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OVEREXPRESSION OF P-GLYCOPROTEIN AND ALTERATIONS IN TOPOISOMERASE II IN P388 MOUSE LEUKEMIA CELLS SELECTED *IN VIVO* FOR RESISTANCE TO MITOXANTRONE

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Abstract—The overexpression of P-glycoprotein (PGP) and alterations in DNA topoisomerase II (TOPO II) were evaluated in mouse leukemia P388 cells selected *in vivo* for mitoxantrone (MTT) resistance (P388/MTT) and compared to doxorubicin (DOX) resistant (P388/DOX) or vincristine (VCR) resistant (P388/VCR) models. Among a panel of TOPO II inhibitors which included etoposide (VP-16), DOX, MTT and 4′-[(9-acridinyl)-amino]methanesulfon-*m*-anisidide (m-AMSA), the relative resistance compared to parental sensitive P388/S cells was: P388/DOX > P388/MTT > P388/VCR. All the resistant sublines exhibited minimal cell kill (<20%) at vincristine concentrations > 100-fold the IC_{50} for P388/S cells. In a soft-agar colony-forming assay, the modulation of cytotoxicity in P388/MTT cells by the calmodulin inhibitor trifluoperazine following a 3-hr drug treatment demonstrated a marked potentiation in cell kill with MTT, VP-16, DOX and m-AMSA but not VCR. Immunoblotting data revealed that while PGP was not detectable in P388/S cells, the overexpression of PGP was apparent in P388/MTT cells and the relative expression between the resistant sublines was: P388/DOX > P388/MTT > P388/VCR. Although the amount and DNA cleavage activity of TOPO II in nuclear extracts from P388/VCR cells were comparable to those in P388/S cells, they were markedly lower in both P388/DOX and P388/MTT cells. However, decatenation activity of TOPO II in nuclear extracts was comparable between the sensitive (P388/S) and resistant sublines (P388/MTT, P388/DOX, and P388/VCR). Results from the present study demonstrated that P388 cells selected for resistance to mitoxantrone exhibit changes in TOPO II and overexpression of PGP similar to P388/DOX cells, while vincristine resistant cells only overexpress PGP. Since therapeutic strategies are primarily designed to interfere with PGP-mediated drug efflux, the choice of agents for modulating resistance in tumors which overexpress PGP versus tumors which overexpress PGP with altered TOPO II could be different.

Resistance to antitumour agents is a significant problem in the chemotherapeutic management of human neoplastic diseases. Tumor cells selected for resistance to a single agent can often express broad cross-resistance to other agents which are dissimilar in structure and/or mechanism of action [1]. This phenomenon of broad cross-resistance is termed multidrug resistance (MDR)§ and tumor cells demonstrating this phenotype often overexpress a M_r 150,000–180,000 dalton plasma membrane glycoprotein termed P-glycoprotein (PGP) [1]. The functional role of PGP in cells with the MDR phenotype is suggested to involve efflux of cellular drug based on homology of amino acid sequence of PGP with bacterial periplasmic proteins [1].

Although the role of drug efflux mediated by PGP is an attractive hypothesis, we have demonstrated that expression of resistance is correlative with alterations in topoisomerase II (TOPO II) function rather than drug accumulation in doxorubicin resistant L1210 cells with the MDR phenotype [2, 3]. Further, potent lipophilic anthracyclines, the accumulation of which is not compromised in MDR cells, also exhibit limited cytotoxic activity [4, 5]. To evaluate whether expression of resistance to mitoxantrone is related to alterations in TOPO II and/or PGP, in the present study we have used P388 mouse leukemia cells selected *in vivo* for resistance to mitoxantrone (MTT) and compared it to other multidrug resistance model systems selected for resistance to doxorubicin (DOX) or vincristine (VCR). Specifically, the sensitive and resistant sublines of P388 mouse leukemia were characterized for: (a) cross-resistance to TOPO II inhibitors [etoposide (VP-16), DOX, MTT, 4′-[(9-acridinyl)-amino]methanesulfon-*m*-anisidide (m-AMSA)] and VCR; (b) modulation of cytotoxicity in mitoxantrone resistant cells by the calmodulin inhibitor trifluoperazine (TFP); (c) PGP and TOPO II content in immunoblots; and (d) decatenation and DNA cleavage activity of TOPO II in nuclear extracts.

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§ Abbreviations: MDR, multidrug resistance; DOX, doxorubicin; VCR, vincristine; MTT, mitoxantrone; VP-16, etoposide; m-AMSA, 4′-[(9-acridinyl)-amino]-methanesulfon-*m*-anisidide; TFP, trifluoperazine; FBS, fetal bovine serum; PGP, P-glycoprotein; and TOPO II, topoisomerase II.

