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A Unique Interaction between Polyamine and Multidrug Resistance (P-glycoprotein) Transporters in Cultured Chinese Hamster Ovary Cells Transfected with Mouse *mdr-1* Gene

Shewan M. Aziz,‖ David R. Worthen,§ Mustafa Yatin,†‡ Kenneth B. Ain,†‡ and Peter A. Crooks§‖*

*Department of Pharmacy Services and Division of Oncology and Hematology, Henry Ford Hospital, Detroit, MI 48202-2689; †Thyroid Cancer Research Laboratory, Medical Service, Veterans Affairs Medical Center, Lexington, KY 40511; ‡Department of Internal Medicine, University of Kentucky Medical Center, Lexington, KY 40536-0082; and §Division of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0082, U.S.A.

ABSTRACT. We have shown that a functional link exists between the polyamine transporter and the multi-drug resistance (MDR) efflux transporter (P-glycoprotein, P-gp) in MDR-positive cancer cells. To further explore the nature of this interaction, we have examined the effect of reduced polyamine transport activity on cellular expression and activity of P-gp acquired by either selection or transfection. Chinese hamster ovary (CHO) cells and their polyamine transport-deficient mutants (CHOMGBG) were transfected with mouse *mdr-1b* gene. The activity of P-gp in these cells was quantified by measuring cellular accumulation of radiolabeled taxol and etoposide in the presence and absence of the P-gp modulator SDZ PSC-833 (valspodar; a semisynthetic undecapeptide derived from cyclosporin D). The *mdr-1b*-transfected CHO cells accumulated 2- to 3-fold less taxol and etoposide than the controls, an accumulation defect reversed by the potent MDR modulator PSC-833. Despite expression of P-gp on the surface of *mdr-1b*-transfected CHOMGBG cells, this classic MDR phenotype was not observed. Similarly, CHO cells, but not CHOMGBG cells, showed MDR activity after selection with doxorubicin as determined by reduced accumulation of radiolabeled taxol. Treatment with 50 μM of reduced polymer of spermine and glutaraldehyde, a selective blocker of the polyamine transport system, reduced MDR activity in *mdr-1*-transfected CHO cells and restored cellular accumulation of etoposide and taxol to control levels, effects not observed in *mdr-1*-transfected CHOMGBG cells. Notably, *mdr-1*-transfected CHO cells were 4- to 16-fold more resistant to the cytotoxic effects of the P-gp substrates doxorubicin, taxol, and etoposide than were the *mdr-1*-transfected CHOMGBG cells. CHO cells transfected with the *mdr-1* gene exhibited a 23% reduction in cellular uptake of [^{14}C]spermidine compared with untransfected controls; spermidine accumulation in CHOMGBG cells was no different than that in untransfected controls. These data suggest that the existence of a functioning polyamine transport system may be a requirement for MDR transporter activity, while the expression of functioning P-gp appears to reduce polyamine transporter activity. BIOCHEM PHARMACOL **56**;2: 181–187, 1998. © 1998 Elsevier Science Inc.

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The polyamines PUT,¶ SPD, and SPM are a ubiquitous group of cationic molecules that play a central intracellular role in cellular growth, differentiation, and neoplastic transformation [1–6]. Polyamines are necessary for increases in DNA, RNA, and protein syntheses. Increases in intracellular polyamine contents are driven principally by a coordinated interaction between *de novo* synthesis and trans-membrane transport pathways [1–11]. Considerable heterogeneity exists in terms of the number of polyamine transporters and the energy sources for polyamine uptake. We have shown that both cultured bovine pulmonary artery smooth muscle cells and rat aortic smooth muscle cells express two transporters: a "non-selective" pathway that imports all three polyamines and a "selective" pathway that imports only SPD and SPM [9, 10].

The development of drug resistance in human tumors is a major obstacle in the curative potential of cancer chemotherapeutics [12, 13]. This phenomenon can be attrib-

‖ Corresponding authors: Shewan M. Aziz, R.Ph., Ph.D., Department of Pharmacy Services, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, MI 48202-2689. Tel. (313) 876-1799; FAX (810) 876-1302; and Peter A. Crooks, Ph.D., Division of Medicinal Chemistry and Pharmaceutics, College of Pharmacy, University of Kentucky, Rose Street, Lexington, KY 40536-0082. Tel. (606) 257-1718; FAX (606) 257-7585.

