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Altered Drug Interaction and Regulation of Topoisomerase IIβ: Potential Mechanisms Governing Sensitivity of HL-60 Cells to Amsacrine and Etoposide

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ABSTRACT

Topoisomerase II (topo II), an enzyme essential for cell viability, is present in mammalian cells as the α- and β-isoforms. In human leukemia HL-60/S or HL-60/doxorubicin (DOX)0.05 cells, the levels of topo IIα- or β-protein were similar in either asynchronous exponential or synchronized cultures. Although topo IIα was hypophosphorylated in HL-60/DOX0.05 compared with HL-60/S cells, both overall and site-specific hyperphosphorylation of topo IIβ was apparent in HL-60/DOX0.05 compared with HL-60/S cells. The phosphorylation of topo IIα and not β was enhanced in the S and G_2 + M phases of HL-60/S cells. In contrast, an increase in the phosphorylation of topo IIβ compared with α was apparent in the G_1 and S phases of HL-60/DOX0.05 cells. The cytotoxicity and depletion of topo IIα or β in cells treated with drug for 1 h revealed that mole-for-mole, amsacrine was 2-fold more effective than etoposide in killing HL-60/S or HL-60/DOX0.05 cells and in depleting the β versus α topo II protein. Present results demonstrate that: 1) hyperphosphorylation of topo IIβ in HL-60/DOX0.05 cells may be a compensatory consequence of the hypophosphorylation of topo IIα to maintain normal topo II function during proliferation, and 2) enhanced sensitivity of HL-60/S or HL-60/DOX0.05 cells to amsacrine may be due to the preferential interaction and depletion of topo IIβ.

The DNA topoisomerases (topo) alter DNA topology for the processing of genetic material and are key targets for the clinically important antineoplastic agents amsacrine (*m*-AMSA) and etoposide (VP-l6) (Chen and Liu, 1994; Watt and Hickson, 1994; Wang, 1996). Although topo I exists as a single 97-kDa protein, topo II has an α- and β-isoform with molecular mass of 170 and 180 kDa, respectively (Watt and Hickson, 1994; Wang, 1996). Although a substantial literature exists on the regulation, function, and drug interactions of the α-isoform, the role of the β-isoform has received substantially less attention (Chen and Liu, 1994; Watt and Hickson, 1994; Wang, 1996).

A role for topo IIβ in cell differentiation was originally proposed by Woessner et al. (1990, 1991). More recently, the altered regulation of topo II and the pronounced up-regulation of topo IIβ during all *trans*-retinoic-induced differentiation of human leukemia HL-60 cells has been reported (Aoyama et al., 1998b). Previous studies (Cornarotti et al., 1996; Dereuddre et al., 1997) have suggested that the α- and β-isoforms of topo II may represent distinct targets that govern differential sensitivity to drugs that poison the enzyme. A more recent study (Herzog et al., 1998) has provided evidence that high levels of resistance to *m*-AMSA in a subline of HL-60 is correlative with the absence of detectable levels of the topo IIβ protein. A notable observation of this study was the finding that the absence of topo IIβ does not interfere with cell proliferation (Herzog et al., 1998).

HL-60 cells that exhibit increased resistance to doxorubicin (DOX) have been isolated (Ganapathi et al., 1996a). Although these resistant cells exhibit decreased drug accumulation due to overexpression of P-glycoprotein, the expression of resistance to topo II poisons is correlative not with intracellular drug levels but with decreased drug-stabilized topo II-DNA cleavable complex formation (Ganapathi et al., 1996a; Aoyama et al., 1998) and functional alterations in topo IIα. The reduced DNA damage also has been found to be related to site-specific hypophosphorylation of topo IIα (Aoyama et al., 1998). Although these changes in drug accumulation and hypophosphorylation of topo II are possibly linked, the differential sensitivity to *m*-AMSA compared with

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ABBREVIATIONS: topo, topoisomerase(s); *m*-AMSA, amsacrine; VP-16, etoposide; HL, human leukemia; DOX, doxorubicin; PAGE, polyacrylamide gel electrophoresis.

VP-16 remains unexplained in the HL-60 cells. In the present study, we have investigated the regulation of topo IIβ as well as the differential sensitivity of the α- and β-isoforms to *m*-AMSA and VP-16 with isoform-specific antisera in sensitive (HL-60/S) and DOX-resistant (HL-60/DOX0.05) HL-60 cells. Results suggest that unlike topo IIα, both overall and site-specific hyperphosphorylation of topo IIβ is observed in the HL-60/DOX0.05 cells. Furthermore, in both the HL-60/S and HL-60/DOX0.05 cells, *m*-AMSA was >2-fold more effective than VP-16 in topo IIβ-stabilized DNA cleavable complex formation (based on band depletion experiments) and cytotoxicity in a soft-agar colony assay.

