Cancer Chemother Pharmacol (1994) 34 (Suppl): S 46–S 52

Cancer Chemotherapy and Pharmacology

Clinical, pharmacokinetic and biological studies of topotecan

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Abstract. The topoisomerase I inhibtor topotecan is a potent water-soluble camptothecin derivative with activity in a wide variety of preclinical models. Topotecan exhibits schedule dependency in vivo, with the greatest activity being observed on repeated dose schedules. On the basis of the initial clinical studies that showed a short plasma half-life, we attempted to prolong drug exposure by giving topotecan as a 24-h infusion weekly. In a phase I trial, we treated 32 patients at doses ranging from 1.0 to 2.0 mg/m^2. The patient population had not been heavily pretreated with chemotherapy and was of good performance status. The incidence of neutropenia, which was dose-limiting, increased sharply with relatively small increments in dose. Doses greater than 1.5 mg/m^2 were associated with nadirs that developed after one to three weekly treatments. A patient with metastatic colorectal cancer had a prolonged partial response. The plasma pharmacokinetics of topotecan (lactone and open forms) was characterized in 21 patients. Mean plasma steady-state drug levels were proportional to the dose and were within the range required to exert cytotoxicity in preclinical models. Plasma elimination curves were fit to a one-compartment model, in which the harmonic mean half-life of topotecan was 3.5 h. The ratio of the lactone to the total drug concentrations was constant throughout, which suggests that for this schedule the total drug concentration may be used as a measure of active lactone exposure. This conclusion is supported by the pharmacodynamic analysis, which revealed a positive correlation of both lactone and total drug steady-state concentrations with bone marrow toxicity. The further investigation of this and other infusional schedules in phase II trials will be conducted. The steady-state concentrations of total drug will be measured in several of these trials to establish its potential role in adaptive dosing using this schedule. Such a strategy is justified by the interpatient variability in toxicity and the steep dose-response curve observed in this study. Preliminary evidence of interpatient variability in the mRNA expression of topoisomerase I in the peripheral mononuclear cells and colon mucosa is presented. Trials are under way using biological endpoints for further selection of patients in whom the use of topoisomerase inhibitors may be therapeutically beneficial.

Key words: Topotecan – Topoisomerase I expression – Camptothecin – Pharmacodynamics

Introduction

Attention has recently focused on the DNA-associated enzyme topoisomerase I as a target for anticancer chemotherapy [1]. The functions of this enzyme in DNA replication, DNA repair, and gene expression are disrupted by members of a novel class of anticancer agents, the camptothecin derivatives [1]. Four semisynthetic camptothecin analogues are currently in clinical trials in the United States, and all appear to have a therapeutic index superior to that of the parent drug, which was found to have unacceptable toxicity in early studies. The earliest developed analogue was irinotecan (CPT-11) [2, 3], which is a prodrug; the active metabolite SN-38 has a prolonged half-life in humans. CPT-11 is predominantly toxic to the gastrointestinal (GI) mucosa, and diarrhea is dose-limiting [2]. Much interest has been engendered by the early phase II trials of this agent that indicate promising activity in lung and colon cancers using a weekly schedule [4, 5].

Paper presented at the Topoisomerase Inhibitors Conference, University of Maryland Cancer Center, 27–30 October 1993, Monterey, California, USA. Supported in part by Bristol Myers Oncology Division

This work was supported in part by grant CA06792 from the National Cancer Institute (NCI, NIH, DHHS) and by an appropriation from the Commonwealth of Pennsylvania

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The 9-dimethylaminomethyl derivative of camptothecin, topotecan, is a water-soluble analogue with comparable preclinical activity [6, 7]. Topotecan does not require metabolic activation but, as with all members of this class, exists in solution as a lactone in a dynamic pH-dependent equilibrium with the inactive ring-opened form. Preclinical studies indicate that topotecan has broad antitumor activity and that this activity is enhanced by the use of schedules of frequent or prolonged administration [6]. The initial phase I trials of topotecan were conducted on an intermittent 5-day schedule [8]. In these trials, the plasma half-life of topotecan was found to be substantially shorter than that of SN-38. With a view to exploring further the apparent schedule dependency of topotecan, we designed a regimen of 24-h infusion repeated weekly. The goals of this study were to determine a dose of topotecan suitable for phase II trials using this schedule, to describe the pharmacokinetics of the lactone and total drug forms in these patients, and to relate the observed toxicity to pharmacokinetic indices. In this paper we summarize the findings of the phase I study [9] and discuss studies ongoing at Fox Chase Cancer Center and in the Eastern Cooperative Oncology Group (ECOG) that are directed at determining the basis of toxicity and of response in patients treated with topotecan.

