Topoisomerase-I activity in ovarian cancers is a predictor of sensitivity to topotecan chemotherapy – an exciting preliminary result

Mu-Hsien Yu¹, Steve R. Roffler², Bing-Mae Chen²

¹Department of Obstetrics and Gynecology, Tri-Service General Hospital and National Defense Medical Center, Taipei, Taiwan
²Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan

Abstract

Purpose: To determine whether the activity of topoisomerase I (topo I), the target of the anti-neoplastic drug camptothecin (CPT), is elevated in ovarian cancers and whether topo I activity is a useful predictor of tumor response to topotecan (Hycamptin; Smithkline Beecham Pharmaceutical, Philadelphia, PA), a CPT analog. Methods: The topo I activities of 21 normal ovaries, 10 ovarian benign tumors and 66 ovarian malignant tumors were assayed by measuring relaxation of super-coiled DNA. Among the patients with ovarian carcinoma, eleven women with recurrent epithelial ovarian cancer who received second-line topotecan chemotherapy every 3 weeks for 6 cycles were analyzed. Results: Mean topo I activities in ovarian cancers (3.81 + 3.09 h⁻¹) were significantly greater than in the ovarian benign tumors and the normal ovaries (0.45 + 0.25 h⁻¹ and 0.53 + 0.46 h⁻¹). Stage 3 and 4 ovarian carcinoma specimens displayed significantly greater topo I activity (4.62 + 3.29 h⁻¹) than stage 1 and 2 tumors (2.30 + 1.97 h⁻¹). Of the 11 patients with recurrent disease, 4 responded to topotecan and 7 did not. The topo I activity in tumor samples of responders (6.45 + 2.32 h⁻¹) was significant greater than that of non-responders (3.59 + 1.83 h⁻¹). Conclusions: Topo I activity is elevated in ovarian cancers compared to the benign ovarian tumors and the normal ovaries. Topotecan may be an appropriate second-line regimen for patients with recurrent ovarian cancers that display high topo I activity. The sensitivity to topotecan chemotherapy may be further predicted by measurement of topo I activity.

Keywords: topoisomerase I, ovarian carcinoma, topotecan, response
Introduction

The gold standard chemotherapy for women with epithelial ovarian cancer is a combination of paclitaxel and a platinum analog. Although this combination has achieved a high response rate, the treatment for some patients is still suboptimal. The majority of patients usually succumb after an initial gratifying response to chemotherapy followed by the emergence of resistant disease and clinical progression. Accordingly, the appropriate choice of a second-line chemotherapy regimen and the confident prediction of tumor response are clearly needed.

Camptothecin and its analogs such as topotecan and CPT-11 have been under extensive investigation for cancer chemotherapy. Topotecan, a water-soluble derivative of CPT, has demonstrated activity against ovarian cancer and is approved by the Food and Drug Administration for the salvage treatment of ovarian cancer (1). However, low response rates (14% to 16%) were obtained in multiple studies with topotecan in patients with relapsed ovarian cancer (2). If response to topotecan could be predicted, patients could be segregated to increase therapeutic benefit and reduce unnecessary toxicity associated with ineffective therapy. Topo-I activity has been reported to play an important role in sensitivity to CPT analog in patients with ovarian cancer (3). The sensitivity to CPT analog also appears to correlate with topo I activity (4,5). We have previously shown that topo I protein levels do not correlate with topo I activity (6). In the current study we therefore measured topo I activity in normal ovary and tumor samples and also studied the relationship between topo-I activity and tumor response to topotecan to clarify whether topo I activity can help in selection of patients who will have benefit from topotecan chemotherapy.

Materials and Methods

Reagents and tissues: Camptothecin and biological reagents were purchased from Sigma Chemical Company (St. Louis, MO). Camptothecin stock solutions were made in dimethylsulfoxide at 2.5 mg/ml, sterilized by filtration and stored at -70°C. Hycamptin was purchased from SmithKline Beecham Pharmaceutical (Philadelphia, PA). Tissue samples including 66 ovarian cancers, 10 ovarian benign tumor (4 teratoma, 2 endometrioma, 3 mucinous and 1 serous cystadenoma), and 21 normal ovaries were obtained from patients who underwent surgery at Tri-Service General Hospital, Taipei, Taiwan. Informed consent was obtained from all subjects. Care was taken to ensure that normal and tumor components of samples were separated during tumor dissection. All specimens were immediately frozen in liquid nitrogen and stored at -135°C. The pathology of all tissues was microscopically confirmed by a pathologist.

