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ORIGINAL ARTICLE

**Nancy L. Lewis · Richard Scher · James M. Gallo
Paul F. Engstrom · Christine E. Szarka · Samuel Litwin
Andrea L. Adams · Deborah Kilpatrick · Diane Brady
Louis M. Weiner · Neal J. Meropol**

Phase I and pharmacokinetic study of irinotecan in combination with raltitrexed

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Abstract *Purpose:* To determine the maximum tolerated dose (MTD) of raltitrexed when given with irinotecan and to evaluate the pharmacokinetics of these two agents when given in combination. *Methods:* Patients with advanced solid tumors received irinotecan intravenously over 90 min on days 1 and 8 of each 21-day cycle, with raltitrexed given intravenously over 15 min after irinotecan either on day 1 (cohorts 1–7) or day 2 (cohorts 8–9). The 39 patients received irinotecan and raltitrexed in cohorts of three to six patients at the following dose levels (mg/m^2): 100/1.0, 100/1.5, 100/2.0, 100/2.5, 100/3.0, 100/3.5, 125/3.0, 75/3.0, 100/3.0. Pharmacologic monitoring of irinotecan and raltitrexed was carried out during cycle 1. *Results:* A total of 39 patients received irinotecan and raltitrexed in cohorts of three to six patients at nine dose levels. The MTD with dosing of irinotecan on days 1 and 8 and raltitrexed on day 1 was 100 mg/m^2 and 3.0 mg/m^2, respectively, every 21 days, with dose-limiting toxicities (DLTs) of fatigue, neutropenia and diarrhea. When raltitrexed was administered 24 h after irinotecan, these doses exceeded the MTD. No pharmacologic interactions were observed between these agents, and no correlations between pharmacokinetic parameters and toxicity were noted. Of 26 patients with colorectal cancer, 6 had objective partial responses (23%). Four of these patients had previously received a 5-FU-based regimen, two for metastatic disease. *Conclusions:* Irinotecan can be safely administered with raltitrexed on a day-1 and day-8 schedule at 100 mg/m^2 and 3.0 mg/m^2, respectively, every 21 days. When raltitrexed was given on day 2, these doses were not tolerated, necessitating a dose reduction of the irinotecan to 75 mg/m^2. This regimen possesses clinical activity in patients with colorectal cancer.

Keywords Irinotecan · Raltitrexed · Phase I clinical trial

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N.L. Lewis (✉) · R. Scher · J.M. Gallo · C.E. Szarka
A.L. Adams · D. Kilpatrick · D. Brady · L.M. Weiner
N.J. Meropol
Division of Medical Science, Fox Chase Cancer Center,
7701 Burholme Avenue, Philadelphia, PA 19111, USA
E-mail: n_lewis@fccc.edu
Tel.: +1-215-2141676
Fax: +1-215-7283639

P.F. Engstrom · S. Litwin · N.J. Meropol
Division of Population Science,
Fox Chase Cancer Center, Philadelphia, PA 19111, USA

Introduction

Irinotecan is a semisynthetic derivative of the plant alkaloid camptothecin with a broad spectrum of antitumor activity [5, 16, 20, 28, 30, 51]. In vivo, it is converted by the hepatic enzyme carboxylesterase to SN-38, a metabolite that contributes significantly to irinotecan's antitumor activity [26, 27]. Like other camptothecins, irinotecan exerts antitumor activity by interfering with DNA topoisomerase I.

Irinotecan has significant antitumor activity against colorectal cancer, small-cell lung cancer, non-small-cell lung cancer, breast cancer, gastric cancer, and gynecologic malignancies [19, 31, 45, 48, 49]. Irinotecan was the first new drug in 40 years to be approved for use in the United States for metastatic colorectal carcinoma. The single-agent response rate with irinotecan is approximately 15% to 30%, with higher response rates generally observed in previously untreated patients [38]. More recently, irinotecan has been incorporated into regimens with 5-fluorouracil (5-FU) and leucovorin for first-line treatment of metastatic colorectal cancer [15, 42]. Despite a 3.5% treatment-related mortality reported with this regimen in subsequent studies, it remains one standard of care with appropriate clinical monitoring [39]. Data from clinical trials evaluating its efficacy in the adjuvant setting have yet to mature. The activity of irinotecan in combination with other agents continues to be evaluated in a variety of malignancies.

The major side effects of irinotecan include nausea, vomiting, myelosuppression with dose-dependent neutropenia, and diarrhea [3]. In three clinical trials of 304 patients with metastatic colorectal cancer, the incidence of grade III/IV diarrhea was approximately 30%. Aggressive treatment with loperamide at the first sign of diarrhea may reduce the incidence of severe toxicity [1, 38, 41].

