[CANCER RESEARCH 55, 4004–4009, September 15, 1995]

Advances in Brief

Cross-Resistance to Camptothecin Analogues in a Mitoxantrone-resistant Human Breast Carcinoma Cell Line Is Not Due to DNA Topoisomerase I Alterations

Chih-Hsin J. Yang,[1] Julie K. Horton,[2] Kenneth H. Cowan, and Erasmus Schneider[3]

Medicine Branch, National Cancer Institute, Bethesda, Maryland 20892

Abstract

We have previously described a mitoxantrone-resistant human breast carcinoma cell line, MCF7/MX, in which resistance was associated with a defect in the energy-dependent accumulation of mitoxantrone in the absence of P-glycoprotein overexpression (M. Nakagawa *et al.*, Cancer Res. *52*: 6175–6181, 1992). We now report that this cell line is highly cross-resistant to the camptothecin analogues topotecan (180-fold), 9-aminocamptothecin (120-fold), CPT-11 (56-fold), and SN38 (101-fold), but is only mildly cross-resistant to the parent compound camptothecin (3.2-fold) and 10,11-methylenedioxy-camptothecin (2.9-fold). Topotecan accumulation was decreased in MCF7/MX cells compared to parental MCF7/WT cells, and there was a corresponding reduction in topotecan-mediated stimulation of the enzyme/DNA complex formation in MCF7/MX cells compared to MCF7/WT cells. No overexpression of the multidrug resistance-associated protein was detected compared to parental MCF7/WT cells. Furthermore, both sensitive MCF7/WT and mitoxantrone-resistant MCF7/MX cells contain equal amounts of DNA topoisomerase I protein, and DNA relaxation activities were equal in both cell lines and inhibited to the same extent by topotecan and camptothecin. Thus, these results suggest a novel mechanism of resistance to topoisomerase I inhibitors in cancer cells.

Introduction

CPT[4] was originally isolated from an extract of the Chinese tree *Camptotheca acuminata* and shown to be active against the murine leukemia cell line L1210 during the 1950s by a natural products screening program sponsored by the National Cancer Institute. Sodium CPT entered Phase I trials in the 1970s and showed a unique spectrum of anticancer activity. However, the development of this drug was hampered by profound hematological and unpredictable gastrointestinal toxicity and hemorrhagic cystitis. The discovery that DNA topoisomerase I is the target of CPT and the subsequent understanding of its mechanism of action, including the fact that accumulation of the active lactone form of the drug in acidic body environments may account for the observed clinical toxicities, led to a renewed interest in this compound. As a result, several CPT analogues including TPT, CPT-11, and 9-AC have entered clinical Phase I and II trials in the 1990s and have shown promising activities in lung, colorectal, ovarian and cervical cancer, acute leukemia, and non-Hodgkin's lymphoma (1). Distinct activities and toxicities have been observed with different CPT analogues, suggesting that despite their similar structures, they may differ in their pharmacological properties.

Cancer cells can acquire resistance to CPTs by different mechanisms including decreased topoisomerase I levels (2), mutations in topoisomerase I (3), decreased metabolic activation of CPT-11 to the active metabolite SN38 (4), and decreased accumulation of some CPT analogues in Pgp-overexpressing cells (5). Furthermore, cells selected for resistance to mAMSA (6), cisplatin (4), or melphalan (7) developed cross-resistance to CPT analogues, presumably reflecting differences in DNA topoisomerase I and/or DNA repair mechanisms in these cells. Here, we describe studies in MCF7/MX cells, a mitoxantrone-resistant variant of the human breast carcinoma cell line MCF7 (8), which indicated that MCF7/MX cells are highly cross-resistant to TPT, 9-AC, CPT-11, and SN38. Comparison of the sensitivities to these compounds in a series of multidrug-resistant MCF7 variant cell lines with different mechanisms of resistance indicated that cross-resistance to these CPT analogues was unique in MCF7/MX cells. Cross-resistance to TPT was associated with decreased TPT accumulation and reduced stimulation of the topoisomerase I/DNA complex formation in MCF7/MX cells, whereas no evidence for DNA topoisomerase I alterations was found in these cells. Thus, these studies suggest the presence of a novel mechanism of resistance to DNA topoisomerase I inhibitors.

Materials and Methods

Materials. TPT, 10,11-mCPT, and 9-AC were obtained from the Drug Synthesis Branch of the National Cancer Institute (Bethesda, MD). CPT-11 and SN38 were a gift from Dr. B. Sinha (National Cancer Institute). CPT was provided by Dr. L. F. Liu (Robert Wood Johnson Medical School, Piscataway, NJ). TPT and CPT-11 were dissolved in water, and 10,11-mCPT, 9-AC, CPT, and SN38 were dissolved in DMSO as stock solutions of 1–10 mM.

