A Phase I Clinical Trial of Sequentially Administered Doxorubicin and Topotecan in Refractory Solid Tumors

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ABSTRACT

Purpose: To determine whether agents that target topoisomerase I and II could be administered sequentially.

Design: A Phase I study was conducted to evaluate sequential treatment with bolus IV doxorubicin followed 48 h later by topotecan given as a 30-min i.v. infusion on 3 consecutive days, with additional cycles of therapy repeated every 3 weeks. Characteristics of the 22 patients entered into the study were: 13 male and 9 female; median age, 49.5 (range 33–66) years; Eastern Cooperative Oncology Group performance status, 0–1; and normal cardiac, hematological, hepatic, and renal function. All patients had received prior therapy (median ≥2 prior regimens).

Results: The maximum tolerated dose of the combination was 25 mg/m² doxorubicin and 5.25 mg/m² topotecan (1.75 mg/m²/day × 3). Neutropenia was the dose-limiting toxicity. Attempts to further escalate the dose using 5 μg/kg granulocyte colony-stimulating factor proved unsuccessful because of thrombocytopenia. Among the 17 patients who were evaluable for response, 6 had a partial response, and 4 showed evidence of disease stabilization. The partial responses occurred in patients with small cell lung cancer (3 of 7), non-small cell lung cancer (1 of 6), esophageal adenocarcinoma (1 of 2), and ovarian carcinoma (1 of 1), and it lasted for 3–6 months. Administration of doxorubicin 2 days before topotecan did not alter topotecan pharmacokinetics. Changes in topoisomerase mRNA levels were observed during chemotherapy.

Conclusions: The sequential combination of doxorubicin followed by topotecan is highly active in several chemotherapy refractory lung, ovary, and esophageal cancers. Despite significant neutropenia, toxicity is manageable and well tolerated. Phase II trials to further evaluate the efficacy of this promising combination regimen against non-Hodgkin’s lymphoma and lung cancer have been initiated.

INTRODUCTION

DNA topoisomerases I and II are nuclear enzymes that are essential for DNA replication, RNA transcription, chromosomal condensation, and mitotic chromatid separation (1). The topoisomerases are important targets for cancer chemotherapeutic agents (2). Camptothecin and derivatives are topoisomerase I poisons that do not affect topoisomerase II (3). The topoisomerase II poisons, such as etoposide and doxorubicin, do not interact with topoisomerase I (4). Topoisomerase-targeted agents stabilize a transient covalent enzyme-DNA complex, which produces DNA strand cleavage and apoptosis (5). Unlike the situation where elevated target enzymes confer resistance to an antineoplastic agent, as with thymidylate synthase and 5-fluorouracil or dihyrofolate reductase and methotrexate, the presence of increased topoisomerase protein levels actually enhances the sensitivity of cancer cells to inhibitors of the enzyme. Similarly, decreases in the level of either topoisomerase I or II may result in diminished sensitivity to the agents targeting the respective enzyme, which may be a clinically relevant mechanism of resistance (6–8).

A preclinical study explored the in vivo therapeutic effect of sequential combinations of topoisomerase I- and II-acting drugs in a murine tumor model system. It was observed that topoisomerase I mRNA and protein levels in the tumor decreased, whereas topoisomerase II mRNA and protein levels rose, after treatment with camptothecins. The reverse effect, a fall in topoisomerase II and concurrent rise in topoisomerase I levels with a topoisomerase II-active drug treatment, was observed. The conclusion was that topoisomerase I and II agents can be combined with greater than additive tumor cytotoxicity and no increase in host toxicity (9).

A Phase I trial of sequentially administered doxorubicin and topotecan was performed on the basis of these observations in preclinical studies. The objectives of the study were to determine the DLT3 and MTD of sequential doxorubicin (day 1) and topotecan (days 3–5) in patients with refractory solid tumor...
malignancies. In addition, the response rate of the patients was determined. Assessing the influence of doxorubicin on topotecan pharmacokinetics and the effect on topoisomerase I and II levels in peripheral blood mononuclear cells was performed.

