ABSTRACT

Camptothecin-resistant DC3F Chinese hamster lung fibroblast cell lines were obtained after mutagenic treatment with ethylmethanesulfonate and subsequent exposure to 1 μM camptothecin (CPT). The most resistant cell line, which was obtained after exposure to CPT for 10 days, was designated DC3F/C-10. Comparison of 50% inhibitory concentration values after 8-h CPT treatments showed that DC3F/C-10 cells were 134-fold resistant to CPT. Resistance was associated with marked reduction of CPT-induced DNA single-strand breaks and DNA-protein cross-links. This reduction was not due to reduced amounts of immunoreactive DNA topoisomerase I protein, although nuclear extracts from DC3F/C-10 cells had less enzyme catalytic activity than those from DC3F cells. Also, fast protein liquid chromatography-purified DNA topoisomerase I from DC3F/C-10 had lower specific catalytic activity than that from DC3F cells. DNA topoisomerase I from DC3F/C-10 was resistant to inhibition of catalytic activity and induction of DNA cleavage by CPT. These results suggest that CPT resistance in DC3F/C-10 cells is due to qualitative alteration of DNA topoisomerase I.

INTRODUCTION

Eukaryotic DNA topoisomerase I is a cellular target of CPT2 (1−3), and one possible mechanism of CPT-induced cell killing is that the drug-stabilized cleavage complexes interfere with replication forks, resulting in replication arrest and fork disassembly (4, 5).

Resistant cell lines are useful for investigating not only the mechanism of CPT-induced cell killing but also the molecular interactions between CPT and DNA topoisomerase I. Two different approaches have been used to generate CPT-resistant cell lines. In one procedure, parental cells are first mutagenized with alkylating agents such as EMS and then selected for drug resistance in the presence of CPT. In the other, cells are exposed to CPT continuously at concentrations that are gradually increased. In the present study we have isolated a highly CPT-resistant DC3F Chinese hamster lung fibroblast cell line selected after mutagenesis with EMS and a 10-day exposure to 1 μM camptothecin ((IT). The most resistant cell line, which was obtained after exposure to CPT for 10 days, was designated DC3F/C-10. Comparison of 50% inhibitory concentration values after 8-h CPT treatments showed that DC3F/C-10 cells were 134-fold resistant to CPT. Resistance was associated with marked reduction of CPT-induced DNA single-strand breaks and DNA-protein cross-links. This reduction was not due to reduced amounts of immunoreactive DNA topoisomerase I protein, although nuclear extracts from DC3F/C-10 cells had less enzyme catalytic activity than those from DC3F cells. Also, fast protein liquid chromatography-purified DNA topoisomerase I from DC3F/C-10 had lower specific catalytic activity than that from DC3F cells. DNA topoisomerase I from DC3F/C-10 was resistant to inhibition of catalytic activity and induction of DNA cleavage by CPT. These results suggest that CPT resistance in DC3F/C-10 cells is due to qualitative alteration of DNA topoisomerase I.

MATERIALS AND METHODS

Chemicals. CPT, HN2, and methotrexate were obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute (Bethesda, MD). Etoposide and teniposide were generously provided by the Bristol-Myers Co. (Syracuse, NY). Vinristine and radiolabeled nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO) and New England Nuclear (Boston, MA), respectively. SV40 DNA and agents for immunoblotting were purchased from BRL (Gaithersburg, MD). All other chemicals were of reagent grade. CPT was dissolved at 10 mM in dimethyl sulfoxide, aliquoted, and kept as stock solution at −70°C.

Cell Culture. DC3F Chinese hamster lung fibroblast cells were grown in monolayer culture in minimal essential medium with Earle's salts (ABI, Columbia, MD) supplemented with 10% heat inactivated fetal calf serum (GIBCO, Grand Island, NY). 0.1 mM nonessential amino acids, 2 mM glutamine, 1 mM sodium pyruvate, 100 units/ml penicillin, and 100 μg/ml streptomycin (control medium) at 37°C in an atmosphere of 95% air and 5% CO2 (6).