Raltitrexed is a quinazoline folate analogue that was developed as a direct and specific inhibitor of thymidylate synthase (TS) [24], with greater in vitro potency against this target than 5-FU. TS is a key enzyme in the de novo synthesis of thymidine triphosphate (TTP), the only nucleotide specifically required for DNA synthesis. The substrate for TS is deoxyuridine monophosphate (dUMP), which forms thymidylate (TMP) through a reductive methylation. Raltitrexed inhibits TS by inhibiting the folate binding site, rather than the binding site for dUMP as in the case of 5-FU. Raltitrexed rapidly enters cells using the reduced folate carrier, and is then polyglutamated by folylpolyglutamate synthetase. Polyglutamation enables raltitrexed to concentrate within cells and these polyglutamates are significantly more potent TS inhibitors than the monoglutamated parent drug [25]. In addition, the cellular retention of the polyglutamated form allows antitumor activity by bolus administration, and has facilitated the adoption of a 3-week bolus schedule used in previous trials.

Phase I trials of raltitrexed have demonstrated dose-related gastrointestinal toxicities, myelosuppression and liver enzyme abnormalities [22]. Phase II trials have demonstrated an activity profile similar to that of 5-FU. Three phase III trials in untreated metastatic colorectal carcinoma patients compared raltitrexed 3.0 mg/m^2 every 3 weeks to 5-FU plus leucovorin [11, 13, 35, 44]. In these trials, grade III/IV toxicities included 6–18% of patients experiencing leukopenia, 10% with diarrhea, and 2–3% experiencing mucositis. Reversible grade III/IV elevation of transaminases occurred in 7–13% of patients. Objective responses between the treatments were similar in each study, and were in the ranges 14–19.8% for raltitrexed and 12.7–18% for 5-FU/leucovorin. Median overall survival was lower in the raltitrexed arms (9.7–10.9 months) when compared to standard 5-FU/LV (12.3–12.7 months).

Various irinotecan treatment schedules have been evaluated, with the most common regimens being once every 3 weeks and weekly [2, 14, 37, 40, 47]. In this trial, irinotecan was administered 2 of every 3 weeks to avoid treatment on day 15, when peak gastrointestinal toxicity of raltitrexed is expected to occur [9].

The activities of irinotecan and raltitrexed have been demonstrated separately in a variety of tumors. Both drugs have similar activity in colorectal cancer, with raltitrexed appearing to be better tolerated than 5-FU, and irinotecan is active in 5-FU-refractory colorectal carcinoma. The lack of complete cross-resistance between irinotecan and 5-FU suggests that a similar effect may be seen between irinotecan and raltitrexed. Thus, a combination of these two agents may lead to significant activity with an acceptable toxicity profile. This phase I trial was undertaken to determine the maximum tolerated dose (MTD) of raltitrexed in combination with irinotecan. In addition, preclinical evidence supports synergy between these agents [4]. This synergy was schedule-dependent and maximal when SN-38 was administered 24 h before raltitrexed. Thus, a schedule employing a 24-h interval between irinotecan and raltitrexed was developed.

Patients and methods

Eligible patients had histologic proof of a malignant solid tumor and had either failed conventional chemotherapy or had a disease for which no standard therapy exists. Patients with recurrent or metastatic gastrointestinal adenocarcinoma with no prior treatment were eligible. A maximum of one prior adjuvant and one prior chemotherapy regimen for metastatic disease were allowed. Patients could not have had prior chemotherapy or radiotherapy within 4 weeks of entry into the study and must have recovered from all toxicities from prior treatments. Prior radiotherapy was limited to 25% or less of the bone marrow-bearing skeleton. Patients could not have received prior irinotecan or raltitrexed therapy. Patients were at least 18 years of age, with a life expectancy of at least 3 months and an ECOG performance status of 0 or 1. Eligibility criteria for bone marrow function included a WBC count of 3.5×10^6/l and a platelet count of 100×10^9/l. A serum creatinine level of less than or equal to 1.8 mg/dl or a creatinine clearance of greater than or equal to 60 ml/min was required. Serum bilirubin levels had to be less than or equal to 1.8 mg/dl. Liver enzyme levels of less than three times the upper limit of normal (ULN) and alkaline phosphatase levels of less than three times the ULN (unless due to metastatic disease, in which case, less than five times ULN) were required.

Pregnant or lactating patients, and patients with other serious medical illnesses, central nervous system metastases or clinically significant ascites or pleural effusions were excluded. Written informed consent was obtained from all patients prior to enrollment. The study was approved by the Fox Chase Cancer Center Institutional Review Board and the Cancer Therapy Evaluation Program of the National Cancer Institute.