**PATIENTS AND METHODS**

**Patient Selection.** Patients had histologically documented metastatic or inoperable malignant solid tumors, for which there was no known curative or standard palliative therapy. Performance status was Eastern Cooperative Oncology Group 0–2. Patients had adequate hepatic, renal, and hematological function determined by a serum glutamate-oxaloacetate transaminase < 2.5 × upper limit of normal, bilirubin < 1.5 mg/dl, creatinine < 1.5 mg/dl, a white blood count > 3,000/µl1, and platelets > 100,000/µl2. At least 3 weeks must have elapsed since radiotherapy or chemotherapy and >1 week since surgery. The cumulative dose of doxorubicin and other anthracyclines received by the patient during all prior treatments could not exceed 300 mg/m². Patients with significant cardiac disease or clinical evidence of congestive heart failure were excluded from the study. Patients were required to have a left ventricular ejection fraction > 50% if prior treatment with doxorubicin.

**Drug Dosage and Administration.** Patients had a history, physical examination, complete blood count, and serum chemistries (including liver function tests and creatinine) performed before therapy and weekly (complete blood counts were performed three times per week). These assessments were repeated at 1 month after the last course of topotecan and doxorubicin and on apparent progression. Documentation of all measurable disease by examination and any appropriate imaging studies (e.g., plain radiograph, computerized tomography, and nuclear medicine scan) was performed before therapy. Disease measurable by examination or plain radiographs was reevaluated after each cycle of therapy. Other imaging procedures required for disease measurement were repeated after two cycles and every two cycles thereafter if applicable.

**Treatment Plan.** Doxorubicin (Adriamycin; Pharmacia Upjohn, Kalamazoo, MI) and topotecan (Hyacptin; Smith Kline Beecham, King of Prussia, PA) were prepared from commercially available supplies, formulated, and administered per institutional guidelines. Doxorubicin was administered as a 1-h i.v. infusion on day 1, and topotecan was delivered as a once per institutional guidelines. Doxorubicin was administered as a 1-h i.v. infusion on day 1, and topotecan was delivered as a once daily 30-min i.v. infusion on days 3, 4, and 5. The starting doses were 25 mg/m² doxorubicin and 1.5 mg/m²/day topotecan. Doses of the two drugs were sequentially escalated, one at a time, by constant increments of 10 mg/m² for doxorubicin and 0.25 mg/m²/day for topotecan, beginning with topotecan. Thus, the second dose level was 25 mg/m² doxorubicin and 1.75 mg/m²/day topotecan, the third dose level was 35 mg/m² doxorubicin and 1.75 mg/m²/day topotecan, days 3–5 (5.25 mg/m² total dose), and so forth. When an ANC < 500/µl occurred in a patient at dose level 3, all subsequent patients were administered G-CSF at 5 µg/kg from day 6 until the ANC > 5,000/µl.

Concurrent supportive care, including narcotics, nonsteroidal anti-inflammatory drugs, and antiemetics, was given as needed. The administration of glucocorticoids as antiemetics for doxorubicin and/or topotecan was permitted only after the failure of other nonsteroidal antiemetic agents.

Three fully evaluable patients were initially entered at each dose level. Dose escalations proceeded as outlined above until a single patient experienced a DLT. DLT was defined as either neutropenia (polymorphonuclear < 500/µl) or thrombocytopenia (platelets < 25,000/µl) for >4 days or irreversible grade 2 or any grade 3–5 nonhematological toxicity (CTC 2.0 criteria).4 When any DLT occurred in a single patient, 3 additional patients were added to that cohort. The occurrence of a second DLT in 2–6 patients established the previous dose as the MTD, unless the DLT was neutropenia. When grade 4 neutropenia of >4 days duration was first observed, 3 additional patients (6 total) were administered G-CSF at 5 µg/kg from day 6 until the ANC > 5,000/µl. If no other DLT occurred, dose escalation continued with all patients receiving G-CSF. When a non-neutropenia DLT was reached, the previous dose was established as the MTD, and 10 additional patients were added.