Preparation of Isolated Nuclei and Nuclear Extracts. The procedures for isolation of nuclei and DNA topoisomerase I purification method were described previously (6, 7). Briefly, exponentially growing cells were rinsed with ice-cold nuclear buffer [150 mM NaCl, 1 mM KH2PO4, 5 mM MgCl₂, 1 mM ethylene glycol bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid, 10 mM 2-mercaptoethanol, 0.1 mM phenylmethylsulfonyl fluoride, 10% (v/v) glycerol, pH 6.4], scraped, and centrifuged. Cells were resuspended in 0.1 volume of ice-cold nucleus buffer, and then 0.9 volume of cold nucleus buffer containing 0.3% Triton X-100 was added. The mixture was gently rotated for 10 min at 4°C. The nuclei were spun down and resuspended in Triton X-100-free nucleus buffer.

To obtain nuclear extracts, all procedures were carried out in the presence of 0.44 trypsin inhibitory unit/ml aprotinin in addition to phenylmethylsulfonyl fluoride. Isolated nuclei were washed once in Triton X-100-free nucleus buffer and resuspended in nucleus buffer containing 0.35 mM NaCl (final concentration). The salt extraction was performed for 30 min at 4°C with gentle rotation. The nuclei were spun and the supernatant was centrifuged again to remove any insoluble materials. The supernatant (nuclear extract) was used for immunoblotting and DNA relaxation assays.

Purification of DNA Topoisomerases. Nuclear extract was first bound to a Q-Sepharose “Fast Flow” (Pharmacia, Uppsala, Sweden) disposable column. The 0.5 M NaCl fraction from the column was then applied to a Mono Q column (Pharmacia LKB Biotechnology, Uppsala, Sweden) for anion exchange chromatography at 4°C. The column was eluted with a 0.15 to 0.5 M NaCl linear gradient. DNA topoisomerase I activity was determined in each fraction by relaxation of SV40 DNA in the absence of ATP, and topoisomerase II activity by the induction of double-strand breaks in the presence of ATP and teniposide. Two peaks of DNA topoisomerase I activity were found at 0.15−0.16 and 0.25−0.28 M NaCl, respectively. SDS-polyacrylamide gel electrophoresis and silver staining showed that the first and second peaks contained M, 68,000 and 100,000 DNA topoisomerase I, respectively (7). Thereafter, these 2 peaks were designated FPLC-purified M, 68,000 and 100,000 DNA topoisomerase I.

For further purification, Mono Q fractions that had DNA topoisomerase activity were applied to 10 ml 15−40% glycerol gradient centrifugation. Gradients were collected from the bottom of the tubes in 0.7-mM fractions. Each fraction was assayed for DNA topoisomerase I and II activities. One unit topoisomerase I activity was defined as the enzyme activity that yielded 50% relaxation of 0.4 μg DNA after 30 min in 30 μl reaction buffer (0.01 M Tris, pH 7.5, 0.05 M KCl, 0.1 M NaCl, 5 mM MgCl₂, 0.1 mM EDTA, 15 μg/ml bovine serum albumin) at 37°C.

Cytotoxicity Assay. Drug sensitivity was determined by colony-forming assays, as described previously (5). Briefly, 1−2 × 10⁴ cells were distributed into 25-cm² flasks. The following day, exponentially growing cells were treated with drugs for the indicated time periods. There-
after, cells were washed 3 times with medium, trypsinized, and seeded as 10^2, 10^3, 10^4 (10^5) in triplicate. Colonies were allowed to grow for 6–8 days. Then culture flasks were washed twice with phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 4.3 mm NaHPO_4, 1.4 mM KHPO_4, pH 7.3), fixed with methanol (95%), and stained with methylene blue (0.05%). Survival fraction was calculated by dividing the plating efficiency of drug-treated cells by that of untreated control cells. The 50% inhibition concentration was calculated as the drug concentration that reduced colony formation to 50% of control. The degree of resistance was expressed as the ratio of the 50% inhibitory concentration of the resistant cell line to that of wild type cells.

