A Phase I and Translational Study of Sequential Administration of the Topoisomerase I and II Inhibitors Topotecan and Etoposide


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ABSTRACT

Because topoisomerase (topo) I- and topo II-targeting agents exert their principal effects on the two major classes of enzymes involved in regulating DNA topology in the cell, there has been considerable interest in evaluating combinations of these classes of agents. In preclinical studies of inhibitors of topo I and topo II in combination, drug scheduling and sequencing have been critical determinants of antitumor activity, with a greater magnitude of cytotoxicity generally occurring when treatment with the topo I inhibitor precedes treatment with the topo II-targeting agent. The underlying mechanism that has been proposed to explain this schedule dependency is compensatory up-regulation of topo II and, therefore, enhanced cytotoxicity of topo II inhibitors in cells treated initially with topo I inhibitors. The feasibility of sequentially administering the topo I inhibitor topotecan (TPT) followed by the topo II inhibitor etoposide to patients with advanced solid malignancies was evaluated in this Phase I and translational laboratory study. Fifty patients with solid neoplasms were treated with TPT doses ranging from 0.17 to 1.05 mg/m²/day as a 72-h continuous (i.v.) infusion on days 1–3 followed by etoposide, 75 or 100 mg/m²/day as a 2-h i.v. infusion daily on days 8–10. The combined rate of severe neutropenia and thrombocytopenia was unacceptably high above the TPT (mg/m²/day)/etoposide (mg/m²/day) dose levels of 0.68/100 and 0.68/75 in minimally and heavily pretreated patients, respectively, and these dose levels are recommended for further disease-directed evaluations of TPT/etoposide on this administration schedule. Successive biopsies of accessible tumors were obtained for determination of topo I and II levels prior to and immediately after treatment with TPT and prior to and immediately after treatment with etoposide in seven patients. The results of these limited studies in tumors did not fully support the proposed mechanistic rationale favoring the development of this particular sequential TPT/etoposide regimen, because only two of the six patients' tumors in whom topo I was successively measured had either modest or substantial decrements in topo I levels following treatment with TPT, and the principal effect of interest, up-regulation of topo II following treatment with TPT, was clearly documented in the tumors of only one of six subjects in whom successive measurements of topo I were performed. Even in view of the notable objective antitumor activity in three subjects, including a complete response in a patient with colorectal carcinoma and partial responses in one patient each with non-small cell lung and gastric carcinomas, the toxicity and ancillary laboratory results do not provide substantial evidence that sequential treatment with TPT and etoposide might be more advantageous than either TPT or etoposide administered as a single agent.

INTRODUCTION

TopoI and topo II are essential nuclear enzymes that modulate the topological state of chromatin DNA by inducing transient single- and double-strand breaks, respectively, and alleviating DNA supercoiling produced during DNA replication and recombination, RNA transcription, and chromosomal decondensation (1–4). Following induction of DNA strand breaks and formation of covalent “cleavable” topo-DNA complexes, the torsional strain in DNA is alleviated via strand passage, and enzymatic reunion occurs (5–9). There is evidence that topo II can function like topo I, albeit less efficiently (10, 11). For example, yeast cells that are completely devoid of topo I can readily propagated, indicating that either topo I or topo II is required for DNA replication (12–14). In addition, high topo II levels and enzymatic activity have been demonstrated to in-

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crease in tumor cells with acquired resistance to topo I-directed agents. This effect is associated with hypersensitivity to topo II inhibitors (15).

