DNA Topoisomerase II α Expression Is Associated with Alkylating Agent Resistance

Joseph P. Eder, Jr., Victor T-W. Chan, Shu-Wing Ng, Nalyzer A. Rizvi, Stergios Zacharoulis, Beverly A. Teicher, and Lowell E. Schnipper

ABSTRACT

Increased expression of DNA topoisomerase IIα has been associated with resistance to certain DNA-damaging alkylating agents, but no causal relationship or mechanism has been established. To investigate this observation, we developed a model of topoisomerase II overexpression by transfecting a full-length Chinese hamster ovary topoisomerase IIα into EMT6 mouse mammary carcinoma. Topoisomerase IIα-transfected cell lines demonstrated continued topoisomerase IIα mRNA and protein expression, which were undetectable in vector-only lines, in stationary phase (G0-G1). The topoisomerase IIα transfectants were ~5–10-fold resistant to the alkylating agents cisplatin and mechlorethamine. Upon release from G0-G1, the topoisomerase IIα transfectants demonstrated more rapid thymidine incorporation and shorter cell-doubling times than control cells. Purified topoisomerase II and nuclear extracts with topoisomerase IIα-decatenating activity bound to cisplatin-treated DNA with significantly greater affinity than to untreated DNA in a cisplatin concentration-dependent manner. These observations suggest that expression of topoisomerase IIα may have a role in cellular resistance to antineoplastic alkylating agents. The mechanism for this may involve increased binding of topoisomerase IIα to alkylating-agent-damaged DNA.

INTRODUCTION

DNA topoisomerase II is a predominantly nuclear protein essential for DNA replication, RNA transcription, chromosome condensation, mitosis, and DNA recombination (1). In addition, topoisomerase II is localized to the AT-rich scaffold attachment regions, the site of gene transcription, where it forms a significant part of the mitotic chromosomal scaffold (2–4). Topoisomerase II exists in two isoforms, a Mr 170,000 α form, which is cell cycle regulated, and a cell cycle-independent β form (5). Most of the functions of topoisomerase II listed above are ascribed to the α isoform; the β isoform may function in rRNA production (1, 6). Topoisomerase IIα (topoisomerase IIα) is II expression produced increased resistance to the DNA-damaging antineoplastic agents mechlorethamine and cisplatin. This increased resistance was associated with increased topoisomerase II binding to cisplatin-damaged DNA.

MATERIALS AND METHODS

Cell Lines and Tissue Culture. The EMT6 murine mammary carcinoma tumor line, usually grown by serial passage in BALB/c mice, was grown in cell culture in Waymouth’s media (GIBCO-BRL), 10% dialyzed fetal bovine serum (Sigma Chemical Co.), and 400 μg hygromycin (CalBiochem), called complete media. Two clones were derived from the original EMT6 cell line by limiting dilution (105–106 cells plated in 96-well plates) and subsequent serial expansion. These cells were grown in Waymouth’s media-10% dialyzed fetal bovine serum only.

Transfection. The CHO topoisomerase IIα gene was isolated as described previously (11). A SalI fragment containing the entire cDNA was fitted with compatible BamHI-linking oligonucleotides and ligated into the BamHI multiple cloning site of the pMPEP 4 mammalian expression vector with a metallothionein IIα promoter (Invitrogen; Fig. 1). EMT6 cells (2 × 106) were transfected by electroporation and selected in complete media. Hygromycin-resistant colonies were isolated after 2–3 weeks, expanded, and frozen stocks were made for storage in the vapor phase of liquid nitrogen.

Analysis of Transfectants. DNA and RNA were isolated, purified, and subjected to Southern and Northern analysis as we described previously, except that the high molecular weight DNA was treated with the restriction endonucleases Xbal and EcoRI (New England Biolabs Ltd.; Ref. 11). The full-length CHO topoisomerase IIα cDNA was randomly primed and used as the probe for these analyses.