Cell Lines. Selection and culture conditions for parental MCF7/WT, mitoxantrone-resistant MCF7/MX (8), doxorubicin-resistant MCF7/ADR (9), etoposide-resistant MCF7/VP (10), and 4-hydroperoxycyclophosphamide-resistant MCF7/4HC (11) cells have been described previously. MCF7/4HC cells were a gift from Dr. B. Teicher (Dana-Farber Cancer Institute, Boston, MA), whereas all other cell lines were developed in our laboratory.

Cytotoxicity Assays. Cytotoxicities were determined using the sulforhodamine B assay after continuous exposure of the cells to various concentrations of the drugs for 7 days as described previously (10).

TPT Accumulation. Since radioactively labeled TPT is not commercially available, the flow cytometry method described by Hendricks *et al.* (5) was used to measure TPT accumulation in MCF7/WT and MCF7/MX cells. Briefly, MCF7/WT and MCF7/MX cells (10^6-10^7 cells) were trypsinized, centrifuged, washed with serum-free IMEM and resuspended in serum-free IMEM at 10^5 cells/ml. Various concentrations of TPT were then added to the cells. After incubation at 37°C for 30 min, when steady-state accumulation was achieved, each sample was subjected to flow cytometry on a FACScan (Becton

Received 6/5/95; accepted 8/3/95.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] To whom requests for reprints should be addressed, and presently at Department of Oncology, National Taiwan University Hospital, 7, Chung-Shan South Road, Taipei, Taiwan, 100.

[2] Present address: Sealy Center for Molecular Science, University of Texas Medical Branch, Galveston, TX 77555.

[3] Present address: Laboratory of Diagnostic Oncology, Division of Molecular Medicine, Wadsworth Center for Laboratories and Research, Albany, NY 12201-0509.

[4] The abbreviations used are: CPT, camptothecin; IMEM, improved MEM; CPT-11, irinotecan, (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin); SN38, 10-hydroxy-7-ethylcamptothecin (the active metabolite of CPT-11); 10,11-mCPT, 10,11-methylenedioxy-camptothecin; TPT, topotecan, 10-hydroxy-9-dimethyl-amino-methyl-camptothecin; 9-AC, 9-aminocamptothecin; Pgp, P-glycoprotein; *MDR1*, multidrug resistance gene 1; MRP, multidrug resistance-associated protein.

Dickinson, Mountain View, CA). A 15-mW argon laser was used to deliver a 488-nm excitation to the cells, and fluorescence in the cells was detected using a 585-nm filter with a band width of 42 nm.

CPT Accumulation. Since the fluorescence of CPT was too weak to be detected by flow cytometry, CPT accumulation was determined using tritium-labeled drug ([^{3}H]CPT, specific activity 29.1 Ci/mmol, obtained from Moravek, Brea, CA) as described previously (8, 10). In brief, MCF7/WT and MCF7/MX cells were incubated with various concentrations of [^{3}H]CPT in serum-free IMEM at 37°C. After 30 min when steady-state accumulation was achieved, the medium was removed, and the cells were washed twice in ice-cold PBS. Cells were then lysed with 0.2 M sodium hydroxide and neutralized with 0.2 M hydrochloric acid. Tris buffer (pH 7.5) was added to the solution, and radioactivity was determined in a liquid scintillation counter. The counts were normalized to the protein content of the cells, which was determined according to the Micro BCA method (Pierce, Rockford, IL).

Western Blotting. Analysis of DNA topoisomerase I protein levels in MCF7/WT and MCF7/MX cells was performed as described previously (8, 10). DNA topoisomerase I antibody was kindly provided by Dr. L. F. Liu (Robert Wood Johnson Medical School).

DNA Relaxation. DNA topoisomerase I activity in MCF7/WT and MCF7/MX cells was determined using the DNA relaxation assay (12). Nuclear extracts were incubated with supercoiled pBR322 plasmid (GIBCO, Grand Island, NY) at 37°C for 30 min. TPT or CPT (1–100 μM) was added to different amounts of protein (0.25–1000 ng in 50 μl reaction buffer) in the reaction. Relaxed and supercoiled DNA were separated in a 1% agarose gel by electrophoresis and visualized by ethidium bromide staining.

K$^+$/SDS Precipitation Assay. The cellular DNA of MCF7/WT and MCF7/MX cells was radioactively labeled by incubation of the cells for 24 h with 1 μCi/ml [^{3}H]thymidine (New England Nuclear, Wilmington, DE). MCF7/WT and MCF7/MX cells (10^5 cells/drug concentration in duplicate) were then treated with increasing concentrations of CPT or TPT for 30 min at 37°C, followed by lysis and determination of cleavable complexes formed by K$^+$/SDS precipitation as described previously (13).

Quantitative Reverse Transcription-PCR for MRP. Levels of MRP mRNA were determined by quantitative reverse transcription-PCR as described previously (10).