Because topo I- and topo II-targeting agents exert their principal effects on two major classes of enzymes involved in regulating DNA topology, which may overlap functionally, there has been considerable interest in combining these two classes of agents. In preclinical studies, combined treatment of neoplasms with topo I and topo II inhibitors has yielded mixed results. The cytotoxic effects of treating cells with the topo I-targeting agents and etoposide simultaneously have been less than additive in a number of cell lines, including hamster lung fibroblasts, HT-29 human colon carcinoma, and human leukemia cell lines (16, 17). One possible explanation for this antagonism is that topo I-targeting agents inhibit nucleic acid synthesis that is required to convert topo II-DNA adducts to cytotoxic lesions (16, 18, 19). On the other hand, the cytotoxic effects of sequential administration of topo II inhibitors or other CPT or its hydrophilic analogue TPT (9-dimethylaminomethyl-10-hydroxycamptothecin) have been at least additive and sometimes synergistic in hamster fibroblasts in vitro, as well as cell lines derived from human leukemia, colon carcinoma, and a variety of other tumor types (18–20). Similar schedule-dependent effects have been noted when CPT analogues are combined with topo II inhibitors in vivo (21, 22). For example, simultaneous administration of TPT and etoposide was no more effective in a murine leukemia model than either drug alone, whereas sequential administration was synergistic (21). Similarly, combinations of the topo I inhibitor irinotecan and the topo II-directed agent doxorubicin failed to demonstrate synergy when the two agents were administered simultaneously, but the administration of irinotecan prior to doxorubicin was synergistic (23, 24). It has been proposed that this synergy might be due to compensatory up-regulation of topo II levels and, therefore, enhanced cytotoxicity of topo II inhibitors in cells treated initially with topo I inhibitors (25).

To date, TPT has demonstrated prominent activity in patients with recurrent or refractory ovarian and small cell lung carcinomas, as well as preliminary activity in many other adult and pediatric malignancies that are somewhat responsive to topo II-directed agents, including non-small cell lung carcinoma, malignant glioma, neuroblastoma, rhabdomyosarcoma, multiple myeloma, lymphoma, leukemia, and sarcoma (26–38). Given the promising results with TPT and other CPT analogues as single agents in Phase I, II, and III trials, efforts directed at determining the optimal means to incorporate these agents into combination regimens are rational at this juncture in their development. This Phase I study evaluated the feasibility of sequential administration of the topo I inhibitor TPT as a 72-h continuous i.v. infusion on days 1–3 at a starting dose of 0.17 mg/m²/day, which was associated with minimal toxicity in a previous Phase I study of TPT as a single agent on this administration schedule (40). Dose escalation was based on a modified Fibonacci scheme and principally involved TPT, which was increased in successive cohorts of new patients to 0.34, 0.51, 0.68, 0.85, and 1.05 mg/m²/day. Etoposide was administered as a 2-h i.v. infusion daily on days 7, 8, and 9. Treatment was administered every 3–4 weeks. Initially, the etoposide dose was to be fixed in all of the patients at 100 mg/m²/day; however, the dose was subsequently decreased to 75 mg/m²/day in heavily pretreated patients due to unacceptable toxicity in such patients treated with etoposide at the higher dose. At least three new patients were treated at each escalated dose level. Intrapatient dose escalation was permitted if a patient had at least stable disease after completing three courses of TPT/etoposide and if one new patient had completed treatment at the next higher dose without DLT. Dose reductions by one level were permitted for patients experiencing DLT. If one of three new patients at any dose level experienced DLT, then a maximum of six new patients were treated at that dose level. The MTD or recommended Phase II dose was defined as the highest dose level that induced DLT in fewer than one-third of new patients (at least two of a maximum of six new patients). DLT was defined as any one of the following events occurring during the first course of treatment: (a) nonhematological toxicity of grade 3–4 severity (except nausea and vomiting associated with suboptimal pharmacological prophylaxis and/or management), (b) ANC <500/μl (grade 4 neutropenia) lasting longer than 5 days, (c) ANC <500/μl associated with fever requiring hospitalization for parenteral antibiotics, (d) hemoglobin ≤6.5 mg/dl, and (e) platelets ≤25,000/μl (grade 4 throm-
Tumor cells were stored frozen at \( -80^\circ \text{C} \) in vials that contained 5 mg of TPT as the base and 100 mg of mannitol per vial. TPT was reconstituted with 2 ml of sterile water to a concentration of 2.5 mg/ml TPT base equivalent. The pH was adjusted to 3.0 with HCl and NaOH. After reconstitution, the solution was diluted with 100–500 ml of 5% dextrose solution, which was infused over 24 h for 3 consecutive days using a Walkmed (Medfusion, Inc., Duluth, GA) ambulatory pump. Etoposide was supplied as the commercially available Vespid (Bristol-Myers Squibb, Princeton, NJ) in 5-ml vials containing 100 mg of drug, 40 mg of polysorbate 80, 3.25 g of polyethylene glycol 300, 10 mg of anhydrous citric acid, and 150 mg of benzyl alcohol (30.5% alcohol). The appropriate dose, which was diluted with 500 ml of 5% dextrose solution to achieve a drug concentration below 0.5 mg/ml to avoid precipitation in aqueous solutions, was infused i.v. over 2 h. Prophylactic antiemetics were not administered routinely.