Camptothecin-induced DNA Damage. SSB and DPC were measured in isolated nuclei by alkaline elution (8). Briefly, nuclei were isolated from [3H]thymidine-labeled cells and were treated with CPT at 37°C for 30 min in nucleus buffer. Reactions were stopped by diluting with ice-cold nucleus buffer that contained the same amounts of CPT as for the drug treatment in order to avoid the reversal of DNA damages at 0°C (8). Nuclei suspensions were then loaded onto polycarbonate filters (Nucleopore Corp., Pleasanton, CA). [3H]Thymidine-labeled nuclei (internal standard), which had been irradiated with 1500 rads, were loaded after the nucleus buffer containing the drug-treated nuclei had dripped through. Lysis was performed with SDS and proteinase K, and elution was carried out with tetrapropylammonium hydroxide:EDTA, 0.1% SDS, pH 12.1. Fractions were collected every 5 min for 30 min. DNA single strand break frequencies were expressed in rad-equivalents.

DPC were assayed by alkaline elution under nondeproteinizing DNA-denaturing conditions. After drug treatment, [3H]thymidine-labeled nuclei were irradiated with 3000 rads on ice. Nuclei were loaded onto polyvinylchloride-acrylic copolymer filters (Metricel DM-800; Gelman Sciences, Ann Arbor, MI) and lysed with SDS in the absence of proteinase K. Filters were rinsed with 0.02 M EDTA, pH 10. DNA was eluted with tetrapropylammonium hydroxide:EDTA, pH 12.1, without SDS. Fractions were collected at 3-h intervals for 15 h. DPC frequencies were calculated according to the bound-to-one terminus model and expressed in rad-equivalents (9).

DNA Topoisomerase I Immunoblotting. Whole cell lysates were made by disrupting cells in electrophoresis sample buffer (2% SDS, 100 mM dithiothreitol, 60 mM Tris, pH 6.8, 0.1% bromphenol blue, 10% glycerol). Both cell lysates and nuclear extracts were analyzed for immunoreactive DNA topoisomerase I using human serum from a scleroderma patient containing anti-M, 100,000 topoisomerase I auto-antibodies (kindly provided by Dr. Earnshaw, Johns Hopkins University) (10). Samples were electrophoresed on SDS-polyacrylamide gels (4–20%), transferred to nitrocellulose, and incubated with the described antibodies. DNA topoisomerase I proteins were detected with streptavidin–alkaline phosphatase (BRL, Gaithersburg, MD). Protein concentration was measured by the Bradford method (Pierce Chemical Company, Rockford, IL).

Topoisomerase I Catalytic Activity. The catalytic activities were examined by DNA relaxation assay using supercoiled SV40 DNA (6, 8). Samples were incubated with 0.4 µg DNA in 30 µl reaction buffer (0.01 M Tris, pH 7.5, 0.05 M KCl, 0.1 M NaCl, 5 mM MgCl_2, 0.1 mM EDTA, 15 µg/ml bovine serum albumin) at 37°C, unless otherwise indicated. Reactions were stopped by adding SDS and proteinase K (1% and 50 µg/ml, respectively). After an additional 30-min incubation at 37°C, the fraction of supercoiled DNA was separated by chloroquine agarose gel electrophoresis. The gels were stained with 2 µM ethidium bromide and destained in 1 mM MgSO_4. Negatives of the gel pictures were scanned with Beckman DU-8B spectrometer in order to quantify the amount of DNA in each topoisomer peak (11).

Camptothecin-induced inhibition of topoisomerase I-mediated DNA relaxation (see Table 2) was computed from the formula:

\[
\text{% inhibition} = 100 \times \frac{(SC_{DE} - 0.5)}{(SC_{O} - 0.5)}
\]

where \(SC_{DE}\) and \(SC_{O}\) represent the fraction of supercoiled DNA measured in the presence of drug and 1 unit enzyme and in untreated DNA, respectively (3).

Sequence Selective DNA Cleavage by Topoisomerase I. The \(^{32}\)P-3'-end-labeled FokI SV40 DNA fragment (137 base pairs), which contains a major CPT-induced topoisomerase 1 cleavage site, was used (12). Reactions were carried out for 15 min at 37°C, stopped by adding SDS and proteinase K, and incubated for an additional 30 min at 37°C. After ethanol precipitation, samples were analyzed by DNA sequencing gel electrophoresis as described previously (12).