Nucleic extracts. Finely diced tissues samples were washed once each with ice-cold phosphate-buffered saline and nuclear buffer (150 mM NaCl, 1 mM KH2PO4, 5 mM MgCl2, 1 mM EGTA, 0.2 mM dithiothreitol, 10% (v/v) glycerol and 0.1 mM PMSF, pH 6.4). Tissues were suspended in nuclear buffer containing 0.3% Triton X-100 and disrupted in a mechanical dounce homogenizer on ice. Nuclei, collected by centrifugation at 1000xg for 10 min, were washed once with nuclear buffer before addition of nuclear
buffer containing 0.25 M NaCl. Nuclei were gently rotated for 30 min at 4°C, centrifuged at 16,000xg for 30 min to remove debris, and immediately assayed for topo I activity or stored at -135°C. At least two independent nuclear extracts were prepared for each sample.

Topo-I relaxation assay. Supercoiled pBR322 plasmid was purified from bacterial cultures by alkaline lysis followed by equilibration centrifugation in a cesium chloride-ethidium bromide continuous gradient for 36 h. Aliquots of supercoiled DNA were stored in absolute ethanol at -80°C. To assay topo I activity, 1.0 µg supercoiled pBR322 was preheated to 37°C in 15 µL reaction buffer (10 mM Tris-HCl pH 7.5, 200 mM KCl, 10 mM MgCl₂, 1 mM EDTA, 1 mg/ml BSA and 1 mM dithiothreitol) before addition of 5 µL nuclear extract diluted in reaction buffer. The reaction was terminated after 30 min by addition of 2 µL 10% SDS. Two µL loading dye (40% sucrose, 0.025% bromophenol blue and 50 mM EDTA) was added and samples were electrophoresed at 3 V/cm for 5 h in a 1% TPE agarose gel containing 0.03% SDS to separate relaxed and supercoiled DNA. Gels were extensively washed with water to remove SDS and stained with ethidium bromide. Gel images were captures on an Eagle eye under UV illumination. The supercoiled DNA band was quantified using the public domain NIH image program (developed at NIH and available at Topo I activity was calculated.

Patients. 11 patients enrolled for Hycamtin chemotherapy were selected from women admitted to Tri-Service General Hospital for recurrent epithelial ovarian cancer between 1999 and 2002. All patients had undergone and failed first-line chemotherapy with paclitaxel and platinum (TP). Patients that displayed resistance to TP and experienced recurrent disease within 6 months after the completion of TP therapy were defined as platinum-resistant; otherwise they were defined as platinum-sensitive. The subjects included 9 patients with serous cystadenocarcinomas, 1 patient with endometrioid cystadenocarcinoma and 1 patient with clear cell carcinoma. Patient ages ranged from 40 to 71 years.

Treatment and response. Eligible patients received 1.5 mg/m² Hycamtin as a 30-minute infusion for 5 consecutive days every 21 days for 4-6 cycles. Treatment was stopped if there was no change in tumor size. The initial doses of Hycamtin were modified in subsequent courses depending on toxicity. No premedication was given to patients. Prophylactic recombinant granulocyte colony-stimulating factor (G-CSF) (filgrastim: Neurogen; Amgen Inc. Thousand Oak, CA) was allowed if the patient experienced grade 3 or 4 neutropenia during the previous treatment course. Tumor size was evaluated by ultrasonography or computed tomography after each course of chemotherapy. All responses required careful review. Complete response (CR) required the complete disappearance of all known measurable and assessable disease on two separate measurements at least 4 weeks apart.
Partial response (PR) was defined as more than 50% reduction in all measurable lesions without the appearance of new lesions for at least 4 weeks. No change (NC) was defined as less than 50% decrease or 25% increase in all measurable lesions without the appearance of new lesions. Progressive disease (PD) was defined as more than 25% increase in measurable disease at a known site, or the appearance of a new lesion. Nonmeasurable disease was assessed with an elevated CA-125 tumor marker.

Statistical analysis. Statistical significance of difference between mean values was calculated using the independent t-test for unequal variances.