**Pretreatment and Follow-up Studies.** Histories, physical examinations, performance status assessments, and routine laboratory studies were performed pretreatment and weekly after treatment. Routine laboratory studies included a complete blood cell count, differential WBC count, electrolytes, blood urea nitrogen, creatinine, glucose, total protein, albumin, calcium, phosphate, total and direct bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, prothrombin and partial thromboplastin times, and urinalysis. A chest X-ray and electrocardiogram were obtained pretreatment and before each course of therapy. Tumor measurements were recorded at baseline and after every two courses. Patients were able to continue treatment if they did not develop progressive disease. The outcome was scored a CR if there was disappearance of all active disease on two measurements separated by a minimum period of 4 weeks, and a PR required at least a 50% reduction in the sum of the products of measurable disease documented by two measurements separated by at least 4 weeks. Progressive disease was defined as at least a 25% increase in the sum of the products of the bidimensional measurements of all measurable disease.

**Topo I and II.** Successive tumor samplings were to be obtained pretreatment, after treatment with TPT (day 3), prior to treatment with etoposide (day 7), and after treatment with etoposide (day 10) in patients with tumors that were amenable to repeated biopsies. Tumor cells from malignant effusions or pleural tissue, as determined by morphological criteria, were separated on a Ficoll Hypaque gradient (Sigma Chemical Co., St. Louis, MO), and cell viability was determined by trypan blue dye exclusion. Tumor cells were stored frozen at \(-70^\circ\text{C}\) in nutrient medium (RPMI 1640 plus 10% DMSO).

### RESULTS

Fifty patients, whose characteristics are displayed in Table 1, received 151 total courses of treatment through six dose levels (Table 2). Seventeen (34%) patients had advanced non-small cell lung cancer. The numbers of new and total patients and courses at each dose level, as well as the corresponding DLTs, are shown in Table 2. The median number of courses administered per patient was 2 (range, 1–14). All patients and 42 of 151 courses were fully evaluable. All nine evaluable courses were due to the fact that the schedule of laboratory evaluations did not conform to the protocol guidelines. All first courses were evaluable for toxicity. Four patients required dose reductions.
due to severe unacceptable myelosuppression. Both severe neutropenia and thrombocytopenia were the principal DLTs of this sequential TPT/etoposide regimen as shown in a function of dose level in Table 2. These toxicities were consistently intolerable above TPT (mg/m²)/etoposide (mg/m²) dose levels of 0.68/100 for minimally pretreated patients and 0.68/75 for heavily pretreated patients.