RESULTS

Selection of CPT-resistant Cell Lines. CPT-resistant DC3F cell lines were established according to the method described by Gupta et al. (13). Chinese hamster lung fibroblast cells (DC3F) were first mutagenized with the alkylating agent EMS (50 µg/ml) for 16 h, which yielded 50% cell survival. The EMS-treated cells were grown for 3 days in normal medium to allow time for mutation fixation. Thereafter, the selection of CPT-resistant mutant cells was carried out by exposure to 1 µM CPT for 10 days; 10^6 mutagenized cells were incubated with CPT without medium change. After a 10-day exposure, cell cultures were washed and allowed to grow without trypsinization in CPT-free medium. Two colonies survived. The surviving colonies were replated and cloned twice to obtain cell lines of single cell origin. These established cell lines were maintained in CPT-free medium. The more resistant cell line was designated DC3F/C-10.

DC3F/C-10 cells grow more slowly than the parental DC3F cells. Doubling times of DC3F and DC3F/C-10 cells are 9.4 ± 1.12 (SD) and 14.8 ± 1.55 h, respectively. Plating efficiency for untreated DC3F and DC3F/C-10 are 70–90% and 60–80%, respectively.

Drug Sensitivity. Because CPT-induced cytotoxicity increases with exposure time (5), we examined CPT-induced cell killing under 3 different conditions: 30-min, 8-h, and continuous exposure (Table 1; Fig. 1). DC3F/C-10 cells were 140-, 134-, and 81-fold resistant following 30-min, 8-h, and continuous exposure, respectively (Table 1). Resistance of DC3F/C-10 cells was stable in the absence of CPT since after 6 months of culture in drug-free medium CPT resistance remained unaltered (Fig. 1). In the present study, we undertook further investigation comparing DC3F/C-10 to DC3F.

DC3F/C-10 cells were examined for cross-resistance and/or collateral hypersensitivity to other antineoplastic agents, including HN2, etoposide, methotrexate, and vincristine (Table 1). The durations of drug treatments and solvents used for dissolution of the drugs were as follows: HN2 (0.5 h, 0.01 N HCl), etoposide (1 h, dimethyl sulfoxide), methotrexate (24 h, 0.05 N NaOH), and vincristine (24 h, H2O). Controls were treated with appropriate amounts of solvent alone. HN2 treatment was carried out in medium containing 1% fetal calf serum and others in control medium. Table 1 shows that DC3F/C-10 cells were resistant to methotrexate and slightly hypersensitive

<table>
<thead>
<tr>
<th>Table 1 Drug sensitivity (50% inhibitory concentration) of camptothecin-resistant cells</th>
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<tr>
<td>DC3F (µM)</td>
</tr>
<tr>
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</tr>
<tr>
<td>CPT (0.5 h)*</td>
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<tr>
<td>CPT (8 h)</td>
</tr>
<tr>
<td>CPT (continuous)</td>
</tr>
<tr>
<td>HN2 (0.5 h)</td>
</tr>
<tr>
<td>Etoposide (1 h)</td>
</tr>
<tr>
<td>Methotrexate (24 h)</td>
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<tr>
<td>Vincristine (24 h)</td>
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* Numbers in parentheses, duration of drug treatment.
* Mean ± SD.
* Measured in nm.
to etoposide and vincristine. DC3F and DC3F/C-10 cells had similar sensitivity to γ-radiations (data not shown).

