Biochemical and Biophysical Research Communications **262,** 121–126 (1999)
Article ID bbrc.1999.1120, available online at http://www.idealibrary.com on IDEAL®

Transport of Phosphatidylcholine in MDR3-Negative Epithelial Cell Lines via Drug-Induced MDR1 P-Glycoprotein

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Received July 8, 1999

Human MDR1 P-glycoprotein (P-gp) is a membrane efflux pump for cytotoxics, whereas MDR3 P-gp is a phosphatidylcholine transporter. We have examined a role for MDR1 P-gp in phosphatidylcholine transport in MDR3-negative epithelial cells that have been induced to express the MDR1 P-gp by exposure to cytotoxics. The accumulation and retention of the fluorescently labelled phosphatidylcholine analogue, C12-NBD-PC, was studied in resistant, KBV1 and MCFadr, and sensitive, KB3-1 and MCF7, cells. Lower accumulation and decreased retention of C12-NBD-PC was evident in resistant cells, e.g., KBV1 accumulated 56%, and MCFadr accumulated 60%, of C12-NBD-PC levels in KB3-1 and MCF7, respectively. Treatment with the MDR1 P-gp inhibitor, verapamil, altered the kinetics of C12-NBD-PC in the resistant cells to more closely follow the pattern of C12-NBD-PC handling by sensitive cells. Comparison of C12-NBD-PC to that of the model MDR1 P-gp substrate, rhodamine-123, indicated phosphatidylcholine turnover kinetics by MDR1 P-gp to be relatively low. The transport by MDR1 P-gp of phosphatidylcholine from inner to outer membrane leaflet may regulate P-gp function and fulfill a role in the MDR1 multidrug-resistant phenotype.

Key Words: **multidrug resistant; MDR1; MDR3; P-glycoprotein; phospholipid transport; cancer.**

Lipids are distributed asymmetrically across the plasma membrane bilayer with the majority of the choline containing lipids, phosphatidylcholine and sphingolipids, located in the outer leaflet, whereas most aminophospholipids, phosphatidylserine and phosphatidylethanolamine, are located in the inner cytoplasmic leaflet (1). The passive transbilayer movement of most lipids is very slow, although exceptions include the transport of diacylglycerol and cholesterol. Several plasma membrane proteins have been identified to act as phospholipid translocators. The aminophospholipid translocase (2) selectively transports phosphatidylserine and phosphatidylethanolamine from the outer to the inner leaflet of the plasma membrane; phosphatidylcholine and sphingomyelin are not recognized substrates for this transporter. Another phospholipid translocator, phospholipid scramblase located in the human erythrocytic plasma membrane, can induce rapid ATP-dependent transbilayer migration of aminophospholipids to the inner leaflet of the plasma membrane in activated, injured, or apoptotic cells exposed to elevated intracellular Ca^{++} (3).

The multidrug resistance (MDR) gene family is a small family of isozymes consisting of two members in humans (MDR1 and MDR3) and three members in rodents (mdr1a, mdr1b, and mdr2). These genes encode for P-gps that are part of the ATP-binding cassette superfamily, the members of which share common structural characteristics and transport a variety of compounds across membranes. In humans only the MDR1 encoded P-gp contributes to the multidrug resistant phenotype, serving as a membrane efflux mechanism to pump cytotoxics out of the cell (4). However, comparison between human MDR1 and MDR3 shows 78% homology in the coding sequence (5). Exchanging homologous segments between MDR1 and MDR3 and undertaking site-directed mutagenesis has enabled identification of critical residues, specifically Q330, V331 and L332 in transmembrane domain 6 of the MDR1 P-gp, that when inserted in the N-terminal domain of MDR3 P-gp affords transport of some, but not all, MDR1 P-gp substrates (6).

Murine mdr2 and human MDR3 share 91% identity at the amino acid level and are found predominantly in normal liver tissue where expression is restricted to

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0006-291X/99 $30.00

the canalicular membranes of hepatocytes (7). Both mdr2 and MDR3 P-gp function as a phosphatidylcholine translocase/flippase and are involved in the secretion of phosphatidylcholine into the bile (12). MDR3 P-gp phosphatidylcholine flippase activity has been shown to be dependent upon the presence of ATP and Mg^{++} and can be inhibited by the P-gp modulator verapamil (13–14). Some cell types, human leukemic and lymphocytic cells (8–10) and *in-vitro* human leukemia cell lines K562/ADR and HL6/ADR, have been shown to express MDR3 concurrently with MDR1 (11).