Results

The topo I activity of nuclear extracts prepared from normal and tumor specimens was assayed by measuring relaxation of supercoiled DNA. Densitometer quantitation of supercoiled DNA band intensity after electrophoresis of DNA on agarose gels allowed estimation of topo I activity. Only band intensities corresponding to between 10-90% DNA relaxation were employed for calculation of topo I activity to prevent saturation of the assay and increase the assay precision. Table 1 summarizes the mean topo I activities measured in ovarian specimens. The mean topo I activity in ovarian carcinoma tumors was significantly (p < 0.0005) greater than in ovarian benign tumors and normal ovaries. In addition, topo I activity was significantly (p < 0.0005) higher in advanced (stages 3 and 4) compared to early (stages 1 and 2) ovarian carcinoma. 48 of 66 ovarian (73%) carcinomas [11 of 23 stages 1 and 2 (48%) and 37 of 43 stages 3 and 4 (86%)] displayed topo I activities greater than 3 standard deviations above the mean values of topo I activity in normal and benign ovarian tissues.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Topo I activity(μg/h)*</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal ovary</td>
<td>0.52 ± 0.46</td>
<td>21</td>
</tr>
<tr>
<td>Benign ovarian tumor</td>
<td>0.45 ± 0.25</td>
<td>10</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>3.81 ± 3.09b,c</td>
<td>66</td>
</tr>
<tr>
<td>Ovarian carcinoma (stage 1-2)</td>
<td>2.30 ± 1.97b,c</td>
<td>23</td>
</tr>
<tr>
<td>Ovarian carcinoma (stage 3-4)</td>
<td>4.62 ± 3.29b,c,d</td>
<td>43</td>
</tr>
</tbody>
</table>

* Activity is expressed as μg supercoiled DNA relaxed per μg nuclear extract in 1h.

b Significantly greater (p < 0.0005) than normal ovary.

c Significantly greater (p < 0.0005) than benign ovarian tumor.

d Significantly greater (p < 0.05) than stage 1-2 ovarian carcinoma.
Of the 11 patients that failed initial platinum-based chemotherapy and were then treated with topotecan as second-line chemotherapy, 4 responded to topotecan (CR, 3; PR, 1) and 7 did not (NC, 2; PD, 5). Table 2 lists the patient characteristics between responders and non-responders.

Table 2 Patient characteristics

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Responder (4)</th>
<th>Non-responders (7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>45 – 53 (47.8)</td>
<td>40 – 71 (54.3)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Mucinous</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Clear cell</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Platinum sensitive</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Platinum resistant</td>
<td>1</td>
<td>4</td>
</tr>
</tbody>
</table>

The response rate of the topotecan in platinum-sensitive patients (3/6) was higher than in the platinum-resistant patients (1/5). Although the topo I activity varied widely between individual samples in these patients, the mean topo I activity of the responders was significantly (p < 0.05) greater than the mean activity of the non-responders (Table 3).

Table 3. Topoisomerase I activities in tumors of responders and non-responders.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Topo I activity($h^{-1}$)</th>
<th>No. of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Responders</td>
<td>6.45 ± 2.32 *</td>
<td>4</td>
</tr>
<tr>
<td>Non-responders</td>
<td>3.59 ± 1.83</td>
<td>7</td>
</tr>
</tbody>
</table>

*Significantly greater (p < 0.05) than non-responder

Discussion

Topo I is the sole cellular target of CPT and related analogs such as topotecan. CPT stabilizes the intermediate covalent complex formed between topo I and duplex DNA, resulting in double-stranded DNA breaks and cell death. Catalytically active topo I must be present in cells for CPT-induced toxicity (7). Moreover, the sensitivity of tumor cells to CPT

18
is believed to be positively correlated with topo I activity (7-10). We therefore examined the activity of topo I in normal and neoplastic ovarian specimens to clarify the relationship between topo I activity and sensitivity to the second-line chemotherapy consisting of topotecan in patients with ovarian cancer.

Topo I activity was 8-fold higher in ovarian carcinomas as compared with benign tumors and 7-fold higher as compared with the normal counterparts. Although mean topo I activities were significantly elevated in ovarian carcinomas, the activity in individual tumors varied widely, ranging from 0.04 to 19.3 h\(^{-1}\). Benign ovarian tumors, with most cells in the resting phase, expressed topo I activities at similarly low levels as normal ovaries. We also found that 73% of ovarian tumors possessed topo I activities greater than three standard deviations above the mean topo I activity in the corresponding normal and benign tissues. An earlier study performed in the Netherlands also found similar elevations of topo I activity (8-fold) in malignant ovarian tumors compared with benign tumors (11) suggesting little variation of topo I activity among diverse populations of patients. Although the absolute topo I activity required for tumor sensitivity to topotecan has not been defined, our results suggest that a major portion of ovarian cancers may have significant sensitivity to topotecan. (12-14). However, almost all trials were salvage therapy for serious patients, making achievement of satisfactory response rates difficult.