**Definition of Disease Response.** For measurable (by exam or X-ray) malignancies, definition of a complete response, partial response, stable disease, and progression was standard and required a duration of at least 1 month (10).

**Pharmacokinetic Studies.** Plasma concentration-time profiles resulting from the third daily dose of topotecan were determined during the first cycle of therapy. Blood specimens (3 ml) were collected in Vacutainer plasma tubes with freeze-dried sodium heparin (Becton Dickinson, Franklin Lakes, NJ) from a vein in the arm that did not receive the drug infusion at the following times relative to the beginning of the infusion: −5, 10, 30, 35, and 45 min and 1–3 and 5 h. Sample tubes were placed on ice and promptly transferred into polypropylene microcentrifuge tubes for immediate centrifugation (2 min, 12,000 × g). Three 100-µl aliquots of plasma from each sample were individually treated with 200 µl of −70°C methanol, then vigorously mixed by vortexing and centrifuged (2 min, 12,000 × g). The protein-free supernatants, afforded within 5 min after obtaining the original sample from the patient, were stored at −70°C until assayed by reversed-phase high-performance liquid chromatography with fluorescence detection, as described previously, with minor modifications (10, 11). The remaining plasma was maintained at −70°C until assayed for total topotecan as reported (10, 11).

A series of six standard solutions of topotecan in plasma at concentrations ranging from 1.1 to 55.6 ng, plus a drug-free sample, was prepared and analyzed together with the study specimens on a daily basis. Parameters defining the best-fit line determined by linear regression analysis of the chromatographic peak area of the drug in the plasma standards, performed with 1/5₀₀₀ weight, were used to calculate drug concentrations in study samples. The concentrations of topotecan lactone and total topotecan in all study specimens were determined in duplicate on separate days. Results were considered to be acceptable if the two concentration values differed from their average by ±10%.

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1. Infinity; CL, total plasma clearance; RT-PCR, real-time PCR; PBMC, peripheral blood mononuclear cell.

parameters were generally all standard curves run during the course of the study. Both assessed by analyzing the interpolated drug concentrations from curve were reassayed in duplicate on appropriate dilution. In-
mulated concentration exceeding the upper range of the standard otherwise, the sample was reassayed. Specimens with an estimated concentration exceeding the upper range of the standard curve were reassayed in duplicate on appropriate dilution. Inter-
day accuracy and precision of the analytical methods were assessed by analyzing the interpolated drug concentrations from all standard curves run during the course of the study. Both parameters were generally ≤10% at all concentrations, except for the lower limit of the standard curves, at which the accuracy was 5.6 and 14.5% of the nominal concentration of topotecan lactone and total topotecan, respectively. Corresponding values of the precision were 12.2% for topotecan lactone and 8% for total topotecan.

Actual sample times were calculated from the beginning of the infusion of drug to the midpoint of each sample collection interval. Individual patient plasma concentration-time data were analyzed by noncompartmental methods using routines supplied in the WinNonlin Version 1.1 software package (Scientific Consulting, Apex, NC). Area under the plasma profiles from time 0 to infinity (AUC) was estimated using the trapezoidal algorithm to the last data point with extrapolation to infinity using the estimated value of the slope of the terminal log-linear disposition phase (−λ). Mean values of pharmacokinetic variables for the entire cohort of patients was calculated as the geometric mean of the individual patient values (12). SDs for the geometric mean values were estimated by the jackknife technique (13).

