A Phase I Study of Topotecan Followed Sequentially by Doxorubicin in Patients with Advanced Malignancies


ABSTRACT

Inhibitors of topoisomerase I and topoisomerase II have demonstrated synergy when administered sequentially in several tumor models while having a diminished antitumor effect when given concurrently. To explore the potential for clinical sequence-dependent synergy, we instituted a Phase I study of topotecan (a topoisomerase I inhibitor) followed by doxorubicin (a topoisomerase II inhibitor) in patients with advanced malignancies.

Thirty-three patients with advanced malignancies or malignancies for whom no standard therapy exists were entered into the study. Topotecan was administered in escalating doses by 72-h continuous infusion on days 1, 2, and 3, followed by a bolus of doxorubicin given on day 5. To explore the hematological toxicity associated with this sequence, bone marrow aspirates were obtained both prior to the topotecan infusion and immediately prior to the doxorubicin in 10 patients to determine by fluorescence-activated cell sorting analysis whether CD34+ cell synchronization was occurring using this sequential schedule.

Dose-limiting hematological toxicity occurred at the first dose-level in three of six patients. Therefore, we defined the maximum-tolerated dose (MTD) below our starting dose-level. Further dose-escalation and a new MTD were defined with the addition of granulocyte-colony stimulating factor (G-CSF). The MTD was, therefore, topotecan 0.35 mg/m²/day continuous i.v. infusion on days 1, 2, and 3, followed by doxorubicin 45 mg/m² on day 5 without G-CSF, whereas the MTD with G-CSF was topotecan 0.75 mg/m²/day by 72-h continuous i.v. infusion, followed by doxorubicin 45 mg/m² i.v. bolus on day 5.

Ten patients with paired bone marrow aspirates obtained before topotecan and before doxorubicin administrations were available for evaluation. In 7 of 10 patients, there was an increase (16.6 ± 2.9% to 25.0 ± 3.5%; P < 0.02) in the proportion of CD34+ cells in S-phase 24 h after the topotecan infusion and prior to doxorubicin compared to the pretreatment values, whereas 1 patient had a decrease in the proportion of CD34+ cells in S phase and 2 patients had no change.

Topotecan and doxorubicin with this sequence and schedule can be given safely; the dose-limiting toxicity is hematological toxicity. Alterations in the fraction of hematopoietic progenitor CD34+ cells in S phase may account for the increased granulocytopenia and thrombocytopenia observed at relatively low dose levels of the combination with and without G-CSF.

INTRODUCTION

Topotecan (9-dimethylaminomethyl-10-hydroxycamptothecin) is a water-soluble derivative in the class of topoisomerase I inhibitors, the camptothecins. Camptothecin analogues inhibit type I DNA topoisomerase through the formation of stable topoisomerase I-DNA cleavable complexes (1). These topoisomerase I-cleavable complexes cause single-strand breaks in cultured cells when they impact with the replication fork in S-phase (2). Topotecan has a broad spectrum of antitumor activity against murine solid tumors, in vitro human cell lines, and in vivo human xenografts (3).

The pharmacological half-life of the active form of topotecan (lactone ring configuration) is relatively short, and the formation of DNA single-strand breaks introduced by topoisomerase I inhibitors is reversible following drug withdrawal. Continuous exposure to topotecan via continuous i.v. infusion increases the exposure of cells to the active lactone ring and may increase the cytotoxic effects of this drug (4–6).

Doxorubicin and the anthracyclines exert their cytotoxic effects through protein-associated breaks in DNA via inhibition of normal topoisomerase II. Topoisomerase II promotes DNA cleavage, unwinding, and reannealing during DNA synthesis, mitosis, and transcription (7, 8). Doxorubicin stabilizes the binding of topoisomerase II to DNA in a complex that prevents reannealing and, hence, results in double- and single-strand breaks. When these breaks are present at vulnerable points in the cell cycle, cytotoxicity occurs (9). Cells resistant to topoisomerase II inhibitors have been shown to have decreased levels of topoisomerase II and increased levels of topoisomerase I (10). Conversely, some human cell lines that are resistant to the camptothecin analogues exhibit collateral drug sensitivity to topoisomerase II inhibitors, presumably due to increased topoisomerase II levels (11, 12).

