Phase I Clinical and Plasma and Cellular Pharmacological Study of Topotecan without and with Granulocyte Colony-stimulating Factor

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ABSTRACT

Topotecan, a semisynthetic water-soluble analogue of camptothecin, inhibits human topoisomerase I (topo I). We performed a Phase I clinical and plasma pharmacological study of topotecan administered by 24-h continuous infusion without and with granulocyte colony-stimulating factor (G-CSF). We also measured topo I-DNA complexes in peripheral blood mononuclear cells (PBMCs) in an attempt to correlate formation of topo I-DNA complexes in patients treated with topotecan with toxicity and/or response. One hundred four courses of topotecan at doses of 2.5–15.0 mg/m² were administered to 44 patients with solid tumors. The maximum tolerated dose without G-CSF was 10.0 mg/m²; granulocytopenia was the dose-limiting toxic effect. The maximum tolerated dose could not be increased with G-CSF because of severe thrombocytopenia. Plasma pharmacology was obtained in 11 patients treated at 12.5 mg/m² and 15.0 mg/m². The topotecan lactone end-infusion plasma levels correlated strongly with the area under the curve. Lactone elimination was biexponential with a mean t₁/₂α of 28 min and a t₁/₂β of 3.8 h at 12.5 mg/m². Topo I-DNA complexes were measured before and after treatment in PBMCs from seven patients. Pretopotecan topo I-DNA complexes were available on two additional patients treated at 15 mg/m². The mean increase in topo I-DNA complexes at the end of the topotecan infusion was 1.25 times the pretreatment value. There was a statistically significant relationship (P = 0.02) between lack of disease progression and the level of topo I-DNA complexes measured in PBMCs before therapy. For Phase II studies of minimally treated adults with solid tumors, the recommended topotecan starting dose administered by 24-h continuous infusion is 10 mg/m² without G-CSF.

INTRODUCTION

Topotecan [(S)-dimethylaminomethyl-10-hydroxycamptothecin hydrochloride, NSC 609669, SK & F 1048641, a water-soluble analogue of camptothecin, is an inhibitor of human topo I (1)]. Like camptothecin, topotecan exerts its antitumor activity by stabilizing covalent topo I-DNA complexes that are thought to initiate a biochemical cascade leading to cell death (2–4). The antitumor activity of topotecan has been demonstrated in several animal models, including murine tumors that are relatively refractory to most established antineoplastic agents (5–7). Several Phase I studies performed in patients with solid tumors using various schedules of topotecan administration have been reported (8, 9) and summarized (10). In patients with nonhematological malignancies, neutropenia was the DLT regardless of the schedule of drug administration. Other reported adverse effects included thrombocytopenia, vomiting, alopecia, fatigue, anemia, and anorexia.

Because neutropenia was the major toxic effect encountered in Phase I trials of topotecan, and because the camptothecin analogues were shown to have significant dose-response effects, studies were initiated to increase the MTD of topotecan administered over 30 min daily for 5 days by using G-CSF. Saltz et al. (11) were not successful in this endeavor because of the rapid emergence of thrombocytopenia and fatigue. However, Rowinsky et al. (12) were able administer topotecan in doses of 2.0–2.5 mg/m² when G-CSF was initiated 24 h after the last dose of topotecan. Whether addition of G-CSF can significantly enhance the therapeutic index of topotecan is currently unknown.

In a series of recently completed and ongoing Phase II trials, topotecan has demonstrated antitumor activity in small cell lung cancer (13) and ovarian cancer (14). Most of the reported studies evaluating clinical efficacy have used short i.v. infusions for 5 consecutive days every 3 weeks. However, the activity of topotecan against human solid tumor xenografts increased significantly when the drug exposure was increased (15). This observation is consistent with data showing that cytotoxicity to camptothecin was increased in S-phase cells (16).

Because topo I is the cellular target for topotecan and inhibition of topo I is the probable mechanism by which topotecan exerts its cytotoxicity, many investigators suggested that the early clinical development of topotecan could be facilitated by