**Hematological Toxicity.** Both severe neutropenia and thrombocytopenia were the principal DLTs of sequential treatment with TPT and etoposide. The effects of this regimen on ANC and platelet counts are displayed in Table 3. There was no evidence of cumulative hematological toxicity in patients treated repeatedly at the same dose level. The onsets of neutropenia and thrombocytopenia were relatively late, with ANC and platelet count nadirs typically observed on days 16–22 and 15–19, respectively. At the 0.85/100 mg/m²/day dose level, there were two episodes of dose-limiting myelosuppression during course 1, including grade 4 neutropenia lasting 7 days and grade 4 thrombocytopenia resulting in a fatal gastrointestinal hemorrhage. At this dose level, ANC nadirs were below 500/μl in five of six minimally pretreated patients during 8 of 17 total courses. Five of six patients experienced grade 4 neutropenia during course 1. Four of these five patients received further treatment at this dose level without evidence of cumulative toxicity. With regard to severe thrombocytopenia, platelet counts decreased to below 25,000/μl in two of six minimally pretreated patients during 2 of 17 courses. A single minimally pretreated patient who was treated at the next highest TPT/etoposide dose level, 1.05/100 mg/m², also experienced prolonged, dose-limiting neutropenia and grade 4 thrombocytopenia during course 1. The next lower TPT/etoposide dose level (0.68/100 mg/m²/day) was much better tolerated by minimally pretreated patients. AlthoughANCs decreased to below 500/μl in two of six minimally pretreated subjects treated with TPT/etoposide 0.68/100 mg/m² day, no individual experienced DLT out of a total of 21 courses.

<table>
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<th>TPT (mg/m²/day)</th>
<th>Etoposide (mg/m²/day)</th>
<th>No. of patients (new)</th>
<th>No. of courses</th>
<th>ANC &lt;500/μl for &gt;5 days</th>
<th>ANC &lt;500/μl + fever</th>
<th>Platelets &lt;25,000</th>
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<th>New patients with any DLT: total new patients</th>
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* min, minimally pretreated; hvy, heavily pretreated. Heavily pretreated is defined as: (a) at least three prior chemotherapy regimens, (b) irradiation to >10% of bone marrow, or (c) at least two prior chemotherapy regimens consisting of carboptin or nitrosoureas.

* nonhemc, nonhematological toxicity.

* Patient expired during neutropenic sepsis.

* Patient expired due to a gastrointestinal hemorrhage.

Heavily pretreated patients were much more prone to developing severe hematological toxicity compared to minimally pretreated patients as demonstrated by the development of grade 4 neutropenia in three of six heavily pretreated new patients (including two episodes during course 1) at the first dose level, in which a relatively low dose of TPT, 0.17 mg/m²/day, was administered in combination with etoposide, 100 mg/m²/day. The occurrence of this severe toxicity in heavily pretreated patients at such a low TPT dose indicated that the prospects for further dose escalation were limited, and, therefore, the dose of etoposide was decreased to 75 mg/m²/day in heavily pretreated patients to permit further TPT dose escalation. The duration of grade 4 neutropenia at dose levels in which TPT doses were below 0.85 mg/m²/day was always brief (<5 days); however, one heavily pretreated patient treated at the TPT/etoposide dose level of 0.34/75 mg/m²/day developed a small bowel obstruction due to progressive disease and grade 4 neutropenia and fever, which resulted in his expiration on day 22. Dose-limiting thrombocytopenia was never noted during course 1 in heavily pretreated patients treated with TPT doses below 0.85 mg/m²/day. However, dose-limiting effects were consistently observed in heavily pretreated patients treated with TPT/etoposide at the 0.85/75 mg/m²/day dose level. Six of seven patients experienced grade 4 neutropenia during course 1, of which two episodes were dose limiting. One patient had an ANC nadir below 500/μl for more than 5 days; another subject developed severe neutropenia with fever requiring hospitalization for parenteral antibiotics and subsequently died due to sepsis on day 16. Platelet counts were less than 25,000/μl in three heavily pretreated individuals treated at this dose level. The next lower TPT/etoposide dose level, 0.68/75 mg/m², was much better tolerated by heavily pretreated patients with no dose-limiting neutropenia experienced in 11 total courses. There was one episode of dose-limiting thrombocytopenia at this dose level.