Inhibitors of topoisomerase I and topoisomerase II appear
to act synergistically when administered sequentially in several tumor models, whereas they have a diminished antitumor effect when given concurrently (11, 12). One proposed mechanism of synergy for sequential treatment with camptothecin analogues followed by doxorubicin is that the camptothecins lead to an increase in the tumor S-phase cell population, a decrease in the G1 cell population, and an increase in topoisomerase II levels, making the cells more susceptible to doxorubicin cytotoxicity (11, 13). A proposed mechanism for the antagonism observed with the concurrent administration of camptothecins and a topoisomerase II inhibitor include the inhibition of DNA and RNA synthesis, both of which are necessary for topoisomerase II binding to DNA (9, 14, 15). Preclinical evidence of antagonism of cytotoxicity has been reported with the concurrent administration of camptothecin with topoisomerase II inhibitors including etoposide, 4'-(9-acridinylamino)-methanesulfon-m-aniside, and daunorubicin in the HL-60 cell line (12). In nude mice bearing human tumor xenografts, the sequential administration of the camptothecin analogue CPT-11, followed by a 24-h washout interval then followed by doxorubicin, was associated with synergistic cytotoxicity, whereas the concurrent administration of CPT-11 and doxorubicin was associated with significant antagonism (13).

The single-agent Phase I study of 72-h infusional topotecan reported a recommended Phase II dose level at 1.6 mg/m² per day; the dose-limiting toxicity was granulocytopenia and thrombocytopenia (16). Anticipating possible additional toxicity of the combination, the starting dose in our study was just under the starting dose level of the single-agent topotecan Phase I study using the 72-h schedule. Previous experience with doxorubicin doses at 45 mg/m² i.v. given every 3 weeks in combination with other cytotoxic agents demonstrated antitumor activity and good tolerability (17). The principle goal was to escalate the dose of topotecan with this combination.

In this Phase I study of topotecan and doxorubicin, we explored the combination of topoisomerase I and II inhibitors using the sequential administration of topotecan by 72-h continuous infusion, followed 24 h later with doxorubicin by i.v. bolus in an effort to exploit the potential for synergistic treatment schedules observed in preclinical models.

PATIENTS AND METHODS

Eligibility. Patients age 18 years and older with histologically or cytologically confirmed advanced cancer who had been treated previously with up to four prior chemotherapy regimens were eligible for this study. Patients must have failed all potentially curative therapy prior to this study. Written informed consent was obtained before treatment according to NCI2 and the Walter Reed Army Medical Center Investigational Review Board guidelines. All patients were required to have a normal baseline cardiac left ventricular ejection fraction and an Eastern Cooperative Oncology Group performance status of 0, 1, or 2 at study entry. Patients were excluded if they had central nervous system metastases; a history of cardiac disease; had exceeded 360 mg/m² of prior doxorubicin therapy; or had undergone prior camptothecin therapy, autologous bone marrow transplantation, or prior treatment within 6 months with nitrosoureas, melphalan, or mitomycin C. Concurrent irradiation and prior irradiation to the mediastinum were not allowed. Laboratory requirements included a WBC count ≥3,500/µl, platelets ≥100,000/µl, serum bilirubin less than 1.8 mg/dl, aspartate aminotransferase and alanine aminotransferase not to exceed three times the upper limit of normal, and serum creatinine less than 1.5 mg/dl. Pretreatment evaluation included history and physical examination with tumor measurements; chest X-ray; ECG; CBC with differential, serum chemistries, and liver function tests; staging studies appropriate to define the extent of metastatic disease including computed tomography scan, ultrasound, bone scan, and plain radiographs of suspicious areas seen on bone scan, as clinically indicated; and a determination of resting baseline cardiac left ventricular ejection fraction by radionuclide ventriculography. Restaging studies were performed after the second cycle of therapy and then after every subsequent third cycle in responding patients or those with stable disease. A complete blood count was obtained on all patients every Monday, Wednesday, and Friday while patients were in the study to monitor hematological toxicity.