Results

Drug Sensitivities of MCF7 Cells. Representative survival curves for MCF7/WT and MCF7/MX cells after incubation with CPT and various analogues for 7 days are shown in Fig. 1, whereas Table 1 lists the average 50% inhibitory concentration values for different CPT analogues in parental drug-sensitive and several different drug-resistant MCF7 cell line variants with different mechanisms of resistance. As shown in Table 1, MCF7/MX cells were highly cross-resistant to four different CPT analogues, namely TPT (180-fold), 9-AC (120-fold), SN38 (101-fold), and CPT-11 (56-fold), but only mildly cross-resistant to the parent compound CPT (3.2-fold) and 10,11-mCPT (2.9-fold). Furthermore, none of the other drug-resistant MCF7 cell lines investigated showed more than a 5-fold cross-resistance to any of the CPTs studied (with the exception of CPT-11 in the Pgp-overexpressing MCF7/ADR cells). Thus, it appears that the mechanism(s) responsible for resistance to mitoxantrone in MCF7/MX cells is also highly effective against certain CPT analogues.

TPT Accumulation. Using radioisotope-labeled [^{14}C]mitoxantrone, we have previously shown that resistance of MCF7/MX cells to mitoxantrone was associated with reduced intracellular drug accumulation (8). To test whether resistance to TPT may also be due to a drug accumulation defect, we measured TPT accumulation using the flow cytometry method previously described by Hendricks *et al.* (5). As shown in Fig. 2, *A-C*, MCF7/MX cells accumulated less TPT than MCF7/WT cells. The difference in TPT accumulation between MCF7/WT and MCF7/MX cells was 5.5-fold at 10 μM and 2.3-fold at 50 μM. Furthermore, in a separate experiment there was no detectable uptake in MCF7/MX cells at 5 μM TPT whereas TPT-derived fluorescence was readily measurable in MCF7/WT cells at the same concentration (data not shown).

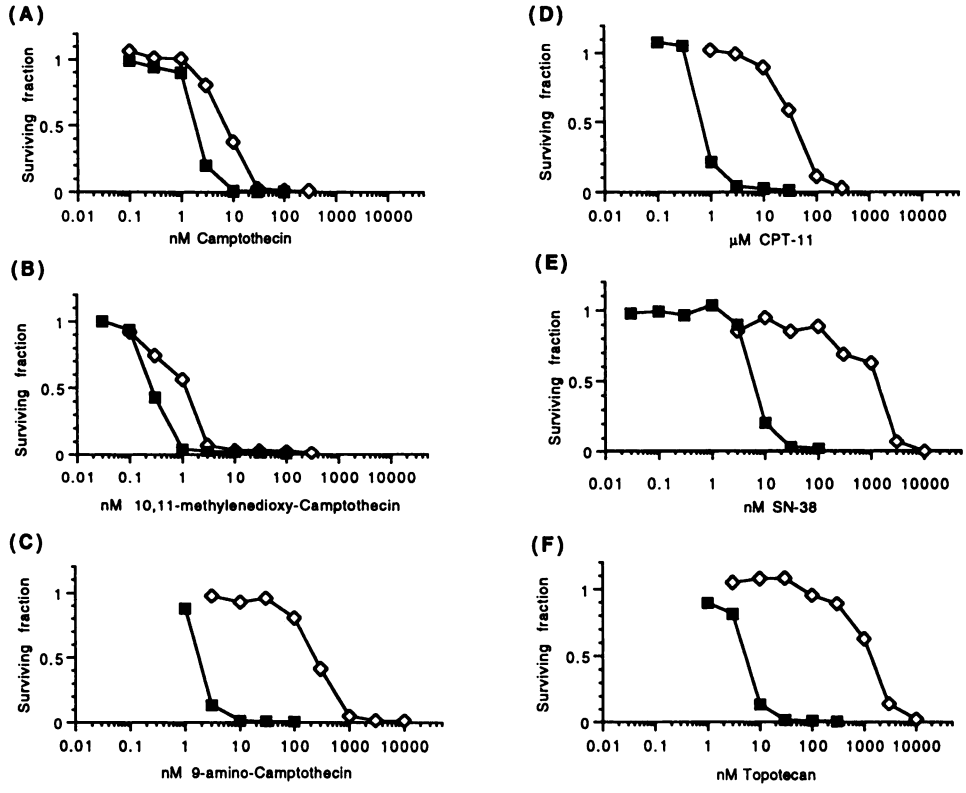

Fig. 1. Survival curves of MCF7/WT (■) and MCF7/MX (◇) cells with six CPT analogues: *A*, CPT; *B*, 10,11-mCPT; *C*, 9-aminocamptothecin; *D*, CPT-11; *E*, SN38; *F*, TPT. Relative survival was determined after 7 days of continuous drug exposure using the sulphorhodamine B assay as described in "Materials and Methods."

