Biochemical Pharmacology, Vol. 52, pp. 1569–1576, 1996.

ISSN 0006-2952/96/$15.00 + 0.00
PII S0006-2952(96)00561-8

Increased Efflux of Vincristine, but not of Daunorubicin, Associated with the Murine Multidrug Resistance Protein (MRP)

Christopher A. Slapak, Robin L. Martell,†‡ Masanori Terashima* and Stuart B. Levy‡§*

‡Center for Adaptation Genetics and Drug Resistance and the Departments of Molecular Biology and Microbiology and of Medicine, Tufts University School of Medicine and the New England Medical Center, Boston, MA 02111; and the *Department of Medicine, Division of Cancer Pharmacology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA 02115, U.S.A.

ABSTRACT. The multidrug resistance protein (MRP) is a membrane protein that mediates altered transport of cytotoxic drugs. Although MRP overexpression has been described in doxorubicin-selected human tumor cell lines, the murine PC-V10 and PC-V40 cell lines are members of the only reported series of vincristine-selected cell lines that overexpress *mrp*. Western blotting, using an antiserum developed against human MRP, demonstrated high-level expression of murine MRP primarily in the plasma membranes in each of the vincristine-selected cell lines. Only PC-V160, selected for high level resistance, demonstrated concomitant overexpression of the P-glycoprotein. As compared with parental cells, each of the drug-selected cell lines demonstrated an energy-dependent, decreased net accumulation of vincristine without any changes in the initial rates of vincristine influx. However, there was an enhanced rate of vincristine loss, 2.3-fold from the PC-V40 cell line and 3.9-fold from the PC-V160 cell line. Selective plasma membrane permeabilization with digitonin equalized vincristine accumulation among the parental, the PC-V40, and the PC-V160 cell lines. No intracellular pH differences were detected among the cell lines. Despite high-level MRP expression, daunorubicin accumulation and the rate of daunorubicin loss in the PC-V40 cells were the same as that observed in parental PC4 cells. Fluorescence microscopy demonstrated no difference in the pattern of subcellular daunorubicin accumulation between parental and PC-V40 cells. These studies demonstrate that murine MRP, overexpressed and found predominantly in the plasma membrane of vincristine-selected PC-V40 cells, is associated with an energy-dependent increased efflux of vincristine, but not with efflux or altered distribution of daunorubicin. BIOCHEM PHARMACOL 52;10:1569–1576, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. MRP; vincristine resistance; drug efflux

Acquired tumor insensitivity to chemotherapeutic agents is a major obstacle to achieving improved outcomes in clinical oncology. The occurrence of simultaneous resistance to more than one class of chemotherapeutic agents, known as multidrug resistance, further limits their usefulness. The best characterized form of multidrug resistance is that linked to the P-glycoprotein: a multifunctional transport protein whose expression results in simultaneous resistance to anthracyclines, *Vinca* alkaloids, epipodophyllotoxins, and taxanes [1, 2]. Expression of the more recently identified MRP[‖] also results in the acquisition of multidrug resistance, presumably through changes in drug transport [3–5]. That drug-selected cell lines may express simultaneously both P-glycoprotein and MRP is an important consideration in designing clinical trials aimed at reversing multidrug resistance [6–8].

We have reported previously a series of MEL cells selected for progressive vincristine resistance [7]. Cells selected for low or intermediate-level vincristine resistance, as represented by the PC-V10 and PC-V40 cell lines, overexpressed transcripts of the murine homolog of *mrp* in the absence of murine *mdr1* or *mdr3* expression. Only with selection for high-level vincristine resistance was a cell line observed (PC-V160) that expressed murine *mdr3* associated with continued high-level expression of *mrp*. We now report the characterization of the murine MRP in the vincristine selected drug-resistant cell lines. Associated with overexpression of murine MRP were enhanced rates of vincristine efflux with no measurable change in rates of vincristine influx. In contrast to human MRP, expression of murine MRP in these cell lines was not associated with anthracycline efflux or subcellular redistribution.

† The first two authors contributed equally to this manuscript.

§ Corresponding author: Dr. Stuart B. Levy, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111. Tel. (617) 636-6764; FAX (617) 636-0458.

‖ *Abbreviations:* MRP, multidrug resistance protein; *mdr*, murine multidrug resistance (P-glycoprotein) gene; MEL, murine erythroleukemia; Dg/azide, 2-deoxyglucose/sodium azide; DNP, 2,4-dinitrophenol; and BCECF-AM, 2′,7′-bis-[2-carboxyethyl]-5-[and-6-]carboxyfluorescein, acetoxymethyl ester.

Received 1 March 1996; accepted 7 June 1996.