¶ *Abbreviations:* CHO, Chinese hamster ovary; CHOMGBG, polyamine transport-deficient Chinese hamster ovary; [^{14}C]SPD, ^{14}C-labeled spermidine; FBS, fetal bovine serum; MDR, multi-drug resistance; MEM, minimum essential medium alpha; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P-gp, P-glycoprotein; poly-SPM, reduced polymer of spermine and glutaraldehyde; PUT, putrescine; SPD, spermidine; and SPM, spermine.

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uted to several mechanisms, including MDR [12–15]. The typical intrinsic or acquired MDR phenotype includes cross-resistance to several classes of drugs and is associated with the expression of a membrane protein, P-gp. P-gp functions as a membrane efflux pump, which transports antineoplastic agents out of cancer cells, in a manner similar to the polyamine influx transporter, which carries polyamines into cancer cells [12–15]. Reversal or modulation of MDR has been achieved using a wide variety of pharmacologic agents including calcium channel blockers, cyclosporins, cardiovascular drugs, steroid analogs, antibiotics, calmodulin inhibitors, and antimalarials [12–21]. Most of these agents reverse MDR by competitively inhibiting the binding of chemotherapeutic agents to P-gp, a mechanism unrelated to their primary chemotherapeutic action [12–21].

We recently synthesized an SPM-glutaraldehyde conjugate (poly-SPM), which selectively blocks the polyamine transport system in pulmonary artery smooth muscle cells [22]. Our results demonstrate that poly-SPM blocks polyamine transport, depletes cellular polyamine content, and exerts cytotoxic effects in several cultured human cancer cell lines and their MDR-positive counterparts [23, 24]. Interestingly, the MDR variants were 2- to 4-fold more resistant to the cytotoxic effects of poly-SPM than their parental cell lines. In addition, MDR-positive cells had higher K_m and lower V_{max} values for polyamine transport than those generated in wild-type cells [24]. Poly-SPM reversed the MDR phenotype, decreasing the IC_{50} values of the chemotherapeutic agents doxorubicin, taxol, vinblastine, and etoposide, to those levels observed in the parental cell lines. This effect was accompanied by an increased cellular accumulation of these P-gp substrates [23]. No such effect was observed in the parental cell line, which does not express *mdr-1* gene product [23]. Poly-SPM had no influence on the IC_{50} of non-MDR chemotherapeutic agents such as cisplatin. Collectively, these findings suggest that a relationship exists between the polyamine influx transporter system and the MDR efflux pump.

In the present study, we have further explored the nature of the interaction between the polyamine and MDR transporters by examining the effect of the absence of a functioning polyamine transport system on the activity of the MDR transporter acquired by either selection or transfection. CHO cells and their polyamine transport-deficient mutants (CHOMGBG) were either selected with doxorubicin or transfected with mouse *mdr-1b* gene. We then examined the effect of the absence of the polyamine transporter on the expression and activity of the MDR-1 transporter. The effect of P-gp expression on the activity of the polyamine transporter was also examined.

MATERIALS AND METHODS

Materials

MEM, EDTA, trypsin, and penicillin/streptomycin solution were purchased from Life Technologies. FBS was provided by HyClone. PBS, glutaraldehyde, and other drugs and chemicals used in this study were purchased from the Sigma Chemical Co. Radiochemicals and related supplies were purchased from the Amersham Corp. All plasticware used for tissue culture was obtained from Costar.

Preparation of the SPM Conjugate

The reduced polymeric glutaraldehyde conjugate of SPM was prepared as previously described [22, 23]. Briefly, a solution (1 mL) of 1 mM of SPM · 4HCl in 0.2 M of PBS was added to 1 mL of 0.2 M of PBS containing glutaraldehyde (3%, v/v) at pH 7. The mixture was incubated at 37° for 1 hr. $NaBH_4$ (10 mg) was added to reduce the imine functionalities, and the reaction was left to stand at ambient temperature for 30 min. Then the mixture was dialyzed (lower molecular weight cutoff: 12,500–15,000) against 0.2 M of PBS.

