Attenuated Topoisomerase II Content Directly Correlates with a Low Level of Drug Resistance in a Chinese Hamster Ovary Cell Line

Charles D. Webb, Michael D. Latham, Richard B. Lock, and Daniel M. Sullivan

ABSTRACT

A new multiple drug-resistant Chinese hamster ovary cell line, CHO-SMR3, has been isolated which demonstrates a direct correlation between reduced cellular topoisomerase II activity (5-fold reduction) and a low level of resistance (3- to 7-fold) to topoisomerase II inhibitors. This cell line, initially selected for resistance to 9-(4',6-O-ethylidene-β-D-glucopyranosyl)-4'-demethyllepipodophyllotoxin, exhibits cross-resistance to other topoisomerase II inhibitors including 4'- (9-acridinylamino)methanesulfon-m-anisidide, doxorubicin, and mitoxantrone. The resistant cells show a 4.5-fold decrease in topoisomerase II by immunoblotting when compared to wild-type cells. Drug uptake studies reveal equivalent equilibrium intracellular concentrations of VP-16, 4',6-O-ethylidene-β-D-glucopyranosyl)-4'-demethyllepipodophyllotoxin in the resistant and parental cells. The catalytic activity of topoisomerase II (decatenation of kinetoplast DNA) is 5-fold less in the drug-resistant cell line relative to wild-type Chinese hamster ovary cells. Drug-induced DNA damage, measured as either formation of DNA double-strand breaks or covalent DNA-enzyme complexes, is 4-fold less in the resistant cell line. Finally, Northern blot analysis demonstrates a 5-fold reduction in topoisomerase II mRNA isolated from log phase CHO-SMR3 cells. These findings suggest that a reduced level of topoisomerase II is likely to be the sole mechanism of drug resistance in this novel cell line.

INTRODUCTION

Mammalian DNA topo II is a ubiquitous enzyme which acts to alter the topological state of DNA and thereby facilitates such vital cellular functions as segregation of chromatids during mitosis (1, 2) and replication of DNA (3). The mechanism of topo II action involves binding to DNA with subsequent formation of a transient DNA double-strand break through which a second DNA duplex may pass. Strand passage is then followed by religation of the original cleaved strand of DNA. One enzyme-mediated event (cleavage, stand passage, and religation) thus changes the DNA linking number by 2. (For a more detailed description of the mechanisms of topo II activity see Refs. 4 and 5.) Topo II has also been shown to play an important role as a structural protein within the nucleus, both as part of the nuclear scaffold and matrix (6, 7) as well as in the structural organization of nuclear chromatin (8).

Because of the vital importance of this enzyme to proliferating cells, it also provides a good target for antineoplastic drug therapy (9). Indeed, the cytotoxic effect of many chemotherapeutic agents has been found to result from the inhibition of topo II activity. These drugs include both intercalating agents such as doxorubicin, m-AMSA, mitoxantrone, and actinomycin D, as well as nonintercalating drugs such as the epipodophyllotoxins VP-16 and teniposide (10-12). These drugs appear to act by inhibiting DNA religation, resulting in an apparent increase in DNA damage (13, 14). Drug-induced DNA damage measured as double-strand breaks has been shown to correlate well with the cytotoxic effects of these drugs (15). Likewise, the drug-mediated formation of precipitable DNA-enzyme covalent complexes has also shown good correlation with cytotoxicity in CHO cells (16).

Numerous cell lines have been generated which demonstrate resistance to topo II inhibitors. The mechanisms of resistance in these cell lines have primarily involved either an alteration in drug uptake or efflux (17, 18) (e.g., cells which exhibit amplification of the mdrl gene with overexpression of the P-glycoprotein drug efflux pump) or altered topo II activity which may be the result of either a quantitative (19) or qualitative change in the enzyme itself (20-22). Usually, the multiple drug resistance exhibited by a particular cell line is the result of a combination of these factors. In this paper, however, we will discuss a recently isolated drug-resistant CHO cell line which appears to have a single mechanism of resistance to topo II inhibitors. It is a novel cell line in that it has a low level of drug resistance to topo II-active drugs, and the level of resistance, as well as the attenuated catalytic and cleavage activities, appears to depend solely on decreased nuclear topo II.