PCR Primers and Products. A RT-PCR assay of poly(A)+ RNA was used to detect the CHO topoisomerase IIα RNA. Two sets of priming oligonucleotides were synthesized (DNA International). The first set was complementary to the cDNA made from the first chain synthesis of a poly(A)+ RNA sequence at a consensus ATPase site surrounding nucleotide 1478, which was identical in the mouse and CHO. The oligomers are 5'-CCAAACCTGTGAT-GATGCCA-3' and 5'-CTGAAGACCCACAATCT-3'. The second set of primers is complementary to a poly(A)+ RNA segment that includes the 3' terminal portion of the metallothionein IIα promoter, the polylinker sequence (5'-GGTACCAGCTGCTAGCAAG-3') and the most 5' nucleotides of the CHO topoisomerase IIα poly(A)+ RNA (5'-ATCAGTGGGAAAACCGGAG-3').

Poly(A)+ RNA was isolated from exponentially growing and serum-deprived cells as described previously (11). DNA was synthesized from 1 μg of poly(A)+ RNA with the Superscript cDNA Pre amplification kit (GIBCO-BRL) in the presence of random hexamer primers. Five μl (of 20 μl) of the first-strand cDNA preparation were added to the PCR mixtures containing 1.0 μM of each primer pair, 200 μM dNTPs in 50 μl (total volume) of reaction buffer [50 mM KCl, 10 mM Tris-HCl (pH 8.3), 3.0 mM MgCl2] and 2.5 units Ampli-Taq polymerase (Perkin Elmer Cetus). The mixture was denatured at 94°C, annealed at 52°C, and extended at 72°C (1 min each), followed by a 10 min extension on a DNA thermal cycler for 30 cycles.
TOPOISOMERASE II EXPRESSION AND CISPLATIN RESISTANCE

Fig. 1. The vector used in the transfection studies is shown. It consists of the full-length CHO topoisomerase II ligated into the multiple cloning BamHI site of the pMEP4 mammalian expression vector (Invitrogen).

(Perkin Elmer Cetus). PCR products were analyzed on a 1.6% agarose gel with 5 μg/ml ethidium bromide.

Cell Synchronization and Proliferation Studies. Cell proliferation assays, done in triplicate at least three times, were performed by seeding 5 x 10^4 cells in 35-mm culture dishes in complete media and counting daily by hemocytometer.

Cells synchronization was performed by serum deprivation (without media changes) for 96 h. The effectiveness of cell synchronization was measured by counting replicate plates at 96 and 120 h and by determination of cell cycle distribution. DNA histograms were measured by FACS. Cells were washed twice with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4·7H_2O, and 1.4 mM KH_2PO_4), fixed with ice-cold ethanol, kept on ice for 1 h, concentrated by centrifugation, washed again with cold PBS, resuspended in 0.1 mg/ml propidium iodide, treated with 5 μg/ml of RNase A at 37°C for 20 min, and protected from light before FACS.

For measurements of DNA synthesis, cells were washed in PBS twice and serum-free Waymouth’s medium/hygroycin was added. [3H]methyl-thymidine incorporation into DNA was measured by adding complete media and 0.6 μCi/ml of [3H]thymidine to serum-deprived, synchronized cells. Cells were harvested at selected time points by 0.5% SDS lysis, and DNA was precipitated by 10% trichloroacetic acid at 4°C. Lysates were collected on glass fiber filters (Whatman GF/C) and counted on a Beckman LS 6000 scintillation counter.

Drug Sensitivity Studies. Clonogenic survival studies of antineoplastic agent treatment were performed after 96 h of serum deprivation. Etoposide, novobiocin, cisplatin, and meclohydroethamine were prepared as described earlier (11). Cisplatin, meclohydroethamine, and etoposide exposures were for 1 h. Cells were then washed free of drug and incubated for 23 h. Cisplatin- and meclohydroethamine-treated cells were placed back in conditioned serum-free media to allow for DNA damage repair (14). Etoposide-treated cells were placed into complete media at the time of drug treatment and for the incubation period. Novobiocin exposure was for 24 h in serum-free media. Afterward, cells were suspended by treatment with trypsin, counted, and placed into complete media and overlaid with 0.5% soft agar. Cells were allowed to grow for 6–14 days until colonies ≥50 cells were present. Colonies were fixed with acetic acid:methanol, agar was washed away with hot water, stained with crystal violet, and counted. Data were analyzed for mean and SEM of surviving colonies using Macintosh SYSTAT 5.1 and graphed with Macintosh Cricket Graph.