Table 1 *Fifty percent inhibitory concentration values and relative resistance factors of various camptothecin analogues in MCF7 cell line variants*

	MCF7/WT (nM)	MCF7/MX (nM)	MCF7/ADR (nM)	MCF7/VP (nM)	MCF7/4HC (nM)
CPT	2.2 ± 0.5	7.1 ± 1.5 (3.2)[a]	3.1 ± 0.1 (1.4)	3.1 ± 0.01 (1.4)	2.4 ± 0.8 (1.1)
10,11-mCPT	0.34 ± 0.02	1.0 ± 0.1 (2.9)	0.32 ± 0.01 (0.94)	0.35 ± 0.02 (1.0)	0.35 ± 0.02 (1.0)
9-AC	2.0 ± 0.5	240 ± 30 (120)	4.0 ± 0.1 (2.0)	5.4 ± 0.8 (2.7)	1.9 ± 0.2 (0.95)
CPT-11	750 ± 170	42000 ± 4000 (56)	10500 ± 4800 (14)	3000 ± 400 (4.0)	1400 ± 600 (1.9)
SN38	0.66 ± 0.18	67 ± 26 (101)	1.62 ± 0.65 (2.4)	2.0 ± 0.5 (3.0)	0.59 ± 0.19 (0.89)
TPT	6.5 ± 2.8	1200 ± 100 (180)	24 ± 8 (3.7)	17 ± 2 (2.6)	6.8 ± 2.6 (1.0)
Mitoxantrone	0.158 ± 0.0025	(3932)[b]	N.D.[c]	(2.8)[d]	N.D.
Doxorubicin	15.5 ± 0.50	(11.4)[b]	(192)[e]	(9.1)[d]	(1.5)[f]
VP-16	166 ± 8.7	(10.6)[b]	(175)[e]	(28.4)[d]	N.D.
Pgp	–	–	+++[e]	–[d]	N.D.
MRP	+	+	+	+++[d]	N.D.

[a] Relative resistance factors (in parentheses) of various camptothecin analogues in parental and several drug-resistant MCF7 cell line variants. For comparison, the relative resistance factors for the selecting agents and the Pgp and MRP expression status are also given.
[b] Ref. 8.
[c] N.D., not determined.
[d] Ref. 10.
[e] Ref. 9.
[f] Ref. 11.

CPT Accumulation. Since CPT-derived fluorescence was too weak to be detected by flow cytometry, we used [^{3}H]CPT to examine CPT accumulation in MCF7/WT and MCF7/MX cells over a wide range of concentrations (Fig. 2*D*). CPT accumulation in MCF7/WT cells was approximately 2-fold higher than that in MCF7/MX cells at concentrations from 3 to 100 nM, and the difference became negligible at higher concentrations (10 to 100 μM; data not shown).

DNA Topoisomerase I. The cytotoxicity of a particular CPT can be correlated in a dose-dependent manner with the level of drug-induced enzyme/DNA complex formed within a cell. In good agreement with the results from the accumulation studies, we found that there was a marked reduction in the stimulation of topoisomerase I/DNA complex formation by TPT in MCF7/MX cells as compared to that in MCF7/WT cells (Fig. 3). In contrast, the formation of enzyme/DNA complexes was equally sensitive to CPT stimulation in MCF7/MX cells as in MCF7/WT cells.

Western blot analysis indicated that DNA topoisomerase I protein levels in MCF7/WT and MCF7/MX cells were similar (Fig. 4*A*). Furthermore, there was also little difference in the catalytic topoisomerase I activity in nuclear extracts from both cell lines (Fig. 4, *B* and *C*). Thus, incubation of 10 ng nuclear extracts from either MCF7/WT or MCF7/MX cells with 500 ng supercoiled pBR322 resulted in nearly complete relaxation of the supercoiled DNA, while 2 ng of the same extract from either cell line had only a small effect on pBR322. The sensitivity of both extracts to TPT or CPT inhibition was also examined at different concentrations of protein and drugs (Fig. 4, *B* and *C*). TPT at 10, 30, and 100 μM showed the same extent of inhibition of relaxation of supercoiled DNA by nuclear extract

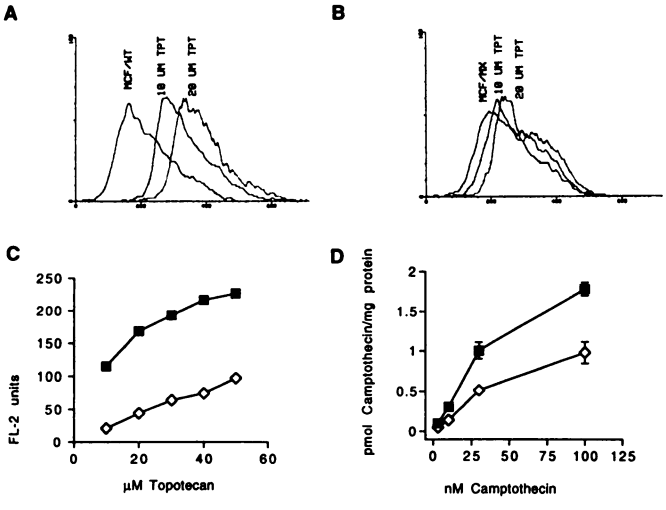

Fig. 2. Drug uptake into MCF7/WT and MCF7/MX cells. *A-C*, TPT levels in MCF7/WT and MCF7/MX cells after 30-min incubation with different drug concentrations as determined by flow cytometry. *A* and *B*, a representative example of the actual fluorescence-activated cell sorting result with MCF7/WT cells (*A*) and MCF7/MX cells (*B*) at 0, 10, and 20 μM TPT. *C*, *ordinate* shows the difference between the mean FL-2 units of MCF7/WT (■) or MCF7/MX (◇) cells treated with different concentrations of TPT and untreated MCF7/WT or MCF7/MX, respectively. *D*, CPT levels in MCF7/WT (■) and MCF7/MX (◇) cells after 30-min incubation with different concentrations of [^{3}H]CPT.