Drug Preparation and Administration. Topotecan (SmithKline Beecham, King of Prussia, PA) and G-CSF (Neupogen; Amgen, Thousand Oaks, CA) were supplied by the Division of Cancer Treatment, NCI. Doxorubicin was obtained commercially. Topotecan was diluted prior to administration with either 5% dextrose USP (DSW) or with 0.9% NS and was considered stable for at least 24 h.

For ambulatory pump preparation, a quantity of the reconstituted solution appropriate to provide a 24-h supply of topotecan was instilled into a Medication Reservoir Cassette (Pharmacia Deltec, Inc., St. Paul, MN) with 0.9% NS to a total volume (QSAD) of 50 ml. Topotecan was given i.v., infused over 24 h daily for 3 days. A fresh supply of drug was prepared for each day of the three-day infusion and was administered via a CADD-1 (Pharmacia Deltec, Inc.) ambulatory infusion pump. Doxorubicin was reconstituted with sodium chloride injection USP (0.9% NS) or sterile water for injection USP to give a final concentration of 5 mg/ml. Doxorubicin was given 24 h following the completion of the topotecan infusion as an i.v. bolus over 5–10 min.

G-CSF was supplied as a 300 µg/ml solution in 2-ml single-use vials. Patients were taught to self-administer G-CSF as a s.c. injection. G-CSF 5 mg/kg/day was given in the evenings starting no sooner than 24 h after the doxorubicin was administered and continued until the WBC count was ≥10,000/µl on two successive determinations after the expected nadir.

Hematological DLT was determined during the first cycle of therapy and was defined as lack of recovery of blood counts (granulocyte count, ≥1,500/µl; platelet count, ≥90,000/µl) by day 21, which subsequently resulted in a delay of the next cycle, or duration of granulocyte nadir of <500 cells/µl for greater than 5 days, or a single platelet count <20,000/µl. Because dose-limiting hematological toxicity occurred at the first dose-
the failure to recover neutrophils and platelets by day 28 for all dose levels. Further patient accrual was initiated at a reduced dose of topotecan, where a new MTD was defined. Further dose-escalations proceeded with the addition of G-CSF. Nonhematological DLT was defined also during cycle 1 as any grade 3 or 4 toxicity (NCI Common Toxicity Criteria) in any category except nausea or vomiting. Febrile neutropenia was not considered a DLT in this study based on the availability of G-CSF in the management of febrile neutropenia and our experience with other combination Phase I studies at the Medicine Branch, NCI. The MTD was defined as the highest dose level at which no more than two of six patients experienced DLT. Treatment cycles were repeated every 21–28 days provided recovery of blood counts as defined above had occurred and provided any nonhematological toxicity had resolved to grade 1 or less.

Patients with bidimensionally measurable lesions were evaluated for antitumor response. A complete response was defined as the complete disappearance of all clinically detectable disease including measurable and evaluable sites for a minimum of 4 weeks, whereas a partial response required a ≥50% decrease in the sum of the products of the longest perpendicular dimensions of measurable lesions for at least 4 weeks without an increase (>25%) in the size of any lesion known to contain malignant disease or without the development of new lesions. Progressive disease was defined as an increase of ≥25% in the size of any lesion or the development of any new lesions.