MATERIALS AND METHODS

Cell Lines and Culture

Vincristine-selected sublines were derived by selecting the parental PC4 cell line in final vincristine concentrations of 10, 40, and 160 ng/mL [7]. The fold-resistance to vincristine (vcr) and doxorubicin (dox) was measured previously as follows: PC-V10: 11-fold (vcr), 1.1-fold (dox); PC-V40: 42-fold (vcr), 6.0-fold (dox); and PC-V160: 215-fold (vcr), 31-fold (dox) [7]. The cell lines were grown for 1 month in the absence of vincristine prior to further studies.

Vincristine Accumulation and Uptake Studies

Cells (1×10^6/mL) were incubated in PBS-glucose (1.0 g/L) at 37° with 25 nM [^{3}H]vincristine (4.3 to 8.6 Ci/mmol; Amersham, Arlington Heights, IL) for 60 min. In some experiments, cells were incubated in PBS in the presence of Dg (50 mM)/azide (15 mM), DNP (200 μM), or monensin (5 μg/mL) for 15 min prior to the addition of vincristine. To terminate drug accumulation, duplicate 200-μL aliquots were removed and centrifuged immediately through silicone oil (density 1.040 to 1.045; William F. Nye Co., New Bedford, MA) as described [9, 10].

For uptake studies, cells were washed and resuspended in a final [^{3}H]vincristine concentration of 25 nM. At 0, 1.5, 3.0, 5.5, 10, and 60 min after resuspension, cell-associated vincristine was assayed as above. Data are expressed as the percentage of cell-associated radioactivity measured in parental cells after 60 min of drug accumulation.

Effect of Digitonin on Vincristine Accumulation

Cells (2×10^6/mL) were incubated in 25 nM [^{3}H]vincristine in PBS at 37° for 60 min. Digitonin (Sigma, St. Louis, MO) was added to a final concentration of 20 μM 5 min prior to the end of the [^{3}H]vincristine incubation.

Vincristine Efflux Studies

Cells (2×10^6/mL) were incubated in medium at 37° with [^{3}H]vincristine. To obtain equimolar intracellular drug levels, [^{3}H]vincristine was added to PC4-WT cells to a final concentration of 25 nM, PC-V40 cells to 50 nM, and PC-V160 cells to 100 nM. After 60 min, cells were washed and resuspended to 1×10^6 cells/mL at 37°. At indicated intervals, triplicate samples were centrifuged through silicone oil, and cell-associated radioactivity was determined. The rate coefficients, k (min^{-1}), for vincristine efflux (0–3 min) were obtained from the negative value of the slope from a linear regression analysis of a semilogarithmic plot of the fraction of vincristine remaining in the cells versus time.

Daunorubicin Accumulation and Efflux Studies

Net anthracycline accumulation was assayed by flow cytometry after a 60-min incubation in daunorubicin (Wyeth-Ayerst Laboratories, Philadelphia, PA) at 0.2 μM as previously described [11]. Additional accumulation studies were performed after 60, 90, 120, 150, and 180 min of exposure to 0.1 μM [^{3}H]daunorubicin (Dupont–New England Nuclear, Boston, MA; 0.9 Ci/mmol). Accumulation was terminated by sedimenting through silicone oil. For efflux studies, cells were exposed to 0.1 μM [^{3}H]daunorubicin for 60 min before resuspending in drug-free medium at $t = 0$ as described above. Efflux was terminated, and cell-associated radioactivity was determined by pelleting cells through silicone oil.

Fluorescence Photomicrographs

Cells, incubated in 500 ng/mL of daunorubicin for 60 min, were washed and kept on ice until examination by fluorescence microscopy. Photographs were taken through an Olympus BH-2 fluorescence microscope (Olympus Optical Co., Tokyo, Japan).

Measurement of Intracellular pH

Intracellular pH was determined using BCECF-AM (Molecular Probes, Eugene, OR). PC4-WT, PC-V40, and PC-V160 cells were exposed to growth medium (control) or the following conditions (75 min each): (1) PBS, (2) Dg/azide, and (3) DNP. Cells were resuspended in accumulation buffer (50 mM KPO_4, 125 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, pH 7.4) with 3 μM BCECF-AM for 30 min. The intracellular pH was determined from the values of the emission ratios obtained at 505 nm/439 nm after excitation at 439 nm using a Perkin–Elmer LS-F fluorescence spectrophotometer. A calibration curve was derived by adjusting extracellular pH after equalizing intracellular and extracellular pH through the addition of 5 μM nigericin in K^+-enriched medium.

Plasma Membrane Isolation and Western Blot Analysis

Membrane enriched proteins were prepared using sucrose gradient fractionation, and enrichment was determined as described [12]. Protein was quantified [13], and 10 μg/lane was resolved by SDS–PAGE in a 6.0% polyacrylamide gel [14]. The protein was electroblotted overnight at 4° to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH). After blocking, the blots were incubated either with antiserum MRP-6KQ diluted 1:500 (provided by M. Center, Kansas State University, Manhattan, KS) or with monoclonal antibody C219 (Signet Laboratories, Dedham, MA) at 1 μg/mL. They were then treated with an appropriate peroxidase-conjugated second step (Amersham) before being developed with an Enhanced Chemiluminescence kit (Amersham). Films were scanned with an LKB Ultrascan XL laser densitometer (LKB Instruments Inc., Gaithersburg, MD).