Treatment

Irinotecan was manufactured by Pharmacia Upjohn and available in 5-ml vials containing 100 mg. Raltitrexed was formulated as a lyophilized powder at 2 mg/vial. Both drugs were provided by the National Cancer Institute, Division of Cancer Treatment and Diagnosis, Bethesda, Md. Irinotecan was administered intravenously (i.v.) over 90 min. Raltitrexed was administered as an i.v. infusion over 15 min. Initially raltitrexed was given immediately after irinotecan on day 1 for cohorts 1–7. After preclinical evidence supporting schedule-dependent synergy became available [4], subsequent cohorts (8–10) received raltitrexed on day 2, 24 h after irinotecan administration.

Cohorts of three to six patients were treated at the following irinotecan/raltitrexed dose levels (mg/m^2): 100/1.0, 100/1.5, 100/2.0, 100/2.5, 100/3.0, 100/3.5, and 125/3.0. Three patients were treated initially at each dose level. If none of the patients experienced a dose-limiting toxicity (DLT), then the dose was escalated to the next higher level for three additional patients. If one of the three patients experienced a DLT, three additional patients were accrued at the same dose. If two or more of the six total patients in a given dose level experienced DLT, then the MTD was exceeded and additional patients were treated at the next lower dose level. Therefore, the MTD was defined as the dose level below that at

which two or more of six patients experienced DLT. The study was amended to include additional cohorts treated on the modified schedule at the following irinotecan/raltitrexed dose levels (mg/m^2): 75/3.0, and 100/3.0. DLTs were defined as neutropenic fever, nonhematologic toxicity of grade 3–4 (except nausea or vomiting), or hematologic toxicity of grade 4 (NCI-CTC, version 1.0).

Prior to beginning treatment, patients underwent a complete medical history, physical examination and tumor measurements if measurable disease was present. The pretreatment evaluation also included a complete blood count, a biochemical screening profile, carcinoembryonic antigen or other tumor marker, a chest roentgenogram and electrocardiogram. On a weekly basis during the study, CBC, biochemical profiles and a toxicity assessment were performed. Tumor measurements were obtained every 6 weeks. Response was defined according to World Health Organization criteria [33].

Dose modifications for toxicity were mandated as follows: for grade 3 hematologic toxicity, the raltitrexed dose was decreased by 25% and for grade 4 hematologic toxicity, irinotecan was decreased by 25% and raltitrexed by 50%. For day-8 irinotecan dosing, grade 2 hematologic toxicity resulted in a 25% dose reduction and the day-8 dose was omitted for grade 3 or 4. For grade 3 nonhematologic toxicity, raltitrexed was decreased by 25%, and for grade 4 nonhematologic toxicity, the irinotecan was decreased by 25% and raltitrexed by 50%.

Pharmacologic monitoring

For irinotecan and metabolite analyses, serial blood samples were collected into heparinized tubes predose, at 30, 60 and 90 min (end of infusion), and also at postinfusion times of 5, 15, 30, 45 and 60 min, and 2, 4, 8, 12, 18 and 24 h on day 1 of the first cycle only. Urine was collected over two 12-h periods from 0 to 12 h and from 12 to 24 h. For raltitrexed, blood samples were collected predose, at 15 min (end of infusion), and at postinfusion times of 5, 15, 30, 45, 60 and 75 min, and 1 h 45 min, 3 h 45 min, 7 h 45 min, 11 h 45 min and 23 h 45 min in cycle 1. Plasma was separated from all blood samples by centrifugation, and stored in polypropylene tubes at –80°C until analysis by HPLC. The total volume of urine was measured and approximately 20 ml was transferred to polypropylene tubes and stored at –80°C until analysis by HPLC.

Plasma samples for irinotecan and SN-38 were analyzed by HPLC using a protein precipitation method based on previously published methods [36, 50]. Patient plasma samples were prepared by the addition of 20 µl internal standard solution (1.25 µg/ml camptothecin) and 20 µl methanol to 100 µl plasma. Plasma standards were prepared by adding 10 µl of each standard (irinotecan and SN-38) and 20 µl of the above internal standard solution to 100 µl blank plasma. Proteins were precipitated by the addition of 100 µl cold methanol and 100 µl 7% perchloric acid (PCA). Following centrifugation, aliquots of the resultant supernatant were loaded onto the HPLC system.