**Pharmacodynamics.** Peripheral blood samples were collected from the patients before doxorubicin treatment, before the first dose of topotecan, and after the third dose of topotecan. Mononuclear cells were isolated by a standard density gradient centrifugation technique (Ficoll-Paque; Pharmacia Biotech). After the centrifugation, mononuclear cells were transferred from the Ficoll layer and washed with HBSS to remove platelets. Routinely, ~20 × 10⁶ leukocytes per each blood collection were isolated. Total RNA was extracted from the blood cells by RNA STAT-60 (Tel-Test, Inc.). cDNA was synthesized from each of 1 μg of total RNA sample using TaqMan Reverse Transcription reagents (Perkin-Elmer Corp) according to the manufacturer’s protocol. The resulting first-strand cDNA was diluted and used as template for real-time quantitative PCR analysis. PCR reactions were performed using SYBR Green kit (Perkin-Elmer Corp) monitored the increase in fluorescence during the complete amplification process. The expression of the housekeeping gene, cyclophilin 33A, was used to normalize for variances in input cDNA. The sequences of the primer pairs for each gene are as follows: topoisomerase I, 5'-GGCA-GAGAGCTGTAGCCCTGT-3' and 5'-CCTGCTCTCACAGAGCCTTG-3'; topoisomerase II α, 5'-TTCTTGAGCCCT-TCACGAC-3' and 5'-AGGCTGCAATGGGTGACCTTC-3'; topoisomerase II β, 5'-GCACTGACCTGGGTGAACAA-3' and 5'-GGCAGTTTCTGACCTTCTTCTTTT-3'; and cyclophilin 33A, 5'-ACCAAGCCGCTGTTGTACGT-3' and 5'-TT-TGTGCTGCACTTCTTCTGC-3'. All primer pairs were selected with the help of PrimerExpress 1.0 software (Perkin-Elmer Corp). All samples were performed in duplicate. Standard curves demonstrated a difference of +/−10% on duplicate standards.

**RESULTS**

The characteristics of the 22 patients enrolled into the study are summarized in Table 1. The median age was 49.5 years (range, 33–66). Eighteen patients were fully evaluable for toxicity. Two patients were withdrawn before receiving topotecan because of rapid disease progression, 3 had evaluable but non-measurable disease, and single patients received G-CSF at dose levels 1 and 2 because of remoteness from medical care. Eight patients had small cell lung cancer, 7 had non-small cell lung cancer, 3 had esophageal cancer, and the remaining patients had a variety of solid tumors. All patients had failed at least one prior combination chemotherapy regimen. 10 patients had received more than two prior chemotherapy regimens, and 13 had received prior radiation therapy.

Significant nonhematological toxicity was infrequent. Grade 3 nausea only, nausea and vomiting, and fatigue was observed in single patients. Alopecia was universal. Toxicity during cycle 1 was predominantly hematological (Table 2). Grade 4 neutropenia occurred in 1 patient at dose level 1, 6 patients at dose level 2, and 4 patients at dose level 3. For this study, dose-limiting neutropenia required an ANC < 500/μl and a duration of >5 days. This occurred at dose level 3. G-CSF, 250 μg/m², was then added to all subsequent patients at dose level 3. Despite the use of G-CSF, 3 additional patients demonstrated Grade 4 neutropenia, and 4 patients developed Grade 4 thrombocytopenia. Eight patients were then added to dose level 2 to further expand experience with this dose. No patient required hospitalization or antibiotics for neutropenia.

Seventeen patients were evaluable for response (Table 3). Overall, 6 of 17 patients (35%) demonstrated partial responses, lasting 3–6 months. Partial responses were observed in 3 of 7 patients with small cell lung cancer, 1 of 6 patients with non-small cell lung cancer, and single patients with esophageal adenocarcinoma and ovarian carcinoma. Table 4 reviews the
Sequential Doxorubicin and Topotecan

The mean CL of topotecan lactone (topotecan (H11005) P = 0.31) determined at each dose level (two-tailed, single factor ANOVA). The mean CL of topotecan lactone (35.5 ± 21.8 liters/h/m², n = 9) and total topotecan (16.1 liters/h/m², n = 11) in patients treated with the third 1.75 mg/m² daily dose of the drug is comparable with previously reported values for the 30-min i.v. infusion schedule (14–17). There were no significant correlations (i.e., r > 0.4) between the CL, Cmax, or AUC of topotecan and patient characteristics, pretreatment laboratory values, or toxicity during cycle 1. In addition, there was no significant difference between the Cmax or AUC values in the group of 10 patients that showed some evidence of response to therapy as compared with those with continuous disease progression.