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The abbreviations used are: topo I, topoisomerase I; DLT, dose-limiting toxicity; MTD, maximum tolerated dose; G-CSF, granulocyte colony-stimulating factor; AGC, absolute granulocyte count; AUC, area under the curve; PBMC, peripheral blood mononuclear cell; CV, coefficient of variation.
developing assays that quantify the drug-target interaction in clinical material and their subsequent correlation with toxicity or response. Studies quantifying topo I levels in human material showed that the levels of the enzyme are higher in some tumor tissues than in normal tissues (17), supporting the hypothesis that the difference in topo I levels between tumor and normal tissues could be exploited to improve the therapeutic index of topotecan. In addition, topo I levels measured in tumors frequently correlated with cytotoxicity. For example, baby hamster kidney cells engineered to overexpress topo I are hypersensitive to camptothecin (18), and conversely, some camptothecin-resistant tumor cell lines express decreased levels of topo I (19). However, factors other than cellular topo I content can affect the degree of cytotoxicity observed after exposure to camptothecin or its analogues (20). Direct quantification of drug-induced topo I-DNA complexes in tumor or surrogate cells could circumvent this problem and potentially provide direct information for predicting individual responsiveness to topotecan or other topo I inhibitors.

In this report, we outline the clinical and pharmacological results of a Phase I study of topotecan administered by 24 h continuous infusion every 21–28 days without and with G-CSF. This schedule was chosen because of the cell cycle specificity of topotecan and the rapid plasma clearance of the active lactone form of the drug. We also sought to correlate topo I inhibition measured in PBMCs with topotecan end-infusion plasma concentrations, AUC, clinical toxicity parameters, and antitumor efficacy after exposure to topotecan.

PATIENTS AND METHODS

Patient Selection

All 44 patients had histologically confirmed advanced solid tumors refractory to standard treatment. Patients were eligible for treatment if they had Zubrod performance status ≤2, survival expectancy greater than 12 weeks, age > 15 years, AGC ≥1,500 cells/mm³, platelet count ≥150,000 cells/mm³, adequate liver function (bilirubin level ≤1.5 mg/100 ml), and adequate renal function (creatinine level ≤1.5 mg/100 ml). The patients could not have received chemotherapy or radiotherapy for 3 weeks before study entry and had to have recovered from the toxic effects of any prior therapy. Because cystitis was a significant toxicity reported in clinical trials of the parent compound camptothecin, patients with > 6 red blood cells/high-power field on urinalysis, a history of hemorrhagic cystitis, or prior pelvic irradiation were not eligible for the study. Signed informed consent was obtained from all patients.

Study Design

A minimum of three patients without prior exposure to topotecan were treated at each dose level. Patients treated with doses that had no toxic effects were allowed to receive subsequent courses at higher doses; however, if patients developed toxicity, the dose was not increased. Near the MTD, additional patients were treated to more completely define acute and cumulative toxicities. Once the MTD without G-CSF was established, the dose of topotecan was further escalated in 25% increments in combination with G-CSF initiated 24 h after completion of the topotecan infusion. G-CSF was administered daily by s.c. injection of 5 μg/kg/day for 14 days or until the AGC was ≥5,000 cells/mm³. Patients who tolerated therapy continued to receive treatment as long as their tumors showed no evidence of progression. All patients were evaluated for toxicity. Patients were formally evaluated for antitumor response after every two courses of therapy. For patients with measurable disease, standard response criteria were used (21). The criteria for removing patients from the study included disease progression, patient noncompliance, request to withdraw, and the development of unacceptable toxicity. For hematological toxicities, the MTD was defined as the dose of topotecan associated with a granulocyte count <500 cells/mm³ or platelet count <50,000 cells/mm³ in one-third of the patients receiving their first course of therapy at that level. For hematological toxic effects, the National Cancer Institute common toxicity criteria were used (22).

Drug Administration

The starting dose of topotecan was 2.5 mg/m² and was administered by continuous i.v. infusion over 24 h. Subsequent courses were given every 21 days. Because mild myelosuppression was encountered at the starting dose, subsequent doses were escalated in approximately 25% increments. The final doses chosen for each level were rounded off to simplify calculations. The appropriate dose of topotecan was reconstituted and diluted in 450 ml of 5% dextrose in water and infused with an ambulatory pump over 24 h. The patients were clinically assessed at least once every 3 weeks, and hematological parameters were monitored twice weekly or daily if the granulocyte or platelet count fell below 1,000 or 50,000 cells/mm³, respectively. Biochemical parameters were checked weekly.

Plasma Pharmacology

Blood samples (10 ml in heparinized tubes) were obtained immediately before infusion, 3, 6, 23, and 24 h after the start of infusion, and 5, 15, 30, 60, 120, 180, 240, 360, 480, and 660 min after the end of infusion. The blood was briefly centrifuged at 2000 × g and the plasma was then removed for immediate analysis.