Plasma concentrations of SN-38 glucuronide (SN-38G) were also determined using β-glucuronidase to hydrolyze SN-38G to SN-38 and repeating the SN-38 analyses. Internal standard solution (20 µl) was added to the plasma samples (100 µl) as above followed by the addition of 20 µl of an aqueous solution of β-glucuronidase (200 U). Samples were vortexed and incubated for 2 h at 37°C, and then treated by the protein precipitation method as described above.

Aliquots (30 µl) of urine samples were prepared by the addition of 40 µl internal standard solution (1.25 µg/ml camptothecin) and 60 µl methanol. Urine standards consisted of 75 µl urine, 40 µl internal standard solution and 30 µl of each standard, CPT-11 and SN-38. Urine standards and samples were then diluted with 200 µl PBS and 200 µl 7% PCA under mixing. The HPLC conditions were analogous to those used for plasma samples.

Aliquots of plasma and urine extracts (75 µl) were loaded onto an autosampler tray and processed on a Hewlett-Packard 1050 Series HPLC system fitted with both a Zorbax SB-C8, analytical column (5 µm, 150×4.6 mm) and guard column (5 µm, 12.5×4.6 mm). Solutes (irinotecan and SN-38) were eluted by an isocratic mobile phase (acetonitrile/0.025 *M* triethylamine buffer, 3:7 v/v) pH 4.2 (adjusted with H_3PO_4) at a flow rate of 1.0 ml/min and a total run time of 9 min. All solutes were detected by fluorescence at the following programmed excitation/emission wavelengths: irinotecan and camptothecin at 372/425 nm, SN-38 at 372/535 nm.

All methods measured total concentrations (lactone plus hydroxy acid forms) of irinotecan, SN-38, and SN-38G. The methods afforded a lower limit of quantitation of about 4 n*M* for irinotecan and 6 n*M* for SN-38 and an accuracy and precision of 10% or less for both drugs.

Raltitrexed plasma samples were prepared for analysis by the addition of 10 µl methanol to 100 µl plasma. Calibration standards were prepared by adding 10 µl of various known amounts of raltitrexed methanolic solutions to 100 µl blank plasma. Plasma proteins were precipitated by the addition of 200 µl cold methanol under mixing conditions for 60 s. Samples were then centrifuged for 5 min at 15,000 *g* and 25-µl aliquots of the supernatant were placed onto the HPLC system. The HPLC system (Hewlett-Packard 1050 Series) contained a C6 analytical column (Spherisorb C6, 5 µm, 150×4.6 mm) and guard column (5 µm, 10×4.6 mm). Raltitrexed was eluted with an isocratic mobile phase (40% methanol/0.175 *M* acetic acid) at a flow rate of 1.5 ml/min with a run time of 10 min and UV detection at 346 nm. The assay sensitivity was 25 ng/ml with accuracy and precision values of 10% or less.

Noncompartmental analyses were used to determine all pharmacokinetic parameters for irinotecan, SN-38, SN-38G and raltitrexed using the WinNonlin program (WinNonlin version 3.0; Pharsight Corporation, Mountain View, Calif.). The observed plasma concentration-time data for each species was viewed on semilog coordinates to graphically estimate the duration of the terminal elimination phase that was used in a linear regression algorithm of the natural log of concentration versus time values. Subsequently, a linear-log trapezoidal numerical integration method was used to calculate the area under the drug concentration-time curve (AUC) from time zero to infinity, and the area under the first moment concentration-time curve (AUMC). The irinotecan and raltitrexed AUC and AUMC values for each patient were used to calculate individual total clearance (CL) and the volume of distribution at steady-state (Vss) values by standard formulas [21]. The Cmax and terminal elimination half-life ($t_{1/2}$) values were also measured. For SN-38 and SN-38G, the AUC, $t_{1/2}$, and Cmax values were determined for each patient. Renal clearances of irinotecan and SN-38 were calculated from the total amount of each compound in urine divided by their respective AUC values. Finally, the biliary index was calculated for each patient as $AUC_{irinotecan} \times AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ as previously described [23].

Relationships between pharmacokinetic parameters and measures of toxicity were investigated. Specifically, a sigmoid Emax model was used to evaluate relationships between different AUC indices and maximum diarrhea grade as well as the percentage decrease in ANC at the nadir. In addition, the relationship between the biliary index and grade of diarrhea was examined. CL and Vss values of both irinotecan and raltitrexed were analyzed for dose-independence using an analysis of variance with P values < 0.05 indicating statistical significance.

Results

Enrolled onto this phase I study were 39 patients. Patient characteristics are shown in Table 1. There were 20 male and 19 female patients, with 56% having a performance status of 0. Their median age was 62 years (range 30 to 80 years). Of the 39 patients, 94% had metastatic tumors of the gastrointestinal tract, 80% had had prior therapy, and 20% had had more than one regimen.