Results from this study demonstrated that altered TOPO II accompanied by overexpression of PGP is apparent in P388 cells selected for resistance to MTT or DOX, whereas P388 cells selected for resistance to VCR only exhibit overexpression of PGP.

MATERIALS AND METHODS

The parental sensitive (P388/S), doxorubicin resistant (P388/DOX), and mitoxantrone resistant (P388/MTT) P388 mouse leukemia cells [6, 7] were provided by Dr. Randall Johnson, SmithKline Beecham Pharmaceuticals, King of Prussia, PA. The vincristine resistant (P388/VCR) subline of P388 mouse leukemia [8] was provided by Dr. Lee Wilkoff, Southern Research Institute, Birmingham, AL. The drug resistant P388 mouse leukemia cells selected *in vivo* were maintained *in vitro* as suspension cultures. Briefly, ascites cells from DBA/2 mice implanted with sensitive or resistant sublines of P388 leukemia were cultured in RPMI 1640 medium (M.A. Bioproducts, Walkersville, MD), supplemented with 2 mM L-glutamine, 10% fetal bovine serum (FBS) (Sterile Systems Inc., Logan, UT) and 10 μM 2-mercaptoethanol. Doubling times *in vitro* of the sensitive and resistant sublines were 12–14 hr.

Amsacrine (m-AMSA) was provided by Dr. Ven Narayanan, Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. VP-16 was a gift from Bristol Laboratories, Wallingford, CT. Mitoxantrone was a gift from Dr. Frederick Durr, Lederle Laboratories, Pearl River, NY. Stock solutions of DOX, VCR and MTT were prepared in sterile glass distilled water, while m-AMSA and VP-16 were prepared in dimethyl sulfoxide (DMSO).

SV40 and λ Hind III DNAs, Ban I, Hpa II, and EcoR I restriction endonucleases, T4 polynucleotide kinase, and agarose were purchased from Bethesda Research Laboratories, Gaithersburg, MD. Calf alkaline phosphatase was purchased from New England Biolabs, Inc., Beverly, MA. [γ-^{32}P]ATP and [^{3}H]dT-kinetoplast DNA were purchased from New England Nuclear Research Products, Boston, MA, and Lofstrand Laboratory, Gaithersburg, MD, respectively. The XAR-5 film for autoradiography was from the Eastman Kodak Co., Rochester, NY.

Cytotoxicity in vitro. A soft-agar colony-forming assay [4] was used to determine the cytotoxic effects of DOX, MTT, m-AMSA, VP-16 and VCR in the P388/S, P388/DOX, P388/MTT and P388/VCR cells. Working dilutions of the various drugs were prepared in RPMI 1640 supplemented with 2 mM L-glutamine and 10% FBS. The final concentration of DMSO for VP-16 or m-AMSA treated cells was <1%. Log phase cultures of P388/S, P388/DOX, P388/MTT and P388/VCR cells in RPMI 1640 supplemented with 10% FBS were treated (2×10^6 cells total, at 1×10^6 cells/mL) with 0.001 to 100 μM VP-16, DOX, VCR, MTT, or m-AMSA for 3 hr at 37° in a humidified 5% CO_2 plus 95% air atmosphere. Modulation of cytotoxicity in P388/MTT cells was similarly determined by treating with VP-16, DOX, MTT, m-AMSA or VCR in the absence or presence of 5 μM TFP. Following treatment, cells were washed twice with drug-free RPMI 1640 plus 10% FBS and recovered by centrifugation (100 *g*). Control and treated cells were plated in triplicate at a density of 1×10^4 cells/35 × 10 mm Petri dish and incubated for 5–7 days at 37° in a humidified 5% CO_2 plus 95% air atmosphere; colony counts were determined as described previously [4]. The plating medium was RPMI 1640 supplemented with 2 mM L-glutamine, 20% FBS and 10 μM 2-mercaptoethanol. Under these conditions, the colony count in untreated controls of sensitive and drug resistant sublines was 2000–3500, corresponding to a colony-forming efficiency of 20–35%.

Immunochemical detection of P-glycoprotein. A plasma membrane enriched-microsomal fraction prepared from log-phase cultures of cells from the sensitive and resistant sublines was used to analyze PGP content [3]. Control cell lines for the detection of PGP included the colchicine resistant Chinese hamster ovary CH^{R}C5 and the parent sensitive counterpart AUXB1 [9]. Briefly sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) of plasma membrane fractions (50 μg protein/sample) was carried out as originally described by Laemmli [10] and modified by Greenberger *et al.* [11]. The gel was western blotted as described previously [3]. Monoclonal antibody C-219 [12] was used to probe the nitrocellulose for PGP followed by detection with ^{125}I-labeled-goat anti-mouse IgG as described earlier [3].