Drug Analysis. Samples were extracted using C18 solid phase-extraction cartridges that were preconditioned with 3 ml of methanol followed by 1 ml of buffer A (0.01 M NaH2PO4, pH 6.3) and 1 ml of buffer B (0.1 M NH4PO4/1 mM diocetyl sodium sulfosuccinate, pH 6.5). Aliquots (500 μl) of sample or standards were added to 1 ml of buffer B, applied to the extraction cartridge, rinsed with an additional 1 ml of buffer B, then rinsed twice with 1 ml of buffer A. The samples were then eluted with 1 ml of a solution consisting of methanol (750 μl) and buffer A (250 μl). The eluate was vortexed for 10 s, and 100 μl aliquots were then analyzed in duplicate. The remaining 800 μl of eluate were acidified with 6 μl of 2% phosphoric acid and after overnight refrigeration were analyzed for total topotecan (hydroxy acid and lactone).

Separation was achieved by reverse-phase high-pressure liquid chromatography with C18 guard and analytical columns (Nova-Pak 4 μm particle size, 3.9 × 150 mm; Waters Corp., Milford, MA) and a mobile phase consisting of 625 ml of methanol, 312 ml of water, 5 ml of triethylamine, 40 ml of 250 mM NH4PO4/1 mM diocetyl sodium sulfosuccinate, and 23 ml of
1 M sodium phosphate buffer; the pH of the solution was adjusted to 6.3 with phosphoric acid. The flow rate was 1.0 ml/min. Topotecan was detected with a fluorescence detector (Waters Model 470) with excitation at 375 nm and emission at 470 nm.

Retention times, peak heights, and peak areas were calculated with a Maxima model 825 data analysis system (Waters Corp.). Drug concentrations were calculated by using the peak area of unknown versus the peak area of calibration standards extracted from patient plasma prepared just before analysis. Calibration curves were linear from 1 to 500 ng topotecan/ml; drug extraction efficiency averaged 98 ± 6% and the intra- and interassay CV was <10%.

**Pharmacokinetic Analysis.** Parameters were calculated by fitting the appropriate compartmental model to individual patient plasma concentration time data using a weighted nonlinear least-squares regression analysis with ADAPT II modeling software (Biomedical Simulation Resource, University of Southern California, Los Angeles, CA). The AUC was calculated using the formula

\[ \text{AUC} = \int_0^\infty c(t) \, dt \]

Measurement of topo I-DNA Complex Formation in PBMCs

The assay for detection of Topo I-DNA complexes was based on studies indicating that protein-bound DNA is retained on glass fiber (23–25) and nitrocellulose (26) filters when applied to these filters in a buffer of low ionic strength. Under these conditions, DNA not associated with protein passes through the filter. The filter-bound DNA can be quantified by hybridization to a radiolabeled Aku DNA probe (27). This assay was developed in our laboratory and recently validated with HLA60 cells (28).

Blood samples (40–50 ml) were obtained from patients before (pretreatment sample) and at the end of the 24 h infusion (posttreatment sample). Blood cells were separated from plasma by centrifugation and resuspended in cold PBS. PBMCs were isolated using a Ficoll-Hypaque gradient (Pharmacia, Piscataway, NJ). The cells were then harvested and diluted in autologous serum and cold PBS for counting.

Immediately after separation from whole blood, three aliquots of 1 × 10^6 cells obtained before and after the topotecan infusion were lysed with 100 μl of 1.25% SDS and 5 mM EGTA, pH 8, at 65°C. A separate fraction of the isolated pretreatment cells was resuspended at 5 × 10^5 cells/ml in Iscove’s modified Dulbecco’s medium before treatment in vitro with 100 μM topotecan or vehicle (deionized water) for 1 h at 37°C in 5% CO₂. After drug treatment, 1 × 10^6 cells were centrifuged at 13,000 × g for 1 min (in duplicate or triplicate), and the medium was aspirated. The cells were then immediately lysed as above. The lysates were vigorously vortexed and incubated at 65°C for 10 min before the DNA was sheared by passing it through a 27-gauge needle. One ml of protein-binding buffer (0.4 μM guanidine HCl; 10 mM Tris, pH 8; 10 mM EGTA, pH 8; 0.01% Sarkosyl; 0.3 μM NaCl; 10 mM MgCl₂) heated to 65°C was added to the lysed cells, and the lysates were applied to nitrocellulose filters using a dot blotter. Under the buffer conditions described, only protein-bound DNA was retained on the filters. The DNA was fixed to the nitrocellulose filters by heating at 80°C for 2 h in a vacuum oven.