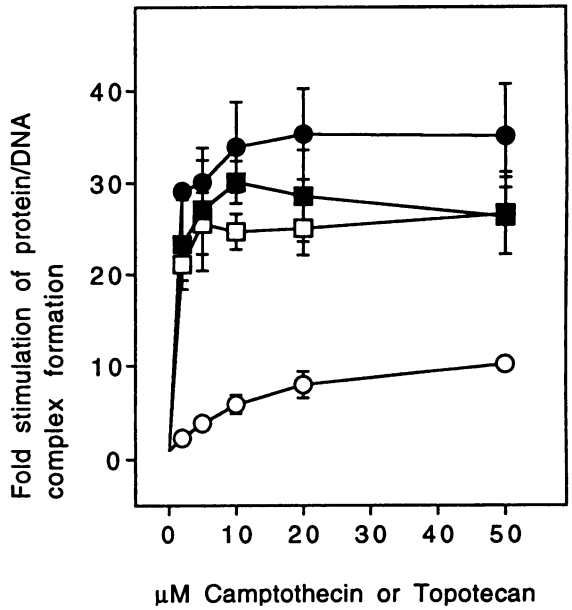

Fig. 3. K^+/SDS precipitation assay with MCF7/WT and MCF7/MX cells. The formation of topoisomerase I/DNA cleavable complexes in MCF7/WT (●, ■) and MCF7/MX (○, □) cells was examined after [^{3}H]thymidine-labeled cells had been exposed to various concentrations of CPT (■, □) or TPT (●, ○) for 30 min at 37°C. The results shown are the means ± SE of three independent experiments.