MATERIALS AND METHODS

Materials

The WT and drug-resistant cell lines were grown in monolayer and suspension cultures at 37°C in a minimal essential media (Gibco Laboratories, Grand Island, NY) with 5% heat-inactivated fetal bovine serum (Gibco) in the presence of 5% CO₂, Penicillin (100 IU/ml) and streptomycin (100 μg/ml) were added to all culture media.

Sigma Chemical Co. (St. Louis, MO) supplied ethyl methanesulfonate, camptothecin, vincristine sulfate, Nonidet P-40, RNase A, DNase I, aprotinin, leupeptin, pepstatin A, chymostatin, and antipain HCl. VP-16 was generously supplied by Bristol-Myers Co. (Wallungford, CT), and [3H]VP-16 (400 μCi/mmol) was supplied by Moravek Biochemicals, Inc. (Brea, CA). m-AMSA was obtained from the Drug Synthesis and Chemistry Branch of the National Cancer Institute. Lederle Laboratories (Pearl River, NY) generously provided mitoxantrone, and doxorubicin was obtained from Adria Laboratories (Columbus, OH). Nitrogen mustard was obtained from Merck, Sharp and Dohme (West Point, PA). RNA ladder, dithiothreitol, and phenylmethylsulfonyl fluoride were supplied by Bethesda Research Laboratories (Gaithersburg, MD). ICN Biomedicals, Inc. (Costa Mesa, CA) supplied the [14C]thymidine (58 μCi/mmol). The enhanced chemiluminescence Western blotting detection system was obtained from Amersham (Arlington Heights, IL).

Methods

Isolation of VP-16-resistant CHO Cell Lines

Drug-resistant CHO cell lines were generated by the method previously described by Gupta (23). Briefly, 5 x 10⁶ monolayer WT log phase CHO cells in 30 ml media (75-cm² flask) were treated with ethyl...
washed three times with 30 ml sterile buffer A (150 mM NaCl, 5 mM potassium phosphate, pH 7.4) to remove the ethyl methanesulfonate. The cells were allowed to recover for 10–14 days. During this recovery phase, the cells were maintained at log phase density by resededing 2–3 times/week after being trypsinized from the flask. The mutagenized cells (5 × 10^6 cells in 10 ml media) were then distributed to several 20 × 100 mm Falcon plastic Petri dishes (Becton Dickinson, Lincoln Park, NJ) and allowed to attach overnight. After continuous exposure to a low concentration of VP-16 (1 µg/ml) for 10–14 days, the Petri dishes with 1–2 surviving colonies/plate were washed gently three times with 10 ml of room temperature sterile buffer A to remove loosely adherent cells. The colonies were then aspirated from the dish with a 200-µl sterile pipet tip and transferred into 10 ml fresh media in a 25-cm² flask. The drug-resistant cell lines were then screened for both resistance to the cytotoxic effects of VP-16 by colony-forming assays as well as for resistance to VP-16-induced DNA damage by alkaline elution (see below). Cell lines of interest were next cloned (70 cells seeded in a 96-well plate), and five clones from each cell line were then restudied for VP-16 sensitivity. The CHO-SMR₃ cell line was isolated in this manner.

**Cytotoxicity Assays**

Drug and radiation sensitivities were determined by colony-forming assays as previously described (16), with the following modifications. Five hundred log phase cells in 10 ml media were allowed to attach overnight to a 100-mm plastic Petri dish and were then exposed to various concentrations of antineoplastic drugs or doses of radiation. The duration of drug treatment (empirically determined to give a 2–3 log cell kill) and solvent used for dissolution of the drugs were as follows: VP-16, camptothecin, and m-AMSA, 4 h, dimethyl sulfoxide; mitoxantrone and doxorubicin, 1 h, H₂O; vincristine, 18 h, H₂O; and nitrogen mustard, 1 h, H₂O. Controls were treated with the appropriate amount of solvent alone. Following drug treatment, the plates were washed three times with 10 ml sterile 37°C buffer A and then incubated in 10 ml of drug-free media. Colonies were allowed to grow for 7–10 days, stained with 2% crystal violet in methanol, and counted. The degree of resistance was calculated as the ratio of the resistant cell 50% inhibition drug concentration to the WT 50% inhibition drug concentraton.