The amount of DNA on the filters was determined by hybridization to Aku DNA radiolabeled by random priming with [32P]dCTP by using the Amersham Multiprime Labeling System (Amersham Corp., Arlington Heights, IL). Hybridization of the Aku probe (provided by Dr. Grady Saunders, M. D. Anderson Cancer Center) to DNA on the filter was quantified by using a Betascope 603 apparatus (Betagen, Waltham, MA). Topotecan-induced topo I-DNA complexes were expressed as the ratio of the average cpm of samples treated with topotecan divided by the average cpm of samples treated with water.

**Statistical Analysis**

Statistical methods included distribution plots and scatter plots. Associations between pairs of numeric variables were assessed using Spearman’s rank correlation (R_s). Associations between numeric and categorical variables were assessed using Wilcoxon’s rank sum test. All P values were two tailed.

**RESULTS**

**Phase I Trial**

Between April 1990 and July 1993, a total of 44 patients were enrolled; 33 patients received topotecan without G-CSF and 11 topotecan with G-CSF. The patients’ characteristics are listed in Table I. The median age for all patients was 56 years. There were slightly more women than men, and because of the promising preclinical work of Giovanella et al. (17), we chose mostly patients with adenocarcinoma of the colon. Forty of 44 patients (91%) had received prior chemotherapy (median, two regimens), and 13 patients (30%) had received prior radiation therapy to extrapelvic sites. One hundred four courses of topotecan (median, two courses per patient) were administered at doses ranging from 2.5 mg/m² to 15.0 mg/m². Treatment toxicity was evaluated in all patients; response could be evaluated in all patients except one.

**Hematological Toxicity.** The hematological toxic effects of all topotecan courses administered without and with G-CSF are summarized in Table 2. At doses above 3 mg/m², individual patients treated at some levels experienced granulocyte and platelet nadirs below 500 and 50,000 cells/mm³, respectively. In general, the patients experiencing hematological toxicity at the lower dose levels had extensive prior exposure to...
Table 2 Summary of topotecan doses and hematologic toxicity

<table>
<thead>
<tr>
<th>Level</th>
<th>Topotecan dose (mg/m²)</th>
<th>G-CSF</th>
<th>No. of Patients</th>
<th>Courses</th>
<th>Median nadir AGC, x 10⁹/mm³ (range)</th>
<th>Median duration of neutropenia, days</th>
<th>Median nadir platelet count, x 10⁹/mm³</th>
<th>Median duration of thrombocytopenia, days</th>
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<td>3</td>
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<td>1.9 (1.0-3.4)</td>
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<td>No</td>
<td>4</td>
<td>11</td>
<td>1.7 (1.1-3.1)</td>
<td>128 (47-368)</td>
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<td>4</td>
<td>No</td>
<td>7</td>
<td>16</td>
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</tr>
<tr>
<td>3</td>
<td>5</td>
<td>No</td>
<td>6</td>
<td>10</td>
<td>1.7 (0.2-3.4)</td>
<td>173 (55-226)</td>
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<td></td>
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<tr>
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<td>6.25</td>
<td>No</td>
<td>3</td>
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<td>3</td>
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<td>145 (93-188)</td>
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<tr>
<td>6</td>
<td>10</td>
<td>No</td>
<td>4</td>
<td>7</td>
<td>1.1 (0.4-1.7)</td>
<td>118 (24-151)</td>
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<tr>
<td>7</td>
<td>12.5</td>
<td>No</td>
<td>6</td>
<td>11</td>
<td>1.0 (0.0-1.7)</td>
<td>164 (9-286)</td>
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<tr>
<td>8</td>
<td>12.5</td>
<td>Yes</td>
<td>10</td>
<td>17</td>
<td>1.2 (0.0-5.5)</td>
<td>41 (13-121)</td>
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<tr>
<td>9</td>
<td>15</td>
<td>Yes</td>
<td>6</td>
<td>8</td>
<td>1.4 (0.0-3.3)</td>
<td>41 (12-104)</td>
<td>7</td>
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</tbody>
</table>

*a Days with AGC <1000 cells/mm³.

*b Days with platelet count <50,000 cells/mm³.