Table 1. Patient characteristics

No. of patients	
Total	39
Male/female	20/19
Age (years)	
Median	62
Mean	60.7
Range	30–80
Performance status	
0	22
1	17
Prior therapy	
None	8
Adjuvant only	8
Adjuvant and metastatic	8
Metastatic only	15
Tumor type	
Colorectal	26
Pancreatic	3
Cholangiocarcinoma	3
Other	7

Patients were treated at nine different dose levels, as listed in Table 2. For the first seven cohorts, raltitrexed was administered immediately following irinotecan on day 1. Preclinical evidence supporting a schedule-dependent synergy between these agents was initially reported while this trial was ongoing [4], and a protocol amendment permitted subsequent cohorts to receive raltitrexed on day 2, 24 h after irinotecan administration. With the schedule change, the dose of irinotecan was initially reduced to 75 mg/m^2 and subsequently escalated to 100 mg/m^2.

A total of 229 cycles were administered. The median number of cycles per patient was 5 (range 1 to 17). Although one patient did not receive the day-8 irinotecan dose in cycle 1 due to neutropenic fever, all 39 patients were considered evaluable for toxicity. Of the remaining 38 patients, one patient in cohort 7 had a 25% dose reduction of day-8 irinotecan for an absolute neutrophil count of 1400/μl and one patient in cohort 8 had a 1-week delay in receiving day-8 irinotecan in order to undergo a transfusion of packed red blood cells for a hemoglobin of 7.8 g/dl. All first cycle toxicities by dose level are listed in Tables 3 and 4. The most common nonhematologic toxicities were nausea and vomiting (64%), diarrhea (69%) and fatigue (84%). Hematologic toxicities included 29 patients (74%) with grade 1 or 2 anemia. Only three patients (7.7%) experienced grade 3 anemia, and one patient had grade 3 thrombocytopenia. Neutropenia was the most prevalent hematologic toxicity and was mild (grade 1 or 2) in 25.6% of patients, but dose-limiting in five patients (12.8%) with two patients experiencing neutropenic fever.

Toxicities requiring dose modification (delay, reduction or omission) in subsequent cycles occurred in 16 of the 39 patients. The reasons for dose modification and the number of patients experiencing these toxicities are outlined in Table 5. The two patients under the miscel-

Table 2. Patient cohorts and DLTs

Dose level	No. of patients	Irinotecan (mg/m^2)	Raltitrexed (mg/m^2)	DLTs	
					No.
1	3	100	1.0	Grade 3 diarrhea	1
2	6	100	1.5		
3	3	100	2.0		
4	3	100	2.5		
5	3	100	3.0		
6	6	100	3.5	Grade 3 nausea, vomiting, fatigue; grade 4 neutropenia	2
7	6	125	3.0	Grade 3 nausea; grade 3/4 fatigue; grade 4 diarrhea, vomiting, neutropenia	2
8[a]	6	75	3.0	Grade 4 neutropenia	1
9[a]	3	100	3.0	Grade 3 fatigue; grade 3/4 diarrhea; grade 4 neutropenia	2

[a]Irinotecan on day 1, day 8, and raltitrexed on day 2

Table 3. Incidence and grade of nonhematologic toxicities for cycle 1

Dose level	No. of patients	Diarrhea				Nausea				Vomiting				Fatigue			
		1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	3	3	–	–	–	3	–	–	–	2	–	–	–	2	1	–	–
2	6	2	–	1	–	4	–	–	–	–	1	–	–	4	–	–	–
3	3	2	–	–	–	2	–	–	–	1	–	–	–	3	–	–	–
4	3	1	2	–	–	1	–	–	–	–	–	–	–	2	–	–	–
5	3	1	–	–	–	1	–	–	–	1	–	–	–	2	1	–	–
6	6	1	2	–	–	3	–	1	–	–	1	1	–	3	–	2	–
7	6	2	1	–	2	3	–	1	–	1	2	–	1	1	1	1	1
8	6	5	–	–	–	3	1	–	–	–	–	–	–	6	–	–	–
9	3	–	–	1	1	1	–	–	–	1	–	–	–	1	1	1	–

Table 4. Incidence and grade of hematologic toxicities for cycle 1

Dose level	No. of patients	Anemia				Neutropenia				Thrombocytopenia			
		1	2	3	4	1	2	3	4	1	2	3	4
1	3	1	1	1	–	1	–	–	–	–	–	–	–
2	6	6	–	–	–	1	–	–	–	1	–	–	–
3	3	–	3	–	–	1	–	–	–	–	–	–	–
4	3	2	–	–	–	–	2	–	–	–	–	–	–
5	3	3	–	–	–	–	–	–	–	–	–	–	–
6	6	1	4	1	–	2	–	1	1	1	–	1	–
7	6	2	2	–	–	–	1	–	1	1	–	–	–
8	6	2	1	1	–	1	–	–	1	–	–	–	–
9	3	–	1	–	–	1	–	–	–	–	–	–	–