Statistical Analysis

Standard error bars are presented for values on the accumulation, influx, and efflux graphs. Student's paired t-test

was used to compare differences after 60 min of vincristine accumulation or after 3 min of vincristine efflux. Statistics were calculated with software programs 1-2-3 (Lotus Development Corp., Cambridge, MA) or StatView (Brainpower Inc., Calabasas, CA).

RESULTS

Analysis of Vincristine Accumulation

[^{3}H]Vincristine accumulation was assayed after a 60-min drug exposure. An approximately 35% decreased vincristine accumulation was noted in PC-V10 cells compared with the parental cell line (Fig. 1A). Progressively less vincristine accumulation was noted in the PC-V40 and PC-V160 cell lines.

Analysis of Daunorubicin Accumulation

Anthracycline accumulation, assayed by flow cytometry using daunorubicin, was found to be the same in parental, PC-V10, and PC-V40 cells (Fig. 1B). Only PC-V160 cells had markedly decreased daunorubicin accumulation (Fig. 1B). Similar findings were noted after a 60-min accumulation of [^{3}H]daunorubicin: PC-V40 cells accumulated 113 ± 7% and PC-V160 cells accumulated 53 ± 13% of the amount of daunorubicin as parental cells. Increasing daunorubicin exposure times to 3 hr (with intermediate sampling at 90, 120, and 150 min) produced no effect on the daunorubicin accumulation profile in the PC-V40 cells, as compared with the parental cell line, at each time point (data not shown).

Effect of Inhibitors on Vincristine Accumulation

To determine whether vincristine transport was associated with an energy-dependent process, the cell lines were pretreated with Dg/azide or DNP prior to vincristine accumulation. All the cell lines demonstrated a marked increase in vincristine accumulation in response to Dg/azide or DNP pretreatment (Fig. 2). Monensin, an ionophore known to disrupt vesicular proton gradients, but not to uncouple oxidative phosphorylation [15], caused little if any change in the level of vincristine accumulation in PC-V40 cells; modestly increased vincristine accumulation in PC-V160 cells; and modestly decreased accumulation in parental cells (Fig. 2).

Effect of Digitonin Permeabilization

To demonstrate that the observed vincristine accumulation differences were not due to differences in intracellular drug binding, cells were selectively permeabilized with the detergent digitonin, which has been shown to selectively permeabilize the plasma membrane [16, 17]. After digitonin treatment, vincristine accumulation (mean ± SEM) was approximately equal in PC-V40 cells (100 ± 2%) and PC-