Materials and methods

Drugs and chemicals. Topotecan was supplied in glass vials containing either 1 or 5 mg free topotecan base/ml in 2.2 ml diluent stabilized with D-gluconic acid, monopotassium salt (anhydrous, USP), and adjusted to pH 3.0. The drug was reconstituted in 0.9% sodium chloride or 5% dextrose to a concentration of 6.7–500 μg/ml. Other reagents were obtained from Sigma unless noted otherwise.

Patient population. Patients eligible for this study had a histologic diagnosis of a malignant solid tumor and had exhausted the standard therapeutic options for their disease or had a malignant disease for which no established therapy exists. The were 18 years of age or older and had an ECOG performance status of 0–2. They had adequate bone marrow (white cell count, ≥4,000/mm^3; platelet count, ≥100,000/mm^3), liver (bilirubin, ≤1.5 /dl), and kidney (creatinine, ≤1.5 mg/dl) function. Patients had recovered from all toxicities of prior treatment and had received no prior chemotherapy or radiotherapy within 4 weeks of entry into this study (8 weeks for drugs with delayed toxicity such as nitrosoureas or mitomycin). All patients gave written informed consent in accordance with federal, state, and institutional guidelines.

Patients were monitored with complete blood counts, biochemical profiles, physical examination, and X-rays and scans as required for tumor measurement. The occurence of grade 2 or worse toxicity before the completion of four weekly doses led to subsequent doses being withheld until the toxicity had resolved and to dose reduction as described elsewhere [9]. Results are reported using the Consensus Toxicity Criteria (1988 Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Md.).

Treatment plan. Topotecan was diluted in 0.9% saline at a concentration of 10–500 μg/ml or in 5% dextrose (6.7–330 μg/ml) and was given as a continuous intravenous infusion over 24 h. The starting dose of topotecan was 2 mg/m^2, equivalent to 0.025 of the dose lethal to 10% of the population (LD_{10}) in mice given bolus doses. Because of granulocytopenia, the next level was decreased to 1 mg/m^2, and subsequent doses were escalated by 0.25 mg/m^2. The maximum tolerated dose was defined as the dose of topotecan at which fewer than one-third of the patients would experience grade 3 or 4 toxicity.

Pharmacokinetics studies. Blood samples were drawn into heparinized (green-top) Vacutainer tubes and were obtained before treatment; at 3, 6, 9, 12, 15, 18, and 24 h (end of the infusion); and at 5, 15, and 30 min as well as 1, 2, 4, 6, 12, and 24 h postinfusion. The samples were rapidly cooled by immediately placing the collection tube in a dry ice/isopropanol bath. Two 1.5-ml aliquots were transferred to polypropylene microcentrifuge tubes (Marsh Biomedical Products, Inc., Rochester, N. Y.) and centrifuged at 12,000 *g* for 30 s. From each tube, 500 μl plasma was transferred to new microcentrifuge tubes and extracted by the addition of 500 μl cold acetonitrile (kept in a dry ice/isopropanol bath) and 20 μl 10% zinc sulfate solution. The precipitated proteins were removed by centrifugation, and the clear supernatant was stored at –80° C. Plasma samples spiked with topotecan were extracted exactly as described for the patients samples and were used for standard curves. A 100-μl sample of the supernatant was injected into the high-performance liquid chromatographic (HPLC) system. To the duplicate of each sample, 20 μl 20% phosphoric acid was added to convert hydroxy acid topotecan to the lactone, and 100 μl of this mixture was injected onto the HPLC column. Topotecan quality-control (QC) samples made from a separate weighing of topotecan were prepared on the day of dosing for each subject. Care was taken to ensure that the sample-handling procedures maintained the maximal recovery of topotecan [9].

