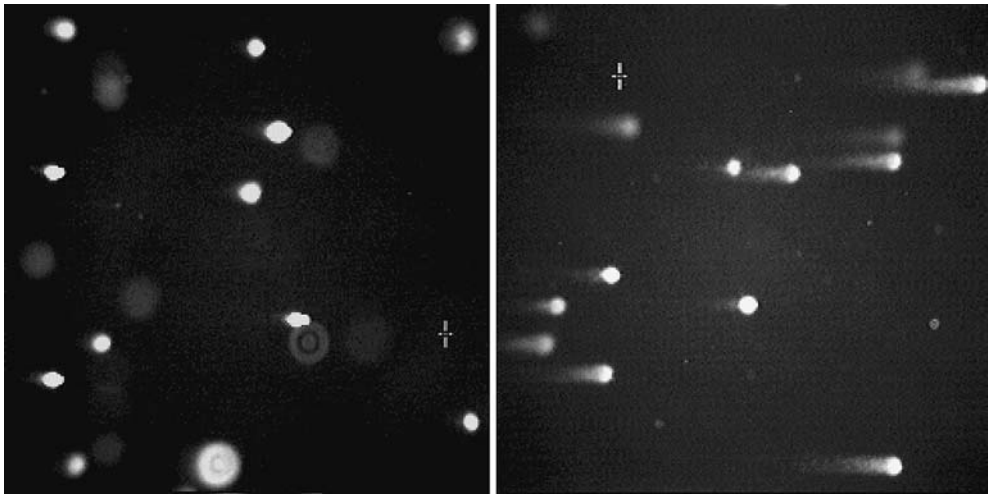

Fig. 5 Example of irinotecan-induced DNA damage assessed by the comet assay in samples taken before the start of the first irinotecan infusion (*left*) and 6 h after the end of the infusion (*right*). DNA damage causes formation of small DNA fragments that migrate more rapidly than intact DNA in an electrical field. The resultant movement of DNA appears as a tail (*comet*) upon visualization with a DNA specific stain (SYBR Green)

trations of SN38 (determined for each dose cohort) varied from 9.0 ng/ml to 25.3 ng/ml, corresponding with 0.023–0.065 μM SN38, and mean steady state concentrations from 3.3–13.3 ng/ml, corresponding with 0.007–0.027 μM SN38. These concentrations are thus well within the range of the SN38 concentrations that resulted in synergistic drug interaction with Ara-C in vitro (0.001 and 0.003 μM, Fig. 1). The final pharmacokinetic model is shown in Fig. 3, with an example of a typical patient fit. The model consists of two compartments (plasma and peripheral) for irinotecan, with linear conversion of irinotecan in plasma to the SN38 metabolite. SN38 is fitted using a one-compartment model, with linear elimination from the body.

Differential equations described each compartment. The concentration of irinotecan in the central (plasma) compartment ($CPT11_c$) was