Bone Marrow CD34+ Processing. After informed consent, bone marrow aspirates were collected in preservative-free heparin from 12 patients both prior to topotecan treatment and on day 5 of treatment (just prior to doxorubicin). Bone marrow aspirates were obtained from two patients at dose level −1, four patients from dose level 2, and six patients from dose level 3. Cells were stained with Turk’s stain and counted manually. Mononuclear cells were separated with lymphocyte separation medium (Organon Teknika, Westchester, PA), frozen, and stored in liquid nitrogen. Paired pre- and posttreatment aliquots from each patient, assayed concurrently, were thawed, washed in fluorescence-activated cell sorting buffer, consisting of HBSS without phenol red (Life Technologies, Inc.) supplemented with 0.1% sodium azide and 0.2% human serum albumin and stained with CD34-FITC (HPCA-2; Becton Dickinson Immunocytochemistry Systems, San Jose, CA). To mildly fix surface antigens, paraformaldehyde (to a final concentration of 0.25%) was added during vortexing to cells suspended in PBS (Life Technologies, Inc.); cells were fixed for 1 h at 4°C. Cells were permeabilized by incubation for 15 min at 37°C in 0.2% Tween 20 (Sigma Chemical Co., St. Louis, MO) in PBS, washed, and resuspended in fluorescence-activated cell sorting buffer containing 10 μg/ml propidium iodide (Sigma) and 11 Kunitz units/ml of RNase A (Sigma). Cells were analyzed on a FACsort (Becton Dickinson Immunocytochemistry Systems) using the Lysis II program for data acquisition and analysis. The cell cycle profile was assessed on an FL-2A histogram (propidium iodide incorporation) of CD34+ cells by acquiring cells delimitated simultaneously by three collection gates: FSC versus SSC dotplot to exclude clumps and debris; FL-2W versus FL-2A dotplot to exclude doublets; and FL-1H (CD34-FITC) versus SSC to compare with an isotypic control. A histogram marker was set symmetrically on the G0-G1 peak to calculate the percentage of CD34+ cells in S, G2, and M phase. No mathematical model was used to compensate for the G0-G1 and S-phase cell overlap. Mean values were compared by paired analysis using each patient’s mean values as their own controls and analyzed using a two-tailed t test. The full methodology and results have been submitted in a separate manuscript.

RESULTS

A total of 33 patients were entered into the study, and all patients were evaluable for toxicity. The dose-escalation scheme and the number of patients entered at each level are shown in Table 1. A total of 126 cycles of sequential topotecan and doxorubicin were administered with a median of three cycles per patient (range, 1–11). The patient characteristics are summarized in Table 2. Fifteen males and 18 females with a median age of 59 years (range, 25–74) received therapy. Twenty-seven patients had received prior chemotherapy, and the median number of prior regimens was two (range, 0–4). Six patients received prior mitox-
Topotecan followed by Doxorubicin in Advanced Malignancies

Hematological Toxicity. Dose-limiting hematological toxicity occurred in three of the six patients treated at the starting dose level of topotecan, 0.50 mg/m², per day for 72 h of continuous i.v. infusion followed 24 h later by doxorubicin, 45 mg/m², on day 5 without G-CSF. This DLT was characterized by failure to recover neutrophils ≥1500 cells/μl by day 21 in three patients; in addition, two of these patients developed grade 4 neutropenia for >5 days. Further patient entry was undertaken at a reduced dose level of topotecan, 0.35 mg/m², per day over 72 h of continuous i.v. infusion and doxorubicin, 45 mg/m², i.v. on day 5. Dose-limiting hematological toxicity was not observed at this new level. However, the granulocyte nadir was late occurring at a median of day 20 (range, 16–23 days); therefore, therapy could be delivered only every 28 days. The patients entered at this reduced dose level received a median of one prior chemotherapy regimen (range, 0–3). One of five patients experienced grade 3 fatigue at this reduced dose level; therefore, this level was defined as the MTD of the combination without G-CSF. At this MTD without G-CSF, 24 cycles of therapy were administered to six patients. One patient required a dose reduction, whereas two patients were dose-escalated by one level on cycles 2 and 4.