Severe effects of this regimen on the RBCs were frequently observed. Anemia was cumulative and generally more common...
in patients treated at the higher dose levels. Similar to the effects of TPT/etoposide on ANC and platelet counts, heavily pretreated individuals were much more prone to the development of severe anemia. Three heavily pretreated patients developed grade 4 anemia (hemoglobin ≤6.5 mg/dl) at relatively low TPT/etoposide dose levels (0.17/100 mg/m²/day (one patient during course 1 and a second patient during course 4) and 0.51/75 mg/m²/day (one patient during course 2)), whereas anemia of this severity was not observed in minimally pretreated patients. Overall, 18 of 50 (36%) patients, including 9 of 25 (36%) heavily pretreated and 9 of 25 (36%) minimally pretreated patients, experienced either grade 3 or 4 anemia at some time during treatment.

Nonhematological Toxicity. Except for nausea and vomiting, nonhematological toxicities associated with this TPT/etoposide regimen were generally mild to moderate in both minimally and heavily pretreated patients. Nausea and/or vomiting occurred in 74% of patients, the majority of whom did not receive prophylactic treatment with antiemetics. Typically, symptoms worsened progressively until the 3rd day of TPT treatment, abated on day 5 or 6, and then resumed during treatment with etoposide on days 7–9. Severe nausea and/or vomiting responded well following treatment with parenteral hydration, dexamethasone, and/or serotonin antagonists. Nausea and/or vomiting were dose limiting (grade 4) in two instances; one event was experienced by a minimally pretreated patient at the 0.51/100 mg/m²/day dose level. The second occurred in a heavily pretreated patient at 0.85/75 mg/m²/day dose level. Other relatively common nonhematological adverse effects of mild to moderate severity included anorexia in 14 of 50 (28%), fatigue in 13 of 50 (26%), and both mild diarrhea and fever in the TPT pretreatment period in 6 of 50 (12%).

Tumor topo Studies. The effects of this sequential regimen of TPT followed by etoposide on intratumoral levels of topo I and II in seven subjects who had successive biopsies performed are depicted in Figs. 1–4. The specimens were adequate to permit successive measurements of topo I and II in six and four patients, respectively. Representative Western blots for topo I and II are shown in Figs. 3 and 4. With regard to the effects of the 72-h continuous infusion of TPT treatment on topo I, topo I was essentially unchanged in three subjects and decreased in the other three subjects on day 3 (or day 4) to 32% (ovarian cancer), 60% (non-small cell lung cancer), and 90% (non-small cell lung cancer) compared to pretreatment levels. On day 7, 3 days following TPT and immediately before treatment with etoposide, topo I levels had either returned to pretreatment levels (all three subjects with decrements documented on day 3) or increased relative to pretreatment values (all three subjects with no change documented on day 3). Interestingly, treatment with etoposide resulted in decrements in topo I from day 7 to day 10 in all of the subjects.

The hypothesis that topo I inhibition due to treatment with TPT would possibly result in a compensatory increase in topo II, thereby increasing the susceptibility of tumors to subsequent treatment with etoposide, was evaluated in successive biopsies from four patients (Figs. 2 and 4). Contrary to this hypothesis, topo II levels did not increase in any tumor evaluated immediately following TPT treatment (day 4). Instead, topo II levels were either unchanged (three patients) or lower (one subject) compared to pretreatment values. Immediately prior to treatment with etoposide (day 7), topo II levels increased modestly (30%) in only one patient, whereas levels were either unchanged or moderately lower in the other subjects. Treatment with etoposide resulted in only a modest decrement (17%) in topo II levels relative to pretreatment in one individual, whereas levels were either modestly higher or unchanged in the other three patients.

Antitumor Activity. Three patients experienced major antitumor responses. A 35-year-old male with colorectal carcinoma whose metastatic disease involving the liver had progressed during prior treatment with chemotherapy regimens consisting of both 5-fluorouracil/leucovorin and 5-fluorouracil/mitomycin C, experienced a CR lasting 10 months following treatment with TPT/etoposide at the 0.68/75 mg/m² dose level.