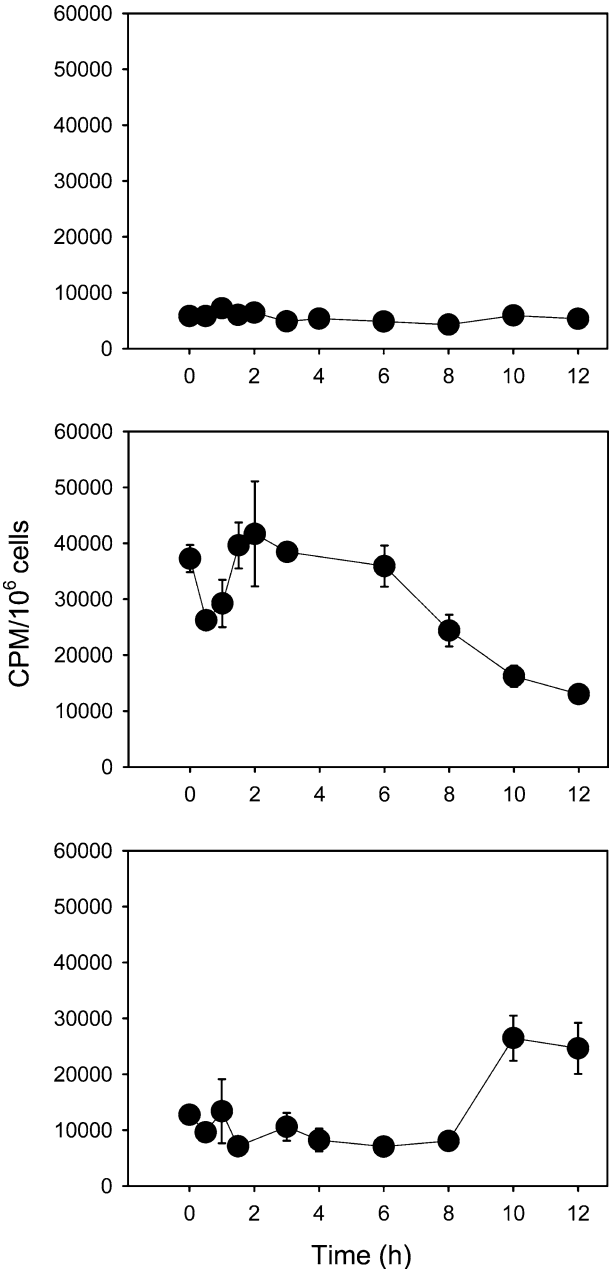

Fig. 6 Representative examples of time kinetic changes in DNA synthesis rates, demonstrating no change (*top*), decrease (*middle*) and increase (*bottom*). Each data point is the mean of triplicate samples, with standard errors

$$\frac{\mathrm{dCPT11_c}}{\mathrm{d}t} = \frac{\mathrm{Dose}}{K_0} + \mathrm{CPT11_p}(K_{21}) - \mathrm{CPT11_c}(K_{12} + K_{13}),$$

where K_0 is the rate of intravenous infusion, $\mathrm{CPT11_p}$ is the concentration of irinotecan in the peripheral compartment, $\mathrm{K_{21}}$ is the rate constant describing movement of irinotecan from the peripheral to the central compartment, K_{12} is the rate constant describing movement of irinotecan from the central to the peripheral compartment, and K_{13} the rate constant for conversion of irinotecan to SN38. The concentration of irinotecan in the peripheral compartment was described by

$$\frac{\mathrm{dCPT11_p}}{\mathrm{d}t} = \mathrm{CPT11_c}(K_{12}) + \mathrm{CPT11_p}(K_{21}),$$

and the concentration of SN38 in the central (plasma) compartment ($\mathrm{SN38_c}$) was described by

$$\frac{\mathrm{dSN38_c}}{\mathrm{d}t} = \mathrm{CPT11_c}(K_{30}) - \mathrm{SN38_c}(K_{30}),$$

where K_{30} is the elimination rate constant for SN38 out of the body.

The above model characterized both irinotecan and SN38 pharmacokinetics well. The median r^2 for irinotecan was 0.92, and for SN38, 0.82. Pharmacokinetic parameter estimates are summarized in Table 2. Both irinotecan and SN38 demonstrated linear pharmacokinetics over the doses used in this study, with AUC and Cmax values both increasing approximately in proportion to dose (Fig. 4). Similarly, clearance did not appear to change continuously with dose, with median irinotecan clearances of 24.8, 23.4, and 20.6 l/h/m^2 and SN38 apparent clearances of 117.1, 62.7, and 73 l/h/m^2 for the 10, 15, and 20 mg/m^2 doses, respectively ($P > 0.4$ for all comparisons of dose by clearance, Kruskal-Wallis). The mean (CV%) half-life of irinotecan was 6.2 (39) h, and the mean (CV%) half-life of SN38 in plasma was 13.4 (57) h.

DNA damage

Irinotecan-induced DNA damage could be evaluated by the comet assay in seven patients, but could not be evaluated in the other seven because of high levels of DNA damage in the untreated control samples. Figure 5 shows representative examples of comet assay results in a pre-treatment sample and a sample following the start of irinotecan infusion. DNA damage was observed as early as 2 h after the start of the irinotecan infusion and was generally heterogeneous, as evidenced by variable 'comet tail' lengths within samples. No obvious correlations were observed between the extent of DNA damage and irinotecan dose, nor time point ($P > 0.05$, Pearson's correlation).

DNA synthesis rates

DNA synthesis rates could be evaluated following the first irinotecan dose in eight patients. Pre-treatment DNA synthesis rates varied widely among patients, ranging from 499 to 20,509 (median, 6,337) CPM/10^6 cells. DNA synthesis rates decreased, increased or remained constant in different patients during the 12-h sampling period (Fig. 6). In general, high pre-treatment DNA synthesis rates were associated with a steady decrease over the subsequent sampling time, while a low initial synthesis rate was associated with no change or a late increase in DNA synthesis rate. Differences between

the minimum and maximum rates within the 12-h sampling periods ranged from 1.6- to 12.6-fold (median, 2.6). No correlations could be established with any of the other pharmacokinetic or pharmacodynamic parameters studied. Five patients were studied during multiple treatment cycles; DNA synthesis rates were similar or higher during subsequent cycles, but were never lower.