RT-PCR for topoisomerase I, topoisomerase IIα, and topoisomerase IIβ was performed in a subset of 7 patients (Fig. 2). Only 3 of 7 patients demonstrated a rise in topoisomerase I mRNA levels after doxorubicin, although 4 of 7 patients had relative decreases in topoisomerase I mRNA levels after topotecan. Topoisomerase IIα mRNA levels followed the expected pattern of decline after doxorubicin in 4 of 7 patients and were again baseline on day 5 in 5 of 7 patients. Topoisomerase IIβ mRNA levels showed a more consistent pattern of behavior. Levels rose, albeit only slightly in 1, 48 h after doxorubicin in 6 of 7 patients and were at or above baseline on day 5 in 6 of 7 patients. Although only a retrospective observation, 3 patients with small cell lung cancer (002, 003, and 013) had partial responses, and each had a rise in topoisomerase IIβ mRNA levels after topotecan, which was seen in only 1 of 4 patients without a response. Changes in topoisomerase mRNA were not significantly related to any topotecan pharmacokinetic variable in this small group of patients.

Table 3 Responses

| Disease            | Patients | Evaluable | Outcome
<table>
<thead>
<tr>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell lung</td>
<td>8</td>
<td>7</td>
<td>PR: 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD: 1</td>
</tr>
<tr>
<td>Non-small cell lung</td>
<td>7</td>
<td>6</td>
<td>PR: 1</td>
</tr>
<tr>
<td>Esophageous</td>
<td>3</td>
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<td>PR: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD: 1</td>
</tr>
<tr>
<td>Ovarian</td>
<td>2</td>
<td>2</td>
<td>PR: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD: 0</td>
</tr>
<tr>
<td>Colon</td>
<td>1</td>
<td>1</td>
<td>PR: 0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD: 0</td>
</tr>
<tr>
<td>Stomach</td>
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<td>1</td>
<td>PR: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD: 0</td>
</tr>
</tbody>
</table>

* PR, partial response; SD, stable disease.

DISCUSSION

DNA topoisomerases I and IIα are targets for many clinically important antineoplastic agents. The drugs that interact with topoisomerase I, the camptothecins, are structurally distinct from the topoisomerase II-interacting agents of the anthracycline, epipodophyllotoxin, and anthrancenedione classes. No clinically useful agent that preferentially targets topoisomerase IIβ has been developed to date.

Topoisomerases I and IIβ are expressed constitutively throughout the cell cycle, whereas the expression of topoisomerase IIα occurs only during the S and G2-M phases (18). However, agents interacting with any topoisomerase exhibit cell cycle cytotoxic specificity (19). Drug resistance has been studied with agents interacting with each topoisomerase. Most reports are concerned with mechanisms operative before drug-receptor (topoisomerase) interaction. Decreased cellular accumulation via drug membrane efflux transporters has been observed in vitro (20). Point mutations have been identified in both topoisomerase I and IIα in cell lines but not in primary tumor specimens (21). Slowing progression through the cell cycle may confer resistance to topoisomerase inhibitors. In addition, it has been reported that topoisomerase IIα levels decreased in clinical specimens of small cell lung cancer that were serially obtained from the same patient before and after treatment with etoposide. However, very few studies involving primary tumor biopsy specimens have been performed, and no direct correlation has been made between topoisomerase levels and tumor response (22). Recent investigations into post-target events, such as DNA repair and apoptotic signaling, are interesting but not validated in clinical specimens (23). It is probable that resistance to topoisomerase-active agents is multifactorial (24).

An immediate decrease in the protein levels of the targeted topoisomerase has been observed (25). Recent preclinical observations suggest a possible coordinate balance between topoisomerase IIα and topoisomerase I protein and mRNA levels (9). Administration of topoisomerase I- and IIα-acting drugs could be sequenced to maximize tumor cell kill in a mouse model system without a concomitant increase in host toxicity. This series of observations was the basis for this clinical trial. The sequence of doxorubicin followed by topotecan was extrapolated from the mouse studies.