**Filter Elution**

Drug-induced DNA damage in whole cells was quantified as DNA double-strand breaks by filter elution performed at pH 9.6 as previously described by Kohn (24). [³²P]Thymidine-labeled log phase CHO cells were treated with various concentrations of VP-16 for 1 h at 37°C. The cells were then washed two times with cold buffer A and scraped from the flasks in Hanks’ buffered salt solution with 0.02% Na₂EDTA. The treated cells were then resuspended in cold media and assayed for DNA double-strand breaks.

**Uptake of [³²P]VP-16**

WT and CHO-SMR₃ cells were grown to log phase concentrations in suspension cultures. Equal numbers of each cell line were then incubated with [³²P]VP-16 at concentrations of 10, 25, and 50 µM for 1 h at 37°C. Equilibrium drug concentrations were determined as previously described (16). The results are expressed as cpm/mg dry weight cells.

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**Nuclear Extracts**

DNA topoisomerase II was extracted from the nuclei of CHO cells as the decatenation of networks of kinetoplast DNA isolated from *Cricidida fasciculata* as described previously (26). Agarose gel electrophoresis of released minicircles was used to determine the top II activity present in the extracts. Topoisomerase I activity was also assayed in nuclear extracts as relaxation of supercoiled pUC18 plasmid DNA (27). The 20 µl reaction mixture contained pUC18 DNA (20 µg/ml), 50 mM Tris-HCl (pH7.5), 85 mM KCl, 0.5 mM MgCl₂, 5 mM dithiothreitol, 0.5 mM Na₂EDTA, bovine serum albumin (0.1 µg/ml), and variable amounts of the nuclear extracts. The reaction was allowed to proceed at 30°C for 30 min and then was terminated by the addition of 5 µl of a solution of 2% SDS, 0.05% bromophenol blue, and 50% glycerol. The samples were then electrophoresed in a 1% agarose gel, and the isoforms of DNA were visualized by staining the gel with ethidium bromide (5 µg/ml). Photographs of the stained gel were taken under UV illumination.

**Northern Blot Analysis**

*Escherichia coli* containing complementary DNA probe λ hTOP2 for human top II subcloned into the EcoRI site of pUC18 plasmid DNA (27). The 20 µl reaction mixture contained pUC18 DNA (20 µg/ml), 50 mM Tris-HCl (pH7.5), 85 mM KCl, 10 mM MgCl₂, 5 mM dithiothreitol, 0.5 mM Na₂EDTA, bovine serum albumin (0.1 µg/ml), and variable amounts of the nuclear extracts. The reaction was allowed to proceed at 30°C for 30 min and then was terminated by the addition of 5 µl of a solution of 2% SDS, 0.05% bromophenol blue, and 50% glycerol. The samples were then electrophoresed in a 1% agarose gel, and the isoforms of DNA were visualized by staining the gel with ethidium bromide (5 µg/ml). Photographs of the stained gel were taken under UV illumination.

**Detection of Topoisomerase II by Western Blot**

Both whole CHO cells and nuclear extracts were assayed for immunoreactive DNA topoisomerase II using our polyclonal rabbit anti-recombinant Hela top II antibody (20). Log phase cell lines (1–2 × 10^6) were pelleted at 13,000 × g for 5 min at 4°C and then treated with 90 µl of DNase/RNase solution (0.5 mg/ml DNase I-0.25 mg/ml RNase A-10 µM MgCl₂-50 mM Tris, pH 7.4–2 mM phenylmethylsulfonyl fluoride and 20 µg/ml of antipain, aprotinin, chymostatin, leupeptin, and pepstatin A) and 10 µl 2.5% SDS and placed on ice for 60 min. Next, 60 µl of SDS solution (8% SDS-0.4 M dithiothreitol-40% glycerol and the above protease inhibitors) were added, and the mixture was boiled in a water bath for 5 min. Nuclear extracts (50–100 µg protein) were diluted with one-third volume of SDS solution and boiled for 5 min. All samples were then electrophoresed on gradient SDS-polyacrylamide gels (7.5–10% resolving), transferred to nitrocellulose at 0.75 A overnight at 4°C, and then incubated with the above anti-topo II antibody. DNA topoisomerase II was detected by either the Bio-Rad immunoblot assay (goat anti-rabbit alkaline phosphatase conjugates) or by the Amersham enhanced chemiluminescence detection system (donkey anti-rabbit horse-radish peroxidase secondary antibody) according to the recommendations of the manufacturer. The autoradiograms were evaluated by densitometry to quantify the top II signals from the respective cell lines.