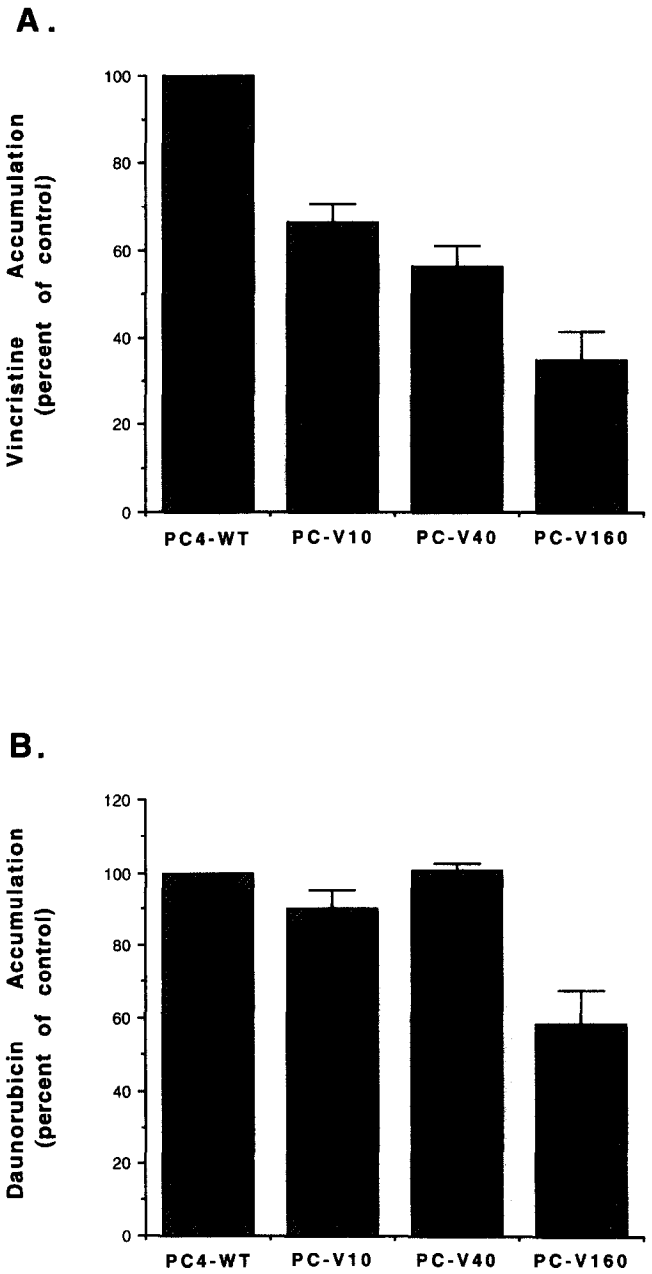

FIG. 1. Accumulation of vincristine and daunorubicin in the parental and drug-resistant cell lines. In part A, cell-associated radioactivity after a 60-min exposure to [^{3}H]vincristine was determined for each cell line and is reported as a percentage of accumulation observed in parental cells (PC4-WT) (1150 ± 110 cpm, mean ± SEM). The net vincristine accumulation in each of the vincristine-selected sublines was significantly less than in parental cells ($P < 0.02$, PC-V10; $P < 0.0005$, PC-V40 and PC-V160). In part B, cell-associated fluorescence related to anthracycline accumulation after a 60-min daunorubicin exposure (0.2 μM) was measured by flow cytometry. The mean channel number was converted to a linear value [11] and is reported by flow cytometry. The mean channel number was converted to a linear value [11] and is reported as a percentage of the fluorescence value observed in parental cells (38.9 ± 2.6, mean ± SEM). Values are means ± SEM of at least three experiments.

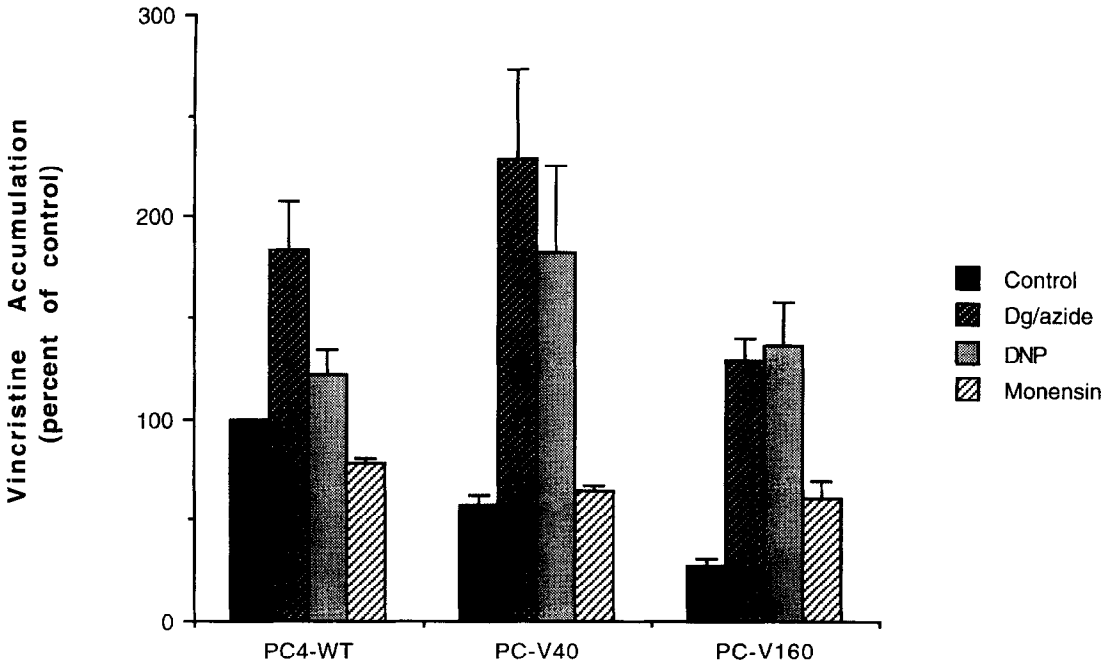

FIG. 2. Effect of energy inhibitors on vincristine accumulation in parental and drug-resistant cells. The effects of a 15-min treatment with 2-deoxyglucose/sodium azide (Dg/azide, 50 mM/15 mM), 2,4-dinitrophenol (DNP, 200 μM), or monensin (5 μg/mL) prior to a 60-min vincristine exposure were determined. All results are reported as the percentage of vincristine accumulation observed in parental PC4 cells without an energy inhibitor (cpm ± SEM = 1510 ± 60). Values are means ± SEM of a minimum of three experiments.

V160 cells (117 ± 1%) as compared with that in digitonin-treated parental cells.