Cell Culture

CHO cells and their related polyamine transport-deficient variants, CHOMGBG (provided by Dr. Wayne Flintoff, University of Western Ontario), were grown in 75 cm^3 tissue culture flasks containing 25 mL of MEM supplemented with 10% FBS, 100 units/mL of penicillin, and 100 μg/mL of streptomycin. Cells were maintained at 37° in a humidified atmosphere containing 5% CO_2 and subcultured every 5–7 days. Cell counts and viability were determined by hemocytometry using the trypan blue dye exclusion method.

DNA Transfection

The pSVK3 plasmid containing the mouse *mdr-1b* gene (provided by Dr. Kevin Ross, Pharmacyclics) was transfected into wild-type CHO and polyamine transport-deficient CHOMGBG cells using lipofectin reagent and the Gibco BRL procedure, as adapted from a previously reported technique [25]. Twenty-four hours after transfection, the medium was replaced, and cells were allowed to grow for 48 hr. Analogously treated control cells were transfected with the same vector without the *mdr-1* insert.

Flow Cytometry

Cellular expression of P-gp expression was quantified with FACScan flow cytometry. Isolated cells were incubated at 4° for 30 min in human serum diluted 1:1 with PBS at a concentration of 1×10^6 cells/1 mL. The cells were washed with PBS and incubated with either MDR-specific monoclonal antibody (Signet 4ET3, Signet Laboratories) or the negative control antibody (mouse anti-human IgG, Biosource) for 30 min at 4°. Both antibodies were used at a dilution of 1:30. Goat serum containing fluorescein isothiocyanate-labeled goat antimouse IgG (TAGO, Biosource) was added to cells at a 1:30 dilution for 30 min at 4° in the dark. Flow cytometry analysis was carried out using a FACScan (Becton-Dickinson) flow cytometer equipped

with 15-mW argon laser. The percentage of cells staining positive for the MDR gene products and the relative levels of MDR expression were determined by measuring the geometric fluorescence intensity of bound fluorescein label.

Selection with Doxorubicin

CHO and CHOMGBG cells were seeded in standard culture medium, and then were exposed gradually to increasing concentrations of doxorubicin in the culture medium as follows: cells were cultured in medium containing 0.1 μM of doxorubicin for 1 week, 0.25 μM for 1 week, 0.75 μM for 1 week, and then 2 μM on the fourth and subsequent weeks [26, 27]. Viability of control CHO cells, CHOMGBG cells, and their doxorubicin-selected counterparts was determined by an MTT absorbance assay and trypan blue dye exclusion assay conducted simultaneously with the taxol accumulation study.

Polyamine Transport

Polyamine uptake and metabolism were examined as described previously [10, 22]. Transfected cells were rinsed with fresh, serum-free MEM, 1 mL of serum-free MEM was added to each well, and the cells were allowed to acclimate for 1 hr at 37°. Then [^{14}C]SPD was added to each well (final concentration 10 μM), and the cells were incubated for 30 min at either 4° or 37°. The [^{14}C]SPD uptake medium was aspirated, and the cells were placed on ice and rinsed with ice-cold PBS. The cells were digested for 1 hr at room temperature in 1 N of NaOH, 400 μL of the cell digest was neutralized with 400 mL of 1 N acetic acid, 400 μL of distilled H_2O was added to 10 mL of scintillation fluid (3a70B, RPI, Inc.) and radioactivity was determined using a Packard liquid scintillation counter. The specific component of polyamine uptake was calculated by subtracting the polyamine uptake rate values at 4° from those generated at 37°. Protein content was determined using an adaptation of the Bradford method [24]. The effect of 50 μM of poly-SPM on [^{14}C]SPD uptake in CHO and CHOMGBG cells was also examined. Cells were treated with poly-SPM 5 min prior to the addition of [^{14}C]SPD, and analogous uptake experiments were conducted.