Table 5. Reasons for treatment-related dose modifications and delays in subsequent cycles

Reason	No. of patients	No. of dose delays	No. of dose reductions	No. of doses held
Neutropenia	6	4	15	1
Diarrhea	6	4	9	0
Fatigue	3	1	3	1
Nausea/vomiting	3	2	3	1
Neutropenic fever	2	1	3	0
Anemia	2	2	0	0
Miscellaneous	2	2	0	0

laneous category had a dose delay for an ongoing urinary tract infection and in order to work up a cough. Of the 229 cycles administered (two doses per cycle), only 4 doses were held, 13 delayed and 26 initially reduced due to treatment-related toxicity.

DLTs are listed in Table 2. In the first seven cohorts treated on the initial day-1 and day-8 dosing schedule, there were five DLTs. One patient in cohort 2 had grade 3 diarrhea, necessitating the enrollment of an additional three patients at this level. This patient's stool specimen was positive for *Clostridium difficile* toxin. No further DLTs were reported until dose level 6 (irinotecan 100 mg/m^2 and raltitrexed 3.5 mg/m^2), with two of six patients experiencing neutropenic fever and grade 3 fatigue. One of these patients died while hospitalized for toxicity after completing one cycle. This patient, diagnosed with stage IV cholangiocarcinoma, was admitted for severe diarrhea and dehydration as well as grade 4 neutropenia. He died after sudden-onset dyspnea and hypoxia, which was consistent with a pulmonary embolism; a postmortem examination was not performed. Two additional DLTs were reported in cohort 7. Thus the MTD with day-1 dosing was 100/3.0 mg/m^2.

When raltitrexed 3.0 mg/m^2 was administered 24 h after irinotecan, the irinotecan dose was initially decreased to 75 mg/m^2, and no DLTs were noted in three patients. However, when the irinotecan was dose escalated to 100 mg/m^2, two of the three patients had DLTs. None of the three patients treated at these doses on the initial dosing schedule had severe toxicity. Although the protocol did not require an expansion of cohorts at the MTD, an amendment was made to add three additional patients at dose level 8 (irinotecan 75 mg/m^2 and raltitrexed 3.0 mg/m^2) to better characterize toxicity when raltitrexed was administered 24 h after irinotecan. One patient on this additional cohort experienced grade 4 neutropenia. Therefore, when using the modified regimen with raltitrexed administered on day 2, the MTD is irinotecan 75 mg/m^2 and raltitrexed 3.0 mg/m^2.

Antitumor activity

Of 35 patients with measurable disease, 16 had radiographic stabilization of their disease, 8 experienced a partial response, as defined by a 50% or greater decrease in the sum of the products of two perpendicular diameters of all measured lesions, and 11 progressed. All patients responding had the response confirmed at 4 weeks with the exception of one patient who declined further follow up. Of the four patients not evaluable for response, three were taken off study secondary to DLTs prior to evaluation. One patient had peritoneal carcinomatosis seen intraoperatively, with no measurable radiographic disease.

Of the eight patients demonstrating a partial response, six were diagnosed with colon cancer, one with pancreatic cancer, and one with cholangiocarcinoma. Five of the eight responders were treated on the modified schedule, and six of the responders had received prior therapy with 5-FU and/or gemcitabine.

Pharmacokinetics

Complete plasma irinotecan, SN-38 and SN-38G concentration-time profiles were obtained in 30 patients at the 100-mg/m^2 and 125-mg/m^2 dose levels. Pharmacokinetic parameters are listed in Table 6. Both total clearance (mean 19.90 ± 5.50 l/h per m^2) and volume of distribution at steady-state (mean 130.89 ± 40.0 l/m^2) for irinotecan were dose-independent, as expected given the relatively narrow dose range and consistent with the findings of previous investigations [8]. Renal clearance of irinotecan averaged 3.29 ± 1.25 l/h per m^2, representing about 16% of drug elimination. Both SN-38 and SN-38G pharmacokinetic properties were consistent with those previously reported with higher SN-38G

Table 6. Pharmacokinetic parameters of irinotecan, SN-38 and SN-38G (*CL* clearance, *Vss* volume at steady state, *AUC* area under the concentration-time curve, $t_{1/2}$ half-life, *Cmax* maximum concentration, *Tmax* maximum exposure, *NA* not available)