Table 3 Topotecan 24-h infusion, maximum tolerated dose

<table>
<thead>
<tr>
<th>Level</th>
<th>Topotecan dose, mg/m²</th>
<th>G-CSF</th>
<th>Patients with AGC &lt;500 cells/mm³</th>
<th>Patients with platelets &lt;50,000 cells/mm³</th>
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</thead>
<tbody>
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<td>12.5</td>
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<tr>
<td>9</td>
<td>15</td>
<td>Yes</td>
<td>3/5</td>
<td>5/5</td>
</tr>
</tbody>
</table>

*a For hematological toxic effects, the MTD was defined as the dose of topotecan associated with a granulocyte count <500 cells/mm³ or platelet count <50,000 cells/mm³ in one-third of the patients receiving their first course of therapy at that level.

*b Per number of patients treated.

alkylating agents (nitrosoureas and carboplatin) and/or radiotherapy to >25% of marrow-containing bone.

Table 3 outlines the hematological toxic effects experienced by patients following their first courses of therapy used to calculate the MTD. On the basis of these data, the MTD was judged to be 10 mg/m². Only three patients were treated at the MTD because of the lack of toxicity at lower doses and because the dose levels above 10 mg/m² had been so extensively explored.

Once the MTD without G-CSF was identified, G-CSF was added to the therapy of 10 patients receiving 12.5 mg/m² topotecan. Six of these 10 patients received their first courses at this level. For patients receiving their first course of topotecan at 12.5 mg/m² with G-CSF, only one of six patients experienced a granulocyte nadir of <500 cells/mm³. However, four of these six patients experienced platelet nadir values of <50,000 cells/mm³ (Table 3), although considering all patients treated at this level (Table 2), the median platelet nadir was only 41,000 cells/mm³. For all courses administered at 12.5 mg/m² with G-CSF, the median nadir AGC was 1,200 cells/mm³ (range, 0.0-5500), but the median duration of neutropenia (days with AGC below 1000 cells/mm³) was brief (3 days compared to 8 days without G-CSF) and did not result in fever or infection or delay therapy. Despite these optimistic results, the fact that the median platelet nadir in this cohort of 10 patients was 41,000 cells/mm³ suggested that thrombocytopenia might limit additional escalation with G-CSF.

To explore whether the topotecan dose could be further increased with G-CSF, six patients were treated with 15 mg/m² topotecan with G-CSF. Five patients received their first course of therapy at this level, and a total of six patients were treated. Three of the five first-course patients had AGC nadir values <500 cells/mm³. All five of the first-course patients also experienced platelet nadir values <50,000 cells/mm³, with three of these five patients experiencing platelet nadir values of <20,000 cells/mm³. One of these patients had an episode of grade 4 hematuria that required transfusion of platelets. Four patients who received 15.0 mg/m² topotecan also had grade 2 or 3 anemia not related to blood loss. Therefore, despite evidence that the granulocytopenia induced by topotecan could be partially ameliorated by G-CSF, thrombocytopenia and eventually granulocytopenia became dose limiting using a 24-h continuous infusion of topotecan.

Nonhematological Toxicity. The nonhematological toxic effects were generally mild, well tolerated, and reversible. Nausea and vomiting were the most common toxic effects encountered: 19 of 44 (43%) patients experienced nausea during the first course of therapy. Except in one patient, all episodes of
the AUC range was 260-1450 pg/liter (568-3166 nMh), and considerable interpatient variability in AUC and clearance values (mean AUC values increased in proportion to dose: 629 pg/liter/h (66.3 nM; lactone plasma concentrations at the end of the infusion were: (t50% of patients). Both laboratory abnormalities were frequently observed at topotecan doses less than 10 mg/m², suggesting that these abnormalities may have been secondary to progressive disease rather than a drug-related toxic effect. There were rare complaints of fever, chills, and abdominal pain related to the infusion.

**Response.** Forty-three patients were evaluated for response. One patient with colon cancer (treated at 8 mg/m²) had a minor response. 10 patients (23%) had no change in tumor volume lasting through at least four courses of therapy, and 33 patients (75%) experienced progressive disease while receiving treatment. No partial or complete responses were observed.

**Pharmacokinetics**

Pharmacokinetic parameters were determined for 11 patients (13 courses) treated with the highest topotecan doses studied: 12.5 mg/m² (8 courses, 4 without G-CSF and 4 with G-CSF) and 15.0 mg/m² (5 courses, all with G-CSF). Topotecan lactone pharmacokinetics are summarized in Table 4. The mean lactone plasma concentrations at the end of the infusion were: 30.6 ng/ml (66.8 nm; CV, 23%) at 12.5 mg/m² without G-CSF, 30.4 ng/ml (66.3 nm; CV, 53%) at 12.5 mg/m² with G-CSF, and 40.2 ng/ml (87.6 nm; CV, 45%) at 15 mg/m² topotecan. The mean AUC values increased in proportion to dose: 629 µg/liter/h (1373 nMh) at 12.5 mg/m² and 996 µg/liter/h (2174 nMh) at 15 mg/m². However, as indicated in Figs. 1 and 2, there was considerable interpatient variability in AUC and clearance values within each dose level studied. For example, at 15 mg/m² the AUC range was 260-1450 µg/liter/h (568-3166 nMh), and the range for clearance values was 10.3-57.7 liters/h/m². Despite this variability, topotecan lactone end-infusion plasma levels correlated strongly with AUC (R² = 0.88; P = 0.002), as demonstrated in Fig. 3.