Camptothecin-Induced DNA Damage. Previous reports showed that the cytotoxicity of camptothecin and its derivatives is related to the amount of CPT-induced cleavable complexes (3, 14). Cleavable complexes can be detected in drug-treated cells by alkaline elution (8). Using this assay, we measured first CPT-induced SSB in whole DC3F and DC3F/C-10 cells. DC3F/C-10 cells were found to produce markedly less SSB after 30-min treatment than DC3F cells (data not shown). In order to determine whether lower intracellular drug level was responsible for the low levels of SSB in DC3F/C-10 cells, we prepared isolated nuclei and compared the amounts of SSB and DPC produced after 30-min treatment with CPT (Fig. 2). In DC3F nuclei, CPT produced approximately similar amounts of SSB and DPC over a range of drug concentrations, as expected for topoisomerase I-mediated DNA breaks (8). In contrast, no DNA damage was detectable in isolated nuclei from DC3F/C-10 cells (Fig. 2). These results indicate that CPT resistance in DC3F/C-10 cells is due to lack of DNA topoisomerase I inhibition by CPT.

Quantitative Analysis of DNA Topoisomerase I by Immunoblotting. We examined intracellular immunoreactive DNA topoisomerase I proteins by Western blotting in order to determine whether quantitative reduction of the target enzyme contributed to CPT resistance. Serial dilutions of whole cell lysates and 0.35 M NaCl nuclear extracts were electrophoresed on SDS-polyacrylamide gel, transferred to nitrocellulose, and reacted with anti-topoisomerase I antibodies from the serum of a scleroderma patient.

Fig. 3 (left) shows that cell lysates from DC3F/C-10 had a slightly greater amount of immunoreactive M, 100,000 protein than those from sensitive DC3F even though there were several cross-reactive bands. When nuclear extracts were immunoblotted, DC3F/C-10 extracts were approximately 2-fold more reactive to the anti-M, 100,000 topoisomerase I antibodies (Fig. 3, right). These results indicate that CPT resistance is not correlated with quantitative reduction of DNA topoisomerase I in DC3F/C-10 cells.

DNA Topoisomerase I Activity. The catalytic activity of 0.35 M NaCl nuclear extracts was measured by a DNA relaxation assay using supercoiled SV40 DNA (12). Various amounts of nuclear extracts were incubated with or without CPT at 24°C for 10 min. Fig. 4 shows that nuclear extract from DC3F has a greater DNA relaxing activity than that from DC3F/C-10 cells. Fifty % DNA relaxation was obtained with 0.25 μg protein from DC3F nuclear extract, while approximately 8-fold more DC3F/C-10 nuclear protein was required to catalyze the same degree of relaxation. Furthermore, catalytic activity of nuclear extract from DC3F cells was inhibited by CPT, whereas that from DC3F/C-10 cells was not.

Additional experiments using 2.75 μg of DC3F/C-10 nuclear extract, which generated 70% relaxed DNA, were performed with higher drug concentrations (up to 500 μM CPT). CPT was still inactive at inhibiting DNA topoisomerase I catalytic activity in nuclear extract from resistant cells (data not shown). In order to investigate whether some DC3F/C-10 factor was inhibiting DNA topoisomerase I enzymatic activity, 0.5 μg protein of nuclear extracts from DC3F was mixed with either 0.5 μg or 2.75 μg protein from DC3F/C-10 nuclear extracts and reacted with DNA under the same conditions as described above. Based on the dose-response curves in Fig. 4, 37 and 15% of supercoiled form were expected after the reactions, respectively. Actually, these mixtures catalyzed DNA relaxation and reduced the supercoiled form to 34 and 12%, respectively. Thus, nuclear extracts from DC3F/C-10 cells did not inhibit the catalytic activity of nuclear extracts from DC3F cells. These results strongly suggest that nuclear extracts from DC3F/C-10 contain no inhibitory factor and that qualitative alteration(s) of DNA topoisomerase I exist in DC3F/C-10 cells.

DNA topoisomerases I from DC3F and DC3F/C-10 were further purified from nuclear extracts by anion exchange chromatography (FPLC) as described in “Materials and Methods.” Fig. 5 shows the kinetics of DNA relaxation by M, 68,000 and 100,000 DNA topoisomerases I. As in the case of nuclear extracts, CPT did not affect the catalytic activity of either the M, 68,000 or 100,000 DNA topoisomerases I from DC3F/C-10 cells.