High-performance liquid chromatography. Plasma concentrations of topotecan (lactone and total drug) were determined by a modification of the method of Beijnen et al. [10]. The chromatographic system consisted of a Hewlett-Packard (Palo Alto, Calif.) HP-1090 Series A liquid chromatograph equipped with an autoinjector/autosampler and an HP-1046A fluorescence detector. Detection by fluorescence was carried out at an excitation wavelength of 382 nm and an emission wavelength of 523 nm. Samples were injected onto a Hewlett-Packard Hypersil ODS C18 reverse-phase analytical column (5 μm; 100-×4.6 mm inside diameter) equipped with a 15×3.2-mm, 7-μm Newguard C18 guard column (Applied Biosystems, Inc., San Jose, Calif.). The isocratic mobile phase consisted of 0.005 *M* dioctyl sulfosuccinate, 0.023 *M* sodium phosphate, 0.56% triethylamine, 53% methanol, and 40% water and was run at a flow rate of 1.5 ml/min. Standard curves were plotted as the peak area versus the concentration of topotecan. Concentrations of the inactive hydroxy acid from were calculated by subtracting values obtained for topotecan (lactone) from values recorded for total topotecan.

Pharmacokinetic analysis. Plasma topotecan concentration versus time curves were fitted to the monoexponential equition

$$C(t) = Ae^{-\alpha t}$$

and were corrected for the length of infusion (24 h) using NONLIN84 [11]. The steady-state volume of distribution (V_{SS}), total body clearance (Cl_{tot}), area under the plasma concentration versus time curve (AUC), and elimination half-life were calculated from standard pharmacokinetic equations [12]. C_{SS} was calculated from *infusion rate/*Cl_{tot}.

The relationship between pharmacokinetic indices and pharmacodynamic effects were best described using the sigmoid E_{max} (Hill) equation, of the form

$$\% \text{ Decrease in ANC} = \frac{100 \times (\text{Param})^h}{(\text{Param}_{50})^h + (\text{Param})^h}$$

where the estimated parameters are the Hill constant (*h*) and the pharmacokinetic parameters (AUC_{50}, C_{SS50}, and $dose_{50}$) that produce the half-maximal effect. Comparisons were made between the topotecan dose (mg/m^2) and the topetecan (both total and lactone) AUC and C_{SS} and neutropenia. Neutrophil toxicity was quantified as the percentage of decrease in the absolute neutrophil count (ANC) measured on treatment days 15, 22, and 29 as compared with the pretreatment values using the formula

$$\% \text{ Decrease in ANC} = \frac{\text{Pretreatment ANC} - \text{nadir ANC}}{\text{Pretreatment ANC}} \times 100.$$

The data were fit to the Hill equation using PCNONLIN [11].

Table 1 Patients' characteristics

Patients entered/evaluable	32/31
Sex (M/F)	16/16
Median age (range)	52 (40–78) years
Performance status:	
0	6
1	26
Primary sites:	
Colorectal	16
Lung	6
Pancreas	5
Other	5
Prior treatment:	
Chemotherapy	23
Chemotherapy/radiotherapy	5
Biologicals	1
None	3

Quantitation of topoisomerase I expression by polymerase chain reaction-RNA assay. For quantitation of topoisomerase I expression, the polymerase chain reaction (PCR)-reverse transcriptase assay was employed as described by Horikoshi and co-workers [13]. For each sample, 100 ng total RNA was used for RNA reverse transcription. Varying amounts of cDNA from the reverse transcriptase reactions were used as the substrate for PCR amplification of topoisomerase I and β-actin. The PCR reaction mixtures [25 µl total volume, containing 1–10 nl cDNA, 12.5 pmol of each of the appropriate primer pairs, 100 m*M* TRIS (pH 8.3), 500 m*M* KCl_2, 0.01% gelatin, 0.5 µl 10 m*M* aqueous deoxynucleotide triphosphate solution, 3.75 µl 12.5 m*M* $MgCl_2$, and 0.63 units *Taq* DNA polymerase] were amplified for 35 cycles at 94° C for 1 min, 55° C for 1 min, and 72° C for 1 min. The PCR primers with the T_7 polymerase promoter sequence TAATACGACTCA CTA TA attached to their 5′ ends were as follows: β-actin primers BA-67 T_7-"GGGAGA" GC GGG AAA TCG TCGT GCG TGA CATT (bases 2104–2127) of the β-actin genomic sequence, located in exon 3 [14], and BA-68 GATGGAGTT GAAGGTA GTTTCGTG (bases 2409–2432) of the β-actin genomic sequence, located in exon 4 [14], were used as an internal control; the primers for topoisomerase I were TOPO 20 T_7-"GGAG" AAG CAG AGG AAG TAG CT (bases 966–985) and TOPO 21 GCT CAA CTG TTT CCG AGC TT (bases 1152–1171) [15]. For T_7 polymerase transcription, 3 µl PCR product was used with 22 µl of a master solution containing transcription buffer [40 m*M* TRIS-HCl (pH 7.5), 12 m*M* $MgCl_2$, 1 m*M* spermidine]; 1 m*M* each of the ribonucleotides adenosine triphosphate (ATP), guanosine triphosphate (GTP), cytidine triphosphate (CTP), and uridine triphosphate (UTP); 0.1 *M* dithiothreitol; 10 units RNA guard; 2.5 µCi [α-^{32}P]-CTP (3000 Ci/mmol); and 50 units T_7 RNA polymerase solution. The mixture was incubated for 1 h at 37° C.