**Assays for DNA Topoisomerase II and Topoisomerase I Catalytic Activity**

Top II catalytic activity was measured in nuclear extracts of WT and CHO-SMR₃ cells as the decatenation of networks of kinetoplast DNA isolated from *Cricidida fasciculata* as described previously (26). Agarose gel electrophoresis of released minicircles was used to determine the top II activity present in the extracts. Topoisomerase I activity was also assayed in nuclear extracts as relaxation of supercoiled pUC18 plasmid DNA (27). The 20 µl reaction mixture contained pUC18 DNA (20 µg/ml), 50 mM Tris-HCl (pH7.5), 85 mM KCl, 10 mM MgCl₂, 5 mM dithiothreitol, 0.5 mM Na₂EDTA, bovine serum albumin (0.1 µg/ml), and variable amounts of the nuclear extracts. The reaction was allowed to proceed at 30°C for 30 min and then was terminated by the addition of 5 µl of a solution of 2% SDS, 0.05% bromophenol blue, and 50% glycerol. The samples were then electrophoresed in a 1% agarose gel, and the isoforms of DNA were visualized by staining the gel with ethidium bromide (5 µg/ml). Photographs of the stained gel were taken under UV illumination.
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Prehybridization buffer, except for the addition of 10% dextran sulfate. The hybridization step was performed overnight at 42°C with 1 x 10^6 cpm of radiolabeled probe/ml of hybridization buffer. The blots were then washed two times for 15 min in 50 ml 1 x standard saline citrate with 0.1% SDS, followed by two more 15-min washes in 50 ml 0.25 x standard saline citrate with 0.1% SDS. The blot was then exposed to Kodak X-ray film at -70°C for 18-48 h prior to developing. The topo II mRNA signal was quantified by densitometry performed on the autoradiograms, as well as by scintillation counting of the radioactivity present in bands excised from the blot itself. A duplicate gel was run simultaneously and stained with ethidium bromide to demonstrate that equal amounts of WT and CHO-SMR<sub>5</sub> RNA were loaded. Also, the primary gel was stained following the vacuum transfer step to ensure equal transfer of RNA from the two cell lines.

Presentation of Data

All data are the mean of 3-5 experiments and, unless otherwise noted, had an error of ±10%. Immunoblots and autoradiograms are representative of 4-5 experiments; nuclear extracts were obtained on three separate occasions.

RESULTS

Isolation and Growth Characteristics of Drug-resistant CHO-SMR<sub>5</sub> Cells. The CHO-SMR<sub>5</sub> cloned cell line was isolated as described under "Methods." Growth curves of the CHO-SMR<sub>5</sub>-resistant cell line and the WT CHO cell line were obtained, and these demonstrated that both cell lines have a doubling time of 14.5 h when grown in monolayer. Likewise, both cell lines reach plateau density at a concentration of 1 x 10^6 cells/ml when grown in 25-cm<sup>2</sup> flasks in 10 ml of media. Viability of the culture was defined as >95% viability by Trypan blue dye exclusion but with no net gain in cell number.

Drug Resistance Profile of CHO-SMR<sub>5</sub>. The CHO-SMR<sub>5</sub> cell line was selected for resistance to VP-16, and when colony-forming cytotoxicity assays were performed using other topo II inhibitors (doxorubicin, mitoxantrone, and m-AMSA), cross-resistance to each of these drugs was noted (Table 1). The degree of drug resistance observed was in the range of 3- to 7-fold. There was, however, no cross-resistance or collateral sensitivity noted to the alkylator, nitrogen mustard, or to vincristine, a Vinca alkaloid. Likewise, the CHO-SMR<sub>5</sub> cell line demonstrated no resistance to the topoisomerase I inhibitor camptothecin.

Colony-forming assays performed using various dose levels of radiation also showed equivalent cytotoxicity for both the WT and CHO-SMR<sub>5</sub> cells.