Further patient accrual and dose escalation was undertaken using G-CSF, 5 mg/kg, s.c. starting on day 6 until neutrophil recovery occurred. Dose-limiting hematological toxicity of thrombocytopenia <20,000/μl occurred in four of six patients at the dose level of topotecan, 1.0 mg/m², per day for 72 h followed by doxorubicin, 45 mg/m², with G-CSF. In addition, grade 3 fatigue was observed in two of these patients. Additional patients were then entered at the next lowest dose level of topotecan, 0.75 mg/m², per day for 72 h followed by doxorubicin, 45 mg/m², i.v. bolus. One of 11 patients entered at this level experienced DLT (grade 4 thrombocytopenia). Patients entered at this dose-level had a median of one prior chemotherapy regimen (range, 0–3). The MTD of the combination with G-CSF was, therefore, defined as topotecan, 0.75 mg/m² per day for 72 h, followed by doxorubicin, 45 mg/m², i.v. bolus. At this MTD with G-CSF, 50 cycles were delivered to 11 patients. One patient required a dose reduction on cycle 6, whereas three patients were dose-escalated to topotecan 1.0 mg/m² per day for 72 h; however, these patients subsequently required dose reductions on cycles 5, 6, and 7, respectively.

In the absence of G-CSF, sequential therapy with topotecan and doxorubicin resulted in a median time to the neutrophil nadir of 20 days, and the platelet nadir occurred at a median of 16 days. The addition of G-CSF shortened the median time to the WBC and granulocyte nadir to 12 days each. As expected, the use of G-CSF did not change the median number of days to the platelet nadir, which occurred on day 15. Ten of 11 patients treated at the MTD with G-CSF could be treated on day 22. The worst hematological toxicities encountered at each dose level for cycle 1 and overall cycles are summarized in Table 3. A total of 126 cycles were administered, of which 9 cycles were complicated by febrile neutropenia in eight patients.

Nonhematological Toxicity. Nonhematological toxicity was common but mild using this combination and schedule. Three patients developed dose-limiting grade 3 fatigue with their first cycle. This occurred in two patients at the dose level of topotecan, 1.0 mg/m², per day for 72 h and doxorubicin, 45 mg/m², i.v. bolus with G-CSF, and in one patient at the MTD of topotecan 0.35 mg/m² per day for 72 h and doxorubicin 45 mg/m² without G-CSF.

Fatigue was the most common nonhematological toxicity observed over multiple cycles of treatment and required dose reduction in five patients. Nausea occurred in 21 patients with vomiting in 14 patients, despite the use of antiemetics. One patient developed grade 3 stomatitis requiring dose reduction. One patient experienced a reduction in the left ventricular ejection fraction to 32% from a baseline of 55% after a total of 135 mg/m² doxorubicin on this protocol and a 435-mg/m² lifetime total dose of doxorubicin. A central venous access device led to complication by febrile neutropenia in one patient. Microscopic hematuria was detected in one patient. The nonhematological toxicities observed overall cycles are summarized in Table 4.

Antitumor Responses. Thirty-one patients had disease sites that were evaluable for antitumor response. Two patients with metastatic breast cancer and one patient with SCLC attained objective partial responses. Both patients with breast

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**Table 3** Hematological toxicity by dose level for cycle 1 and all cycles

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Granulocytes</th>
<th>Platelets</th>
<th>Hemoglobin</th>
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<td>Cycle 1</td>
<td>All cycles</td>
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<td>1</td>
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<td>2 + G</td>
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<td>3 + G</td>
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<td>18</td>
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<td>3 + G</td>
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* Numbers represent percentage of patients.

a Includes patients who were dose escalated on subsequent cycles.
cancer had received no prior chemotherapy, whereas the patient with SCLC had received prior cisplatin and etoposide. Three minor responses were also observed in patients with breast cancer, primary unknown, and transitional cell carcinoma of the bladder.

Bone Marrow CD34+ Analysis. The effects of topotecan on the bone marrow CD34+ hematopoietic progenitor cells were evaluated by flow cytometry. The topotecan infusion was found to reduce bone marrow cellularity by more than 50% ($P < 0.005$). Furthermore, within that reduced population, the average frequency of CD34+ cells was reduced by 35% ($P < 0.02$). In 10 patients in which the frequency of S/G2-M cells could be evaluated, the overall percentage of CD34+ cells in S/G2-M increased from 16.6 ± 2.9% prior to topotecan to 25.0 ± 3.5% after topotecan ($P < 0.02$). This corresponded to an increase in the percentage of CD34+ cells in the cell cycle in seven patients, little change in two patients, and a decrease in only one patient. Of the four patients experiencing dose-limiting thrombocytopenia in which the CD34+ populations could be evaluated, three showed an increase in the percentage of CD34+ cells in S/G2-M, and one showed a decrease.