After the reaction had been stopped with 0.75 µl 0.5 *M* ethylenediaminetetraacetic acid (EDTA), the transcription mixture was electrophoresed on a 6% urea-denatured polyacrylamide gel at 140 V for 3 h. After the gel had been dried under vacuum, autoradiography was performed overnight. The bands representing the topoisomerase I and β-actin gene fragments were cut out and the radioactivity was counted in a liquid scintillation counter. To normalize the topoisomerase I expression to that of the endogenous standard β-actin, the ratio between the amount of radiolabeled PCR product within the linear amplification range and the amount of endogenous standard was calculated as the ratio of PCR products.

Results

Patients were treated on this study at Fox Chase Cancer Center between February 1991 and April 1992. The demographic characteristics of the patients are summarized in Table 1. The single inevaluable patient was registered on study but refused treatment after receiving a single dose without developing toxicity. All of the patients were of excellent performance status, and the population had not been heavily pretreated. The majority of patients had tumors of gastrointestinal origin.

Table 2 Granulocytopenia as a result of topotecan administration

Dose (mg/m^2)	*n*	Granulocytopenia (grade)				
		0	1	2	3	4
1.0	4	4	–	–	–	–
1.25	5	5	–	–	–	–
1.5	7	7	–	–	–	–
1.75	6	1	–	3	–	2
2.0	9	2	1	1	4	1

As previously reported elsewhere [9], the dose-limiting toxicity of topotecan on this weekly infusional schedule was granulocytopenia (Table 2). At the starting dose of 2.0 mg/m^2, grade 3 or worse granulocytopenia occurred in five of nine patients; all required interruption of treatment before 4 weeks had elapsed. The dose was reduced to 1.0 mg/m^2 and escalated in 0.25-mg/m^2 increments. At 1.75 mg/m^2, two of six patients had grade 4 granulocytopenia. One of these, a 71-year-old man with pancreatic cancer, who had previously been treated with a 5-fluorouracil-containing regimen, became febrile after receiving two doses of topotecan. Despite appropriate management, he progressed to develop septic shock and expired. At this dose, three patients experienced grade 2 granulocytopenia after 1, 2, and 3 weeks, respectively. One patient discontinued treatment after 1 week without showing signs of granulocytopenia but with grade 2 leukopenia. Treatment at this dose level could not be sustained. Recovery of counts to ≥2,000 granulocytes/mm^3 occurred within a median of 7 days in patients with grade 2 toxicity (range, 3–10 days).

At 1.5 mg/m^2, seven patients were treated. No neutropenia exceeding grade 2 was observed. Two patients who had received extensive prior treatment developed grade 4 thrombocytopenia after one and three doses, respectively. Two patients showing no sign of myelosuppression exhibited a decreasing performance status in the face of progressive disease after two and six doses, respectively. Three patients received 5–19 doses without developing toxicity. We believe that 1.5 mg/m^2 is an appropriate dose for phase II trials of topotecan on this schedule. The use of this dose will require careful observation of blood counts before each administration of the weekly dose.

Myelosuppression also manifested as dose-related thrombocytopenia. The incidence and severity of this complication was considerably less than that of granulocytopenia. Two of seven patients treated at 1.5 mg/m^2 experienced grade 4 thrombocytopenia, but of 15 patients treated at higher doses, only one developed grade 3 toxicity. Platelet transfusions were not required and no patient experienced bleeding. Other toxicities (≥ grade 2) observed in this trial included moderate nausea and vomiting in five

Table 3 Pharmacokinetic parameters of topotecan (lactone) and total drug (lactone + hydroxy acid) following 24-h continuous infusion[a]