Immunochemical detection of topoisomerase II. Nuclear extracts from log-phase cultures of sensitive and resistant cells were prepared by a modification [3] of the procedure previously described by Pommier *et al.* [13]. The samples of nuclear extracts (50 μg protein) were solubilized in Laemmli sample buffer [10] by heating at 95° for 4 min and run on SDS–PAGE gels consisting of a 4% stacker and 5% resolving gel. The gel following equilibration for 30 min in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) was western blotted at 4° onto 0.45 μm nitrocellulose membrane for 1 hr at 60 V essentially as described by Towbin *et al.* [14]. The nitrocellulose membrane was then blocked overnight in 3% bovine serum albumin (BSA) in Tris-buffered saline [20 mM Tris, 400 mM NaCl, pH 7.5 (TBS)] at 37° followed by washes (2×) in TBS supplemented with 0.05% Tween 20, pH 7.5 (TTBS). The nitrocellulose was subsequently incubated for 2 hr at 37° with a 1:1000 dilution of antiserum from rabbits injected with gel purified human DNA topoisomerase II C-terminal 1/3 polypeptide [15]. Following washing in TTBS, the nitrocellulose membrane was incubated at room temperature for 2 hr using ^{125}I-goat anti-rabbit IgG (ICN Radiochemicals Inc., Irvine, CA). Autoradiograms were developed at −70° using Kodak XAR-5 film and Lanex screen.

VP-16 induced topoisomerase II mediated DNA cleavage. SV40 DNA was linearized with Ban I restriction enzyme and end-labeled at the 5′ termini with [γ-^{32}P]ATP in the presence of polynucleotide kinase [3, 16]. A second cut was introduced with Hpa II approximately 50 base pairs from the labeling site so that topoisomerase II mediated cleavage sites could be localized. The ^{32}P-end-labeled DNA was

incubated with nuclear extracts in the absence or presence of increasing concentrations of VP-16 for 30 min at 37° [3]. Samples were subjected to agarose gel electrophoresis and autoradiography carried out with XAR-5 film [3].

Decatenation activity of topoisomerase II in nuclear extracts. The catalytic activity of topoisomerase II in nuclear extracts from sensitve and resistant sublines was determined using the ATP-dependent decatenation of kDNA [16, 17]. Serial dilutions of nuclear extracts were incubated with 0.2 μg of [^{3}H]-dT-labeled kDNA for 30 min at 37° [3]. Reaction products were subject to electrophoresis and gels were stained with ethidium bromide. The kDNA and minicircle bands were visualized by UV illumination, cut, and counted by liquid scintillation spectrometry [3].

RESULTS

Survival data based on colony formation in the soft-agar assay following treatment with VP-16, DOX, VCR, MTT and m-AMSA in P388/S, P388/DOX, P388/MTT and P388/VCR cells are presented in Fig. 1. Among the inhibitors of topoisomerase II (VP-16, DOX, MTT and m-AMSA), resistance to these agents was as follows: P388/VCR < P388/MTT < P388/DOX. However, the P388/MTT like the P388/DOX and P388/VCR cells exhibited near complete resistance to VCR, at concentrations >100-fold, the IC_{50} for the parental sensitive P388/S cells.

The effect of TFP on the modulation of DOX, MTT, m-AMSA, VP-16 and VCR cytotoxicity in P388/MTT cells is shown in Fig. 2. While TFP was markedly effective in potentiating cell kill in P388/MTT cells treated with the TOPO II inhibitors DOX, VP-16, MTT or m-AMSA, cytotoxicity with VCR was comparable in the absence or presence of 5 μM TFP.

Since overexpression of PGP is frequently associated with the multidrug resistant phenotype [1], plasma membrane enriched microsomal fractions from the sensitive and resistant sublines were analyzed for the presence of PGP. As shown in Fig. 3, PGP was detectable in the resistant CH^RC5 but not the sensitive counterpart AUXB1. While PGP was not detectable in the parental sensitive P388, a novel finding was the detection of PGP in P388/MTT cells. The amount of PGP expression was different among the resistant variants, with maximal amounts of PGP in the P388/DOX cells, while P388/MTT cells had an intermediate level and P388/VCR the lowest. Overall, the increased expression of PGP in the resistant variants was in agreement with the broad cross-resistance observed in the cytotoxicity studies (Fig. 1).