Accumulation Studies

Accumulation studies were carried out as described previously [28]. Transfected cells were cultured for 30 min with either 4.0 μM of [^{3}H]vinblastine or 10.0 μM of [^{3}H]taxol in the presence and absence of unlabeled poly-SPM (50 μM). After 30 min, the cells were washed twice with 1.0 mL of ice-cold PBS and then were lysed with 1.0 mL of 4% SDS in deionized water. The cell lysate was transferred to scintillation vials containing 10 mL of Ecolite scintillation fluid (ICN Biomedicals Inc.). Radioactivity was counted on a Tri Carb C scintillation counter (Packard Co.) and standardized in terms of cellular protein present [24].

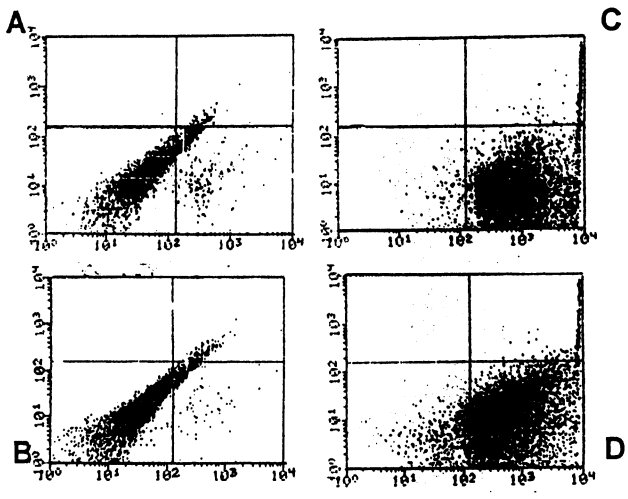

FIG. 1. Wild-type CHO cells (A) and their polyamine transport-deficient variant CHOMGBG cells (B) transfected with either the pSVK3 plasmid containing the mouse *mdr-1b* gene or with the same vector without the *mdr-1b* insert. Results from FACScan flow cytometry revealed that approximately 92% of CHO cells (C) and 89% of CHOMGBG cells (D) were stained positive for the MDR gene product P-gp.

Statistical Analysis

All data are presented as the means ± SEM of three independent experiments conducted in quadruplicate. Comparisons between experimental groups were made using one-way ANOVA. Significant differences between experimental groups were determined using the Newman-Keuls test. In all cases, P values < 0.05 were considered to denote statistical significance.

RESULTS

As previously reported [29, 30] parental CHO cells but not CHOMGBG cells exhibited a temperature-dependent [^{14}C]SPD uptake. Treatment with 50 μM of poly-SPM reduced [^{14}C]SPD uptake in CHO cells by 85% but had no effect on [^{14}C]SPD accumulation in CHOMGBG cells (data not shown).

Wild-type CHO cells and their polyamine transport-deficient variants, CHOMGBG, were transfected with either the pSVK3 plasmid containing the mouse *mdr-1b* gene or with the same vector without the *mdr-1b* insert. FACScan flow cytometry revealed that approximately 90% of both cell types were stained positive for the MDR gene product, P-gp, as shown in Fig. 1. The *mdr-1*-transfected CHO cells expressing functional P-gp showed significantly (*ca.* 23%) lower [^{14}C]SPD uptake than the control cells transfected with the vector without the *mdr-1b* insert. In contrast, [^{14}C]SPD accumulation in *mdr-1*-transfected CHOMGBG cells expressing P-gp did not differ significantly from that of untransfected controls (data not shown).