Materials and Methods

The wild-type HL-60 (HL-60/S) cells were obtained from Dr. Andrew Yen, College of Veterinary Medicine, Cornell University, Ithaca, NY. Cultures of HL-60/S cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum and 2 mM L-glutamine (BioWhittaker, Walkersville, MD) at 37°C in a humidified 5% CO_2 plus 95% air atmosphere. The resistant subline of HL-60 developed by culturing the wild-type cells in increasing concentrations of 0.025 to 0.05 μg/ml DOX has been described previously (Ganapathi et al., 1996b). The DOX-resistant subline (HL-60/DOX0.05) was maintained in the absence of DOX during experimentation. Doubling time in vitro of the HL-60/S and HL-60/DOX0.05 cells was 18 to 20 h.

The enrichment of cells in G_1, S, and G_2 + M phases of the cell cycle was carried out by centrifugal elutriation (Hengstschlager et al., 1997) in a J2–21 centrifuge equipped with a JE-6 rotor (Beckman Coulter, Fullerton, CA). Briefly, cells (2×10^8) were loaded at a rotor speed of 2000 and 1875 rpm for the HL-60/S and HL-60/DOX0.05 cells, respectively. Fractions were collected with incremental increases in flow rate. The cells in each fraction were analyzed for cell cycle phase distribution by flow cytometry (Kawamura et al., 1996). Fractions containing cells in the cell cycle phase of interest were pooled and used for experiments on topo II protein levels and phosphorylation.

The effect of VP-16 or *m*-AMSA on induction of topo II-mediated DNA scission was determined by measuring precipitation of the protein DNA complex by a modification of the SDS-KCl technique (Zwelling et al., 1989; Ganapathi et al., 1996a; Aoyama et al., 1998). Cells were labeled for 24 h with 0.02 to 0.04 μCi/ml of [^{14}C]thymidine, specific activity 53 mCi/mmol (Amersham, Arlington Heights, IL). The labeled HL-60/S cells were treated with 0.1 to 5.0 μM *m*-AMSA or VP-16 for 1 h and processed for measuring DNA damage.

Cytotoxicity studies in vitro were carried out by a soft-agar colony assay (Ganapathi et al., 1996a,b) after exposure to *m*-AMSA or VP-16 for 1 h. The colony-forming efficiency of the HL-60 cells under these conditions was 29%.

Levels of 170 kDa (α) and 180 kDa (β) topo II protein in HL-60/S and HL-60/DOX0.05 cells were determined by SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblotting (Ganapathi et al., 1996). Briefly, extracts from HL-60/S or HL-60/DOX0.05 cells were prepared in radioimmunoprecipitation assay buffer with whole cells or nuclei isolated in nucleus buffer supplemented with 0.3% Triton X-l00 (Ganapathi et al., 1996; Aoyama et al., 1998a,b). Protein content was determined by the Coomassie assay reagent (BioRad, Hercules, CA) and serial dilutions containing equivalent amounts of protein from HL-60/S and HL60/DOX0.05 cells were analyzed by SDS-PAGE (Ganapathi et al., 1996a; Aoyama et al., 1998a,b). After electroblotting onto nitrocellulose, the topo II protein (α and β) was detected after incubation with rabbit polyclonal antibodies that recognize either the 170-kDa (Ganapathi et al., 1996a; Turley et al., 1997; Aoyama et al., 1998b) or the 180-kDa (Turley et al., 1997) topo II followed by ^{125}I-goat anti-rabbit IgG. The specific enhancement by *m*-AMSA or VP-16 of topo II DNA-cleavable complex formation was determined by the band depletion technique (Kawamura et al., 1996). The HL-60/S or HL-60/DOX0.05 cells were treated with 0.1 to 100 μM *m*-AMSA or VP-16 for 1 h at 37°C. Control and treated cells (2×10^6) were lysed in 2× Laemmli buffer, samples were processed by SDS-PAGE and electroblotted onto nitrocellulose (Kawamura et al., 1996), and topo II was detected with a polyclonal rabbit antibody that specifically recognizes the 170- or 180-kDa isoform followed by ^{125}I-goat anti-rabbit IgG (Ganapathi et al., 1996a; Turley et al., 1997; Aoyama et al., 1998). Depletion of topo II due to enhanced cleavable complex formation in treated versus control cells was quantified by a phosphorImager.