	Topotecan dose (mg/m²) Lactone				Topotecan dose (mg/m²) Total			
	1.25	1.5	1.75	2.0	1.25	1.5	1.75	2.0
n	5	7	5	4	5	7	5	4
Css (n*M*)	4.7± 0.6	5.8± 1.2	8.2± 1.8	11.4± 1.3	8.7± 1.5	10.7± 2.7	15.5± 4.0	12.1± 2.1
AUC (n*M* h)	121 ±19.0	139.7±29.0	198.6±43.5	278.2±35.1	210.5±37.5	262.7±65.8	379.6±101.1	304.3±57.3
Cl_{tot} (ml min^{-1} m^{-2})	416.7±68.3	441.8±96.1	365.1±95.3	287.8±36.3				
Half-life (h)[b]	3.7 (2.7–11.7)	3.2 (2.2–4.8)	3.2 (2.2–3.9)	4.0 (3.6–4.6)	3.0 (2.5–3.9)	3.7 (2.4–5.8)	3.5 (2.4–4.8)	3.7 (1.9–7.4)

[a] Data represent mean values ± SD
[b] Values are harmonic means (range)

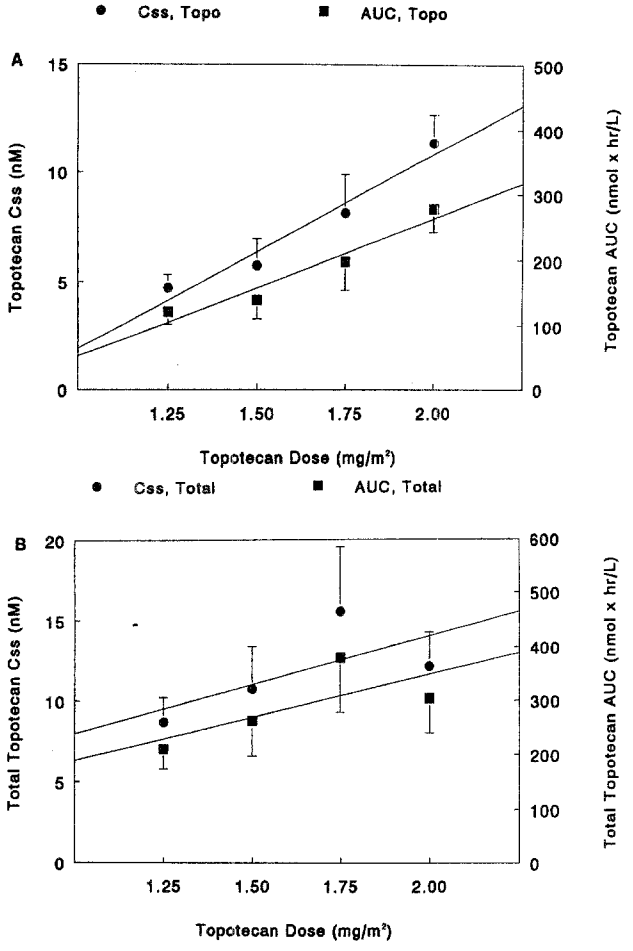

Fig. 1. Linearity of the steady-state concentration (Css) and area under the concentration-time curve (*AUC*) with the dose of topotecan

patients; symptoms were easily controlled by prochlorperazine. Unexplained fever not attributable to infection occurred in three patients. One patient experienced weakness and lethargy that may have been drug-associated, and the only evidence of hepatotoxicity was seen in a single patient who had a grade 2 elevation in alkaline phosphatase that resolved after cessation of the drug.

We have described extensively the assay for plasma topotecan [9]. The plasma pharmacokinetics of both the lactone form and total topotecan were determined following the first drug dose in 21 patients. The results are given in Table 3. Steady state was reached at between 12 and 15 h into the 24-h infusion. From the end of the infusion, the plasma elimination of both the lactone form and total topotecan was monoexponential and declined in parallel, with the half-life being about 3.5 h. Total clearance (Cl_{tot}) was 350 ml min^{-1} m^{-2} for the lactone form and 240 ml min^{-1} m^{-2} for total topotecan. Linear regression analysis revealed that both Css and AUC increased linearly with dose for the total (r = 0.5) and lactone (r = 0.9) forms (P <0.05), supporting a dose linearity of topotecan pharmacokinetics in this dose range (Fig. 1). There was excellent agreement between values calculated for Css using *infusion rate/Cl_{tot}* and Css values calculated by averaging the concentrations measured at 15, 18, and 24 h during the infusion. Using *infusion rate/Cl_{tot}*, mean (± SD) Css values were 11.5±2.8 n*M* for total topotecan and 7.3±2.7 n*M* for topotecan (lactone). By averaging plasma concentrations at 15, 18, and 24 h, mean (± SD) Css values of 11.5±3.4 and 7.2±3.0 n*M* were found for total topotecan and topotecan lactone, respectively, illustrating that similar results are obtained for Css values using either method of calculation.