In our study, topo I activity positively correlated with tumor burden and disease stage. Advanced tumors (stages 3 and 4) displayed significantly higher topo I activity than limited disease (stages 1 and 2). This same situation was also observed in cervical cancer in our previous study (6). A similar trend of increasing topo I protein levels in advanced colon carcinoma compared to early stage disease was reported (15). However, no correlation was found between disease stage and topo I activity in colorectal and prostate cancers in another study (16). Our results suggest that topotecan treatment may be more effective against advanced ovarian carcinoma compared with early disease.

It is accepted that histology evaluation does not provide information in regard to chemotherapy response. The current approach to choose a specific regimen for second-line chemotherapy takes into account the tumor response to front-line therapy (17, 18, 19). However, the choice is usually also a matter of personal bias to some extent. An interesting finding of our study was that mean topo I activity was significantly higher in the topotecan-responders than in the non-responders. These results suggest that topo I activity may be useful in predicting tumor responses to topotecan. Patients with higher topo I activity in their tumors may have a higher probability of benefit whereas patients resenting tumors with lower topo I activity may be spared ineffective therapy. Several studies have reported that the \textit{in vitro} sensitivity of tumor cells to topo inhibitors is correlated with nuclear topo I levels (5, 7, 20). Some earlier clinical studies also found that topo I activity was significantly greater in responders than non-responders and that topo I activity was enhanced by exposure to cisplatinum (3, 21). We did not observe a higher response rate in the
cisplatinum-resistant ovarian cancers than in the sensitive ones (1/5 vs 3/6). It may therefore be rational to incorporate topotecan into current front-line combination chemotherapy. Our results support the concept that topo I activity may be a predictor of the sensitivity to topo I inhibitors. Given the overlap in topo I activities between responder and non-responders, it will be important to identify additional determinants of topotecan response to avoid ineffective topotecan therapy and its associated toxic side effects.

Acknowledgements

This work was supported in part by the Tri-Service General Hospital and the National Science Council of Taiwan (NSC 90-2314-B-016-131 & NSC91-2314-B-016-084)

References

clonal isolates to camptothecin. Cancer Res, 52:525


卵巢癌組織中 Topoisomerase-I 之活性可作為腫瘤對化療藥物 topotecan 反應的預測指標—令人興奮的初步結果

余崇賢 ¹, Steve R. Roffler², 陳炳樺 ²

¹國防醫學院三軍總醫院婦產部
²中央研究院生物醫學科學研究所

摘要

Camptothecin(CPT) 與其同質物 topotecan 已被積極地研究作為抗癌的化療治劑。然而，目前 topotecan 用於治療複發卵巢癌的效果不佳。如果腫瘤對化療的反應可以預測，適合的患者將可得到治療的好處，不適合的患者可以避免無效治療的毒性。本研究即為偵測卵巢癌中 topoisomerase-I(topo-I)之活性是否升高並評估 topo-I 之活性是否可作為腫瘤對化療藥物 topotecan 反應的預測指標。

將 21 個正常卵巢、10 個良性卵巢腫瘤、和 66 個卵巢癌組織以測量放鬆超旋 DNA 的方式來偵測 topo-I 之活性。另外在十一位接受 topotecan 化療的復發卵巢癌患者，我們分析腫瘤之 topo-I 之活性與腫瘤化療反應間的關係。

結果顯示，卵巢癌組織中 topo-I 活性的表現 (3.81 ± 3.09 h⁻¹) 明顯高於良性卵巢腫瘤及正常卵巢組織中的表現 (0.45 ± 0.25 h⁻¹ and 0.53 ± 0.46 h⁻¹)。在第三期和第四期的卵巢癌組織中 topo-I 活性的表現 (4.62 ± 3.29 h⁻¹) 明顯地較第一期和第二期的卵巢癌組織中 topo-I 活性的表現 (2.30 ± 1.97 h⁻¹) 為高。在十一位複發卵巢癌患者，四位有反應，七位反應不佳。呈現反應的腫瘤組織中 topo-I 的活性 (6.45 ± 2.32 h⁻¹) 較反應不佳的腫瘤組織中 topo-I 的活性 (3.59 ± 1.83 h⁻¹)有較高的表現。

因此，初步的結果顯示 topotecan 化療對那些腫瘤組織中具有高 topo-I 活性的復發卵巢癌患者應是適當的第二線選擇用藥。