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Report

Topoisomerase I levels in white blood cells of patients with ovarian cancer treated with paclitaxel–cisplatin–topotecan in a phase I study

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Topotecan stabilizes the topoisomerase I (Topo I) cleavable complex. We measured Topo I levels in white blood cells of patients with ovarian cancer treated with topotecan. Topotecan was given i.v. daily ×5 q 3 weeks in combination with paclitaxel (1 day before topotecan) and cisplatin (just prior topotecan). Our aim was to correlate Topo I levels to pharmacokinetics and toxicity. Topo I levels were determined using Western blotting and were expressed relative to the Topo I level present in 10 μg cell lysate of the human IGROV1 ovarian cancer cell line. We found no correlation between Topo I levels and (non-)hematological toxicity. Topo I levels after the fifth topotecan infusion were significantly negatively correlated with the AUC of topotecan ($R=-0.64$, $p=0.026$), in contrast with Topo I levels prior to ($R=-0.25$, $p=0.4$) and after ($R=-0.30$, $p=0.3$) the first topotecan infusion. Topo I levels after the fifth topotecan infusion (48±27%, mean±SD) were higher than Topo I levels prior to and after the first topotecan infusion (3.0±4.7 and 2.7±3.6%, respectively) ($p=0.001$). In conclusion, we detected a significant inverse correlation between Topo I level and topotecan AUC at day 5, and we found increasing Topo I levels during a daily ×5 schedule of treatment with topotecan. [© 2002 Lippincott Williams & Wilkins.]

Key words: Pharmacokinetics, topoisomerase I, topotecan, Western blotting, white blood cells.

Introduction

Camptothecin and analogs are an important class of anti-cancer drugs that have demonstrated a broad spectrum of anti-tumor activity.[1] The camptothecin analog topotecan is currently registered in many European countries and in the US for the second-line treatment of ovarian cancer.[2] Topotecan and other camptothecin analogs exert their anti-tumor activity by stabilizing covalent Topoisomerase I (Topo I)–DNA cleavable complexes.[3–5] Collisions between the stabilized Topo I cleavable complexes and advancing replication forks result in replication fork arrest and double-strand breaks which leads to cell death.[3,4] The formation of the Topo I cleavable complex is reversible; it exists only in the presence of a camptothecin-like drug.[5,6] Cells in the S phase are about 100–1000 times more sensitive to the cytotoxic effect of Topo I inhibitors than cells in other phases of the cell cycle.[7] *In vitro* experiments have shown that cellular resistance to Topo I inhibitors may be associated with a low level of Topo I or with mutations in the gene encoding for it.[5,8] Increased Topo I levels in tumor tissue is frequently correlated with cytotoxicity in preclinical models.[9,10] Furthermore, Topo I activity has been found to correlate with the clinical response to Topo I inhibitors.[11–13] Hochster *et al.*[14] and Liebes *et al.*[15] found a negative correlation between Topo I level in tumor tissue and the area under the curve (AUC) of topotecan in plasma.

Topo I levels may thus be a useful predictor of resistance or sensitivity to topotecan and can yield useful information for the design of future treatment schedules with topotecan, improving efficacy and preventing toxicity. We measured Topo I

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in mononuclear white blood cells (WBC) of patients treated with topotecan in an attempt to correlate Topo I levels to pharmacokinetic parameters, toxicity and clinical response. Topotecan was given as a 30-min daily × 5 infusion every 3 weeks in combination with paclitaxel (1 day before topotecan) and cisplatin (just prior topotecan). A complete plasma pharmacokinetic evaluation of topotecan, cisplatin and paclitaxel was available.[16]

Material and methods

Patients and study design

The combination of paclitaxel, cisplatin and topotecan was administered as first-line therapy in patients with advanced ovarian cancer. Paclitaxel was administered over 24 h (day 1) followed by cisplatin over 3 h (day 2). On day 2 topotecan was administered following completion of the cisplatin infusion. Topotecan was administered as a 30-min infusion daily for 5 consecutive days (days 2–6). Standard premeditation before paclitaxel administration consisted of dexamethasone, clemastine and cimetidine. Prehydration before cisplatin was started during paclitaxel infusion. The following dose levels of paclitaxel/cisplatin/topotecan (in mg/m^2/day) were evaluated: 110/50/0.3 without granulocyte colony stimulating factor (G-CSF) (dose level 1), 110/50/0.3 (dose level 2), 110/75/0.3 (dose level 3) and 110/75/0.4 (dose level 4) with G-CSF starting at day 6. Treatment was repeated every 3 weeks. Patients characteristics are listed in Table 1. The study protocol was approved by the Medical Ethics Committee of the hospital and all patients gave written informed consent.