Our earlier studies had identified the importance of changes in DNA cleavage activity of TOPO II in cells with the MDR phenotype [3, 18]. To characterize the possible alterations in TOPO II, the amount and activity (catalytic and DNA cleavage) of TOPO II were determined in nuclear extracts from P388/S, P388/DOX, P388/MTT and P388/VCR cells. The immunoblotting data in Fig. 4 demonstrate that the 170 kDa and 180 kDa isoforms of TOPO II were detectable in the sensitive and resistant cells. The recognition of the 170 kDa and 180 kDa isoforms of TOPO II by the antiserum for TOPO II was independently confirmed using antisera specific for the individual isoforms [19]. Antisera raised against column purified calf thymus TOPO II has also been demonstrated to simultaneously recognize the 170 kDa and 180 kDa isoforms of TOPO II [20]. Levels of TOPO II in the P388/MTT cells were lower than those in P388/S cells. Among the other resistant sublines, TOPO II levels in P388/VCR cells were comparable to levels in P388/S, and the P388/DOX cells had levels lower than those in P388/S or P388/VCR but similar to those in P388/MTT cells.

The differences in the amount of TOPO II in nuclear extracts from the sensitive and resistant sublines was also reflected in the VP-16 stimulated cleavage of ^{32}P-end-labeled SV40 DNA (Fig. 5). The VP-16 concentration-dependent DNA cleavage pattern in nuclear extracts from P388/MTT cells was markedly lower compared to that in P388/S cells. Nuclear extracts from P388/VCR cells exhibited DNA cleavage activity comparable to that of P388/S cells while with P388/DOX cells the DNA cleavage patterns were similar to those of P388/MTT cells. Reduced drug stimulated DNA cleavage by nuclear extracts from P388/DOX or P388/MTT cells versus P388/S and P388/VCR cells was also observed in preliminary studies (data not shown) when assayed using a fixed concentration of m-AMSA in the presence of increasing concentrations of nuclear extract suggesting that observed changes in DNA cleavage were not due exclusively to the reduced levels of TOPO II.

Since the ATP-dependent decatenation of kDNA is specific to TOPO II [17], this assay was also utilized to measure differences in TOPO II activity in nuclear extracts from the sensitive and resistant variants. Results for decatenation activity of nuclear extracts from the sensitive and resistant sublines are outlined in Fig. 6 and Table 1. In general, decatenation activity in the P388/MTT and other resistant (P388/DOX and P388/VCR) sublines was comparable to that in P388/S cells.

DISCUSSION

The expression of resistance to antitumor drugs of diverse structure and/or mechanism of action is frequently referred to as the multidrug resistant (MDR) phenotype [1]. This phenomenon of broad cross-resistance is suggested to be due primarily to reduced cellular drug accumulation mediated by overexpression of PGP [1]. While the role of reduced drug accumulation may offer a straightforward explanation for the resistance to mechanistically different drugs, in a variety of MDR model systems, it has been difficult to correlate the level of resistance with a decrease in drug levels [2, 4, 5]. The lack of correlation between cellular drug levels and resistance is particularly apparent with the anthracycline group of agents [2, 4, 5].

In this study, we have attempted to characterize the role of changes in PGP and/or TOPO II in P388 tumor cells selected for resistance to MTT compared to P388 cells selected for resistance to the classical