### Table 3 Hematological toxicities

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<th>Dose level</th>
<th>Etoposide (mg/m²/day)</th>
<th>No. of patients (new)</th>
<th>No. of courses</th>
<th>ANC &lt;1,000</th>
<th>ANC &gt;500/µl</th>
<th>ANC &lt;500/µl &gt;5 days</th>
<th>ANC &lt;25,000/µl fever</th>
<th>Platelets &gt;50,000/µl</th>
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* min, minimally pretreated; hvy, heavily pretreated. Heavily pretreated is defined as: (a) at least three prior chemotherapy regimens, (b) irradiation to >10% of bone marrow, or (c) at least two prior chemotherapy regimens consisting of carboplatin or nitrosoureas.

^ Patient expired during neutropenic sepsis.

^ Patient expired due to a gastrointestinal hemorrhage.
carcinoma and hepatic metastases had a PR lasting 8 months following treatment with TPT/etoposide at the 0.68/100 mg/m²/day level. Finally, a PR lasting 6 months was observed in a 74-year-old male with non-small cell lung cancer with metastases following treatment with TPT/etoposide at the 0.85/100 mg/m²/day dose level. The patient’s disease had progressed during prior chemotherapy with docetaxel. Minor responses were also noted in a 26-year-old heavily pretreated male with a metastatic rhabdomyosarcoma whose malignant ascites, obstructive nephropathy, and tumor-related peripheral edema partially resolved for 8 months and in a 65-year-old male with 5-fluorouracil-refractory colorectal carcinoma who experienced complete resolution of malignant ascites that lasted 4 months.

DISCUSSION

Both topo I and II inhibitors have broad antitumor activity and are active as single agents in the treatment of a wide array of adult and pediatric solid and hematological malignancies (47, 48). Because topo I and II-targeting agents exert their principal effects on the two major classes of enzymes involved in regulating DNA topology within the cell, there has been considerable interest in the effects of combining these two classes of agents. In preclinical combination studies of these two classes of agents, drug scheduling and sequencing appeared to be critical determinants of antitumor activity (16, 17, 21–23). The cytotoxic effects of treating cells with topo I-targeting agents and etoposide simultaneously have been noted to be less than additive in several different tissue culture cell lines (16, 17). In contrast, the cytotoxic effects have been at least additive when CPT analogues and etoposide are administered sequentially (21–23). This study was performed to determine the feasibility of sequentially administering the topo I inhibitor TPT followed by the topo II inhibitor etoposide (TPT/etoposide) in adults with advanced solid neoplasms. In addition to traditional Phase I objectives such as characterizing the principal toxicities and MTDs and recommending doses for subsequent Phase II studies, the study also sought to document the sequential behavior of topo I and II, particularly whether TPT treatment results in topo I and II up-regulation in malignant tumors during treatment with TPT/etoposide.

Myelosuppression, consisting of both neutropenia and thrombocytopenia, was the principal DLT of sequential treatment with TPT as a continuous 72-h i.v. infusion followed 3 days later by etoposide administered as a brief infusion i.v. daily for 3 consecutive days. The combined rate of severe neutropenia and thrombocytopenia was unacceptable above the TPT/etoposide dose levels of 0.68/100 and 0.68/75 mg/m² in minimally and heavily pretreated patients, respectively, and these dose levels are recommended for further disease-directed evaluations. The recommended dose of TPT in this regimen is 42.5% of the MTD dose of single-agent TPT on an identical administration schedule. In another Phase I study of the TPT/etoposide regimen that used an alternate sequential schedule, in which patients with solid malignancies were treated with escalating doses of TPT as a 30-min infusion daily for 5 consecutive days (days 1–5) followed by a fixed dose of oral etoposide 20 mg daily on days 5–15 every 4 weeks, severe, prolonged neutropenia was the principal toxicity, and the MTD for TPT was 1.0 mg/m²/day (49). Although the MTD and recommended Phase II doses of TPT in both TPT/etoposide regimens are substantially lower than the single-agent MTDs and recommended Phase II doses for TPT given as a single agent on an identical administration
The results of these studies did not reveal a substantial compensatory increase in intratumoral topo II levels at the time of treatment with etoposide in patient labeled 0 in Fig. 1. Gels were loaded with polypeptides from $5 \times 10^5$ non-small cell lung cancer cells/lane for a representative patient treated at TPT/etoposide 0.68/100 mg/m²/day. After SDS-PAGE and transfer to nitrocellulose, samples were treated with antibodies to topo I. VP-16, etoposide.