Cellular accumulation studies of the ^{3}H-radiolabeled MDR substrates taxol and etoposide were carried out in

TABLE 1. Effect of a polymeric conjugate of spermine (poly-SPM) on the cellular accumulation of [^{3}H]taxol in CHO cells and their polyamine transport-deficient variants (CHOMGBG) transfected with the *mdr-1* gene

Cell line	Cellular accumulation* (pmol/mg protein)	
	Control	Transfected
[^{3}H]Taxol only		
CHO	378 ± 18	135 ± 20†
CHOMGBG	390 ± 25	390 ± 30
[^{3}H]Taxol + cyclosporin-A (4 μM)		
CHO	390 ± 18	391 ± 17‡
CHOMGBG	380 ± 18	370 ± 29
[^{3}H]Taxol + PSC-833 (2 μM)		
CHO	375 ± 21	380 ± 22‡
CHOMGBG	395 ± 17	450 ± 60
[^{3}H]Taxol + poly-SPM (50 μM)		
CHO	389 ± 32	494 ± 95‡
CHOMGBG	395 ± 18	385 ± 80

*Values are means ± SEM of three independent experiments conducted in quadruplicate.

†$P < 0.01$, control CHO vs transfected CHO.

‡P 0.01, transfected CHO + cyclosporin A, transfected CHO + PSC-833, or tranfected CHO + poly-SPM.

CHO and CHOMGBG cells transfected with the *mdr-1* gene. When compared with untransfected controls, CHO cells transfected with the *mdr-1* gene showed a 64% reduction in the accumulation of taxol (Table 1), while etoposide accumulation was reduced by 50% (Table 2). The accumulation of taxol and etoposide in CHOMGBG cells transfected with the *mdr-1* gene was no different than that of control cells transfected with the vector without the *mdr-1* insert (Tables 1 and 2). Treatment with 2 μM of SDZ PSC-833 (valspodar, Novartis Corp.), a specific modulator of P-gp, reversed MDR activity in CHO cells and restored the accumulation of taxol and etoposide to the level seen in control cells (Tables 1 and 2). Exposure to 4 μM of cyclosporin-A, another competitive P-gp substrate, also restored taxol accumulation in *mdr-1*-transfected CHO cells back to the level of untransfected controls (Table 1). Notably, neither MDR modulator affected cellular accumulation of taxol and etoposide in *mdr-1*-transfected CHOMGBG or untransfected CHOMGBG controls.

TABLE 2. Impact of a polymeric conjugate of spermine (poly-SPM) on the cellular accumulation of [^{3}H]etoposide in CHO cells and their polyamine transport-deficient variants (CHOMGBG) transfected with the *mdr-1* gene

Cell line	Cellular accumulation* (pmol/mg protein)	
	Control	Transfected
[^{3}H]Etoposide only		
CHO	120 ± 4	60 ± 12†
CHOMGBG	108 ± 14	104 ± 14
[^{3}H]Etoposide + PSC-833 (2 μM)		
CHO	111 ± 7	98 ± 10‡
CHOMGBG	105 ± 17	100 ± 9
[^{3}H]Etoposide + poly-SPM (50 μM)		
CHO	104 ± 3	102 ± 8‡
CHOMGBG	102 ± 2	101 ± 6

*Values are means ± SEM of three independent experiments conducted in quadruplicate.

†$P < 0.01$, control CHO vs transfected CHO.

‡$P < 0.01$, transfected CHO vs transfected CHO + PSC-933 or transfected CHO + poly-SPM.

Cellular accumulation studies were carried out with [^{3}H]-radiolabeled taxol in control CHO and CHOMGBG cells and in cells selected with doxorubicin. As compared with control cells, CHO cells but not CHOMGBG cells selected with doxorubicin showed a significant reduction in the accumulation of taxol, suggesting MDR activity. Taxol accumulation in control CHO cells and doxorubicin-selected CHO cells was 350 ± 2.5 and 170 ± 3.2 pmol/mg protein, respectively. In contrast, CHOMGBG cells cultured for as long as 2 months in doxorubicin-containing medium failed to display this classic MDR accumulation defect seen in analogously selected CHO cells. MTT and trypan blue dye exclusion assays showed that a 30-min exposure to the taxol concentration used in this study did not affect cell viability.

To further characterize the modulation of resistance exerted by poly-SPM in MDR cells [24], cellular accumulation studies were carried out with [^{3}H]-radiolabeled taxol and etoposide in the presence of 50 μM of poly-SPM. As compared with untransfected controls, poly-SPM completely reversed the accumulation defect of [^{3}H]taxol (Table 1) and [^{3}H]etoposide (Table 2) in *mdr-1*-transfected CHO cells, but did not affect their accumulation in CHOMGBG cells.