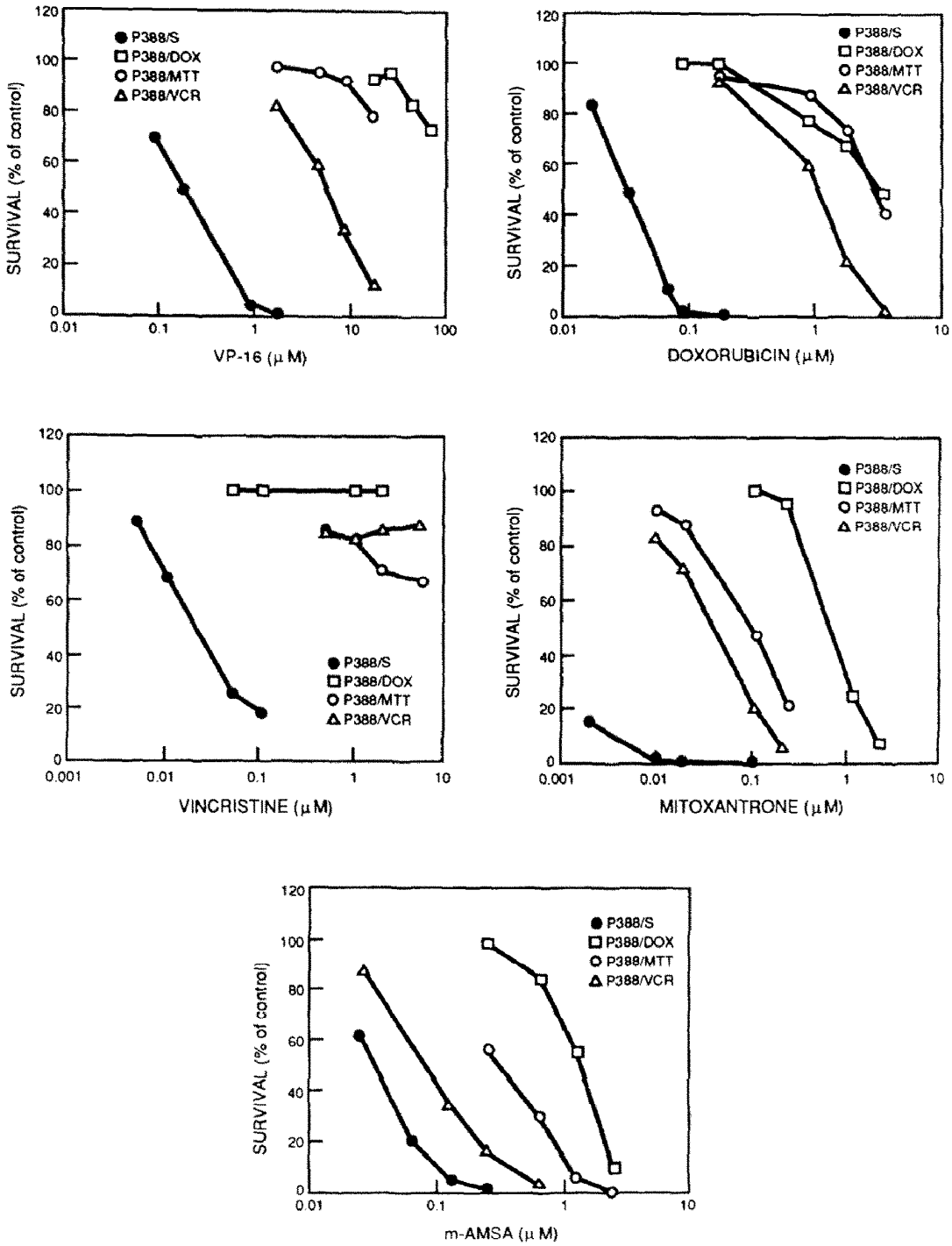

Fig. 1. Cytotoxic effects of VP-16, doxorubicin, vincristine, mitoxantrone and m-AMSA in P388/S, P388/DOX, P388/MTT and P388/VCR cells treated for 3 hr. A total of 2×10^6 cells (1×10^6 cells/mL) was used for control and treated samples. Cells (control and treated) were subsequently plated at a density of 1×10^4 cells per 35×10 mm Petri dish, and the colony count in the untreated control for sensitive or resistant sublines was 2000–3500, corresponding to a colony-forming efficiency of 20–35%. Each point is the mean value from at least triplicate experiments; standard deviation $< 15\%$.

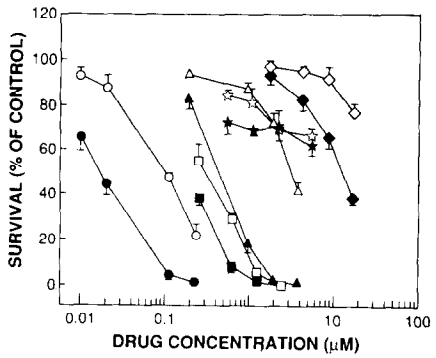

Fig. 2. Effect of TFP on the modulation of DOX, MTT, m-AMSA, VP-16 and VCR cytotoxicity in P388/MTT cells. The cytotoxic effects of MTT (○, ●), m-AMSA (□, ■), VP-16 (◇, ◆) VCR (☆, ★) and DOX (△, ▲) in the absence (open symbols) or presence (closed symbols) of 5 μM trifluoperazine (TFP) were measured in P388/MTT cells treated for 3 hr. A total of 2×10^6 cells (1×10^6 cells/mL) was used for control and treated samples. Cells (control and treated) were subsequently plated at a density of 1×10^4 cells/35 × 10 mm Petri dish, and the colony count in the untreated control for sensitive or resistant sublines was 2000–3500, corresponding to a colony-forming efficiency of 20–35%. Each value is the mean ± SEM of triplicate experiments.