Fig. 3 Western blots depicting relative levels of topo I in tumor biopsies sampled before treatment with TPT, posttreatment with TPT, before treatment with etoposide, and posttreatment with etoposide in patient labeled 0 in Fig. 1. Gels were loaded with polypeptides from $6 \times 10^6$ colon carcinoma cells/lane for a representative patient treated at TPT/etoposide 0.85/100 mg/m². After SDS-PAGE and transfer to nitrocellulose, samples were treated with antibodies to topo I. VP-16, etoposide.

Fig. 4 Western blots depicting relative levels of topo II in tumor biopsies sampled before treatment with TPT, posttreatment with TPT, before treatment with etoposide, and posttreatment with etoposide in patient labeled 0 in Fig. 2. Gels were loaded with polypeptides from $6 \times 10^6$ colon carcinoma cells/lane for a representative patient treated at TPT/etoposide 0.85/100 mg/m². After SDS-PAGE and transfer to nitrocellulose, samples were treated with antibodies to topo II. VP-16, etoposide.

schedule, the potential synergistic effects of these two agents, which should be considered, may render such dosing comparisons irrelevant (26–38). At least two Phase I studies of sequential TPT and etoposide have been performed in adults with refractory acute leukemia. The National Cancer Institute of Canada conducted a Phase I trial of sequential TPT administered as a continuous 5-day i.v. infusion followed by etoposide as a brief i.v. infusion given daily on days 6–8 in adults with acute myeloid leukemia (50). In this study, Crump et al. (50) reported that grade 3–4 mucositis precluded further dose escalation above TPT/etoposide 1.5/100 mg/m²/day, which was the recommended Phase II dose of this regimen. Severe (grade 4) neutropenia and thrombocytopenia also occurred during all of the courses. Similarly, Cooper et al. (51) treated patients with relapsed leukemia with continuous 72-h infusions of TPT followed 24 h later by 5 consecutive days of etoposide, 100 mg/m²/day, as a brief i.v. infusion. As expected, grade 4 neutropenia and thrombocytopenia occurred in all of the patients, but several patients who were treated with TPT at the 1.1 mg/m²/day dose level also developed severe mucositis and hyperbilirubinemia. In both studies, CRs, albeit transient, were observed in drug-refractory patients; however, alternate drug administration schedules have not been evaluated to date.

The present study presented an opportunity to evaluate the effects of the sequential TPT/etoposide regimen on topo I and II levels in the actual target malignant tissues of patients with solid neoplasms instead of in surrogate tissues (e.g., PBMCs). Although the actual numbers of tumors that were successively biopsied were small, the results of these laboratory studies do not fully support the principal proposed mechanistic rationale for evaluating this particular sequential TPT/etoposide regimen. The results of these studies did not reveal a substantial compensatory increase in intratumoral topo II levels at the time of treatment with etoposide. In fact, only two of the six tumors of subjects in whom topo I was successively measured had either modest or substantial decrements in topo I levels, and the principal effect of interest, topo II up-regulation in response to treatment with TPT, was documented in only one subject. Several investigators have consistently observed decrements in topo I in PBMCs of patients during treatment with TPT. Perhaps the most profound example of this phenomenon was reported by Hochster et al. (52), who demonstrated that the percentage of free topo I in PBMCs progressively decreased during weeks 1, 2, and 3 in solid tumor patients treated with TPT as a continuous 21-day i.v. infusion. However, the median percentage decrease in free TPT was modest after 1 week of treatment (median, 26%), and these effects were not significant until weeks 2 and 3 [median percentages of change, 45% (P = 0.10) and 77% (P = 0.016); Ref. 52]. The depletion of topo I was shown to be due, in part, to the formation of high molecular weight cleavable complexes and not solely to down-regulation in its production.