Elimination of topotecan lactone was biexponential. The mean t₁-α was 28 min and t₁-β was 3.8 h at 12.5 mg/m² topotecan, whereas at 15 mg/m² the t₁-α was 36 min and t₁-β was 5.0 h. Fig. 4 shows a representative concentration-time curve for a patient treated at 12.5 mg/m² and compares total topotecan with topotecan lactone. On average, at the end of the infusion, 52% of the topotecan was present as lactone in each

### Table 4: Topotecan lactone pharmacokinetics

<table>
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<tr>
<th>Patient</th>
<th>G-CSF</th>
<th>Dose, mg/m²</th>
<th>Vc, l/m²</th>
<th>Vd, d/m²</th>
<th>t₁-α, min</th>
<th>T₁/2, h</th>
<th>Cl, l/h/m²</th>
<th>Topotecan plasma level, ng/ml</th>
<th>AUC, µg/liter · h</th>
<th>AUC, nm · h</th>
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* Topotecan lactone plasma level (ng/ml) measured at the end of the 24-h infusion.
sample; however, 24 h after completion of the infusion, the ratio of lactone to total topotecan fell to less than 50%.

**Pharmacodynamic Analyses**

Detailed pharmacodynamic analyses were carried out using the pharmacological and clinical data obtained from the 13 courses of therapy administered to patients receiving topotecan at doses of 12.5–15 mg/m². A significant negative correlation was observed between topotecan AUC and nadir hemoglobin levels ($R_a = -0.56; P = 0.05$). Weak negative correlations were also demonstrated between topotecan lactone AUC and WBC, AGC, and platelet nadirs (AUC versus WBC nadir: $R_a = -0.46, P = 0.11$; AUC versus AGC nadir: $R_a = -0.45, P = 0.11$; AUC versus platelet nadir: $R_a = -0.47, P = 0.10$). There was no correlation between topotecan lactone AUC or topotecan lactone end-infusion plasma levels and response to topotecan therapy.

**Formulation of topo I-DNA Complexes in PBMCs**

Using PBMCs as an accessible surrogate to estimate the effect of topotecan on tumor cells or marrow stem cells, we explored relationships between toxicity, efficacy, and formation of topo I-DNA complexes in PBMCs obtained before and after treatment with topotecan from three patients treated with 12.5 mg/m² and four patients treated with 15 mg/m². Pretopotecan topo I-DNA complexes were available on two additional patients treated at 15 mg/m². Table 5 summarizes these data. The mean increase in topo I-DNA complexes at the end of the topotecan infusion was 1.25 times the pretreatment value.

A series of correlative analyses were performed in an effort to associate formation of topo I-DNA complexes with pharmacokinetic or clinical parameters. A clear relationship could not be established between the calculated ratio of topo I-DNA complexes formed after exposure to topotecan divided by the topo I-DNA complexes before therapy and any of the following clinical parameters: nadir WBC, nadir AGC, nadir platelet count, nadir hemoglobin values, or anticancer response. However, interesting trends suggested a possible relationship between the topo I-DNA ratio and topotecan lactone end-infusion plasma levels or topotecan lactone AUC (data not shown). Although we were unable to show a relationship between topotecan lactone end-infusion plasma levels or topotecan lactone AUC and anticancer response (data not shown), the level of topo I-DNA complexes measured in PBMCs before therapy was significantly ($P = 0.02$) higher in those patients ($n = 3$), with no change in measurable disease for at least four courses of therapy (median cpm, 2824) as compared to those patients ($n = 6$) with progressive disease while receiving topotecan (median cpm, 803).