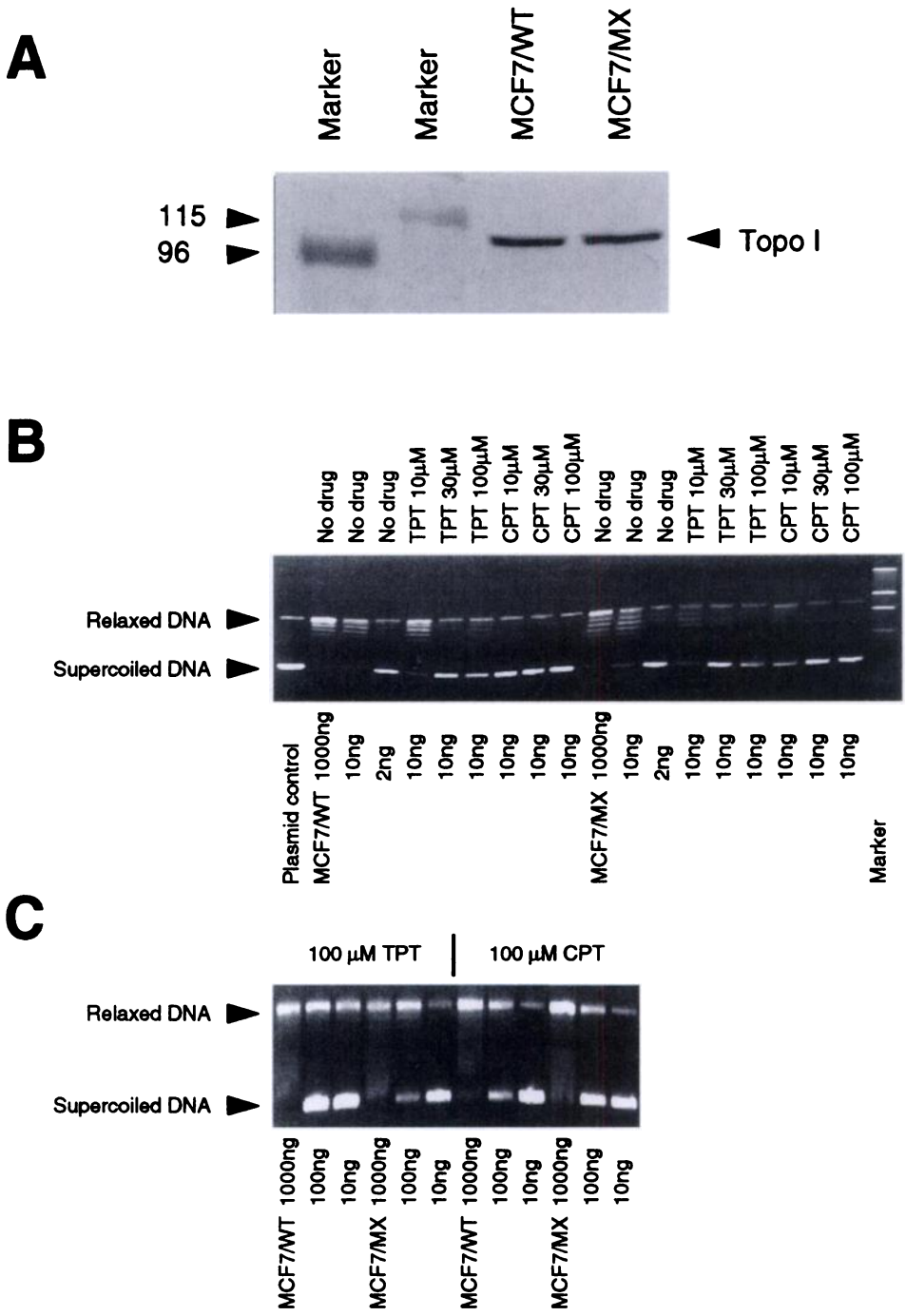

Fig. 4. DNA topoisomerase I levels and activities in MCF7/WT and MCF7/MX cells. *A*, immunoblotting of topoisomerase I in MCF7/WT and MCF7/MX cells. Fifty μg nuclear extract were added to *each lane*, separated by SDS-PAGE, and detected with topoisomerase I antibody. *B* and *C*, pBR322 DNA relaxation by different amounts of nuclear extract protein from MCF7/WT and MCF7/MX cells and inhibition by various concentrations of TPT and CPT.

protein from either MCF7/WT or MCF7/MX cells. A similar result was obtained with CPT, although the relaxation activity from both cell lines appeared to be somewhat more sensitive to the parent form of the drug. Thus, there appeared to be no major qualitative or quantitative difference in DNA topoisomerase I catalytic activity or sensitivity between the two cell lines.

Discussion

Cancer cells exposed to certain chemotherapeutic agents can acquire cross-resistance to other structurally or functionally related or unrelated drugs, a phenomenon known as multidrug resistance. In this report we demonstrate that MCF7/MX cells that had been selected for resistance to the topoisomerase II inhibitor mitoxantrone (4000-fold; Ref. 8) were highly cross-resistant to several clinically available topoisomerase I inhibitors, namely, TPT, 9-AC, and CPT-11. Cross-resistance to these drugs ranged from 50 to 200-fold. In contrast, MCF7/MX cells were only slightly less sensitive than MCF7/WT cells to the parent compound CPT and 10,11-mCPT (3-fold), and only a relatively small degree of cross-resistance was observed against two other topoisomerase II inhibitors, doxorubicin and VP-16 (10-fold; Ref. 8). This differential cross-resistance of MCF7/MX cells to some, but not all CPTs was surprising since the mechanisms that have been proposed to confer resistance to topoisomerase I inhibitors, such as decreased topoisomerase I levels and activity or loss of sensitivity of the enzyme due to mutations, are thought to affect all CPTs similarly (1). Although changes in topoisomerase I have been frequently ob-

served in cell lines selected for CPT resistance, few reports exist in which such changes and cross-resistance to CPT analogues have been described previously in cell lines selected for resistance to non-topoisomerase I inhibitors. Similarly, in this report we have found that cross-resistance of MCF7/MX cells to CPT analogues was not due to any apparent quantitative or qualitative alterations of topoisomerase I. Although it has been suggested that mutations of topoisomerase I may result in differential sensitivity to different CPT analogues (3), we consider this possibility to be unlikely in MCF7/MX cells since there was no difference between the sensitivity of nuclear extracts from MCF7/WT or MCF7/MX cells to TPT- or CPT-mediated inhibition of DNA relaxation activity (Fig. 4, *B* and *C*). Furthermore, although there was a marked reduction in TPT-induced stimulation of enzyme/DNA complex formation in MCF7/MX cells compared to MCF7/WT cells, the relative difference in the stimulation of cleavable complex formation (5.7-fold at 10 μM and 3.4-fold at 50 μM TPT) was very similar to the relative difference in TPT accumulation at the same concentrations (5.5-fold at 10 μM and 2.3-fold at 50 μM TPT). Thus, these results suggest that mechanisms other than the alteration of topoisomerase I are responsible for the resistance to some CPT analogues.