Topoisomerase-I activity

Topo-I activity could be evaluated in pretreatment samples from seven patients, while low peripheral blast cell counts or low nuclear protein extraction efficiency precluded analysis in the other seven. Topo-I activity varied greatly among patients, ranging from no activity to 6.25 ng. Of two patients evaluated before multiple treatment cycles, one had similar topo-I activity before the start of each of two treatment cycles, while the other had similar activity before the first, second and third cycles, but markedly decreased activity before the fourth (Fig. 7).

Discussion

We report administration of irinotecan in a 5-day schedule alternating every 12 h with HiDAC in the treatment of AML refractory to or in relapse following HiDAC and anthracycline therapy. Irinotecan was administered first based on preclinical demonstration of synergy using this approach [6, 38] and in vitro drug sensitivity assays confirmed a synergistic interaction between SN38 and Ara-C in a human AML cell line model. DNA damage and DNA synthesis inhibition by irinotecan were observed at doses well below the established MTD, consistent with previous observations that irinotecan has anti-tumor activity at doses well below its MTD [6, 38].

Irinotecan could be safely administered in doses up to 30 mg/m^2 daily on a 5-day schedule alternating with HiDAC. Two CRs were seen among 14 patients, demonstrating activity of this regimen. The two CRs are noteworthy, given that the patients had AML refractory to or in relapse following HiDAC and anthracycline therapy. This response rate was consistent with that in the irinotecan/gemcitabine study. The MTD of irinotecan was not reached in our study, and response rates may be further improved by longer administration schedules and/or higher doses in future studies.

The pharmacokinetics of irinotecan and SN38 were similar to those in previous reports of studies of higher doses in patients with solid tumors [7, 16]. The dispositions of both irinotecan and SN38 were adequately characterized by a single pharmacokinetic model, which was similar to the model proposed by Ma and colleagues for low-dose irinotecan administered to pediatric patients [25]. Both irinotecan and SN38 appeared to follow linear pharmacokinetics, although the range of doses available for this evaluation was narrow. The low rate of irinotecan-related toxicities and the relatively low CR rate may have precluded identification of relationships between these factors and pharmacokinetic and/or pharmacodynamic measurements.

Purported determinants of response studied here did not correlate with outcome. Although pretreatment topo-I activity did not correlate with response to the initial course of therapy, loss of topo-I activity was seen in serial treatment courses in individual patients.

In conclusion, the combination of irinotecan and HiDAC in the treatment of AML has acceptable toxicity and a measurable response rate at irinotecan doses below MTD. In view of the importance of drug scheduling, including both sequence [6, 38] and timing [28] of drug administration, for the antitumor activity of irinotecan

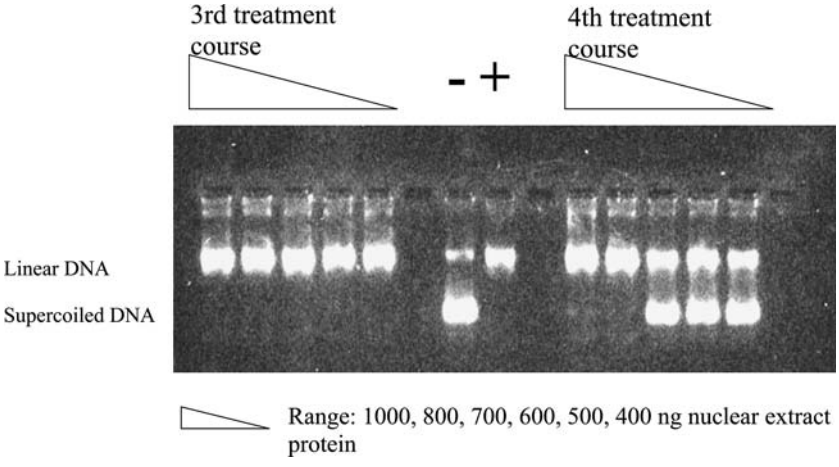

Fig. 7 Change in topoisomerase-I activity over multiple treatment cycles. Topo-I activity decreased markedly before the fourth treatment course. Before the third treatment course, titration down to a level of 400 ng nuclear protein resulted in complete relaxation of supercoiled to linear DNA, while before the fourth treatment course 700 ng nuclear protein did not completely convert the supercoiled DNA. Negative (complete assay with no topoisomerase-I added) and positive (linear DNA only) controls are shown in the *middle two lanes*

and the observed clinical responses in poor prognosis patient groups in this and other studies [2, 29, 31, 39], further clinical studies are warranted to optimize drug doses and administration schedules.

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