Neutropenia was the major toxicity encountered in this study. Grade 4 neutropenia (ANC < 500/μl) was observed at all dose levels but was not associated with fever or hospitalization in any patient. At dose level 3, 35 mg/m² doxorubicin and 5.25 mg/m² topotecan, the duration of grade 4 neutropenia exceeded 5 days, and G-CSF was added to all subsequent patients at that dose level. G-CSF did not prevent significant neutropenia (observed in 3 other patients at dose level 3); however, grade 4 thrombocytopenia was then observed in addition to neutropenia. Other than the expected alopecia inherent in the use of intermittent doxorubicin, no nonhematological toxicity was observed. The pharmacokinetics of topotecan were unaffected by the prior administration of doxorubicin. Doxorubicin (25 mg/ m²) represents 40–60% of the usual dose in combination regimens. The total topotecan dose of 5.25 mg/m² represents 70% of the single-agent dose and exceeds the dose administered in...
combination regimens with paclitaxel or with cyclophosphamide and carboplatin with stem cell support (26). The multiples of the individual doses of doxorubicin and topotecan in combination are in the order of most clinical combination chemotherapy regimens.

Partial responses were seen at every dose level. Three of 7 patients with small cell lung cancer had partial responses, lasting 6 months. None of these patients had received topotecan in the past, so the responses cannot necessarily be attributed to this particular sequence of two individually active agents. Responses were also seen in non-small cell lung cancer, esophageal cancer, and ovarian cancer. Whereas conclusions about efficacy are not appropriate in a Phase I study, especially a study designed to combine two clinically active agents, the responses imply no lack of activity using doxorubicin and topotecan in this sequence.

The effect of sequential topoisomerase I- and II-active agents on the levels of topoisomerase I, IIα, and IIβ mRNA was measured in PBMCs before any drug, 48 h after doxorubicin but before topotecan and immediately after topotecan. RT-PCR measurement of mRNA was used based on our preclinical experience where it was comparable with protein measurement and perhaps less subject to experimental variability from topoisomerase-DNA complex formation (25). It was assumed that adequate drug uptake would occur in PBMCs within the vascular compartment, because both drugs are myelosuppressive. Whereas the effect of this sequential therapy in cancer cells is obviously the most critical factor in determining the benefit to this approach, nontransformed PBMCs are more accessible and homogenous from the perspectives of drug uptake, cell cycle, oxygenation, and other physiological parameters, which may confuse the interpretation of the findings.

Table 4 Characteristics of patients responding to Adriamycin and Topotecan

<table>
<thead>
<tr>
<th>Prior regimens</th>
<th>Best response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell lung cancer</td>
<td></td>
</tr>
<tr>
<td>#1 Etoposide/Platinum</td>
<td>CR (early CNS relapse)*</td>
</tr>
<tr>
<td>#2 Etoposide/Platinum</td>
<td>PR</td>
</tr>
<tr>
<td>#3 Ifosfamide/carb/Ifos</td>
<td>CR</td>
</tr>
<tr>
<td>High-dose Cytoxan, BCNU, Platinum</td>
<td></td>
</tr>
<tr>
<td>Non-small cell lung cancer</td>
<td></td>
</tr>
<tr>
<td>#4 #4 Taxol/Carboplatin</td>
<td>PD</td>
</tr>
<tr>
<td>#5 5 FU, Cisplatin (neoadjuvant)</td>
<td>Path CR</td>
</tr>
<tr>
<td>Taxotere (for metastatic disease)</td>
<td>PD</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td></td>
</tr>
<tr>
<td>#6 Cytoxan/Platinum/Taxol</td>
<td>CR</td>
</tr>
</tbody>
</table>

* Previous treatment history of the six individual who responded to adriamycin and topotecan.