Table 1. Patient characteristics

	No. of patients
Total patients	21
Patients for Topo I analysis	13 (62%)
Median age (range)	59 (29–71)
WHO performance status	
0	4
1	14
2	3
Histology of ovarian cancer	
serous	10
endometroid	7
adenocarcinoma	3
adenopapillary	1

Pharmacokinetics and cellular pharmacology

Complete pharmacokinetic evaluations of cisplatin bound and unbound, paclitaxel and topotecan in plasma were performed. Furthermore, a complete hematological and non-hematological toxicity and response evaluation was completed. These data were previously described by Herben *et al.*[16]

Topo I protein detection

For the determination of Topo I levels, samples were taken prior to and 2 h after the end of the topotecan infusion at day 2, and 2 h after the end of the topotecan infusion at day 6. The heparinized blood samples (30 ml each) were collected on ice. Immediately after collection of the blood samples, mononuclear cells were isolated using either a Ficoll gradient (Lymfoprep; Pharmacia, Uppsala, Sweden) or CPT-vacutainer tubes (Vacutainer CPT; Becton Dickinson, Mountain View, CA) according to the manufacturer's description and stored at −80°C. For Western blotting, cells were denatured by sonification in 62.5 mM Tris, 10% glycerol, 2.5% SDS, 5% β-mercaptoethanol, 0.005% bromophenol blue and 0.5 mg/ml Pefablock. A small amount of cell lysate was used to determine protein concentration according to the method of Bradford.[17] A large batch of the human IGROV1 ovarian cell line was prepared and divided in small aliquots after homogenization and denaturation for use as a reference. After separation of 10–30 μg of the proteins on a 7.5% polyacrylamide gel, proteins were electrophoretically transferred to nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Topo I proteins were detected using a human topoisomerase I antibody (TopoGEN, Columbus, OH) and spots were visualized using the ECL assay (Amersham Life Sciences, 's-Hertogenbosch, The Netherlands). Topo I levels were expressed relative to the Topo I level present in 10 μg cell lysate of the human IGROV1 ovarian cancer cell line.

Pharmacokinetic–pharmacodynamic analysis

For the pharmacokinetic–pharmacodynamic analysis both the absolute Topo I level and the percentage of Topo I level of the pre-treatment value were used. Differences in Topo I levels between patients with different responses were evaluated using one-way analysis of variance (ANOVA) combined with the least significant difference (LSD) method and Student's

t-test was used to calculate the *p* value of significant differences. Statistical analysis was performed with SPSS (version 6.1 for Windows; SPSS Inc). The level of significance (p) was set at 0.05. All tests for significance were two-tailed. Relationships between Topo I levels and categorical toxicity and response data were explored using the Spearman rank correlation test. Relationships between Topo I levels and the AUC of topotecan, cisplatin and paclitaxel were explored using the Pearson correlation test and scatter plots. Relationships between Topo I levels and myelosuppression were explored using Pearson correlation test and scatter plots of the Topo I levels versus the percentage decrease in WBC, absolute neutrophil count and platelet count. The percentage decrease in blood cells is defined as: [100 × (pre-treatment value — nadir value)]/pre-treatment value. The data were fit using (log)-linear and sigmoidal maximum effect (Emax) models using the software package WinNonlin (version 3.0; Pharsight, Mountain View, CA). Only data obtained during the first course were used.

Results

Patients

Patient characteristics are listed in Table 1. Topo I levels were determined in 13 of 21 patients: five patients at dose level 1, three patients at dose level 2, three patients at dose level 3 and two patients at dose level 4.

Topo I levels and pharmacokinetics

A summary of the overall pharmacokinetics and Topo I levels is listed in Table 2 and Figure 1. We found a significant increase of Topo I levels at day 6 in WBC of patients compared to levels at day 2, before and after administration of topotecan (Figure 1) ($p<0.001$ and $p<0.001$, respectively). No correlation between Topo I levels and paclitaxel and cisplatin pharmacokinetics could be found (data not shown). At day 6, 2 h after topotecan administration Topo I levels and the AUC of topotecan were significantly inversely correlated ($R=-0.64$, $p=0.026$) (Figure 2). However, at day 2, before and 2 h after topotecan administration, no correlation between the AUC of topotecan and Topo I levels could be found (Table 2).

Table 2. Pharmacokinetics of paclitaxel, cisplatin and topotecan

Dose level	*N*	Dose (mg/m^2)			G-CSF	AUC[a] (h · nmol/l)			Relative Topo I level (%)[a,b]		
		paclitaxel	cisplatin	topotecan		paclitaxel ($\times 10^3$)	cisplatin ($\times 10^3$)	topotecan	1	2	3
1	5	110	50	0.3	−	4.9 (1.1)	14.3 (1.0)	16.9 (1.1)	1.4 (2.2)	1.9 (2.2)	47 (31)
2	3	110	50	0.3	+	3.5 (0.3)	13.5 (2.8)	14.3 (2.4)	2.2 (2.1)	0.6 (0.7)	68 (37)
3	3	110	75	0.3	+	2.9 (0.9)	19.8 (5.5)	11.7 (2.5)	5.7 (9.6)	5.5 (5.6)	54 (13)
4	2	110	75	0.4	+	4.5 (0.2)	19.6 (0.3)	22.6 (2.6)	4.0 (3.6)	3.2 (4.6)	22 (19)

[a]Mean data; numbers in parentheses show standard deviation.
[b]1: day 2, after paclitaxel and cisplatin, before topotecan infusion; 2: day 2, 2 h after topotecan administration; 3: day 6, 2 h after topotecan administration.