Previous reports have suggested that the sensitivity of cells to some CPT analogues, in particular TPT, might be affected by classical Pgp-mediated multidrug resistance, whereas the parent CPT was not affected by this mechanism. For example, Hendricks *et al.* (5) showed that TPT accumulation and concomitant sensitivity were reduced in a Pgp-expressing cell line and that these effects were reversible by the Pgp antagonist quinidine. Also, Chen *et al.* (14) have demonstrated that TPT was a substrate for Pgp. Furthermore, results reported by Mattern *et al.* (15) have suggested that expression of Pgp may affect the cytotoxicity of many CPTs including TPT, 9-AC, CPT-11, and SN38. Together, these reports indicate that intracellular drug accumulation is a determinant in cellular resistance to CPTs. Thus, it is likely that the reduction in TPT accumulation in MCF7/MX cells compared to MCF7/WT cells is, at least in part, responsible for the observed cross-resistance to TPT and presumably the other analogues. However, this is not mediated by Pgp since our previous studies have shown that there is no detectable *MDR1*/Pgp expression in these cells (8). Furthermore, with the exception of CPT-11, MCF7/ADR cells that are known to highly overexpress Pgp (9) are less than 4-fold cross-resistant to TPT or any of the other CPTs tested (Table 1). Thus, Pgp does not appear to cause the mitoxantrone/TPT resistance phenotype.

Another protein that has been shown to affect drug resistance and accumulation is the recently characterized MRP (16). However, MRP mRNA levels in MCF7/MX cells were similar to those in parental MCF7/WT cells (Table 1). This is in good agreement with a recent report by Futscher *et al.* (17) that indicated that none of four independently mitoxantrone-selected cell lines exhibited MRP overexpression. Furthermore, we have found that MRP-overexpressing MCF7/VP cells (10) were maximally 4-fold cross-resistant to any of the CPTs studied (Table 1). Thus, it appears unlikely that TPT cross-resistance in MCF7/MX cells is associated with MRP overexpression.

Cancer cells exposed to cisplatin (4), 4′-(9-acridinyl-amino)methanesulfon-*m*-anisidide (6), or melphalan (7) have been shown to develop cross-resistance to DNA topoisomerase I inhibitors. For instance, human small cell lung cancer cells N417/AMSA were 28-fold cross-resistant to CPT, despite a 3-fold increase in DNA topoisomerase I activity. In contrast, TPT cross-resistance in the melphalan-resistant human rhabdomyosarcoma xenograft TE-671 MR was associated with decreased topoisomerase I levels. Finally, the 12-fold cross-resistance to CPT-11 of the cisplatin-resistant human ovarian carcinoma cell line HAC2/0.1 was found to be due to the decreased conversion of CPT-11 to its active metabolite SN38. Only a limited number of mitoxantrone-resistant cell lines has been described. Two of those, namely, MCF7/Mitox (18) and EPG85–257 (19), appear to have a phenotype similar to the MCF7/MX cell line described in this report. Each of these cell lines displays relatively specific resistance to mitoxantrone and each exhibits reduced drug accumulation in the absence of Pgp expression. Cross-resistance to CPT analogues apparently has not been examined in any of these other mitoxantrone-resistant cell lines. Thus, it will be interesting to find out whether those independently selected mitoxantrone-resistant cells exhibit a similar pattern of cross-resistance to CPT analogues as the MCF7/MX cells.

A number of Phase I and II studies of TPT, CPT-11, and 9-AC are currently under way in cancer patients and preliminary results are encouraging in several types of cancer. However, the results in the present report suggest that different CPT analogues may be handled differently at the cellular level and that resistance to CPT analogues may develop when cancer cells are exposed to clinically available topoisomerase II inhibitors. Therefore, understanding of the underlying mechanism(s) of TPT resistance in MCF7/MX cells may help in the rational design of clinical trials involving these drugs. Furthermore, the search and discovery of possible drug-resistance modulating agents may help to increase the efficacy of these CPT analogues.

MCF7/WT breast cancer cells exposed to mitoxantrone acquired a high degree of resistance to mitoxantrone and cross-resistance to TPT, CPT-11, SN-38, and 9-AC, but displayed little cross-resistance to CPT and 10,11-mCPT. There were no apparent quantitative or qualitative alterations in DNA topoisomerases I and II. In contrast, resistance to both mitoxantrone and TPT appeared to be associated with decreased drug accumulation. Since MCF7/MX cells do not overexpress either *MDR1* or MRP, the defect in these cells seems to involve a novel mechanism of multidrug resistance that affects the accumulation and sensitivity of mitoxantrone and TPT.

Acknowledgments

We thank Dr. Fran Hakim and Robert W. Robey for their help with the flow cytometry.