**DISCUSSION**

Topotecan is a water-soluble analogue of camptothecin that appears to have schedule-dependent anticancer activity. A 5-day short infusion schedule is currently well into Phase II testing with activity documented in a number of tumor types, including lung and ovarian cancer (13, 14). However, preclinical studies suggest that longer exposure to topotecan and other topo I inhibitors may
increase efficacy (15, 17). In this Phase I trial, the clinical, pharmacological, and cellular effects of a 24-h continuous infusion of toptecan are reported. On the basis of the patient population treated in this trial, the MTD was 10 mg/m². The DLT was granulocytopenia. However, in agreement with a recently reported study using the same infusion length and schedule, measurable effects on granulocytes and platelets were encountered at all doses administered (Table 2; Ref. 29). In the study reported herein, patients experiencing myelosuppression at lower dose levels often had extensive prior exposure to chemotherapy and radiation, but there were exceptions. Because we did not conduct pharmacological analyses at the lower dose levels, it is unclear whether the toxic effects documented in specific patients could be related to variable interpatient pharmacokinetics. Thus, in general agreement with the trial of Van Warmerdam et al. (29), our recommended Phase II starting dose for toptecan administered over 24 h every 3 weeks to previously untreated patients is 8–10 mg/m². At these recommended doses, the planned cumulative dose intensity of the schedule of 24 h every 3 weeks is approximately that of the schedule of 5 consecutive days (1.5–2.0 mg/m²/day).

At 12.5 and 15 mg/m², toptecan was administered with G-CSF in an effort to increase the dose that could be safely administered. With G-CSF, the duration of granulocytopenia at 12.5 mg/m² toptecan was shortened from 8 to 3 days but thrombocytopenia became dose limiting. Grade 2–3 anemia and thrombocytopenia were encountered at all doses administered. With G-CSF, the duration of granulocytopenia at 15 mg/m² was also documented at 15 mg/m² and was the toxic effect most closely correlated with toptecan lactone AUC. Consistent with other reports (29, 30), anemia and thrombocytopenia were more common with the 24-h schedule than with the 5-day, 30-min infusion schedule (8, 11). Our inability to significantly increase the dose of toptecan with G-CSF was consistent with the observations of Saltz et al. (11) but conflicts with those of Rowinsky et al. (12), who reported that with a schedule of toptecan administered i.v. over 30 min daily for 5 days, the dose could be increased beyond the MTD by routine use of G-CSF.

Nonhematological toxic effects, including nausea, vomiting, fatigue, diarrhea, and alopecia, occurred in a significant proportion of patients; however, these effects were easily managed and appeared to be independent of dose. Mucositis, the dose-limiting toxic effect in a 5-day continuous infusion trial of toptecan in acute leukemia (31), was encountered in only one patient.

The mean plasma clearance of 29.6 liters/h/m² in this trial was similar to that documented in a pediatric study of toptecan administered by 24-h continuous infusion (30). However, significant interpatient variability (range, 9.7–70.2 liters/h/m²) was demonstrated. It is possible that the differences documented in clearance contributed to the variable toxic effects encountered at each dose level studied. The drug elimination data generated in our Phase I study was best fit by a two-compartment model with, a mean t₁/₂β of 4.3 h (reviewed in Ref. 9). A recently published adult dose-escalation trial of toptecan administered by 24-h infusion has suggested that the elimination of toptecan may be saturable (29). In support of this conclusion, the authors indicate that the concentration-time profiles from the start of the infusion until 24 h postinfusion were not well characterized by multicompartment linear models. Additionally, at the 10.5 mg/m² (highest) dose level the AUCs and steady-state concentration values versus dose displayed a nonlinear deviation. Likewise, at the highest dose level, mean drug clearance was only 35% of that reported for doses from 2.5 to 8.4 mg/m².

Although these data suggest possible saturable processes, there are several other potential explanations. First, given the small number of experimental subjects at each dose level, there is the possibility of undue influences of outliers in estimating "mean" pharmacokinetic parameters. For instance, mean AUCs in our study (Fig. 1) differed significantly between dose levels; however, the range and distributions were very similar. Second, at the lowest dose levels, it is often difficult to follow plasma drug concentration-time profiles long enough to accurately estimate pharmacokinetic parameters such as AUC. Finally, given the previously demonstrated interindividual differences in drug disposition for toptecan, one would need a much larger population to determine true nonlinear drug disposition.

We found that a two-compartment linear model adequately described both the infusion and postinfusion plasma concentra-
tion-time data in all cases in our study. Topotecan lactone clearances in our study at 12.5 and 15.0 mg/m² were similar to the values reported by other authors, using similar drug doses (8, 9, 11, 30). The suggested nonlinearity detected in this study may in fact be an artifact of the inability to accurately estimate pharmacokinetic parameters at low dose levels due to the limitations of drug assays.