Topoisomerase II Assays. Nuclear extracts were prepared after the method of Dignam et al. (17). DNA was removed by 6% polyethylene glycol purification, and protein concentration was determined (18).

Decatenation activity of kinetoplast DNA by 30 μg of nuclear extract was measured by using the topoisomerase II decatenating kit from Topogen (Columbus, OH), which is a modification of the method of Marini et al. (19).

Western analysis of total cellular topoisomerase II IM, 170,000 protein was performed on nuclear extracts prepared as above (17) and then electrophoresed in a 6% polyacrylamide gel following the method Kaufman et al. (20). After electrophoresis, 10 μg/sample lane of total protein were used for exponentially growing cells and 50 μg/sample lane for stationary phase cells. The antibody used was a rabbit polyclonal antihuman topoisomerase II p170 (Topogen). Detection was enhanced via chemoluminescence (ECL; Amersham).

Glutathione and Metallothionein Assays. To examine any effect of the metallothionein promoter in the pMEP construct used in the transfections, total and protein sulfhydryl concentrations were measured as described previously (21).

DNA-binding Assay. An assay was developed to measure nuclear proteins, including topoisomerase II, binding to cisplatin-adducted DNA. This assay was a modification of the potassium-SDS assay used to measure topoisomerase II binding to DNA (22). The purified p340 plasmid (23) was linearized by XhoI restriction endonuclease digestion and [32P]labeled (24).

Aliquots were then exposed to cisplatin at 10^-3 m (or H_2O for untreated controls) for 2 h at 37°C. Equal amounts of DNA were incubated for 1 h at 37°C with nuclear extracts (30–70 μg of protein), purified human topoisomerase II protein (1.25–10 units; Topogen, Columbus, OH) or combinations of both in the decatenating assay reaction buffer, which includes 0.5 μM ATP and 30 μg BSA as a protein control. DNA protein complexes were precipitated with 0.1 M KCl and 1.5% SDS, washed three times, and scintillation counted as described previously (14). Experiments were repeated four times under optimal conditions and without ATP and magnesium as a negative control.

RESULTS

Characterization of EMT6-transfected Cell Lines. Three transfected cell lines emerging as hygromycin-resistant colonies contained a full-length, unarranged CHO topoisomerase II by Southern hybridization (Fig. 2). Dialyzed serum was used during the hygromycin selection process to reduce activation of the expression vector serum response element, which might produce increased topoisomerase II activity, a protein which produces illegitimate recombination and may select against stable transfecants (25). The XhoI digested produced a 2.7-kb fragment representing the 3' 900 bp of the human metallothio-nein Ila promoter sequence, the intervening polylinker region and the most 5' 1.8 kb of the CHO topoisomerase II. The EcoRI digest produced a 4.6-kb fragment, which represents almost the entire CHO topoisomerase II cDNA. Because of the close similarity between mouse and CHO topoisomerase II cDNA, some slight cross-hybridization with the mouse sequence was seen in all cell lines. The TLC 09, 14, and 62 had full-length CHO topoisomerase II cDNA, some slight cross-hybridization with the mouse sequence was seen in all cell lines. The TLC 09, 14, and 62 had full-length CHO topoisomerase II cDNAs as represented by specific bands of the expected size. The transfected topoisomerase II gene in transformed cell lines TCL14 and TCL62 was amplified (~6–10 copies). The EMT6 vector transfected only cell line VL24 (EMT6 cells transfected with the pMEP vector only as a control did not show the same pattern of specific hybridization). The cell line VL66 was derived from EMT6 cells transfected with the pMEP vector and the full-length CHO topoisomerase II; however, the hybridization pattern (Fig. 2, Lanes 5 and 6) showed that only a partial fragment of the CHO topoisomerase II integrated in this particular cell line. VL66 was investigated further as a vector-only control transfec-tant (non-CHO topoisomerase II producing) cell line, thus the VL designation.