Analysis of Vincristine Uptake

Uptake studies were performed to determine if differences in drug influx contributed to reduced drug sensitivity. When early time points were sampled (0 to 5.5 min), parental, PC-V40, and PC-V160 cells showed no detectable difference in the initial accumulation of vincristine (Fig. 3A). In PC-V40 cells, no difference in drug accumulation was detected up to 10 min (Fig. 3A), but after 60 min these cells demonstrated a ~40% decreased accumulation compared with parental cells (Fig. 1). However, in PC-V160 cells, by 10 min the total accumulation of vincristine was nearly half of that in parental cells. Little additional vincristine accumulation was observed in these cells up to 60 min.

Analysis of Vincristine Loss

The rate of [^{3}H]vincristine loss was measured in PC4-WT, PC-V40, and PC-V160 cells after the cells had accumulated drug during a 60-min incubation. Drug loss, as analyzed in a vincristine retention versus time plot, approached a near linear relationship over the first 3 min (Fig. 3B). This was followed by a further, but non-linear, loss of drug over 5–15 min (data not shown). Both resistant cell lines showed enhanced rates of drug loss compared with the parental cell line. When measured over the first 3 min, the rate of vincristine loss in the PC-V40 cell line was 2.3 times faster than that of parental cells; in the PC-V160 cell line, the rate of loss was approximately 3.9 times faster than that of the PC4 cells. The rate coefficients (k) were measured as: PC4-WT: 0.030 (R = 0.75); PC-V40: 0.068 (R = 0.96); and PC-V160: 0.116 (R = 0.95).

Analysis of Daunorubicin Loss

The rate of [^{3}H]daunorubicin loss was similarly examined in PC4-WT, PC-V40, and PC-V160 cells after an initial 60-min drug incubation. No difference in daunorubicin loss between the parental and the PC-V40 cell lines was noted during 30 min of study. For example, 5 min after beginning daunorubicin loss measurements, drug levels in the PC4-WT and PC-V40 cells were, respectively, 98 and 99%, and at 30 min were 76 and 70% of the t = 0 levels. By contrast, PC-V160 cells retained 68% of the daunorubicin after 5 min, that decreased to 45% after 30 min.

Analysis of Subcellular Daunorubicin Accumulation

Redistribution of anthracyclines away from the nucleus into a vesicular compartment has been associated with anthracycline-selected cell lines that overexpress MRP [6, 18, 19]. Fluorescence microscopy was used to assess daunorubicin distribution in parental and PC-V40 cells. After a 60-min drug exposure, both parental PC4 and PC-V40 cells demonstrated daunorubicin accumulation primarily in the nucleus (Fig. 4). Discreet, focal accumulation into a vesicu-