Parameter	Irinotecan (mg/m^2)		SN-38 (mg/m^2)		SN-38G (mg/m^2)	
	100	125	100	125	100	125
CL (l/h/m^2)	20.3 ± 5.8	18.5 ± 4.1	NA	NA	NA	NA
Vss (l/m^2)	135.1 ± 41.6	113.9 ± 29.9	NA	NA	NA	NA
AUC(μ*M*·h)	9.2 ± 2.8	11.0 ± 1.6	0.72 ± 0.49	0.45 ± 0.17	2.84 ± 2.33	3.55 ± 1.42
$t_{1/2}$ (h)	7.2 ± 1.3	7.4 ± 1.6	9.6 ± 15.5	6.8 ± 15.8	17.7 ± 6.9	15.5 ± 9.1
Cmax (μ*M*)	2.0 ± 0.52	2.7 ± 0.47	0.06 ± 0.02	0.08 ± 0.03	0.21 ± 0.13	0.25 ± 0.10
Tmax (h)	1.31 ± 0.32	1.30 ± 0.29	1.56 ± 0.47	1.29 ± 0.65	2.84 ± 0.51	3.55 ± 0.56

plasma concentrations (mean Cmax 0.22 ± 0.13 μ*M*) being observed than of the active metabolite SN-38 (mean Cmax 0.060 ± 0.027 μ*M*).

Analysis of pharmacokinetic-pharmacodynamic relationships between biliary index, drug exposure indices (i.e. AUC values of irinotecan, SN-38 and raltitrexed), and grade of diarrhea did not reveal any significant correlations. However, based on the relatively narrow dose and AUC ranges, it was not unexpected that an Emax model could not be delineated.

Table 7 provides the mean pharmacokinetic parameters for raltitrexed based on the serial plasma concentration-time data from 36 patients. The raltitrexed mean clearance of 3.03 ± 2.06 l/h per m^2 is similar to values previously reported [10]. Since raltitrexed undergoes primary hepatic elimination and its clearance is considerably less than liver blood flow, it can be classified as a low hepatic clearance drug whose clearance rate is dependent on plasma protein binding and hepatic enzymatic activity. Raltitrexed has a small volume of distribution (mean Vss 14.34 ± 8.67 l/m^2) relative to total body water, consistent with minimal tissue uptake and binding. The elimination half-life of about 2.5 h is sufficiently lower than other terminal elimination phase half-lives [32]. This can most likely be attributed to the more limited blood sampling schedule (last sample at 24 h), whereas depiction of long terminal half-lives are associated with measurement of raltitrexed plasma concentrations for up to 500 h after dosing [10]. The elimination half-life of 2.5 h observed in this study coincides with a secondary distribution phase half-life. Nonetheless, the potential error this might introduce in the estimation of total clearance and volume of distribution seems minimal as the terminal phase raltitrexed concentrations were low and the fractional contribution of this phase to the total areas (AUC and AUMC) was low. Both total clearance and volume of distribution values indicated dose-independence, although there was a trend towards a reduced clearance at the high dose levels.

Discussion

Both irinotecan and raltitrexed have been shown to have activity in colorectal cancer. Furthermore, preclinical models suggest synergy between these two agents. Given their different mechanisms of cytotoxic action they are not likely to have cross-resistance. Thus the combination of raltitrexed and irinotecan has the potential for similar activity to that of the combination of irinotecan and 5-FU with an improved therapeutic index. The data presented demonstrate that raltitrexed and irinotecan may be safely administered at doses at or near their respective MTDs as single agents. Conflicting data exist regarding the schedule-dependent synergy in preclinical models. In colorectal cancer cell lines, Matsui et al. have shown that pretreatment with raltitrexed increases the amount of DNA-bound topoisomerase I up to 4-fold and the DNA-damaging capabilities of karenitecin, another camptothecin derivative, by up to 15-fold [32]. Work by Aschele et al. suggests that SN-38 followed by raltitrexed produces a better synergistic cell kill than the reverse sequence [4]. More recent studies in animal models have shown markedly improved response rates when 5-FU [6, 34] or capecitabine [7] is administered 24 h after irinotecan. In vivo cell cycle kinetics have demonstrated that 24 h after the administration of irinotecan, a synchronous cell population is traversing the S phase of the cell cycle, actively synthesizing DNA and thus rendering these cells highly susceptible to S phase-specific drugs, such as TS inhibitors [34]. In this study, when irinotecan was administered 24 h prior to raltitrexed, a lower MTD was noted. It is notable that five of the eight responders were treated on this schedule.