Immunoreactive Topoisomerase II. The topo II content of the resistant CHO-SMR<sub>5</sub> cell line was evaluated by Western blotting. Immunoblots performed using whole cell lysates of WT CHO cells and the CHO-SMR<sub>5</sub> cell line revealed a substantially decreased amount of topo II present in the resistant cell line (Fig. 1). The immunoblots were developed using the enhanced chemiluminescence Western blotting detection method (see "Materials and Methods") and demonstrated a 4.5-fold reduction in the topo II signal from the CHO-SMR<sub>5</sub> cell line when compared to the WT CHO cells. Immunoblots performed using 1.0 M NaCl nuclear extracts also demonstrated this same degree of attenuation in topo II content (data not shown). The polyclonal antibody used in Fig. 1 detects topo II of molecular mass 170 kDa. Polyclonal rabbit antibodies raised against synthetic peptides from human topo II sequences for p170 and p180 forms of the enzyme (kindly provided by Dr. Fred Drake, SmithKline Beecham Pharmaceuticals) gave the same results as our antiserum when the p170 antibody was used for immunoblotting (data not shown). Virtually no 180 kDa topo II was detected when whole cell lysates from either sensitive or resistant cells were immunoblotted with the p180 antibody (data not shown).

Drug Uptake Studies. Drug uptake studies using [3H]VP-16 showed no significant difference in uptake of drug between the WT CHO cells and the resistant CHO-SMR<sub>5</sub> cells. When exposed to initial extracellular drug concentrations of 10, 25, and 50 μM VP-16, the equilibrium intracellular drug concentrations in the CHO-SMR<sub>5</sub> cells were 119, 109, and 103%, respectively, relative to uptake measured in WT cells. The resistance of the CHO-SMR<sub>5</sub> cell line is therefore unlikely to involve a mechanism of altered drug uptake or efflux.

Table 1 Drug resistance phenotype of CHO-SMR<sub>5</sub> cells

<table>
<thead>
<tr>
<th>Antitumor agent</th>
<th>Wild-type 50% inhibition concentration&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Resistance&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP-16</td>
<td>3.000</td>
<td>7.0</td>
</tr>
<tr>
<td>Doxorubicin</td>
<td>0.230</td>
<td>3.0</td>
</tr>
<tr>
<td>Mitoxantrone</td>
<td>0.008</td>
<td>3.3</td>
</tr>
<tr>
<td>m-AMSA</td>
<td>0.075</td>
<td>3.1</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>0.790</td>
<td>1.0</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.030</td>
<td>0.9</td>
</tr>
<tr>
<td>Camptothecin</td>
<td>0.183</td>
<td>1.0</td>
</tr>
<tr>
<td>Radiation</td>
<td>392 cGy</td>
<td>1.0</td>
</tr>
</tbody>
</table>

<sup>a</sup>μM concentrations.
<sup>b</sup>fold resistance is the ratio of SMR<sub>5</sub> cell to wild-type cell 50% inhibition concentration values obtained from colony-forming assays.

![Fig. 1. Western blot electrophoresis of WT CHO (Lane 1) and drug-resistant CHO-SMR<sub>5</sub> (Lane 2) cells. One million log phase whole cells were immunoblotted with a rabbit polyclonal anti-topoisomerase II antibody.](image-url)
Catalytic Activity of Topoisomerase II and Topoisomerase I.

The catalytic activity of topo II present in 1.0 M NaCl nuclear extracts of WT and resistant CHO-SMR5 cell lines was examined by the ability of the nuclear extract to decatenate minicircles of DNA isolated from *Crithidia fasciculata*. As shown in Fig. 2, when the extracts are adjusted for equal protein concentrations, 5 times the amount of nuclear extract from the CHO-SMR5 cell line is required to fully decatenate 0.5 μg of kinetoplast DNA (250 ng compared to 50 ng of WT nuclear extract). Therefore, it appears that the decrease in topo II catalytic activity in the resistant cell line directly correlates with the decreased amount of topo II present in CHO-SMR5 nuclei.

A similar assay was performed to evaluate the catalytic activity of topoisomerase I present in the same nuclear extracts. The topoisomerase I assay measures the ability of the nuclear extract to relax a supercoiled form of pUC DNA. Fig. 3 demonstrates that topoisomerase I activity is equivalent in the CHO-SMR5 and WT nuclear extracts.

Quantitative Precipitation of the Covalent Topoisomerase II-DNA Complex. The formation of precipitable covalent DNA-enzyme complexes, in the presence of VP-16, was measured using 1.0 M NaCl nuclear extracts from WT and CHO-SMR5 cells. When an equivalent amount of protein from each nuclear extract was used (75 ng), the nuclear extract from the resistant cell line resulted in formation of fewer covalent complexes than did the WT nuclear extract (Fig. 4), with a difference of 3.5- to 4-fold at most VP-16 concentrations.