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Two sets of primers were used. One set produced a 250-bp fragment that encompasses the 3’ region of the CHO topoisomerase II and is specific for the CHO topoisomerase II poly(A)⁺ RNA. This DNA product is from the highly conserved consensus ATP-binding motif present in topoisomerase II genes. The second set produced a 107-bp fragment that encompasses the most 5’ region of the CHO topoisomerase II and is specific for poly(A)⁺ RNA transcribed from the CHO topoisomerase II cDNA (Fig. 3C). The three cell lines tested expressed topoisomerase II poly(A)⁺ RNA, but only TCL62 expressed CHO topoisomerase II poly(A)⁺ RNA. At 1 μM CdCl₂ (Sigma) there was little enhancement of CHO topoisomerase II expression after 24 h, and a decrease was observed at 10 μM. VL66 (incomplete CHO topoisomerase II transfectant) had no evidence of CHO topoisomerase II poly(A)⁺ RNA production, consistent with the lack of integration of a full-length CHO topoisomerase II gene.

GST-G₁-arrested cells were analyzed by RT-PCR for the presence of topoisomerase II poly(A)⁺ RNA. Northern analysis of poly(A)⁺ RNA confirmed that the TCLs, TCL14 and TCL62, produced a 5.6-kb RNA, which hybridized to the full-length CHO topoisomerase II cDNA probe and gave little or no background signal in the vector-only VL lines or in untransfected EMT6 cells (Fig. 3A). Because of the similarity of CHO and mouse topoisomerase II and concern about possible ambiguities in the Northern analysis, a second method was developed to detect CHO topoisomerase II transcripts using RT-PCR.

Two sets of primers were used. One set produced a 250-bp fragment from the highly conserved consensus ATP-binding motif present in the CHO topoisomerase II poly(A)⁺ RNA, surrounding bp 1493 (CHO sequence) of the CHO topoisomerase II. This DNA product is a measure of total cellular topoisomerase II poly(A)⁺ RNA present. The second set produced a 107-bp fragment that encompasses the 3’ polylinker of the vector distal to the metallothionein IIa promoter and the most 5’ region of the CHO topoisomerase II and is specific for poly(A)⁺ RNA transcribed from the CHO topoisomerase II cDNA (Fig. 3C). The three cell lines tested expressed topoisomerase II poly(A)⁺ RNA, but only TCL62 expressed CHO topoisomerase II poly(A)⁺ RNA. At 1 μM CdCl₂ (Sigma) there was little enhancement of CHO topoisomerase II expression after 24 h, and a decrease was observed at 10 μM. VL66 (incomplete CHO topoisomerase II transfectant) had no evidence of CHO topoisomerase II poly(A)⁺ RNA production, consistent with the lack of integration of a full-length CHO topoisomerase II gene.

GST-G₁-arrested cells were analyzed by RT-PCR for the presence of topoisomerase II poly(A)⁺ RNA. TCL09, TCL14, and TCL62 demonstrated the continued expression of topoisomerase II poly(A)⁺ RNA derived from the transfected CHO cDNA, and no topoisomerase II poly(A)⁺ RNA was detected in the control transfectant cell line VL24 (Fig. 4). Western analysis of whole cell lysates showed comparable topoisomerase II levels in exponentially growing cells, but only cell line TCL62 had demonstrable topoisomerase II protein (~5–10% of the total present in proliferating cells) under these stationary (G₀-G₁) conditions (Fig. 5A). Topoisomerase II decatenation assays demonstrated persistent catalytic activity in the G₁-arrested TCLs and no activity in the control transfectants (Fig. 5B, Lane 4, at arrow denoting the decatenated kinetoplast DNA).