Sequence Selective DNA Cleavage. Jaxel et al. (12) demonstrated that CPT stimulates a preferential DNA topoisomerase I cleavage site in SV40 DNA. Using a FokI DNA fragment (Fig. 6), FPLC-purified DNA topoisomerases I from both sensitive and resistant cells cleaved DNA at the same position in the presence of CPT (position 4955) as previously reported by Jaxel et al. (12). However, DNA topoisomerase I from DC3F/C-10 was less active in generating DNA cleavage (Fig. 6). Interestingly, both M, 68,000 and 100,000 DNA topoisomerases I from DC3F/C-10 could induce DNA cleavage with similar sensitivity to γ-radiations (data not shown).

Fig. 2. CPT-induced SSB (●, ■) and DPC (○, □) in isolated nuclei from wild type DC3F and CPT-resistant DC3F/C-10 cells. Drug treatments were for 30 min at 37°C, and DNA damage was measured by alkaline elution. Bars, SD of at least 3 independent experiments. As for DPC, 2 independent experiments are shown.
Fig. 3. DNA topoisomerase I proteins in whole cell lysates (left panel) and nuclear extracts (right panel) from wild type DC3F (W) and CPT-resistant DC3F/C-10 (C) cells. Immunoreactive proteins were analyzed by Western blotting using anti-M, 100,000 DNA topoisomerase I antibodies (scleroderma serum). The numbers of cells from which cell lysates were prepared are indicated above each lane. Nuclear proteins were extracted from isolated nuclei with 0.35 M NaCl. The amount of protein loaded is indicated above each lane. Arrows and numbers, migration positions and size of M, markers (kDa).

CPT concentrations, while maximum cleavage was obtained with DC3F DNA topoisomerase I at 10 μM CPT (Fig. 7).

Glycerol Gradient Purified DNA Topoisomerases I. Purified DNA topoisomerases I from DC3F/C-10 had the same molecular weight as those from DC3F, indicating no gross difference in enzymes between DC3F/C-10 and DC3F cells (Fig. 8).

Comparison of these purified topoisomerases I showed that the specific activity of M, 100,000 topoisomerase I from DC3F/C-10 cells was approximately 5-fold less than that from DC3F and that DC3F/C-10 topoisomerase I was resistant to CPT (Table 2).

DISCUSSION

General mechanisms of drug resistance and hypersensitivity can be categorized into 4 groups: (a) alteration of drug uptake, intracellular distribution, and metabolism; (b) functional alteration of intracellular factor(s) that modulate the activity of the target enzyme(s) and/or sensitivity of substrate(s) to drugs; (c) alterations of repair systems involved in removing drug-induced damage; and (d) quantitative and/or qualitative alteration of the target enzyme(s) or substrate(s). In this study, a new highly CPT-resistant cell line has been selected. It probably has utilized the last of these general mechanisms in developing its resistance.

At present, there is no resistant cell line that shows reduced intracellular CPT accumulation as a primary mechanism for drug resistance. Furthermore, CPT does not inhibit [3H]vincristine binding to plasma membrane of Adriamycin-resistant K562 cells (15), and CPT-11 is cytotoxic in pleiotropic drug-resistant tumors, such as vincristine-resistant and Adriamycin-resistant P388 cell lines (16). These observations indicate that CPT is not a substrate for multidrug-resistant drug efflux systems and suggest that CPT derivatives may be useful in overcoming human tumor multidrug resistance. In this study, for the DC3F/C-10 cell line, the finding that no detectable SSB
reported to either increase or decrease DNA topoisomerase I activity. So far, no CPT-resistant or hypersensitive cell line with the above mechanisms has been reported.

Hypersensitivity of the poly(ADP-ribose) polymerase deficient Chinese hamster cell line of Chatterjee et al. (30) may be due to DNA repair defects rather than DNA topoisomerase I alterations, since, in these cells, CPT-induced SSB are similar to those of parental cells. The existence of DNA repair defects in DC3F/C-10 cells has not been investigated in the present study.