DISCUSSION

In this study, we determined the MTD of topotecan and doxorubicin using a novel sequential schedule of the topoisomerase I inhibitor, topotecan, followed by the topoisomerase II inhibitor, doxorubicin. The sequential schedule was chosen based on preclinical data that demonstrated synergy both in vitro and in vivo using this sequence (13). In this trial, we observed unexpected dose-limiting granulocyte toxicity using this sequence at a dose level that represented 31% of the MTD. This corresponded to an increase in the percentage of CD34+ cells in the cell cycle in seven patients, little change in two patients, and a decrease in only one patient. Of the four patients experiencing dose-limiting thrombocytopenia in which the CD34+ populations could be evaluated, three showed an increase in the percentage of CD34+ cells in S/G2-M, and one showed a decrease.

To examine the possible mechanism of this toxicity, we analyzed hematopoietic progenitors in bone marrow aspirates obtained prior to topotecan compared to aspirates obtained immediately prior to doxorubicin administration. Topoisomerase II inhibitors are most cytotoxic to cells in S phase when the topoisomerase II enzyme levels are highest (9). The observed increase in the S/G2-M phases of bone marrow CD34+ cells following topotecan treatment may in part be responsible for the unexpected hematological toxicity observed at low dose levels of this sequence and combination and corresponds to the tumor changes observed in preclinical models (13). The use of G-CSF allowed further dose escalation; however dose-limiting thrombocytopenia was observed within a 2-fold dose-escalation of topotecan and at 47% of the single-agent topotecan MTD without G-CSF. These observations point to possible synergistic toxicity to pluripotent progenitors with this sequence. Whether the increased hematological toxicity with this sequential administration of topotecan and doxorubicin would also be associated with increased antitumor activity is not known.

Topotecan using the same 72-h infusion schedule followed by etoposide has also been reported in preliminary reports to be associated with unexpected hematological toxicity limiting the dose-escalation of topotecan (18). Other topotecan combination Phase I studies using different schedules have demonstrated unexpected hematological toxicity. Lilenbaum et al. (19) observed dose-limiting neutropenia with the combination of topotecan with doses of paclitaxel (80 mg/m² over 3 h) not generally considered particularly myelosuppressive. Further dose-escalation of paclitaxel to clinically relevant doses with this combination required the use of G-CSF. Similarly, dose-escalations of the combination of topotecan with cisplatin in two studies were compromised by neutropenia and thrombocytopenia and limited to 50 and 67% of the single-agent MTD dose of topotecan (20, 21). Pharmacological interactions from the delayed clearance of topotecan when immediately preceded by cisplatin may partly account for the increased hematological toxicity observed with this combination (20).

Alterations in drug clearance were not examined in our study of topotecan and doxorubicin. However, the short half-life of topotecan and the 24-h washout interval between the administration of topotecan and doxorubicin would be expected to lessen any potential pharmacological interactions. Three partial responses were observed in this study at topotecan doses of 0.5 and 0.75 mg/m² per day for 72 h and doxorubicin, 45 mg/m², in breast cancer and SCLC.

Single-agent antitumor activity of topotecan has been demonstrated to date in both SCLC, transitional cell carcinoma of the bladder, and breast cancer; doxorubicin has demonstrated activity in these diseases, making them good candidates for Phase II testing of this combination and sequence. Anthracycline topoisomerase II inhibitors are used in the treatment of acute leukemias and lymphomas, and preliminary antileukemic and antilymphoma activity of topotecan has been demonstrated recently (22, 23). This combination and sequence is, therefore, worthy of Phase II exploration in both solid tumors and hematopoietic malignancies.

REFERENCES


A phase I study of topotecan followed sequentially by doxorubicin in patients with advanced malignancies.