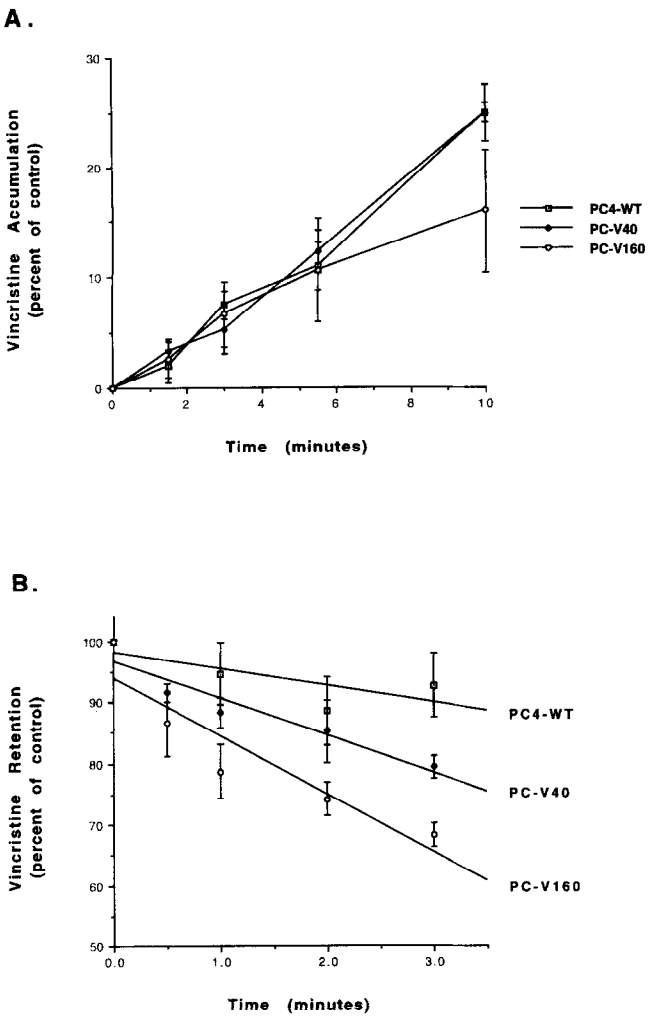

FIG. 3. Analysis of vincristine influx and efflux in parental and resistant cell lines. In part A, parental, PC-V40, and PC-V160 cells (2 × 10^6/mL) were incubated with [^{3}H]vincristine (25 nM final concentration). At the designated time points, cell-associated radioactivity was determined after sedimentation of samples (200 µL) through silicone oil. The data are expressed as the percentage of cell-associated vincristine in parental cells after 60 min of accumulation. Shown is the average uptake (±SEM) within the first 10 min from three experiments each performed in triplicate (cpm ± SEM = WT, 1230 ± 190; PC-V40, 900 ± 80; PC-V160, 170 ± 30). In part B, after a 60-min [^{3}H]vincristine exposure, the cells were washed once and resuspended in drug-free medium at 37°. Cell-associated radioactivity was measured immediately (t = 0). Subsequently, samples (200 µL) were taken at the indicated time intervals and centrifuged through silicone oil. Vincristine retention was calculated as a percentage of cell-associated vincristine present at t = 0 (cpm ± SEM = PC4-WT, 1440 ± 70; PC-V40, 500 ± 60; PC-V160, 540 ± 40). Values are means ± SEM of at least three experiments, each performed in triplicate. By paired *t*-test analysis, the values for PC4-WT vs PC-V40 and for PC4-WT vs PC-V160 at the 3-min time point were significant at $P < 0.05$.

lar compartment was observed as well for both cell lines. Specifically, an expanded vesicular compartment with anthracycline redistribution, as is manifest by U-A10 cells [6], was not observed in the resistant PC-V40 cells.

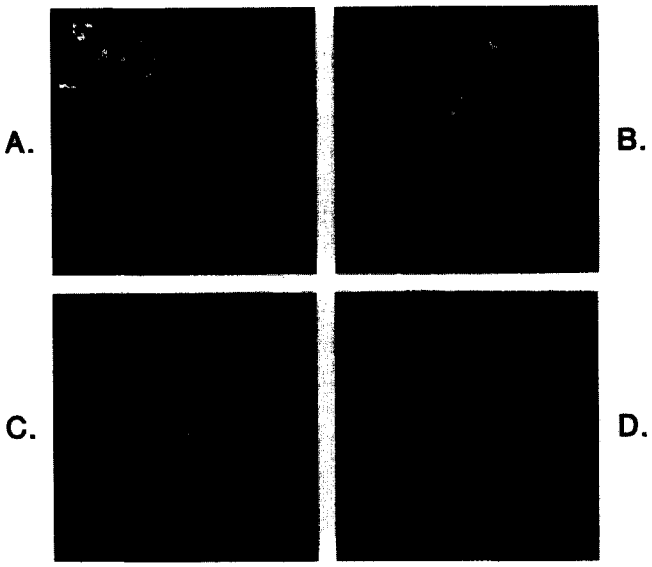

FIG. 4. Daunorubicin distribution in parental and resistant cell lines. Parental PC4 cells (panel A and B) and PC-V40 cells (panels C and D) were exposed to 500 ng/mL of daunorubicin for 60 min; then the cells were washed, visualized by fluorescence microscopy, and photographed. Shown are typical single cells.

Analysis of Intracellular pH

Altered intracellular pH has been suggested to produce drug accumulation differences [20, 21]. The lipophilic ester BCECF-AM was used to estimate intracellular pH in the presence and absence of energy inhibitors in the parental and drug-selected cell lines. No baseline intracellular pH differences were observed between parental PC4 cells and the PC-V40 or PC-V160 cell lines (Table 1). Likewise, treatment in PBS alone or with the energy inhibitors Dg/azide or DNP led to similar levels of acidosis in the three cell lines tested (Table 1).

Expression of MRP

MRP protein levels were examined by western blot analysis using a polyclonal antiserum developed against human MRP [22]. In plasma membrane enriched fractions, a band of approximately 175–180 kDa was detected in parental

TABLE 1. Intracellular pH of parental and resistant cell lines under different experimental conditions

	Intracellular pH			
Cell line	Control	PBS	Dg/SA (50 mM/ 15 mM)	DNP (200 µM)
PC4-WT	7.34 ± 0.06	6.99 ± 0.01	6.90 ± 0.06	6.85 ± 0.01
PC-V40	7.33 ± 0.06	7.06 ± 0.01	6.98 ± 0.04	6.96 ± 0.01
PC-V160	7.30 ± 0.32	6.97 ± 0.02	6.99 ± 0.01	6.95 ± 0.01

Values, obtained using BCECF-AM, are means ± SEM from at least three different experiments. Key: Control growth medium; PBS, phosphate-buffered saline; Dg/SA, 2-deoxyglucose/sodium azide; and DNP, dinitrophenol.