Phosphorylation of topo IIα or topo IIβ in HL-60/S and HL-60/DOX0.05 cells was determined by metabolic labeling with [^{32}P]orthophosphoric acid (Ganapathi et al., 1996a; Aoyama et al., 1998a,b). Nuclei were isolated from the labeled cells, lysed in RIPA buffer (Ganapathi et al., 1996; Aoyama et al., 1998a,b), and topo IIα or topo IIβ protein in lysates containing equivalent number of nuclei, or similar amounts of protein, was immunoprecipitated with rabbit polyclonal antibodies that recognize the 170-kDa (α) or 180-kDa (β) protein (Ganapathi et al., 1996a; Turley et al., 1997; Aoyama et al., 1998b). Details of the technique for metabolic labeling and immunoprecipitation have been reported previously (Ganapathi et al., 1996; Aoyama et al., 1998a,b). Phosphorylated topo IIα or topo IIβ protein levels were determined by densitometric scanning of autoradiograms or by the use of a phosphorImager. Phosphopeptide analysis of the immunoprecipitated 180-kDa (β) topo II was carried out by a modification (Ganapathi et al., 1996a; Aoyama et al., 1998a,b) of the methods described previously (Boyle et al., 1991; Wells et al., 1994). Briefly, the band corresponding to the 180-kDa (β) topo II protein was excised from the dried, unfixed gel, and eluted with 50 mM ammonium bicarbonate, 0.1% SDS, and 0.5% 2-mercaptoethanol overnight. The protein was precipitated with 100% trichloroacetic acid and oxidized with performic acid. Protein samples were digested overnight in *N*-tosyl-L-phenylalanine chloromethylketone-trypsin, and the radioactivity determined by Cerenkov counting. Aliquots of the phosphopeptides reconstituted in pH 1.9 electrophoresis buffer (containing equivalent dpm) were loaded onto thin layer cellulose plates and analyzed by electrophoresis with pH 1.9 buffer in the horizontal dimension, and phosphochromatography buffer in the vertical dimension (Ganapathi et al., 1996; Aoyama et al., 1998a,b).

Results

The effects of VP-16 and *m*-AMSA on the cytotoxic response were determined in HL-60/S and HL-60/DOX0.05 cells with a soft-agar colony assay (Fig. 1). The data indicate that although the HL-60/DOX0.05 cells are >20-fold resistant to VP-16, the magnitude of resistance to *m*-AMSA is only 2-fold. These results suggest that although VP-16 and *m*-AMSA target topo II, the expression of resistance is markedly different depending on their affinity for intercalation with DNA.

We have previously reported that resistance to VP-16 in the HL-60/DOX0.05 cells is not due to alterations in steady-state levels of topo IIα protein and is correlative with the hypophosphorylation of topo IIα (Ganapathi et al., 1996a; Aoyama et al., 1998a). Based on the availability of topo II isoform-specific antisera, and the ability of the technique of band depletion to ascertain drug-stabilized DNA cleavable complex formation with the topo II isoforms, the specific interaction of *m*-AMSA and VP-16 with topo IIα and β was determined. Representative gels and data that summarize the percentage of depletion of each topo II isoform for HL-60/S and HL-60/DOX0.05 cells are shown in Figs. 2 and 3. It is apparent that *m*-AMSA is 10- to 20-fold more potent than VP-16 in depleting an equivalent amount of topo IIβ in either

HL-60/S or HL-60/DOX0.05 cells. In the HL-60/S cells, 50% depletion of topo IIα protein occurred at 1 and 25 μM m-AMSA and VP-16, respectively. However, <50% depletion of topo IIα protein was observed in the HL-60/DOX0.05 cells over the range of drug concentrations tested. The depletion of topo IIβ protein after treatment with the topo II poisons tested was as follows: 1) in HL-60/S cells, 50% depletion was observed at 0.5 μM *m*-AMSA and 10 μM VP-16; and 2) a 50% depletion in HL60/DOX0.05 cells was observed at 1.0 μM *m*-AMSA and 50 μM VP-16. Although *m*-AMSA was superior to VP-16 in depleting the topo II isoforms at lower drug concentrations, maximal depletion of topo II without an early plateau was observed with VP-16. As shown in Table 1, these results on depletion of the topo II isoforms are also consistent with the formation of an equivalent level of drug-stabilized topo II-DNA cleavable complex with 10-fold lower concentrations of *m*-AMSA in the HL-60/S cells.