Table 7. Pharmacokinetic parameters of raltitrexed (*CL* clearance, *Vss* volume at steady state, *AUC* area under the concentration-time curve, $t_{1/2}$ half-life, *Cmax* maximum concentration)

Parameter	Raltitrexed dose level (mg/m^2)					
	1.0	1.5	2.0	2.5	3.0	3.5
CL (l/h/m^2)	4.95 ± 4.70	4.74 ± 2.92	2.74 ± 1.76	1.94 ± 0.48	2.45 ± 0.91	2.47 ± 0.86
Vss (l/m^2)	11.63 ± 9.1	11.15 ± 4.97	14.64 ± 6.07	15.65 ± 10.89	14.46 ± 6.81	17.82 ± 15.63
AUC (μg·h/l)	1091.8 ± 1622.0	459.5 ± 363.4	922.6 ± 459.0	1352.9 ± 573.1	1394.9 ± 573.1	1572.6 ± 540.2
$t_{1/2}$ (h)	0.92 ± 1.16	1.23 ± 1.95	3.09 ± 7.48	5.66 ± 3.03	4.32 ± 2.96	4.64 ± 2.14
Cmax (μg/l)	186.3 ± 45.3	342.2 ± 146.8	922.6 ± 459	1352.9 ± 371.1	1394.9 ± 573.1	1572.6 ± 540.2

In this study, pharmacokinetic parameters for irinotecan, SN-38, and raltitrexed were similar to those previously reported [8, 10] and thus the possibility of a pharmacokinetic-mediated drug interaction is unlikely. There were no significant pharmacodynamic correlations found between irinotecan's biliary index, or other pharmacokinetic measures and the grade of diarrhea. It is plausible that our relatively small sample size with a low incidence of severe diarrhea rendered identification of pharmacodynamic relationships unlikely.

As expected, DLTs seen in this study were neutropenia, diarrhea and fatigue. One patient died with sudden-onset dyspnea and hypoxia, consistent with a thromboembolic event. Raltitrexed has recently been reported to increase platelet counts [52], although this was not the case in this patient.

Of the 35 patients evaluable for response, 8 (22.8%) demonstrated a partial response and 16 (45.7%) experienced stabilization of disease. Of note, five of the eight responders were treated on the modified schedule, with irinotecan administered on days 1 and 8, and raltitrexed on day 2. Six of the responders had been previously treated.

The activity we observed is consistent with other reports of the combination of raltitrexed and irinotecan in gastrointestinal cancers. Ford et al. conducted a phase I trial of irinotecan and raltitrexed in 33 patients with colorectal and gastroesophageal cancers. The recommended dose was irinotecan 350 mg/m^2 i.v. over 30 min followed by raltitrexed 3 mg/m^2 i.v. over 15 min every 3 weeks. Of 30 evaluable patients, 6 (20%) had a partial response and 13 (43%) had stable disease [18]. Similarly, preliminary data from two additional studies have been recently reported. Colucci et al. conducted a phase I/II study with these two agents given biweekly. Irinotecan was administered in escalating doses from 150 to 240 mg/m^2 followed by raltitrexed from 2 to 2.5 mg/m^2. The recommended phase II dose was 210 mg/m^2 and 2.5 mg/m^2, respectively. Of the 17 evaluable patients with advanced colorectal cancer, 6 (35%) had a partial response and 6 (35%) had stabilization of disease [12]. Escudero et al. reported similar preliminary results of an ongoing phase II trial. Irinotecan was administered over 90 min followed 1 h later by raltitrexed as a 15-min infusion every 21 days. Thus far, 49 patients have been enrolled and 32 are evaluable for response. Of these, 13 patients (40.6%) have had a partial response with an additional 12 patients (37.5%) demonstrating stable disease [17].

This collective experience with the combination of raltitrexed and irinotecan suggests that these two agents can be administered together in a variety of regimens, with DLTs including diarrhea and neutropenia. The preclinical data of schedule-dependent synergy suggests that a day 1–2 schedule such as the one we present here should be explored in phase II trials. Although lower doses are required when irinotecan is administered prior to raltitrexed, the response rates, even in those patients who have previously failed 5-FU-based regimens, is encouraging.

Recent evidence suggests that polymorphisms in the methylene-tetrahydrofolate reductase (MTHFR) gene may predict toxicity with the combination of raltitrexed and irinotecan [46]. Thus, treatment selection based on pharmacogenetic factors may be plausible. Given the recently reported potential for fatal toxicity with the combination of 5-FU and irinotecan [29, 39, 43], raltitrexed and irinotecan may provide similar targeting of TS and topoisomerase I with a more favorable toxicity profile than that seen with 5-FU and irinotecan.

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