The MDR activity of P-gp expressed in *mdr-1*-transfected CHO cells is summarized in Table 3. MTT cytotoxicity assays [23] revealed that transfected CHO cells were 3- to 5-fold more resistant to taxol, etoposide, and doxorubicin as compared with untransfected CHO controls, confirming their MDR status. MDR CHO cells were 4.2-, 16.4-, and 5.8-fold more resistant to taxol, etoposide, and doxorubicin, respectively, than were *mdr-1*-transfected CHOMGBG cells. In contrast to the MDR CHO cells, *mdr-1*-transfected CHOMGBG cells were no more resistant to the drugs than their untransfected controls.

DISCUSSION

The results of this study suggest that the presence of a functioning polyamine transport system may be necessary for the MDR P-gp transporter to function. CHO cells with a functioning polyamine transport system transfected with the *mdr-1* gene expressed P-gp to a high degree, and were several-fold more resistant to the cytotoxic effects of the P-gp substrates taxol, doxorubicin, and etoposide as compared with untransfected controls. Uptake studies revealed that *mdr-1*-transfected CHO cells accumulated less than half as much radiolabeled taxol and etoposide as their untransfected controls, an accumulation defect that was

TABLE 3. Summary of the cytotoxicity of taxol (TAX), etoposide (ETP) and doxorubicin (DOX) in wild-type (CHO) and *mdr-1*-transfected (CHO-mdr) Chinese hamster ovary cells and in their wild-type (CHOMGBG) and *mdr-1*-transfected (CHOMGBG-mdr) polyamine transporter-deficient variants

	IC_{50} (μM)			
Drug	CHO	CHO-mdr	CHOMGBG	CHOMGBG-mdr
TAX	4.1 ± 0.32	13.5 ± 1.8	3.7 ± 0.20	3.25 ± 0.24
ETP	0.9 ± 0.03	2.3 ± 0.13	0.12 ± 0.01	0.14 ± 0.01
DOX	4.5 ± 0.60	22.0 ± 1.9	3.5 ± 0.01	3.8 ± 0.05

Data are presented as the means ± SEM of the IC_{50} value of each drug as directly extrapolated from log-linear concentration-response curves generated using the MTT cytotoxicity assay in three independent experiments conducted in quadruplicate.

reversed completely by the classic MDR-modulating P-gp substrates, PSC-833 and cyclosporin A. When P-gp substrate accumulation in *mdr-1*-transfected CHO cells was assayed in the presence of the selective polyamine uptake inhibitor poly-SPM, cellular accumulation of taxol and etoposide was increased to untransfected control levels, indicating that their previously functioning P-gp efflux system was inhibited completely when polyamine transport was blocked. This phenomenon was also noted in previous studies [22, 23, 25], which revealed that selective blockade of the polyamine transport system with poly-SPM reverses the P-gp-associated accumulation defect and increases the cytotoxic activity of MDR substrates in several other MDR cell lines.

In contrast to *mdr-1*-transfected CHO cells, polyamine transport-deficient CHOMGBG cells, though successfully transfected with P-gp, nevertheless failed to display functional MDR or reduced accumulation of P-gp substrates when compared with untransfected CHOMGBG controls. Both control and *mdr-1*-transfected CHOMGBG cells were equally sensitive to the cytotoxic activity of the P-gp substrates doxorubicin, taxol, and etoposide. Cellular accumulation of tritiated taxol and etoposide in transfected CHOMGBG was no different than that in untransfected controls. Further, unlike that in CHO cells, cellular accumulation of P-gp substrates in CHOMGBG cells was unaffected by the MDR modulators PSC-833 and cyclosporin A or by the polyamine transport inhibitor poly-SPM [23, 25].