Quantitative reduction of DNA topoisomerase I seems to be a common mechanism of drug-induced resistance to CPT. Sugimoto et al. (31) found a reduction of cellular DNA topoi-

Table 2 Specific activities and camptothecin sensitivities of M, 100,000 purified topoisomerase I from camptothecin-resistant cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Specific Activity (unit/μg)</th>
<th>CPT Inhibition (%)</th>
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<tbody>
<tr>
<td>DC3F</td>
<td>71</td>
<td>99.9</td>
</tr>
<tr>
<td></td>
<td>67</td>
<td>83.3</td>
</tr>
<tr>
<td>DC3F/C-10</td>
<td>13</td>
<td>11.2</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>0</td>
</tr>
</tbody>
</table>

* One unit topoisomerase I activity catalyzes 50% relaxation of 0.4 μg SV40 DNA after 30 min incubation at 37°C.

* Percent inhibition of 1 unit enzyme in the presence of 10 μM CPT.

The 2 values correspond to 2 independent experiments.
somerase I content in 3 of 4 CPT-resistant cell lines, with purified DNA topoisomerases I from one of these cell lines showing similar enzymatic activity in relaxation of superhelical plasmid pUC13 DNA compared to the parental line. These authors concluded that the most frequently occurring event in the development of CPT resistance was the quantitative reduction of DNA topoisomerase I. CPT-resistant CHO cells (13), P388 cells (32), and human lung cancer cells (33) were also found to have decreased DNA topoisomerase I. In the P388-resistant cell, the DNA topoisomerase I decrease was postulated as due to rearrangement and hypermethylation of the DNA topoisomerase I gene. The DC3F/C-10 cell line differs from these cell lines inasmuch as it has no quantitative decrease in DNA topoisomerase I.

The cell line DC3F/C-10 probably has acquired CPT resistance due to qualitative alterations in the DNA topoisomerase I enzyme. The first example of a cell line in which this occurred has been the human lymphoblastic cell line developed by Andoh et al. (34). Other cell lines with qualitative alterations of DNA topoisomerase I have been reported by Gupta et al. (13) and more recently by Kanzawa et al. (33). Andoh et al. (34) and Kanzawa et al. (33) selected their cell lines by continuous and stepwise exposure to CPT and CPT-11, respectively, while Gupta et al. (13) mutagenized the cells before selection with CPT. DNA topoisomerase I alterations have been well characterized in the cell line of Andoh et al. (34). The mutant enzyme cleaves a DNA topoisomerase I recognition sequence in sequence with 2-fold higher efficiency than the wild type enzyme and forms more stable cleavable complexes (35). Resistance has been attributed to the presence of 2 mutations that cause amino acid changes from aspartic acid to glycine (36).

DC3F/C-10 resistance may also be due to a DNA topoisomerase I mutation since the purified DNA topoisomerase(s) I from those cells have lower specific catalytic activity and are resistant to CPT. Decreased specific activity has not been observed in the previously described CPT-resistant cell lines, suggesting that DC3F/C-10 DNA topoisomerase I may have unique biochemical characteristics.

Our laboratory has already reported that DC3F cells have 2 different DNA topoisomerase I forms, with molecular masses of M, 68,000 and 100,000 (7, 37). In DC3F/C-10, both M, 68,000 and 100,000 topoisomerases I were less active than those from DC3F cells and were resistant to CPT. This result suggests that these 2 topoisomerases I are products from the same gene and that the M, 68,000 form is a fragment of the M, 100,000 topoisomerase I. Although anti-M, 100,000 topoisomerase I antibodies did not show a visible band of M, 68,000 size (Fig. 3), pretreatment of both M, 68,000 and 100,000 topoisomerases I with the antibodies inhibited their catalytic activities (data not shown). At present, it is also unclear whether M, 68,000 topoisomerase I has some biological significance in cells.

Genetic analysis of DC3F/C-10 cells will be necessary to characterize the gene for CPT-resistant DNA topoisomerase I and to investigate, at the molecular level, the alteration that resulted in this resistance. It will be valuable both in further understanding of the DNA topoisomerase I itself and in designing new CPT derivatives.

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Topoisomerase I Alteration in a Camptothecin-resistant Cell Line Derived from Chinese Hamster DC3F Cells in Culture

Akihiko Tanizawa and Yves Pommier


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