MDR drugs DOX and VCR. A remarkable finding was the overexpression of PGP in P388 cells selected *in vivo* for resistance to MTT, in addition to the VCR, or DOX resistant cells. While these results are not surprising for DOX or VCR resistant cells, they are different from those obtained in a human colon carcinoma and the HL-60 cell line selected for resistance to MTT which do not overexpress PGP [21, 22]. Other human tumors selected for resistance to DOX have also been reported to lack PGP overexpression [1, 23–25]. The overexpression of PGP is consistent with existing data demonstrating reduced drug accumulation [26] in the P388/DOX cells, and the patterns of cross-resistance among the resistant sublines suggest a possible relationship to the amount of PGP. A notable finding is the near complete resistance to VCR in the resistant sublines. Based on our recent studies demonstrating a correlative relationship between reduced drug retention mediated by the overexpression of PGP and VCR versus DOX resistance [27], the results from this study with P388/MTT cells further suggest that VCR resistance is related to the presence of PGP. Since VCR is cytotoxic to a sensitive population following short-term exposure for 1–3 hr, and its maximal antitumor activity is cell cycle phase specific, the role of reduced drug retention mediating resistance is also supported by studies demonstrating that VCR cytotoxicity can be enhanced by increasing the length of exposure in resistant sublines which

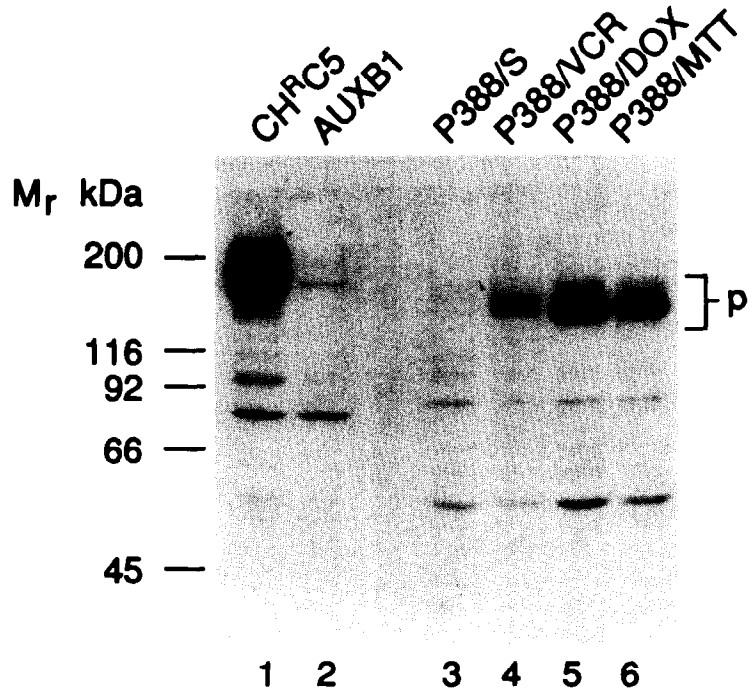

Fig. 3. Analysis of P-glycoprotein(p) levels in sensitive and multidrug resistant P388 mouse leukemia cells. Lane 1, 2, 3, 4, 5 and 6 are colchicine resistant Chinese hamster CH^RC5, sensitive Chinese hamster AUXB1, P388/S, P388/VCR, P388/DOX and P388/MTT cells, respectively. Immunoblotting analysis was carried out with a plasma membrane enriched microsomal fraction (50 μg) which was resolved in SDS–PAGE, transferred to nitrocellulose, and probed with C219 monoclonal antibody [12].