PC4 cells (Fig. 5A). Increased levels of MRP protein were seen in the PC-V10 cell line (~6-fold) with higher levels detected in PC-V40 cells (~18-fold). PC-V160 cells also had increased levels of MRP protein compared with parental cells (~7-fold), though somewhat less than that observed in PC-V40 cells. In membrane fractions enriched for the endoplasmic reticulum, a band of about 175 kDa was also detected in parental PC4 cells (Fig. 5B). Although increased protein levels were seen in PC-V10 cells (1.8-fold), PC-V40 cells (2.3-fold), and PC-V160 cells (1.8-fold), the levels of expression in the endoplasmic reticulum were far less than those observed in the plasma membrane fractions.

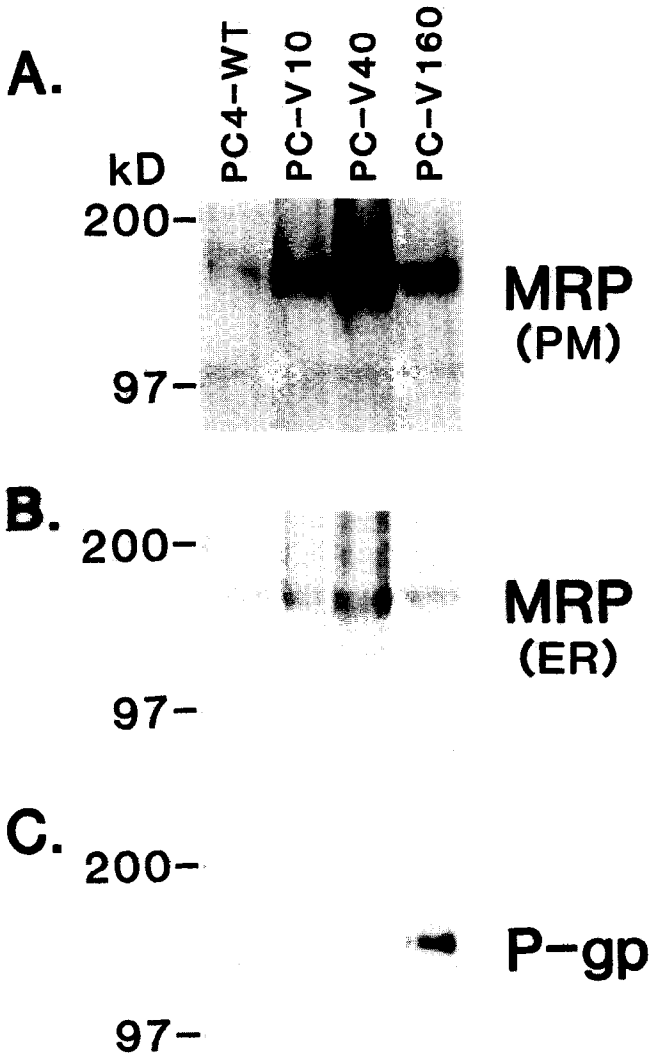

FIG. 5. Expression of MRP and P-glycoprotein in parental and drug-resistant cell lines. Membrane proteins enriched for the plasma membrane (PM) or the endoplasmic reticulum (ER) were prepared by sucrose gradient fractionation, and 10 μg/lane was resolved by electrophoresis on a 6% polyacrylamide–SDS gel. After transfer to a nitrocellulose membrane, western blotting was performed with polyclonal antiserum MRP-6KQ (parts A and B) or monoclonal antibody C219 (part C).

Expression of P-Glycoprotein

Western blot analysis using monoclonal antibody C219 demonstrated a band of about 150 kDa, attributable to *mdr3*, in PC-V160 cells (Fig. 5C) [7]. Prolonged film exposure also revealed a less intense band of 150 kDa in PC4-WT, PC-V10, and PC-V40 cells attributable to the expression of murine *mdr2* (data not shown).

DISCUSSION

The cDNA encoding MRP was first isolated from a doxorubicin-selected cell line that exhibited multidrug resistance, but did not overexpress the P-glycoprotein [23]. Subsequent transfection studies demonstrated that MRP was expressed primarily in the plasma membrane and participated in an outward transport of cytotoxic agents [3–5]. Glutathione conjugation of some substrates has been associated with MRP transport, although whether conjugation is required for export of cytotoxic agents is unclear [24–26].

Cell lines that overexpress MRP have largely, although not exclusively [27, 28], been associated with anthracycline selection. We described vincristine-selected MEL cells in which overexpression of a murine homolog of the human *MRP* gene was detected [7]. This report also gave insight into the temporal relationship between the expression of *mrp* and P-glycoprotein under *Vinca* alkaloid selection. Using antiserum developed against the carboxyl terminus of human MRP (Fig. 5) [20], we now demonstrate the overexpression of a murine protein preferentially located in the plasma membrane fraction of the drug-resistant cell lines.