There were 18, 14, and 12 patients that had pharmacokinetics (performed on day 1) and blood counts determined on days 15, 22, and 29, respectively. The AUC to topotecan (lactone and total) was predictive of the decrease in neutrophil count using the sigmoid E_{max} model. The model-derived values for AUC are closely concordant for the prediction of toxicity on days 15, 22, and 29, suggesting, first, that the model has predictive value and, second, that toxicity is not cumulative. In addition, since the prediction of toxicity is based on the pharmacokinetic analysis on day 1 only, it seems unlikely that the elimination of topotecan changes with repeat dosing in an individual patient. As expected for a drug with such a short half-life, where AUC and Css values would be highly correlated during a 24-h infusion, the pharmacodynamic analysis of the steady-state concentration of topotecan (lactone and total) is almost identical to that of the AUC (Fig. 2). In all cases the Hill equation produced superior fits as compared with the linear relationship.

In this trial, relatively small increments in dose resulted in profound changes in toxicity. Good estimates of neutropenia were also obtained using the delivered dose in the sigmoid E_{max} model. Using this analysis, the dose producing a 50% decrease in neutrophil counts (D_{50}) was 1.66, 1.49, and 1.58 mg/m² on study days 15, 22, and 29, respectively. These values are remarkably close to the re-

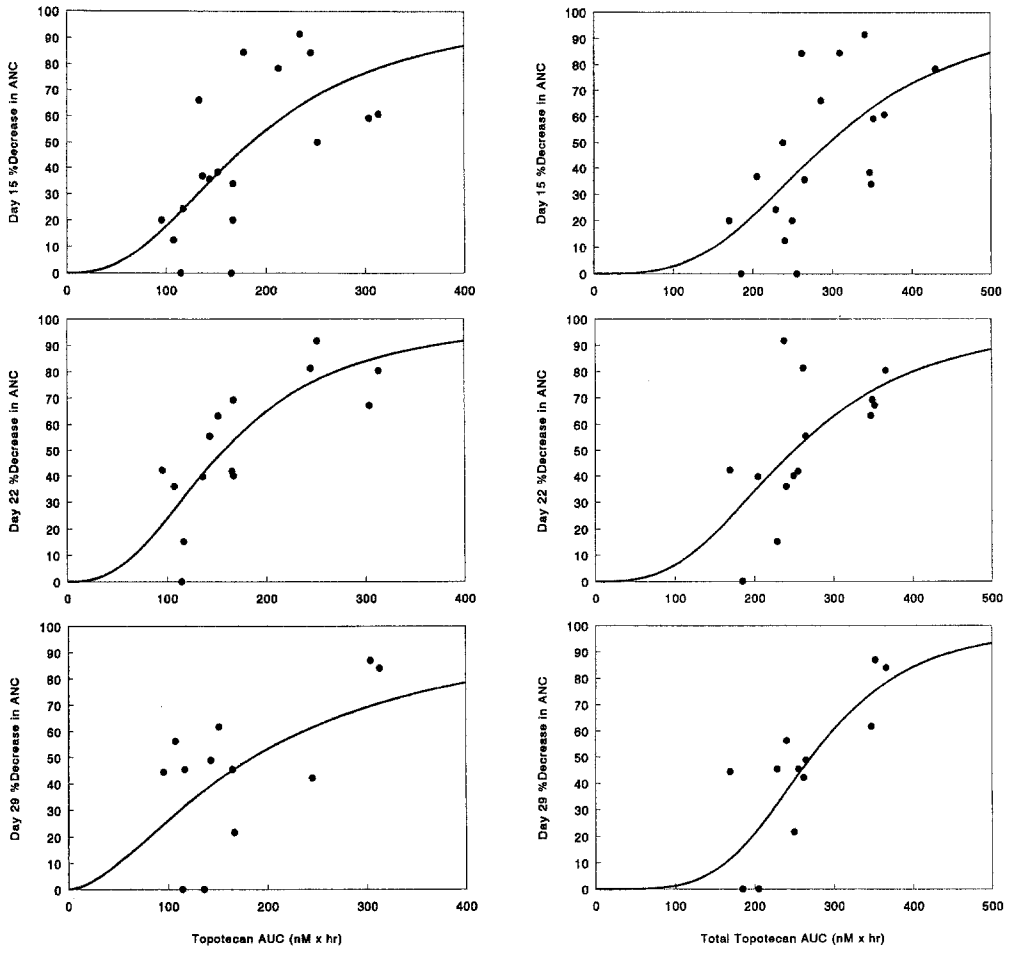

Fig. 2. Relationship between the percentage of reduction in neutrophils versus the area under the curve (*AUC*) of topotecan (lactone) and of total drug (lactone + hydroxy acid) on days 14, 21, and 29 following the initiation of weekly treatment. The curves represent the fit of data as described in Materials and methods

commended phase II dose of topotecan on this schedule. The high values of the Hill constant (ranging from 3.93 to 5.78) reflect the very steep dose-toxicity relationship observed.