Topoisomerase II expression was associated with altered rates of progression through the cell cycle. The doubling time during exponential growth of the CHO topoisomerase II-expressing cell lines TCL14 and TCL62 was approximately 24 h compared to 48 h in the VL24 and VL66 cell lines (Fig. 6A). Because the EMT6 line is not clonal, subclones were isolated by limiting dilution. Two clones, designated A and B, had cell doubling times similar to the control VL cell lines (Fig. 6A). When G₁-synchronized cells were repleted in serum containing complete medium, exposed to a 30 min pulse of [³H]thymidine, and sampled at intervals for 48 h, TCL14 and TCL62 exhibited an enhanced rate (~2-fold) of DNA synthesis and a shorter cell cycle time than did the control VL24 cell line (Fig. 6B).

Various experimental conditions were tested to maximize the difference in topoisomerase II content between the TCLs and the control cells. Because the CHO topoisomerase II construct was expressed constitutively (Fig. 4), this difference was exploited by placing the cells in serum-free media without change for 96 h. This produced a growth-arrested stationary state confirmed by cell counting (no increase in cell number between 96 and 120 h; data not shown). DNA histogram analysis by FACs demonstrated ≥85% of cells with a G₁ phase diploid DNA content after 96 h in serum-deprived conditions (Fig. 7). After serum repletion of these synchronized cells, ≥66% of the topoisomerase II-transfected TCL62 cells were proliferating in...
24 h. Qualitatively similar results were seen in the control transfectant cell lines, allowing for the different cell cycle times.

Chemotherapy Sensitivity Studies. After confirmation that the TCLs continued to express topoisomerase II polyclonal immunoreactive anti-topoisomerase II protein and kDNA-decatenating activity when this was absent or markedly diminished in the control transfectant cell lines, selected cell lines were characterized for their response to chemotherapeutic agents. These cytotoxic assays were completed after 96 h of serum deprivation to maximize the differences in topoisomerase II levels. TCL14 and TCL62 demonstrated substantial resistance to the DNA-damaging antineoplastic alkylating agents mechlorethamine and cisplatin (Fig. 8, A and B). The mechlorethamine and cisplatin resistance was at least 2-4-fold at toxic concentrations (90% inhibitory concentration) but increased (≥4-12-fold) at higher cytotoxic concentrations (90% inhibitory concentration). There was more variation among cell lines for mechlorethamine than among cisplatin cytotoxicity. The relative difference in cisplatin cytotoxicity was comparable in exponentially growing VL24 and TCL62 after serum replacement and resumption of exponential growth (data not shown). The studies completed during serum deprivation and growth arrest compensate for the different growth rates observed in TCL and VL cell lines.

The pMEP plasmid contains the metallothionein IIa promoter. Increased levels of metallothionein and nonprotein sulphydryl (glutathione) content are known to be associated with alkylating agent and cisplatin resistance (26, 27). These parameters were measured in the VL24 and the TCL62 cell lines (Table I). There was no difference between the cell lines and neither sulphydryl species was inducible with up to 10 μM CdCl2 in either cell line.

TCL9, TCL14, and TCL62 were significantly more sensitive (≥10-fold) to the cytotoxic effects of etoposide than the control transfectant VL24 (Fig. 9A). The CHO topoisomerase II transfectants were also more sensitive to the cytotoxic effects of another topoisomerase II inhibitor novobiocin (Fig. 9B). This cytotoxicity was associated with an apoptotic nucleosomal ladder (data not shown).

Binding of Topoisomerase II to Cisplatin-damaged DNA. To investigate the relationship between increased topoisomerase II expression in the TCLs and enhanced survival after cisplatin treatment, the capacity of topoisomerase II to bind to DNA with cisplatin adducts was measured by an in vitro DNA-binding assay. Cisplatin adducted DNA, nuclear extracts with decatennating activity, and purified topoisomerase II protein were used in a modification of the K+-SDS precipitation assay. The p340 plasmid used in these experiments has the advantage that the extent and site of platinum adduct formation include a cleavable complex forming topoisomerase II interactive agent was obviated.