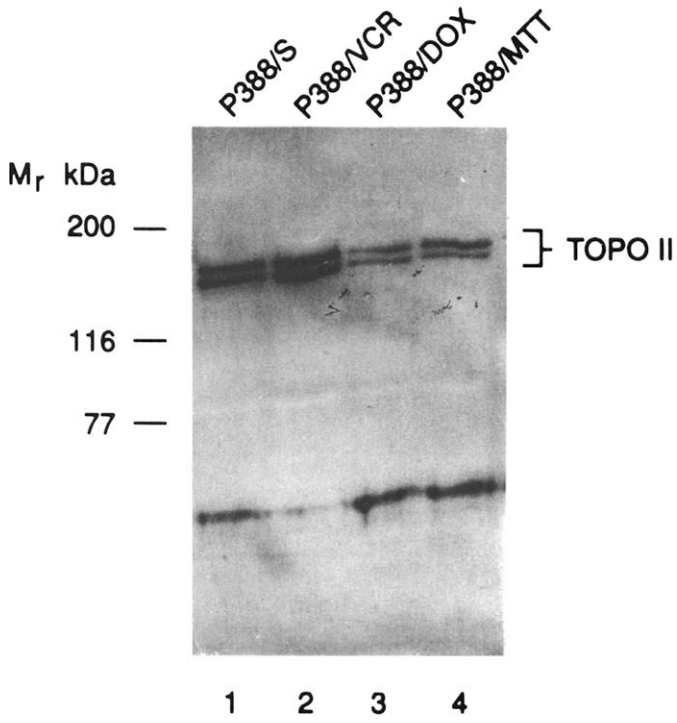

Fig. 4. Analysis of topoisomerase II (TOPO II) levels in sensitive and multidrug resistant P388 mouse leukemia cells. Lane 1, 2, 3 and 4 are P388/S, P388/VCR, P388/DOX and P388/MTT cells, respectively. Immunoblot analysis was carried out with nuclear extracts (50 μg) which were resolved by SDS–PAGE, transferred to nitrocellulose, and probed with antiserum for topoisomerase II.

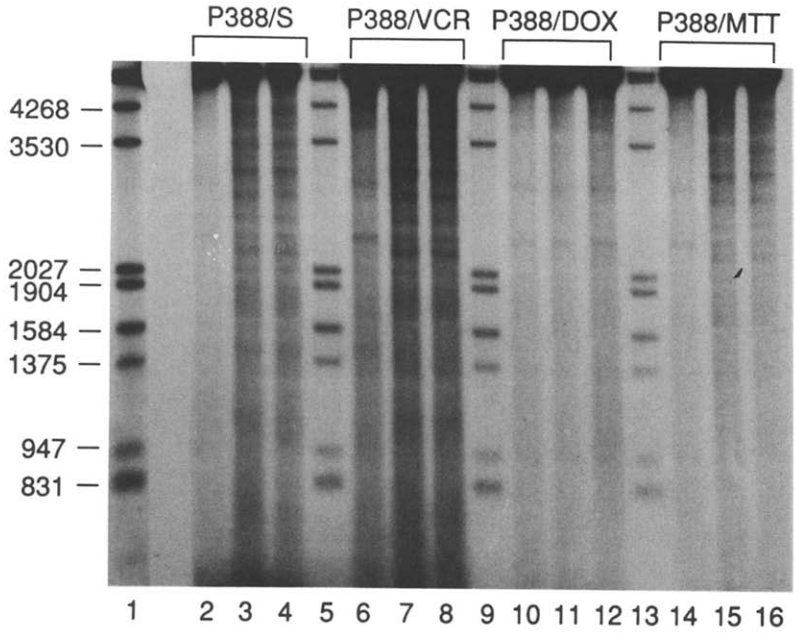

Fig. 5. VP-16 induced cleavage of 5′ ^{32}P-end-labeled SV40 DNA by 1 μg of nuclear extract from P388/S, P388/VCR, P388/DOX and P388/MTT cells. Lanes 1, 5, 9 and 13, λ Hind III EcoR I markers, and numbers on the left represents migration position of markers and size in base pairs. Lanes 2, 6, 10 and 14: no drug; lanes 3, 7, 11 and 15: 1 μM VP-16; and lanes 4, 8, 12 and 16: 10 μM VP-16.

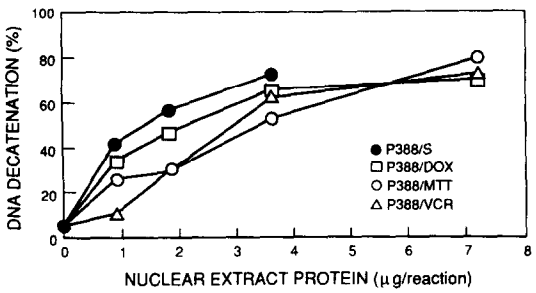

Fig. 6. Topoisomerase II mediated decatenation of kinetoplast (k) DNA by nuclear extracts from P388/S P388/DOX, P388/MTT and P388/VCR cells.

overexpress PGP [8, 26, 27]. Thus, while TFP or other modulating agents can enhance VCR accumulation during short-term exposure (1–3 hr), subsequent efflux mediated by PGP may result in non-cytotoxic cellular drug levels at a sensitive phase of the cell cycle. Overall, overexpression of PGP which is in general a characteristic of VCR or DOX resistant cells was also observed in mitoxantrone resistant P388 cells. Trifluoperazine (TFP), an inhibitor of calmodulin, is markedly effective in potentiating the cytotoxicity of a variety of agents involved in the MDR phenotype [26]. Based on the overexpression of PGP and alterations in TOPO II in the P388/MTT cells, TFP was effective in potentiating the cytotoxicity of TOPO II inhibitors and this is consistent with our observations in other MDR cells with altered DNA cleavage activity of TOPO II [2, 3, 26]. However, since reversal of resistance was not achieved with TFP, it suggests that mechanisms of resistance in P388/MTT cells involve more than overexpression of PGP.