The expression of topoisomerase I appears to be an important predictor of the activity of topoisomerase I inhibitors. In some cell lines, a positive relationship can be shown between the expression of topoisomerase I (protein,

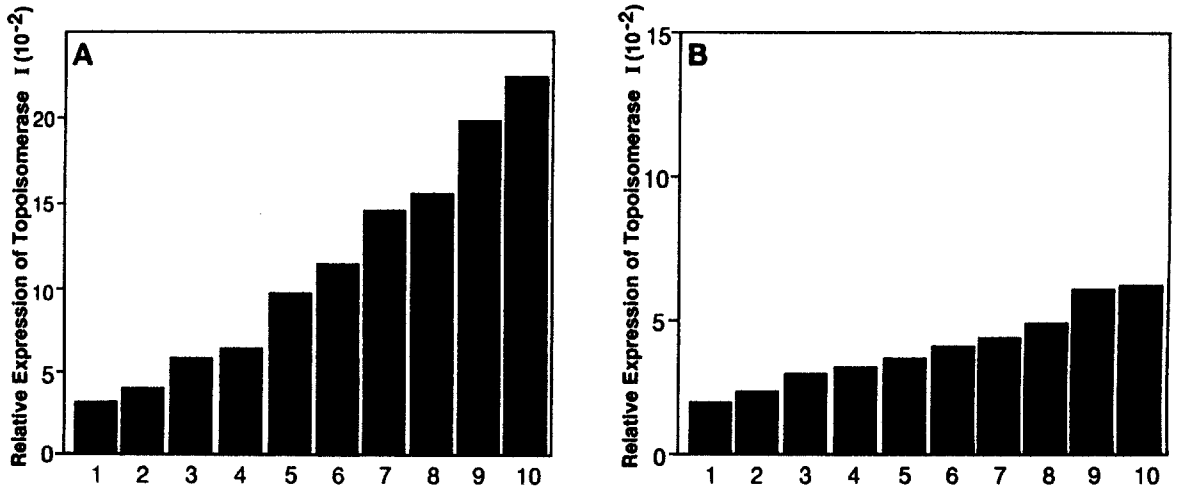

Fig. 3A and B. RNA expression (relative to β-actin) of topoisomerase I in **A** the peripheral mononuclear cells of cancer patients and **B** the colon mucosa of normal subjects undergoing endoscopy

catalytic activity, or mRNA) and the sensitivity to inhibitors [16–18]. Cell lines selected for resistance have a mutated topoisomerase I enzyme [16]. However, other factors undoubtedly contribute to determining sensitivity; other investigators have not found a relationship of susceptibility to inhibition with topoisomerase I expression in cell lines [19]. To determine whether individual variability in expression would relate either to toxicity or to response, we activated a series of studies in association with phase II clinical trials. To provide a baseline, we examined the expression of topoisomerase I mRNA in peripheral mononuclear cells (PMN) and normal colon samples in two groups of patients (Fig. 3). As may readily be observed by inspection, the variability in PMN values was substantially greater than that in colon mucosa. The mean (± SD) relative expression in PMNs was 11.09 ± 6.48 arbitrary units, whereas that in colon mucosa was 3.87 ± 1.29 units. These data demonstrate that substantial interpatient variability in topoisomerase I expression characterizes both the PMN cells of cancer patients and the colon mucosal samples of individuals without cancer who are undergoing routine endoscopy.