We recently utilized the novel polyamine homopolymer poly-SPM [22–24] to test the hypothesis that polyamine transport is a rational pharmacologic target in cancer therapy. Poly-SPM selectively inhibits the polyamine transport system in mammalian cells, depletes cellular polyamine content, and is cytotoxic for several parental and MDR human tumor cell lines [22–24]. Cotreatment with difluoromethylornithine (DFMO), a suicide inhibitor of the L-ornithine decarboxylase (ODC) enzyme, markedly enhances both the depletion of intracellular polyamine content and the cytotoxicity of poly-SPM [24]. MDR tumor cells are more resistant to the cytotoxic effects of poly-SPM and have higher K_m and lower V_{max} values for polyamine transport than those observed in wild-type cells [24]. In addition, poly-SPM modulates MDR activity and increases the cytotoxicity of P-gp substrates in drug-resistant tumor cells. These observations provide indirect evidence for the concept that a functional link may exist between the polyamine influx transporter(s) and the MDR efflux transporter P-gp.

The results of the present study suggest that this relationship between both the polyamine and MDR transporters is more complicated than just a simple functional interaction. The absence of the polyamine transport system does not affect the expression or insertion of P-gp into the cell membrane, yet it renders the MDR transporter nonfunctional. There is substantial homology between the *mdr-1* gene and several other transport proteins present in bacterial cells, including the volume-regulated chloride channel and the bacterial PUT transporter [15, 20, 30–37]. It has been speculated that cytotoxic drug transport and chloride transport could represent two discrete functional states of the same protein [20, 31, 34, 35]. The expression, membrane incorporation, and function of the MDR and polyamine transport systems might be similarly interrelated.

This present study suggests that an interaction may exist between the polyamine transporter and P-gp, one similar to that observed between the MDR transporter and the chloride channel. It has been shown that the expression of P-gp in epithelial cells and in several tumor cells is not associated with increased chloride channel activity [34, 35, 38]. Similarly, the expression of the MDR phenotype in polyamine transport-deficient CHOMGBG cells failed to influence the activity of the polyamine transport system in these cells, while transfection with the *mdr-1* gene actually reduced polyamine transport in CHO cells, as previously noted in MDR human tumor cells [24]. Collectively, these results suggest that induction of the MDR transporter may result in reduced activity of the polyamine transport system. Interestingly, it has been reported that an inverse functional interaction exists between cystic fibrosis transmembrane conductance regulator protein (CFTR) and P-gp [35, 38]. Further studies are warranted to determine if the polyamine transporter is related to P-gp in a manner similar to that suggested for the volume-regulated chloride channel or CFTR and P-gp.

Our previous studies indicate that the specific interac-

tion between polyamine transport and P-gp is the major mechanism responsible for the modulation of MDR activity by poly-SPM in MDR tumor cells. Although modulation of MDR activity by poly-SPM might be mediated through an intracellular interaction of poly-SPM with other MDR mechanisms in MDR-positive cancer cells [12–21], the results of this study show that this is not the case. The finding that the MDR-1 transporter is not functional in CHOMGBG cells further supports our contention that modulation of the MDR transporter activity by poly-SPM is due solely to the inhibition of the polyamine transport system. The results of this study also suggest that modulation of the MDR transporter by poly-SPM is due to polyamine transport blockade, rather than a direct interaction of poly-SPM with the MDR transporter.

Since the absence of the polyamine transport system does not affect the expression or insertion of P-gp into the cell membrane, the presence of a functioning polyamine transport system may be necessary for the active three-dimensional organization of P-gp in the cell membrane. It is also possible that the influx of polyamine transport system substrates, significantly reduced in both CHOMGBG and CHO cells cultured with poly-SPM, is required for the MDR transporter to function. Studies are in progress in our laboratory to determine the effect of the progressive repression of the polyamine transport system on MDR activity.

The results of this study suggest for the first time that an active polyamine transport system is necessary for a functional MDR transporter. Conversely, the induction of both intrinsic and acquired MDR appears to down-regulate the activity of the polyamine transporter. Additional investigation of the unique interaction between these two transporters may lead to the discovery of novel pharmacological targets in the polyamine transport system for improved chemotherapy of resistant tumors.

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