The same assay was performed with various amounts of the nuclear extracts (1.5–150 ng protein) with a constant VP-16 concentration (100 μM). Again, fewer covalent complexes were precipitated from the CHO-SMR5 nuclear extract, with a 4-fold difference noted when compared to the WT (data not shown). These results suggest a direct correlation between the diminished amount of topo II present in the CHO-SMR5 cells and the ability to form covalent enzyme-DNA complexes in the presence of drug.

VP-16-induced DNA Damage. As a measure of VP-16-induced DNA damage in these cell lines, filter elution was performed on whole cells following a 1-h exposure to various concentrations of VP-16. The elution procedure was performed at a pH of 9.6 to assay for the presence of double-strand breaks in the DNA. Double-strand breaks were selected as the end point rather than single-strand breaks to more accurately reflect DNA damage as a result of inhibition of topo II by VP-16, and because DNA double-strand breaks have been shown to have a more direct correlation with cytotoxicity. Fig. 5 shows the results of a representative experiment expressed as the percentage of DNA retained on the elution filter plotted against the sample fraction. As might be expected, fewer DNA double-strand breaks were detected in the resistant CHO-SMR5 cells at each dose level of VP-16 (10–100 μM). The degree of resistance to formation of double-strand breaks is approximately 4-fold at the VP-16 concentrations used (i.e., 100 μM VP-16 is required to cause the same number of double-strand breaks in CHO-SMR5 cells as the 25 μM dose in the WT cells). In addition to the resistance to VP-16-induced DNA scission, CHO-SMR5 cells were also found to be 3-fold resistant to m-AMSA-induced DNA single-strand breaks (data not shown). This degree of resistance to the DNA-damaging effects of m-AMSA correlates well with the sensitivity to this acridine determined by cytotoxicity assays.

DISCUSSION

Investigations into the mechanisms of resistance to topo II inhibitors have shown either an altered drug uptake or efflux in the resistant cell line or a qualitative or quantitative change in the topo II enzyme. In the first case, that of altered drug disposition, the resistant cell line is often found to have overexpression of the P-glycoprotein drug efflux pump which results in the classical multiple drug-resistant phenotype (32). This phenotype is characterized by active pumping of drugs from the cell, partial reversibility of drug resistance by verapamil (a drug which competes for efflux), and, of course, resistance to multiple classes of antineoplastic agents including the anthracyclines (doxorubicin and daunomycin), epipodophyllotoxins (etoposide and teniposide), and the *Vinca* alkaloids (vincristine and vinblastine). This mechanism appears to have no role in the drug resistance exhibited by the CHO-SMR5 cell line as evidenced
Fig. 3. Relaxation of supercoiled pUC18 DNA by the topoisomerase I activity present in 1 M NaCl nuclear extracts of WT and resistant CHO-SMR5 cells. Lane a, 1 μg of λ HindIII DNA run as a standard. Ordinate, kilobase pair markers. Lanes b-j, increasing amounts of WT nuclear extract (0, 10, 25, 50, 100, 250, 500, 750, and 1000 ng protein, respectively); Lanes k-s, increasing amounts of CHO-SMR5 nuclear extract (same amount as in the WT).

Fig. 4. Formation of topoisomerase II-DNA covalent complexes using 1 M NaCl nuclear extracts of WT and CHO-SMR5 cells. The VP-16-mediated formation of covalent complexes between 32P-labeled pUC18 DNA and the topo II enzyme present in nuclear extracts of the WT (○) and CHO-SMR5 (■) cells is shown. In this experiment, 75 ng of protein from both the WT and CHO-SMR5 nuclear extracts were incubated with 32P-labeled DNA and various concentrations of VP-16.

by the presence of equivalent equilibrium intracellular drug concentrations of [3H]VP-16 in both the CHO-SMR5 and WT cells, as well as by the lack of cross-resistance to vincristine. Furthermore, the topo II from the CHO-SMR5 cell line is unlikely to be qualitatively altered since the degree of drug resistance, as well as the catalytic and cleavage activity of the enzyme, all correlate directly with the decreased topo II content. These results suggest that a quantitative change in topo II alone is responsible for the drug resistance of the CHO-SMR5 cell line.