References

1. Slichenmeyer, W. J., Rowinsky, E. K., Donehower, R. C., and Kaufmann, S. H. The current status of camptothecin analogues as antitumor agents. J. Natl. Cancer Inst., *85:* 271–291, 1993.
2. Woessner, R. D., Eng, W-K., Hofmann, G. A., Rieman, D. J., McCabe, F. L., Hertzberg, R. P., Mattern, M. R., Tan, K. B., and Johnson, R. K. Camptothecin hyper-resistant P388 cells: drug-dependent reduction in topoisomerase I content. Oncol. Res., *4:* 481–488, 1992.
3. Tanizawa, A., Bertrand, R., Kohlhagen, G., Tabuchi, A., Jenkins, J., and Pommier, Y. Cloning of Chinese hamster DNA topoisomerase I cDNA and identification of a single point mutation responsible for camptothecin resistance. J. Biol. Chem., *268:* 25463–25468, 1993.
4. Niimi, S., Nakagawa, K., Sugimoto, Y., Nishio, K., Fujiwara, Y., Yokoyama, S., Terashima, Y., and Saijo, N. Mechanism of cross-resistance to a camptothecin analogue (CPT-11) in a human ovarian cancer cell line selected by cisplatin. Cancer Res., *52:* 328–333, 1992.
5. Hendricks, C. B., Rowinsky, E. K., Grochow, L. B., Donehower, R. C., and Kaufmann, S. H. Effect of P-glycoprotein expression on the accumulation and cytotoxicity of topotecan (SK&F 104864), a new camptothecin analogue. Cancer Res., *52:* 2268–2278, 1992.
6. Prost, S., and Riou, G. A human small cell lung carcinoma cell line, resistant to 4′-(9-acridinylamino)-methanesulfon-*m*-anisidide and cross-resistant to camptothecin with a high level of topoisomerase I. Biochem. Pharmacol., *48:* 975–984, 1994.
7. Friedman, H. S., Dolan, M. E., Kaufmann, S. H., Colvin, O. M., Griffith, O. W., Moschel, R. C., Schold, S. C., Bigner, D. D., and Ali-Osman, F. Elevated DNA polymerase α, DNA polymerase β, and DNA topoisomerase II in a melphalan-resistant rhabdomyosarcoma xenograft that is cross-resistant to nitrosourea and topotecan. Cancer Res., *54:* 3487–3493, 1994.
8. Nakagawa, M., Schneider, E., Dixon, K. H., Horton, J., Kelley, K., Morrow, C., and Cowan, K. H. Reduced intracellular drug accumulation in the absence of P-glycoprotein (*mdr*1) overexpression in mitoxantrone-resistant MCF-7 breast cancer cells. Cancer Res., *52:* 6175–6181, 1992.

9. Fairchild, C. R., Ivy, S. P., Kao-Shan, C-S., Whang-Peng, J., Rosen, N., Israel, M. A., Melera, P. W., Cowan, K. H., and Goldsmith, M. E. Isolation of amplified and overexpressed DNA sequences from adriamycin-resistant human breast cancer cells. Cancer Res., *47:* 5141–5148, 1987.
10. Schneider, E., Horton, J. K., Yang, C-H., Nakagawa, M., and Cowan, K. H. Multidrug resistance-associated protein (MRP) gene overexpression and reduced drug sensitivity of topoisomerase II in a human breast carcinoma MCF7 cell line selected for etoposide resistance. Cancer Res., *54:* 152–158, 1994.
11. Frei, E., Teicher, B. A., Holden, S. A., Cathart, K. N. S., and Wang, Y. Preclinical studies and clinical correlation of the effect of alkylating dose. Cancer Res., *48:* 6417–6423, 1988.
12. Hsiang, Y-H., Liu, L. F., Wall, M. E., Wani, N. C., Nicholas, A. W., Manikumar, G., Kirschenbaum, S., Silber, R., and Potmesil, M. DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogs. Cancer Res., *49:* 4385–4389, 1989.
13. Schneider, E., Darkin, S. J., Robbie, M. A., Wilson, W. R., and Ralph, R. K. Mechanism of resistance of non-cycling mammalian cells to 4′-[9-acridinylamino]methanesulphon-*m*-anisidide: role of DNA topoisomerase II in log-and plateau-phase CHO cells. Biochim. Biophys. Acta, *949:* 264–272, 1988.
14. Chen, A. Y., Yu, C., Potmesil, M., Wall, M. E., Wani, M. C., and Liu, L. F. Camptothecin overcomes MDR1-mediated resistance in human KB carcinoma cells. Cancer Res., *51:* 6039–6044, 1991.
15. Mattern, M. R., Hofmann, G. A., Polsky, R. M., Funk, L. R., McCabe, F. L., and Johnson, R. K. *In vitro* and *in vivo* effects of clinically important camptothecin analogues on multidrug-resistant cells. Oncol. Res., *5:* 467–474, 1993.
16. Cole, S. P. C., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M. V., and Deeley, R. G. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science (Washington DC), *258:* 1650–1654, 1992.
17. Futscher, B. W., Abbaszadegan, M. R., Domann, F., and Dalton, W. S. Analysis of *MRP* mRNA in mitoxantrone-selected, multidrug-resistant human tumor cells. Biochem. Pharmacol., *47:* 1601–1606, 1994.
18. Taylor, C. W., Dalton, W. S., Parrish, P. R., Gleason, M. C., Bellamy, W. T., Thompson, F. H., Roe, D. J., and Trent, J. M. Different mechanisms of decreased drug accumulation in doxorubicin and mitoxantrone resistant variants of the MCF7 human breast cancer cell line. Br. J. Cancer, *63:* 923–929, 1991.
19. Dietel, M., Arps, H., Lage, H., and Niendorf, A. Membrane vesicle formation due to acquired mitoxantrone resistance in human gastric carcinoma cell line EPG85–257. Cancer Res., *50:* 6100–6106, 1990.