Discussion

The initial phase II clinical trials of topotecan demonstrate that this drug has promising activity in the treatment of ovarian [20] and small-cell lung cancers [21]. A trial at the M. D. Anderson Cancer Center in a cohort of 28 heavily pretreated patients with ovarian cancer showed a response rate of 14%. The extent of prior treatment makes this a result that merits follow-up in further trials. In small-cell lung cancer, 8 of 13 patients were reported to have responded in an ECOG study. Both trials were conducted using the 5-day schedule. This pattern of activity, although interesting, is substantially different from that obtained using CPT-11, for which activity has been reported in non-small-cell lung and colorectal cancers as well as in small-cell lung cancer [4, 5]. We hypothesize that the major reason for the lack of activity of topotecan in the former tumors may relate to the schedule of administration. The prolonged exposure to topoisomerase I-inhibitory drug follow as treatment with CPT-11 is sought by the weekly infusional schedule of topotecan piloted in this clinical trial. On the basis that an infusional regimen might have a superior therapeutic index, this regimen will be investigated in phase II trials in solid tumors. This method of drug administration has been used extensively by us for other agents; there is a high level of patient acceptance, and all courses may be given in the outpatient setting.

An alternative infusional schedule has been developed by Hochster and colleagues [22], who performed a phase I trial of a 21-day infusion repeated every 5 weeks. This schedule will also be tested in colorectal cancer in the ECOG and in pancreatic cancer at Fox Chase Cancer Center. It is noteworthy that the only responses to topotecan in patients with colorectal cancer have been observed in these infusional studies.

The toxicity data presented in this report are consistent with those derived from the 5-day regimens; neutropenia is dose-limiting, and other side effects are well tolerated [8]. The neutropenia was characterized by a steep dose-response curve, with the transition from minimal to severe toxicity occurring over a relatively small dose increment. Furthermore, at the highest dose, considerable interpatient variability was observed. These data suggest that there may be an advantage to individualizing the dose of topotecan for maximal therapeutic impact. Certainly, there is evidence to support the use of maximally tolerated doses; the striking response data in small-cell lung cancer were obtained using a dose of 2.0 mg/m^2, some 33% higher than the conventional phase II dose [21]. The present data show that the 24-h infusion may be particularly suitable for adaptive dosing. First, this is one of the few studies in which there is a clear relationship between the pharmacokinetics of topotecan and toxicity [9]. Second, the relationship is even better for the total drug than it is for the more difficult to quantitate lactone. The explanation for this finding is likely to lie in the substantial technical difficulties involved in bedside extraction of plasma samples for the lactone assay. The measurement of total drug is conducted when all the samples have been collected and batched; all are processed identically and experimental error is minimized. The practical consequence of this finding is that total drug measurements are likely to be predictive for inclusion in a pharmacodynamic model. Finally, the weekly schedule permits dose adjustments to be undertaken early in the treatment course such that these adjustments may have a real impact on the therapeutic index. Validation of these findings is planned as a part of ongoing phase II studies.

Although pharmacokinetic sources of variability have been demonstrated to be predictive of toxicity, the unusual mechanistic characteristics of topoisomerase I inhibitors should also be considered. Unlike that of many enzyme inhibitors, the toxicity of topotecan is expressed at very low levels of inhibition. The 50% inhibitory concentration (IC_{50}) of topotecan for topoisomerase I is the micromolar range, whereas its cytotoxicity is expressed at nanomolar levels. Sensitivity to topetecan is directly, not inversely, proportional to the topoisomerase content. Thus, overexpression of topoisomerase I renders a cell more sensitive to camptothecin, whereas camptothecin-resistant lines are often found to have a mutated enzyme with diminished function [16–18]. However, this finding is not absolute, and conflicting data have emerged from studies on drug-resistant lines [19]. Additional effects on regulatory pathways are likely to be present in resistant cells, and these remain to be clarified. The available data support the clinical investigation of biochemical and biological determinants of toxicity and response.

The data presented herein demonstrate interindividual variability in the RNA expression of topoisomerase I in normal tissues. The degree of variability is similar to that reported by Husain et al. [23], who found a 66% standard deviation in topoisomerase I protein content in seven normal colon mucosal biopsies. Variability in tumor tissue was substantially greater, and ratios of the content in colon tumor versus normal tissue ranged from 5.0 to 35.0-fold as quantitated by Western blotting [23]. The variability in mRNA content has not been reported, nor has the relationship between the expression in colon versus other

tissues. Szarka and colleagues [24] have recently reported a close relationship between the activity of glutathione transferase in the colon and that in the peripheral mononuclear cells of the same individual. Such a relationship would need to be established for topoisomerase I, however, since the expression of glutathione transferase may be determined by a common exposure to environmental agents, whereas topoisomerase I expression may remain unaffected by such influences [25]. For the toxicity of topoisomerase I inhibitors, the more important relationship is that between peripheral mononuclear cells and marrow stem cells; its existence will be identified in clinical trials.

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