Another possible explanation relates to a phenomenon described by Danks et al. (53), in which topo I undergoes translocation from the nucleus to the cytoplasm during treatment with low concentrations of TPT. Collectively, the results of these studies, particularly those of Hochster et al. (52), suggest that the use of protracted continuous schedules of TPT may be optimal in clinical studies designed to evaluate whether or not treatment with TPT sufficiently decreases topo I levels, resulting in intratumoral topo II up-regulation and increased sensitivity to topo II-targeting agents.

Another factor that should be considered with regard to the lack of a substantial increase in topo II levels following treatment with TPT is the possibility that maximal TPT-induced effects on intratumoral topo I and II may not have been detected due to the specific tissue acquisition scheme used in this study. Tumor biopsies were not obtained in the 3-day period between the end of the TPT treatment and the beginning of etoposide treatment. Indeed, studies by Whitacre et al. (22) have indicated that the duration of the treatment interval between TPT and etoposide may be critical for maximal activity of this regimen. Whitacre et al. treated athymic mice bearing SW 480 human colon cancer xenografts with simultaneous, immediately sequential, and a more widely dispersed sequential administration of TPT and etoposide. Simultaneous drug administration resulted in cytotoxic antagonism in vitro, whereas inhibition of topo I by TPT resulted in a compensatory increase in intratumoral topo IIa that was associated with increased sensitivity of...
tumors to subsequent treatment with the topo II inhibitor etoposide. Furthermore, topo II levels declined 5 days after the last dose of TPT, resulting in the restoration of the original response of the xenograft to etoposide (22). However, topo II up-regulation was maximal at the end of TPT treatment and progressively declined following treatment, indicating that the tissue sampling scheme in the present study was rational from a biological standpoint. Nevertheless, the report by Whitacre et al. (22) emphasizes the critical role of drug scheduling to optimize combination chemotherapy with topo I and II inhibitors. Thus, treatment with TPT immediately before etoposide might be optimal for producing the desired magnitude of regulation of both topo I and II at the time of treatment with etoposide.

Although the present study was the first to evaluate the effects of sequential TPT/etoposide treatment on topo I and II levels in biopsy samples obtained successively during the course of treatment in patients with advanced solid neoplasms. Crump et al. (50) performed similar assessments in the blasts of patients with refractory leukemia who were treated with TPT as a 5-day continuous i.v. infusion on days 1-5 followed by etoposide as a brief i.v. infusion daily on days 6-8. Topo II levels increased in the peripheral blasts within 72 h of starting TPT and returned to near baseline by day 5, whereas topo II levels appeared to decrease in bone marrow blasts on day 5. These observations were coincident with a lower proportion of cells in G0 versus G1 at the end of treatment, although the death and cytolysis of leukemia blasts that undergo topo I depletion may confound the interpretation of such data.

Although the development of sequential TPT/etoposide treatment regimens in malignancies in which both agents have demonstrated significant activity, such as small cell lung carcinoma, and ovarian carcinoma, and leukemia, might be rational from a clinical standpoint, sequential TPT/etoposide regimens have a toxicity profile similar to those of other conventional multiagent regimens used to treat patients with both solid and hematological malignancies. Even in view of the notable objective antitumor activity observed in several patients in this study, the toxicity and efficacy data generated in clinical trials to date fail to provide evidence that sequential TPT/etoposide treatment might be substantially more advantageous than TPT or etoposide administered as single agents. Moreover, studies performed on successive biopsies of solid tumors at relevant times during treatment and paired bone marrow samples harvested before and after etoposide have failed to confirm the initial hypothesis that treatment with TPT will result in the up-regulation of topo II levels in malignant tissue. Instead, it may just as well be concluded that treatment with TPT results in decreased topo II levels in both solid tumors and leukemias in vivo based on the results of this and other pertinent clinical studies performed to date (50, 51). The significance of these findings for further examination of the TPT/etoposide combination remains to be determined.

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A phase I and translational study of sequential administration of the topoisomerase I and II inhibitors topotecan and etoposide.


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