As cisplatin concentration increased over a 100-fold range from 10⁻⁷ to 10⁻³ M, the extent of topoisomerase II binding, as measured by precipitable counts, also increased (Fig. 10A). The TCL62 nuclear extracts, which contain many other proteins in addition to topoisomerase II, also resulted in increased binding as cisplatin adduct formation increased, but this effect reached a plateau between 10⁻⁶ and 10⁻³ M cisplatin. The most striking finding was cooperative concentration-
Bidentate inter- or intrastrand cross-links are the major cytotoxic adduct formed by the nucleophilic alkylating agents; these agents also produce monoadducts, which may undergo depurination and DNA strand breakage or base tautomerization and nucleic acid mispairing (29). Alkylating agents were among the first clinically important antineoplastic agents used and form the core of almost every clinically useful combination chemotherapy regimen (30). Multiple mechanisms of alkylating agent resistance have been described, including altered transport mechanisms, increased glutathione and metallothionein levels, increased intracellular metabolism, and increased DNA repair (31). Unlike the extremely high levels (≥20-fold) of resistance seen in response to natural products and antimetabolites, resistance to the antineoplastic alkylating agents is low level and only rarely ≥10-fold (31).

Increased topoisomerase II levels have been observed in the mechlorethamine resistant Raji HN2 line and a cisplatin-resistant human lung cancer line (12, 13). The Raji HN2 line is 5–10-fold resistant to mechlorethamine at 1 log of cell killing (90% inhibitory concentration) compared to the parental Raji line and has a 3-fold increase in topoisomerase II activity. Both are lost in the absence of mechlorethamine selection pressure (13). In both the Raji HN2 and the cisplatin-resistant lung cancer cell lines, topoisomerase II inhibitors partially reverse resistance to the alkylating agent (32, 15). Although this finding suggests that topoisomerase II contributes to alkylating agent resistance, these cell lines were selected by prolonged serial exposure to the mutagenic alkylating agent itself, a process often associated with the presence of multiple mechanisms of resistance (31). The development of paired cell lines in which one demonstrates stably increased expression of topoisomerase II may provide a model system to evaluate the role of topoisomerase II in alkylating agent resistance.

In these studies, the CHO topoisomerase II α gene was transfected into EMT6 murine mammary carcinoma cells. When transfectants are growth arrested by serum deprivation and endogenous murine topoisomerase II expression decreases, CHO topoisomerase II continues to be expressed. The CHO topoisomerase II transfectants demonstrated up to 10-fold resistance to both cisplatin and mechlorethamine over a wide concentration range, suggesting that topoisomerase II may be a major determinant of alkylating agent resistance. Enhanced sensitivity to etoposide in the topoisomerase II-transfected cell lines indicates

**DISCUSSION**

Antineoplastic alkylating agents, including cisplatin, can be described as prototype DNA-damaging agents (28). Although DNA-dependent DNA precipitation produced by combining topoisomerase II with the nuclear extracts in the presence of cisplatin-adducted DNA. This combination did not enhance DNA precipitation of native, undamaged DNA. Nuclear extracts from serum-deprived VL24 cells, which have no measurable topoisomerase II expression, did not demonstrate cooperative DNA binding under the same conditions (Fig. 10B). Deletion of ATP and magnesium ions from the reaction buffer abolished topoisomerase II binding and cooperative topoisomerase II/nuclear extract binding (Fig. 10C).