The cytotoxicity of topotecan (and other camptothecin analogues) appears to be due to the formation of drug-induced topo I-DNA complexes and the subsequent generation of single-strand DNA breaks (32, 33). We were therefore interested in measuring the amount of topo I-DNA complex formation in readily accessible cells from patients with advanced solid tumors and correlating complex formation with the toxic effects and any evidence of antitumor efficacy encountered during this Phase I trial. To accomplish this goal we used a filter-binding assay recently described by our group to measure the amount of topo-I DNA complex formation in PBMCs from cells obtained before and at the completion of the 24-h infusion. The filter-binding assay used is a measure of the drug susceptibility of the topo I within a cell population to be stabilized in a complex with DNA. The assay detects drug-induced DNA-protein crosslinks based on the differential binding of protein-bound DNA to nitrocellulose filters. It has been shown to correlate with the standard SDS/KCl precipitation assay (28) and was used in a recently completed Phase I trial of topotecan in patients with relapsed acute leukemia (31). However, the clinical significance of the difference in protein-bound DNA measured before and after therapy cannot be unambiguously determined without concurrent measurement of immunoreactive topo I in each patient (28). This could not be consistently determined in this study because the number of cells from each patient was insufficient to perform both the feasibility quantification of topo I-DNA complex formation and immunoblotting. Other studies using different methodologies have also demonstrated that the interaction of topotecan with its cellular target can be measured in PBMCs and tumor cells (34, 35).

Using our filter-binding assay, topo I-DNA complexes measured in PBMCs obtained from patients before and after the 24 h topotecan infusion showed a 25% increase over baseline values. The small number of patients studied prevented a clear correlation between the topo I-DNA complex ratio calculated following exposure to topotecan and clinical parameters such as myelosuppression or objective response; however, trends suggesting relationships between the topo I-DNA complex ratio and topotecan lactone end-infusion plasma levels and topotecan lactone AUC support continued evaluation of the filter-binding assay in subsequent Phase I and II trials of topotecan and other topo I inhibitors. The data suggesting that the absolute value of the pretherapy topo I-DNA complex formation correlated with those patients with nonprogressive cancer through at least four courses of therapy was particularly interesting. Thus, the level of topo I-DNA complex formation measured in an accessible surrogate tissue (PBMCs) may be able to predict those patients that are likely to benefit from treatment with topotecan. However, given the small patient numbers in this trial and the lack of objective responses, much larger numbers of patients treated with topotecan and other topo I inhibitors will need to be studied to establish the validity of this finding.

The observation that a surrogate cell type can be used to predict response to cancer therapy is not without precedent. Reed et al. (36) showed in a group of patients with ovarian and testicular cancer that there was a close correlation between response to cisplatin and formation of cisplatin-DNA adducts in circulating PBMCs. However, in other systems, drug effects measured in PBMCs did not correlate with similar measurements made directly from target malignant cells (37), indicating that each assay needs to be validated on both PBMCs and tumor cells. Circulating PBMCs are composed of variable populations of short-lived cells such as granulocytes and long-lived cells such as T lymphocytes. Studies of topo I levels during granulocyte maturation suggest that topo I levels in circulating granulocytes are much lower than levels in granulocytic precursors (38) or leukemic cells (39). This fact may explain the lack of correlation between the filter-binding assay and myelo-suppression.

In summary, topotecan is well tolerated when administered by 24-h infusion. The MTD is 10 mg/m² and the DLT is granulocytopenia. At the recommended starting dose of 8–10 mg/m² individual patients may also experience drug-related anemia and thrombocytopenia. Topotecan lactone AUC correlated closely with anemia and less well with granulocytopenia or thrombocytopenia. These preliminary studies also suggest that measurement of topo I-DNA complex formation in patients receiving camptothecin analogues should be explored further to address whether the antineoplastic or toxic effects of topo I interactive agents can be predicted for individual patients. This method will be easiest to apply to patients with leukemia, in whom there is ready access to malignant cells in the blood or bone marrow. However, further refinements may allow this assay to be used routinely in patients with solid tumors from whom tumor cells can be obtained from accessible sites, such as ascites or pleural effusions, or by fine-needle aspiration of deeply situated malignant deposits.

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REFERENCES


Clinical Cancer Research

Phase I clinical and plasma and cellular pharmacological study of topotecan without and with granulocyte colony-stimulating factor.

J L Abbruzzese, T Madden, S M Sugarman, et al.


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