Our previous data have demonstrated that topo IIα is hypophosphorylated without changes in the steady-state level of the enzyme in the HL-60/DOX0.05 cells (Ganapathi et al., 1996a). Based on the availability of antisera that efficiently immunoprecipitate the α- or β-isoforms of topo II, we sought to determine the potential for alterations in the protein levels and the phosphorylation of topo IIβ in the resistant cells. As shown in Fig. 4A, the steady-state levels of topo IIβ protein in the HL-60/S and HL-60/DOX0.05 cells were comparable. However, in contrast to the hypophosphorylation of topo IIα, the topo IIβ protein is hyperphosphorylated in the HL-60/DOX0.05 cells (Fig. 4B). The hyperphosphorylation is not a consequence of the differential cellular distribution of topo IIβ because the data in Fig. 4C demonstrate that hyperphosphorylated topo IIβ is detectable both in whole cells and isolated nuclei. Because site-specific phosphorylation of proteins can have important regulatory functions, phosphopeptide mapping of complete tryptic digests of topo IIβ in the HL-60/S and HL-60/DOX0.05 cells was carried out. The data in Fig. 5 demonstrate that the hyperphosphorylation of topo IIβ in the HL60/DOX0.05 cells is site specific. Notably, eight different sites in the topo IIβ protein are hyperphosphorylated in the HL-60/DOX0.05 cells versus HL-60/S cells.

Because the phosphorylation but not protein levels of the α- and β-isoforms of topo II were different in the HL-60/S and HL-60/DOX0.05 cells, the potential for such changes based on cell cycle phase were investigated. The levels of topo IIα and topo IIβ protein in HL-60/S or HL-60/DOX0.05 cells enriched in the G_1, S, and G_2 + M phases of the cell cycle by

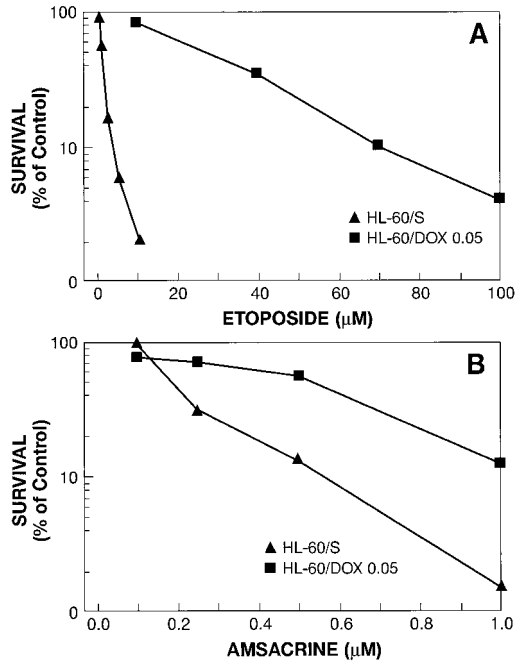

Fig. 1. Cytotoxic effects of VP-16 (A) and *m*-AMSA (B) in HL-60/S and HL-60/DOX0.05 cells. After treatment for 1 h, cytotoxicity was determined by a soft-agar colony assay (Ganapathi et al., 1996a). Data are mean values from at least three experiments. S.D. <15%. HL- 60/S and HL-60/DOX0.05 cells were plated at a density of 1.5×10^4 and 4×10^4 cells, respectively, per 35- × 10-mm Petri dish. Colony-forming efficiency of the HL-60/S and HL-60/DOX0.05 cells was 29 and 10%, respectively.