**Fig. 6.** A, doubling time of cell lines EMT6/clones A and B, VL24 and VL66 (control cell lines, vector transfectants only) and TCL14 and TCL62. Five X 10⁶ cells were plated into 35-mm tissue culture plates and grown in complete media (EMT6 clones were grown in 10% dialyzed serum/Waymouth’s without hygromycin). B, incorporation of [³H]thymidine, 30-min pulse exposure, after addition of complete media after 96 h of serum deprivation.
Table 1  Sulfhydryl levels

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<td>233</td>
<td>288</td>
<td>214</td>
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* ND, not determined.

that topoisomerase II function and the signaling of apoptotic pathways are intact.

An assay developed to investigate topoisomerase II binding to cisplatin-treated DNA demonstrated increased topoisomerase II DNA binding with increased cisplatin concentration. This assay was performed with chromatin-free linearized plasmid DNA and offered the opportunity to control both the amount of cisplatin adducts and topoisomerase II levels. A significant advantage of the assay was that the conditions used eliminated the need for topoisomerase II-acting drugs. Consequently, the protein and topoisomerase II bound to DNA was not influenced by the recruitment of topoisomerase II to DNA by a topoisomerase II-acting drug. The cisplatin concentration effect on topoisomerase II binding is clear, reproducible, and appears stoichiometric.

Resistance to cisplatin DNA damage and cytotoxicity has been shown to be mediated through repair of the DNA damage (reviewed in Ref. 33). Not only have such associations been noted between sensitive and resistant cell lines but also between the number of cisplatin adducts in peripheral leukocytes and clinical response in ovarian and testicular cancers (34, 35). Increased binding of topoisomerase II and the enhanced binding of other proteins that occurs with it suggests that topoisomerase II may be involved in the cellular response to cisplatin-damaged DNA. These data do not address which steps topoisomerase II is associated with in the processing of cisplatin damage.

Topoisomerase II inhibitors of several types enhance alkylating agent cytotoxicity (12, 15, 16, 21) and one of these, novobiocin, has been shown to increase cisplatin interstrand cross-link formation from monoadducts in DNA and impair their removal (36, 37).
observations suggest a possible explanation for the synergistic interaction seen clinically between etoposide and cisplatin. Cisplatin adducts on cellular DNA will "recruit" topoisomerase II to DNA and increase the likelihood of etoposide, topoisomerase II, and DNA interaction, which produces enhanced cytotoxicity. By binding topoisomerase II to DNA at its usual sites, etoposide would also decrease the topoisomerase II available to bind to cisplatin adduct sites, decrease adduct repair, and potentiate the cytotoxicity of that agent in a synergistic fashion. It is also possible that treatment with either agent increases the available target sites for the other agent, perhaps by altering chromatin structure.

We also found that those cells that continue to express topoisomerase II during a G0-G1 stationary state proliferate and replicate DNA more rapidly than do parental controls. Topoisomerase II activity is essential to the rapid completion of chromosome condensation in S phase (1, 38). Control of the state of helical torsion in front of and behind the replicating fork of the DNA synthesis apparatus (and the RNA transcription complex) requires topoisomerase activity, usually a combination of type I and type II topoisomerases (1, 39). The availability of topoisomerase II at the onset of S phase, particularly if a rate-limiting requirement may account for the rapid proliferation rate seen in the CHO topoisomerase II-transfected cells. Under some experimental conditions, DNA repair occurs preferentially during G2 before M phase, preceding DNA repair and a shortened cell cycle time) may be linked by the fact that transcription and DNA repair are themselves intimately linked through the TFIIH complex (reviewed in Ref. 43). Actively transcribed genes are more efficiently and rapidly repaired than are inactive genes. This transcription coupled repair could be greater in the TCLs in either or both G1 or S phase, depending on the genes involved (44). Although it is not certain that topoisomerase II is essential in transcription, given its cell cycle fluctuation, transcription rates may influence or be influenced by the state of helical torsion, of which topoisomeras are a major determinant (45). More studies are necessary to fully understand if and how increased topoisomerase II expression results in enhanced repair of alkylating agent-mediated DNA repair.

REFERENCES

8. Lock, R. B. Inhibition of p34Cdc2 kinase activation, p34Cdc2 tyrosine dephospho-
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