Table 5 Mean pharmacokinetic parameters of topotecan lactone and total topotecan in patients treated with the MTD of 1.75 mg/m² topotecan given as a 30-min i.v. infusion on days 3-5 after a 1-h i.v. infusion of 25 mg/m² doxorubicin on day 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Topotecan lactone</th>
<th>Total topotecan</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Cmax (nm)</td>
<td>89.1 ± 38.7</td>
<td>132.1 ± 65.3</td>
</tr>
<tr>
<td>AUC (nm x h)</td>
<td>135.6 ± 61.6</td>
<td>230.0 ± 100.0</td>
</tr>
<tr>
<td>CL (liters/h/m²)</td>
<td>35.5 ± 21.8</td>
<td>16.1 ± 11.7</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.7 ± 0.9</td>
<td>2.7 ± 0.9</td>
</tr>
<tr>
<td>Vss (liters/m²)</td>
<td>76.6 ± 28.0</td>
<td>42.6 ± 22.3</td>
</tr>
</tbody>
</table>

MRT, mean residence time; Vss, apparent volume of distribution at steady state.

Fig. 1 Plot depicting the distribution of AUC values for topotecan lactone (○) and total topotecan (△) in individual patients treated at each of the three dose levels of the doxorubicin/topotecan combination that were evaluated. Solid bars, mean AUC values.
most consistent pattern of the topoisomerases assayed. Levels rose, even if only slightly, at 48 h after treatment with doxorubicin and remained elevated at the end of topotecan therapy on day 5 in 6 of 7 patients. In 2 patients, this increase exceeded an order of magnitude. Topoisomerase II \( \alpha \) is a target for anthracycline drugs, but changes in its level of expression as a result of therapy have not been reported previously in clinical trials. Nevertheless, murine transgenic cell lines lacking topoisomerase II \( \beta \) have demonstrated greater resistance to mitoxantrone than topoisomerase II \( \beta ++ \) cell lines, suggesting anthracycline cytotoxicity is partially dependent on the topoisomerase II \( \beta \) enzyme, as well as topoisomerase II \( \alpha \) (27).

The significance and reproducibility of this observation is limited by the small sample size and will require larger studies. There was no relationship of changes in topoisomerase mRNA to topotecan pharmacokinetics in this small group of 7 patients.

An additional limitation of the RT-PCR analysis of topoisomerase transcript levels is that this analysis does not address post-transcriptional regulation of topoisomerase protein levels or regulation of cleavable complexes. Indeed, Rubin et al. have demonstrated correlation of the \( C_{\text{ss}} \) of oral camptothecin with decrease in topoisomerase I protein levels. Regulation of topoisomerase I by topoisomerase I poisons is regulated post-transcriptionally and was dependent on both ubiquitin/26S proteasome-mediated regulation and SUMO-1 conjugation of the topoisomerase enzyme (28–31). Importantly, the proficiency of peripheral blood lymphocytes to ubiquinate or sumolyate topoisomerase I is often not representative of the biochemical efficiency of these processes within tumor cells (30). Thus, extrapolation of topoisomerase I protein levels (and indeed RNA levels) within peripheral blood lymphocytes to those of the tumor is potentially inappropriate.

The sequential administration of doxorubicin and topotecan produces acceptable clinical toxicity (myelosuppression) and evidence of antitumor activity, especially in small cell lung cancer. There is no evidence to suggest that doxorubicin altered the pharmacokinetics of topotecan. In addition, there was no apparent relationship between the \( C_{\text{ss}} \) or AUC of topotecan and toxicity or response to therapy. The measure of topoisomerase-I, -II\( \alpha \), and -II\( \beta \) mRNA levels in PBMCs showed a frequent and substantial effect of these agents, although no consistent pattern was observed for either topoisomerase I and topoisomerase II \( \alpha \). Topoisomerase II \( \beta \) mRNA levels showed a more consistent pattern and a suggestion of differing patterns in responding and nonresponding patients. The clinical utility of sequencing doxorubicin and topotecan is currently under investigation in a Phase II trial in non-Hodgkin’s Lymphoma in a national cooperative group trial.

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