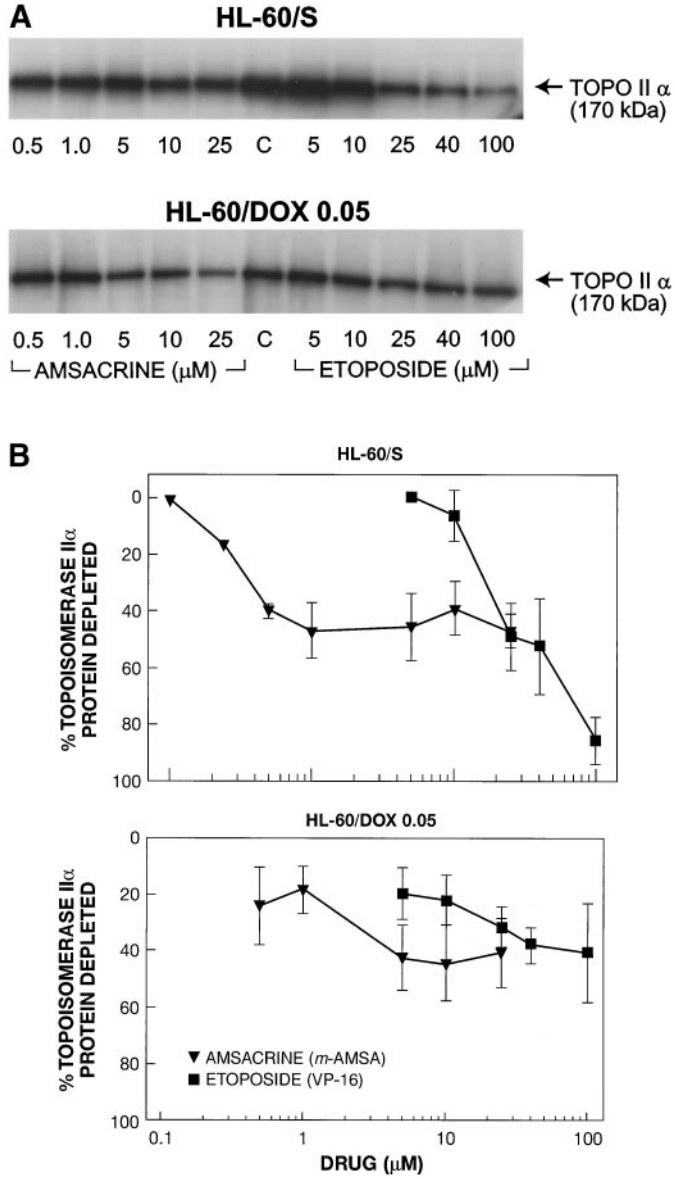

Fig. 2. Band depletion analysis of 170-kDa (α) topo II in HL-60/S and HL-60/DOX0.05 cells treated with *m*-AMSA or VP-16 for 1 h. A, samples of control and treated cells were processed for band depletion analysis as described in *Materials and Methods*. Data are from a representative gel. B, phosphorImager analysis of topo IIα depletion by *m*-AMSA or VP-16 in HL-60/S and HL-60/DOX0.05 cells. Data are mean values from at least three experiments.

centrifugal elutriation are shown in Fig. 6. In a typical experiment, the distribution of cells in the G_1, S, and G_2 + M phase was 43, 44, and 13%, respectively, in asynchronous exponential cultures of HL-60/S or HL-60/DOX0.05 cells. After elutriation the enrichment of cells in the G_1, S, and G_2 + M was 95, 82, and 68%, respectively. Because the topo II protein levels were the lowest in the G_1 phase, the increased expression in the S and G_2 + M phases were expressed relative to the G_1 phase. In both the HL-60/S and HL-60/DOX0.05 cells, the levels of topo IIα protein in the S and G_2 + M phases were 2- and 4-fold higher, respectively. However, a similar cell cycle phase-dependent increase in the level of topo IIβ protein was not apparent, and represented an ~2-fold increase (compared with G_1) in the S and G_2 + M phases.

The cell cycle phase-dependent phosphorylation of topo II isoforms in HL-60/S and HL-60/DOX0.05 cells is outlined in Fig. 7. In HL-60/S cells, an increase (2-fold) compared with the G_1 phase in the phosphorylation of topo IIα but not topo IIβ was observed in the S and G_2 + M phases of the cell cycle. In contrast, although an increase of a lower magnitude was observed with topo IIα in the S and G_2 + M phases, with the HL-60/DOX0.05 cells, the phosphorylation of topo IIβ in the G_1, S, and G_2 + M was similar or greater than that of topo IIα.