The MRP-overexpressing cell lines, PC-V10 and PC-V40, showed a decreased net accumulation of vincristine that was reversed by treatment with energy inhibitors (Fig. 2). These findings suggest that murine MRP functions as a plasma-membrane based drug efflux pump. Treating parental cells with energy inhibitors also resulted in increased vincristine accumulation, suggesting that they, too, possess an intrinsic efflux system. Since the parental cells express a baseline level of MRP (Fig. 5), but not P-glycoprotein, this transporter may be functioning in the parental cells as well.

Monensin, an ionophore known to disrupt intracellular vesicular membrane transport but not to uncouple oxidative phosphorylation [15], had little effect on vincristine accumulation in PC-V40 cells. Thus, a subcellular vesicular transport pathway was likely not involved in the vincristine transport differences displayed by PC-V40 cells. Furthermore, selective plasma membrane permeabilization with the detergent digitonin equalized vincristine accumulation in the cell lines. Therefore, the decreased vincristine accumulation in PC-V40 cells was due to a plasma membrane-based ATP-dependent transport process and not to differences in intracellular drug binding.

Since altered cellular pH has been documented for some drug-resistant cell lines [20, 21] and since some members of the ABC superfamily appear to be ion channels [29], the

relationship between murine MRP expression and intracellular pH was examined. Vincristine contains a basic indole group that is protonated at physiologic pH [30, 31]; differences in intracellular pH would result in changes in net drug accumulation. However, studies utilizing the fluorescent probe BCECF-AM showed no differences in intracellular pH between the parental cell line and PC-V40 and PC-V160 cells (Table 1). The finding that accumulation of daunorubicin, another agent affected by cellular pH, was not reduced in the PC-V40 cell line further supported the BCECF-AM results. Thus, changes in intracellular pH cannot account for the net vincristine accumulation differences between parental and MRP overexpressing drug-resistant cell lines.

There was no difference in the rate of vincristine influx between parental and PC-V40 cells as observed over a 10-min period (Fig. 3A). However, a more than 2-fold increased rate of drug loss was observed in the PC-V40 cells as compared with the parental cell line (Fig. 3B). By contrast, in the PC-V160 cell line, which also expresses the P-glycoprotein, drug uptake differences were apparent after 6–10 min of drug accumulation. Nearly a 4-fold increased rate of drug efflux was noted in PC-V160 cells as compared with parental cells. Taken together, these findings strongly suggest that the drug-resistance phenotype in the MRP expressing PC-V40 cell line is associated with an energy-dependent, increased efflux of vincristine not associated with diminished influx.

PC-V10 cells show no detectable doxorubicin resistance (1.1-fold), and the PC-V40 cell line shows only modest doxorubicin resistance (6.0-fold) [7]. Resistance to another anthracycline, daunorubicin, was only 1.7-fold in PC-V40 (data not shown). Despite high-level MRP expression, anthracycline transport in the PC-V40 cell line was unchanged from parental cells when examined for up to 3 hr. Furthermore, the rate of daunorubicin loss was the same between the parental and the PC-V40 cell line. While some anthracycline-selected, MRP overexpressing cell lines demonstrated a redistribution of drug away from the nucleus into an expanded cytoplasmic vesicular compartment [6, 18, 19, 32], we saw no detectable difference in the pattern of anthracycline subcellular trafficking between the parental and the PC-V40 cell line. Moreover, recent studies showed that reduction of glutathione levels by pretreatment of PC-V10 and PC-V40 with buthionine sulfoximine had no effect on the daunorubicin IC_{50}, but led to increased susceptibility to vincristine and etoposide, again suggesting that the low level anthracycline resistance is not mediated by MRP.¶ These findings suggest that murine MRP expressed in these cell lines may have genetic features distinct from human MRP or may require additional factors for its activity. Selection for vincristine resistance alone may not induce the additional steps required for full expression of the MRP phenotype or, during the process of drug selection, the cells have acquired a mutation in MRP that specifies preferential vincristine resistance. It is of interest that human *MRP* transfected cell lines show increased vincristine over adriamycin resistance [4], while a human *MRP* transfected into murine cells shows the reverse [33]. Moreover, a recent study describes a novobiocin-selected murine cell line which overexpresses *mrp*, has etoposide resistance, not vincristine resistance, and little anthracycline resistance [28].

In this study, we have shown that vincristine-selected MEL cell lines express murine MRP predominantly in the plasma membrane and an energy-requiring, P-glycoprotein-independent outward efflux of vincristine, but not of daunorubicin. Thus, in some cell lines, MRP expression may occur independently of anthracycline resistance.

¶ Draper MP, Martell RL and Levy SB, manuscript submitted for publication.

This investigation was supported, in part, by Clinical Investigator Award CA-01613 from the National Cancer Institute (C.A.S.), USPHS Award T32-C A09429 (R.L.M.), the Arnold D. Imperatore Research Scholarship of the National Leukemia Association (S.B.L.), and CA 593451 from the National Cancer Institute (S.B.L.).

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