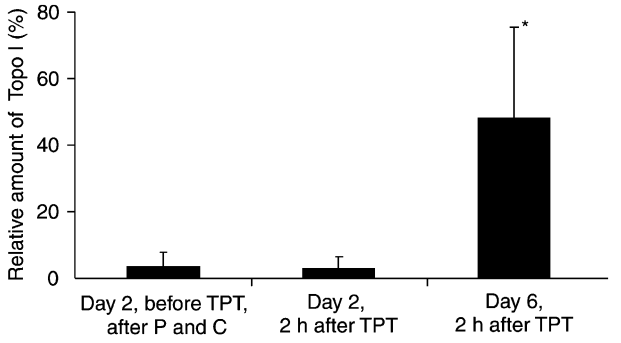

Figure 1. Topo I levels (±SD) of patients treated with topotecan (mean of all dose levels, $N=13$). *Significantly different from day 2 before and after TPT infusion ($p<0.001$).

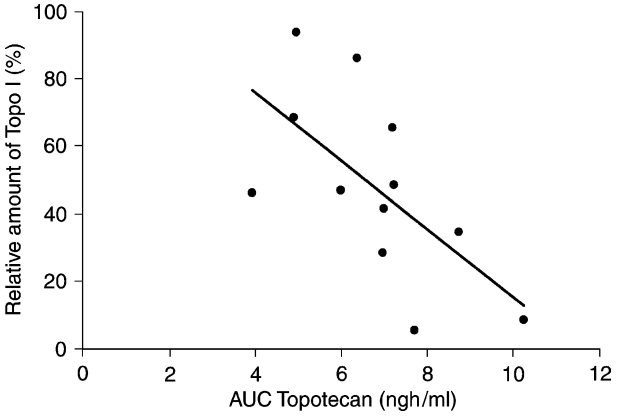

Figure 2. Topo I levels in WBC at day 6, 2 h after topotecan administration correlated to plasma AUC of topotecan of patients in the phase I study of paclitaxel, cisplatin and topotecan. Line represents regression of Topo I levels and AUC topotecan at day 6, 2 h after topotecan ($r=-0.64$, $p=0.026$).

Topo I levels in relation to toxicity and activity

No significant correlation between Topo I levels and hematological toxicity (ANC levels and WBC levels) could be found nor any correlation between response and Topo I levels could be found (results not shown).

Discussion

Topotecan has Topo I as unique target and reversibly stabilizes the Topo I cleavable complex.[3,5,18] We measured Topo I levels in WBC of 13 patients treated with topotecan, in order to investigate the correlation between topotecan pharmacokinetics and Topo I levels, as a clinical and pharmacodynamic endpoint. We found increased Topo I levels on day 6 compared to Topo I levels prior to and 2 h after the first topotecan infusion. From other studies no influence was observed of paclitaxel and cisplatin on Topo I levels (data not shown, unpublished results JHB and JHMS). The increase in Topo I levels could indicate that the sensitivity to topotecan increases after repeated administration. However, these findings are in contrast with other studies, which observed decreased Topo I levels in WBC after (repeated) treatment with topotecan.[9,14,19] The increased Topo I levels after the fifth topotecan infusion in our study was significantly inversely correlated with the AUC of topotecan. This inverse correlation is also found in other studies with topotecan where Topo I levels were measured in WBC or tumor tissue.[9,14,15,19] A possible explanation of this effect is that Topo I is, apparently, down-regulated after high exposure to topotecan.[20] Furthermore, we found no correlation between pre-treatment Topo I levels or differences in Topo I levels and (non-)hematologic toxicity nor could we find any significant relation with anti-tumor activity and Topo I levels. The lack of positive correlations might be caused by the high inter-patient variation in Topo I levels and the limited number of patients. More studies of Topo I levels and Topo I activity in both WBC and tumor tissue are needed to understand pharmacokinetic and dynamic interactions of Topo I and topotecan.

Conclusion

In this limited patient population no correlation between Topo I levels and toxicity endpoints could be observed. However, we found a significant increase in Topo I levels after repeated topotecan exposure. Furthermore, we observed a significant relationship between Topo I levels and topotecan pharmacokinetics as a negative correlation with the AUC of topotecan. Further research is warranted to get more insight into these phenomena and how to use them to design improved treatment schedules with topotecan to enhance efficacy and prevent toxicity.

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