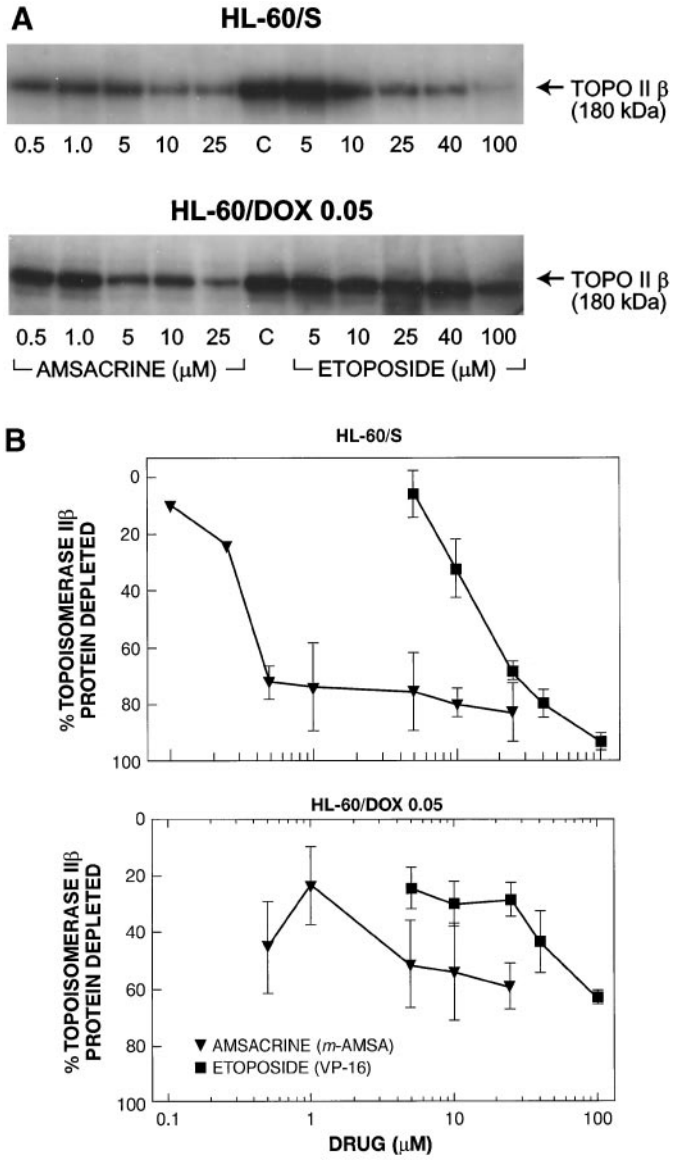

Fig. 3. Band depletion analysis of 180-kDa (β) topo II in HL-60/S and HL-60/DOX0.05 cells treated with *m*-AMSA or VP-16 for 1 h. A, samples of control and treated cells were processed for band depletion analysis as described in *Materials and Methods*. Data are from a representative gel. B, phosphorImager analysis of topo IIβ depletion by *m*-AMSA or VP-16 in HL-60/S and HL-60/DOX0.05 cells. Data are mean values from at least three experiments.

Discussion

Topo II in mammalian cells is present as the α- and β-isoforms (Woessner et al., 1990, 1991; Turley et al., 1997). The two isoforms perform similar catalytic functions related to unknotting and decatenation of DNA, and an essential role for the α-isoform in cell proliferation has been demonstrated

TABLE 1
VP-16 and *m*-AMSA-stabilized Topo II-DNA cleavable complex formation

Drug	*m*-AMSA[a]	V-16[a]
μM		
0.1[b]	5.66 ± 0.7[c]	n.d.[d]
0.25	8.19 ± 1.1	n.d.
0.5	11.62 ± 1.41	2.4 ± 0.2[a]
1.0	n.d.[c]	4.0 ± 0.3
2.5	n.d.	6.5 ± 0.6
5.0	n.d.	9.2 ± 0.5

[a] The precipitated [^{14}C]thymidine in the untreated control cells was 1168 ± 114 dpm and 645 ± 51 dpm for experiments with *m*-AMSA and VP-16, respectively. Values are means ± S.E. from at least three experiments.
[b] HL-60/S cells after labeling with [^{14}C]thymidine were treated with indicated concentrations of *m*-AMSA or VP-16 for 1 h. Drug-stabilized topoisomerase II-DNA complex formation was determined as outlined in *Materials and Methods*.
[c] Fold-increase in drug-stabilized topo II-DNA cleavable complex formation relative to the untreated control. Values are means ± S.E. from at least three experiments.
[d] n.d., not determined.

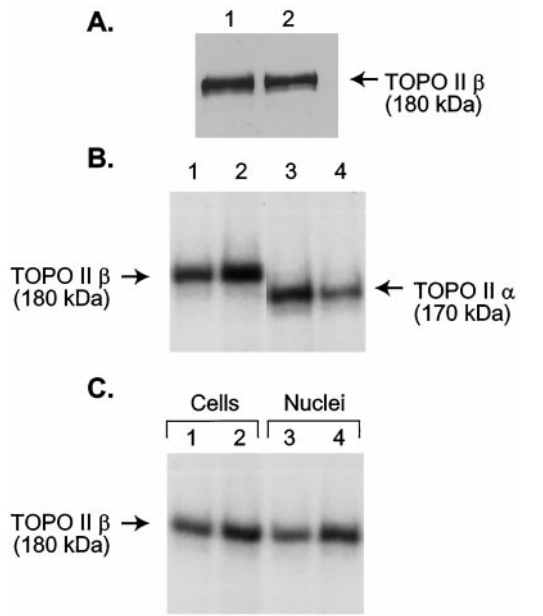

Fig. 4. Analysis of topo II levels by immunoblotting and immunoprecipitation in HL-60/S and HL-60/DOX0.05 cells. A, immunoblot analysis of topo IIβ in HL-60/S (lane 1) and HL-60/DOX0.05 (lane 2) cells; B, phosphorylated topo IIα and topo IIβ in HL-60/S (lanes 1 and 3) and HL-60/DOX0.05 (lanes 2 and 4); C, phosphorylated topo IIβ from HL-60/S (lanes 1 and 3) and HL-60/DOX0.05 (lanes 2 and 4) cells or nuclei.