Alterations in TOPO II levels and/or function have in general been ascribed to cells without the MDR phenotype [28, 29]. While this appears to be generally true for etoposide (VP-16) or m-AMSA resistant cells, tumor cells selected for resistance to DOX or 9-hydroxyellipticine exhibit an alteration in TOPO II and overexpression of PGP [3, 18, 30]. In DOX resistant cell lines with the MDR phenotype, resistance to anthracyclines and other TOPO II inhibitors is correlative with changes in TOPO II activity rather than reduced drug accumulation mediated by PGP [2, 3, 18]. The results from the present study demonstrate that alterations in TOPO II in cells with the MDR phenotype are a characteristic following selection for resistance to inhibitors of TOPO II such as MTT or DOX but not VCR. The TOPO II alterations are manifested in the P388/MTT or P388/DOX cells as decreases in the amount and DNA cleavage activity of the enzyme. The reduced levels of TOPO II in a DOX resistant population of P388 cells have been reported previously [31]. Between the DOX- and MTT-resistant sublines, the greater resistance to TOPO II inhibitors in the P388/DOX cells could be due to differences in the amount of PGP overexpression. Overall, cells selected for resistance to the TOPO II inhibitor MTT can exhibit both altered TOPO II and overexpression of PGP.

In summary, as outlined in Table 1, results from the present study demonstrate that P388 cells exhibit: (1) overexpression of PGP accompanied by changes in TOPO II levels and/or activity following selection *in vivo* for MTT or DOX resistance; and (2) overexpression of PGP without concomitant changes in TOPO II levels or activity following selection *in vivo* for VCR resistance. Since current therapeutic strategies are primarily designed to interfere with PGP mediated drug efflux, the choice of agents for modulating resistance [32, 33] in tumors which overexpress PGP versus tumors which overexpress PGP with altered TOPO II activity could be different.

Acknowledgements—The authors gratefully acknowledge Dr. Randall Johnson, SmithKline Beecham Pharmaceuticals, King of Prussia, PA for providing the parental sensitive, doxorubicin resistant, and mitoxantrone resistant P388 mouse leukemia cells; Dr. Lee Wilkoff, Southern Research Institute, Birmingham, AL for providing the vincristine resistant P388 mouse leukemia cells; Dr. Leroy

Table 1. Characteristics of sensitive and multidrug-resistant P388 mouse leukemia cells

Cell line	IC_{50}* (μM)					Levels of PGP†	TOPO II		
	DOX	MTT	VP-16	m-AMSA	VCR		Levels‡	DECAT§	DSB‖
P388/S	0.034	0.002	0.17	0.025	0.022	−¶	+++¶	1.5	+++¶
P388/MTT	3.45	0.11	17	0.25	5.42	++	++	3.5	++
P388/DOX	3.45	1.15	68	1.27	>5.42 (>90% survival)	++++	+	2.1	+
P388/VCR	0.86	0.02	4.25	0.13	>5.42	+	++++	3.0	+++

* Concentration required for 50% reduction of colonies in soft-agar following 3 hr of treatment.

† P-glycoprotein (PGP) levels were determined by immunoblotting using C219 monoclonal antibody [12].

‡ Topoisomerase II (TOPO II) levels were determined by immunoblotting using IID3 rabbit antiserum to DNA topoisomerase II.

§ Decatenation (DECAT) activity of TOPO II in nuclear extracts was determined using the kinetoplast DNA decatenation assay. Numbers indicate μg nuclear extract for 50% decatenation based on data in Fig. 5.

‖ Double-strand breaks (DSB) induced by nuclear extracts in the presence of VP-16 using ^{32}P-end-labeled SV40 DNA.

¶ Relative levels or activity: −, negative; +, low; ++, medium; +++, high; ++++, very high.

Liu, Johns Hopkins University, Baltimore, MD, for the gift of the antiserum for topoisomerase II; Ms. Ann Levert, Frances Adjei and Carol Dornon for excellent secretarial assistance; and Jim Reed and Jackie Molly of the Art-Medical Illustrations and Photography Department for skilful preparation of the artwork. This work was supported by PHS Grant RO1CA35531 awarded by the National Cancer Institute, Department of Health and Human Services.

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