Several drug-resistant cell lines have been described which demonstrate attenuated levels of topo II. However, in each of these cell lines it has been difficult to discern the exact contribution of the altered topo II level to the drug resistance exhibited by the cell because other mechanisms of resistance have been present simultaneously. For instance, the human nasopharyngeal carcinoma KB cell lines (33, 34) were selected for resistance to VP-16 and exhibited cross-resistance to m-AMSA, mitoxantrone, doxorubicin, and vincristine. Each of the four cell lines isolated had decreased levels of immunoreactive topo II (22–60% of that present in the parental line). Although no overexpression of P-glycoprotein was detected by immunoblotting in any of the cell lines, the equilibrium intracellular concentrations of [3H]VP-16 were found to be reduced in all four cell lines (by 50–75%), and the drug resistance was partially overcome by coincubation with verapamil. These findings suggest that although the reduced levels of topo II are undoubtedly a contributing factor to the multiple drug-resistant phenotype of these KB cells, a drug transport defect is also involved.

Another example of a decreased topo II level in a multiple drug-resistant cell line is found in the P388 murine leukemia cell lines which were found to have diminished levels of topo II resulting from a decrease in the copy number of the normal topo II allele (35). In addition to underexpression of topo II, these resistant cells also overexpress P-glycoprotein, have increased glutathione transferase activity, and may repair drug-induced DNA damage more quickly than parental cells. A 2-fold decrease in immunoreactive topo II has likewise been observed in m-AMSA-resistant P388 cells (19). This appears to be due to inactivation of one topo II allele by gene rearrangement and/or hypermethylation. Interestingly, these cells show a significant increase in topo I activity, an observation which was not made in the present study with the CHO-SMR5 cell line.

The resistant DC3F/9-OHE Chinese hamster lung cell line has a 2- to 3-fold decrease in topo II, along with an as yet unidentified factor which likely modulates topo II activity (36, 37). Also of interest in this particular cell line is the finding that although the immunoreactive topo II is only minimally decreased, the resistance to topo II inhibitors is on the order of 100-fold.

The above-mentioned cell lines all have a diminished level of topo II as a contributing factor to their drug-resistant phenotypes. The ADR-1 cell line, in contrast, exemplifies the effect augmented nuclear topo II has on drug sensitivity (38). ADR-1 is a Chinese hamster ovary cell line which was found to be 3- to 4-fold more hypersensitive to doxorubicin, VP-16, m-AMSA, and mitoxantrone. This degree of hypersensitivity was found to correlate directly with an increased cellular level of topo II. Further evidence to support the direct association between topo II levels and drug sensitivity was found when hybrid cell lines were produced between the ADR-1 and the parent CHO-K1 cells. The hybrids we found to be no longer hypersensitive, and immunoblots revealed a topo II level equivalent to that of the parent cell line.

We feel the CHO-SMR5 cell line is a corollary to the phenomenon described in the ADR-1 cell line. Instead of hypersensitivity resulting from an increased level of topo II, the CHO-SMR5 has a multiple drug-resistant phenotype resulting solely from a diminished topo II level. Like ADR-1 cells, the CHO-
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Fig. 5. Drug-induced DNA double-strand breaks in log phase CHO-SMR5 and WT CHO cells. Parental (A) and drug resistant (B) monolayer CHO cells were treated with VP-16, and the resulting DNA damage was measured as double-strand breaks by the filter elution method. The concentrations of VP-16 are 0 (○), 10 (•), 25 (▲), and 100 μM (▲).

SMR5 line also exhibits a direct correlation between the topo II level and degree of drug sensitivity. In both of these cell lines the quantitative change in topo II is on the order of 3- to 5-fold, resulting in a relatively low degree of hypersensitivity in the ADR-1 cell line and minimal drug resistance in CHO-SMR5 cells. These findings contrast somewhat with a recent report in which two human tumor cell lines, which also express only low levels of resistance to VP-16, showed no detectable modification of topo II (39). Admittedly, CHO-SMR5 cells have a low degree of drug resistance when compared to that of most in vitro derived drug-resistant cell lines, but it is comparable to the degree of resistance demonstrated in cell lines derived from human solid tumor specimens (40, 41) and therefore may be clinically relevant. For these reasons, CHO-SMR5 provides a unique, useful model for the study of drug resistance resulting solely from a diminished topo II level.

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