(Watt and Hickson, 1994; Wang, 1996). Although a precise functional role for the topo IIβ isoform is yet to be demonstrated, its altered phosphorylation during mitosis has been demonstrated (Burden and Sullivan, 1994; Kimura et al., 1994). The evidence that proliferation is not compromised in cells without detectable levels of the β-isoform (Herzog et al., 1998) suggests that it is not essential for cell replication or chromosome segregation. Although the relative abundance of the topo II isoforms varies by cell type (Turley et al., 1997), the contribution of the β-isoform as a determinant of antitumor drug sensitivity is unclear. Based on quantification of protein levels of topo II isoforms in cells selected for resistance to drugs that intercalate with DNA, it appears that higher levels of resistance are correlative with alterations in topo IIβ (Harker et al., 1991; Herzog et al., 1998; Perrin et al., 1998). Although these studies have to a large extent been correlative, transfection studies (Dereuddre et al., 1997) have provided new evidence for a functional role. The data on the markedly higher depletion of topo IIβ by *m*-AMSA versus VP-16 also support the differences observed in levels of resistance to these drugs in the HL-60 cells deficient in the topo IIβ protein (Herzog et al., 1998). The depletion of topo IIβ versus topo IIα at cytotoxic concentrations of *m*-AMSA in the HL-60/S and HL-60/DOX0.05 cells suggests that this differential interaction with the topo II isoforms is responsible for the greater cytotoxic potency of *m*-AMSA in vitro. The data also demonstrate that steady-state levels of topo IIα or β protein are unaltered in the HL-60/DOX0.05 cells. However, unlike the α-isoform, the β-isoform is hyperphosphorylated in the HL-60/DOX0.05 versus HL-60/S cells. It is not clear at the present time whether this change in the phosphorylation

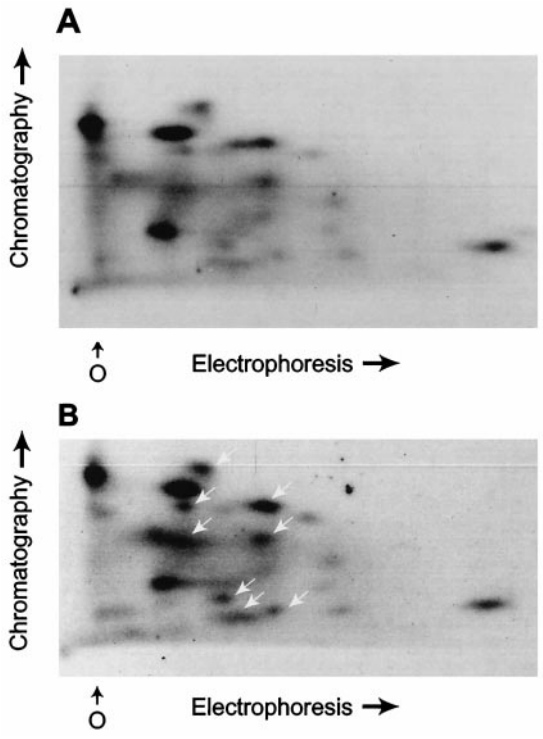

Fig. 5. Representative two-dimensional tryptic phosphopeptide maps of topo IIβ protein from HL-60/S (A) and HL-60/DOX0.05 (B) cells. The sites that are differentially phosphorylated between the HL-60/S and HL-60/DOX0.05 cells are identified by arrows.

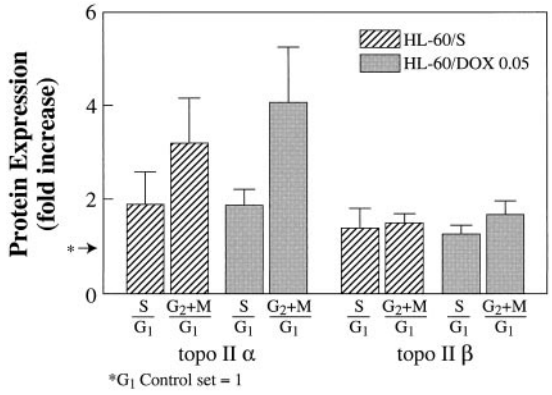

Fig. 6. Analysis of topo IIα and IIβ protein levels by immunoblotting in elutriated G_1, S, and G_2 + M populations in HL-60/S and HL-60/DOX0.05 cells. Data are mean values (±S.D.) from at least three experiments.

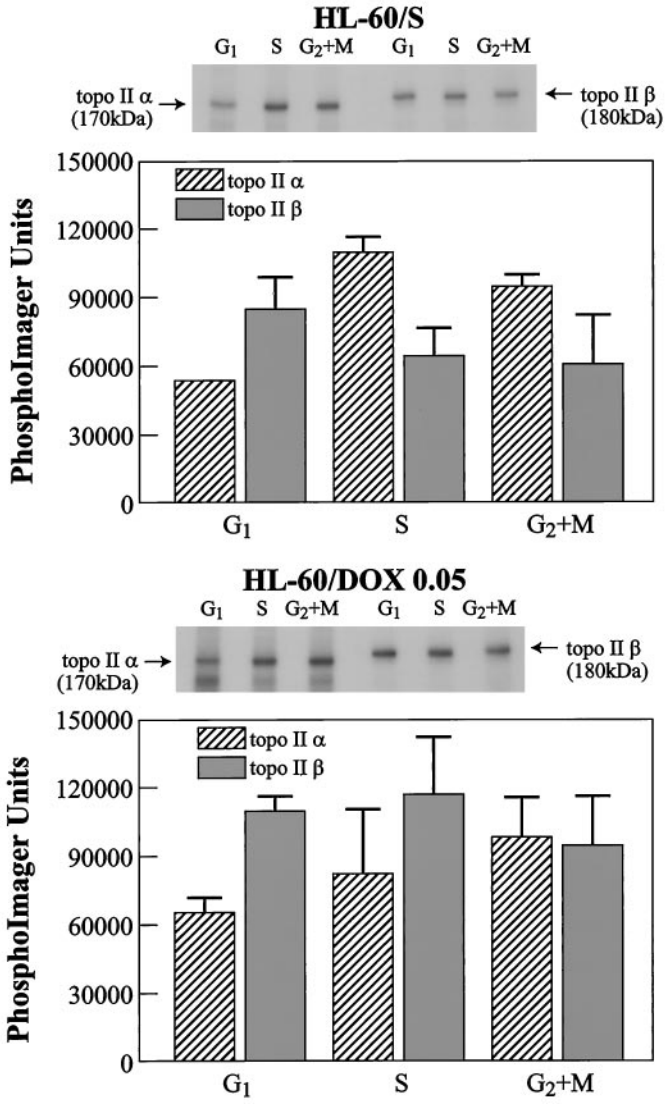

Fig. 7. Determination of phosphorylated topo IIα and IIβ levels by immunoprecipitation in elutriated G_1, S, and G_2 + M populations in HL-60/S and HL-60/DOX0.05 cells. Data are mean values (±S.D.) from at least two experiments. Based on statistical analysis of phosphorImager data, the following differences between the phosphorylation of topo IIα and topo IIβ were found to be significantly different. HL-60/DOX0.05 cells: G_1 phase, topo IIβ > topo IIα, $p = .017$ and HL-60/S cells: S phase, topo IIα > topo IIβ, $p = .045$.

state of the β-isoform is a compensatory response to changes in the α-isoform, but the absence of marked differences in the cell cycle kinetics of the HL-60/S and HL60/DOX0.05 cells supports such a role. Similar to the asynchronous exponential cultures, protein levels of the topo II isoforms in elutriated G_1, S, and G_2 + M populations also were similar between the HL-60/S and HL-60/DOX0.05 cells. It is well recognized that the phosphorylation of topo IIα in the S and G_2 + M phases are critical for cell replication (Watt and Hickson, 1994; Wang, 1996). Although, topo IIα is hypophosphorylated in the HL-60/DOX0.05 cells, no major differences in cell cycle kinetics compared with the HL-60/S cells are apparent. However, a compensatory role of topo IIβ is apparent in the similarities of cell cycle phase-dependent phosphorylation of topo IIβ in the HL-60/DOX0.05 cells and topo IIα in the HL-60/S. The site-specific hyperphosphorylation of topo IIβ in the HL-60/DOX0.05 cells is unique, given the site-specific hypophosphorylation of the α-isoform in these cells. It does not appear that the same site may be involved, due to the distinctly different phosphopeptide maps of the α- and β-isoforms in complete tryptic digests (Aoyama et al., 1998a,b). There also appears to be some consistency in the site-specific hyperphosphorylation of topo IIβ in the HL-60/DOX0.05 cells and in HL-60/S cells induced to differentiate with all *trans*-retinoic acid (Aoyama et al., 1998b).

In summary, the results from this study suggest that the differential sensitivity of HL-60 cells to *m*-AMSA and VP-16 is dependent on the differential interaction of these agents with the topo II isoforms. *m*-AMSA appears to be more effective in interacting with the β-isoform at concentrations, which are at least 10-fold lower than similar concentrations of VP-16 on a molar basis and relevant to induction of a cytotoxic response. The cell cycle phase-specific, as well as site-specific, hyperphosphorylation of topo IIβ in the HL-60/DOX0.05 cells may compensate for the hypophosphorylation of the α-isoform. Furthermore, the hyperphosphorylation of topo IIβ may be functionally involved in the enhanced interaction and reduced level of cross-resistance to *m*-AMSA. Studies are in progress to determine the functional role of site-specific phosphorylation of topo IIβ in drug-stabilized DNA-cleavable complex formation, and in the long term, an understanding of the functional role of the β-isoform could provide new information in exploiting its role as a therapeutic target.

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