A role for MDR1 P-gp in the transport of phosphatidylcholine has previously been reported in haemopoietic cells expressing MDR1 (14) and in a virally transfected recombinant MDR1 expressing epithelial cell line (13). In contrast in this current study we have examined a role for human MDR1 P-gp in phosphatidylcholine transport in MDR3-negative epithelial cells that have been induced to express the MDR1 P-gp multidrug resistant phenotype by prolonged exposure to cytotoxic agents. Specifically, flow cytometric kinetic studies were undertaken examining the accumulation and retention of the fluorescently labelled phosphatidylcholine analogue, 1-palmitoyl-2-[12-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]dodecanoyl] (C12-NBD-PC) in resistant (MDR1 P-gp positive) cell lines, KBV1 and MCFadr, and their respective sensitive (MDR1 P-gp negative) parental cell lines, KB3-1 and MCF7. The absence of human MDR3 in these cell lines was confirmed by lack of MDR3 transcript determined by RT-PCR. The study showed lower accumulation and decreased retention of C12-NBD-PC in the resistant cell lines compared to the respective sensitive cells. Treatment with the MDR1 P-gp efflux pump inhibitor, verapamil, altered the kinetics of C12-NBD-PC in the resistant cells in such a way as to more closely follow the pattern of C12-NBD-PC handling by sensitive cells. In two MDR3 negative epithelial cell lines induced to express the MDR1 P-gp phenotype by exposure to cytotoxics we have confirmed phosphatidylcholine transport by MDR1 P-gp, a role that may fulfill a function in the multidrug resistant phenotype.

MATERIALS AND METHODS

Materials. Cell culture plastics were obtained from Corning Costar (High Wycombe, UK) with DMEM and FBS obtained from Life Technologies-Gibco (Paisley, UK). Dithionite, Verapamil, rhodamine-123, vinblastine, bovine serum albumin and egg yolk phosphatidylcholine were obtained from Sigma Chemical Co (Poole, Dorset, UK); doxorubicin was obtained from Pharmacia & Upjohn (Milton Keynes, UK); C12-NBD-PC was obtained from Molecular Probes (PoortGebouw, Netherlands); the monoclonal anti-human P-glycoprotein antibody, MRK16, was obtained from TCS Biologicals (Oxford, UK), with the corresponding mouse isotypic IgG_{2a} control antibody, and the secondary anti-mouse FITC-conjugated $F(ab')_2$ fragment from Dako (Dako, Cambridge, UK). The sources for RT-PCR materials were as follows: Agarose LE analytical grade and RNasin ribonuclease inhibitor (Promega; Southampton, UK); Ultraspec RNA extraction solution and Ultrapsec DEPC-treated water (Biogenesis; Dorset, UK); Moloney murine leukemia virus reverse transcriptase (MMLV-rt) and dNTPs (Life Technologies-Gibco; Paisley, UK); random hexanucleotide (pdN6) primers (AmPharm, Little Chalfont; UK); Biotaq polymerase (Bioline; UK); oligonucleotide primers were synthesised within the University of Wales College of Medicine on a Beckman DNA synthesiser with HPLC high purity isolation.

Cell lines. The following cell lines were obtained as kind gift from the Imperial Cancer Research Fund (London, UK): human breast carcinoma cells, MCF7, and their highly doxorubicin resistant subline, MCFadr; human nasopharyngeal carcinoma cells, KB3-1, and their highly vinblastine resistant subline, KBV1. Cells were grown in DMEM supplemented with 10% FBS, 100 μg/ml streptomycin and 100 U/ml penicillin G. To maintain the MDR phenotypes, KBV1 and MCFadr cells were cultured in the presence of 1μg/ml vinblastine and 0.1 μg/ml doxorubicin, respectively. Cells were passaged every 3-4 days by trypsin/EDTA disaggregation and, prior to experimentation, the KBV1 and MCFadr cells taken through one round of subculture in the absence of cytotoxics. For experimentation, cells were seeded onto appropriate tissue-culture treated plastic at $30–40 \times 10^3$ cells/cm^2 and studies undertaken at day 5 post-seeding.

Flow cytometric immunofluorescence assay for P-gp expression. Cells were harvested by trypsin/EDTA disaggregation and washed with phosphate-buffered saline containing 1% bovine serum albumin (PBS/BSA). Cells (5×10^5) in PBS/BSA were then incubated on ice in the dark for 30 min with the P-gp antibody MRK16 (2 μl of stock in a final incubate volume of 100 μl) or a mouse isotypic IgG_{2a} control used at equivalent protein concentration. After which cells were washed in PBS/BSA and incubated on ice for 30 min with a 1 in 50 dilution of anti-mouse FITC-conjugated $F(ab')_2$. Cell associated immunofluorescence distributions were obtained from collecting 10,000 events per cell sample using a FACScan flow cytometer (Becton Dickinson; Oxford; UK). The FITC-fluorescence of gated cell populations was analysed using validated analysis software, WinMDI, (Cytonet UK; http://www.uwcm.ac.uk/uwcm/hg/hoy/).

Reverse transciptase-polymerase chain reaction (RT-PCR). 1 μg RNA was extracted (Ultraspec RNA extraction) from the cell lines cultured on tissue culture-treated plastic and reverse transcribed using 200 units of MMLV-rt and 10 pmols of random pdN6 primers in a solution containing Tris and $MgCl_2$ (1.5 mM). cDNA representing 60 ng of RNA was subjected to PCR for human MDR-1, MDR-3 and GAPDH for 30 cycles in a final volume of 50 μl using 1 unit of Biotaq DNA polymerase and 10 pmol/μl of each primer. The primers specific for the human MDR-1 gene (GenBank accession sequence M14758) (15) were forward 5′ CCCATCATTGCAATAGCAGG 3′ (nucleotides 3020-3039) and reverse 5′ GTTCAAACTTCTGCTCCTGA 3′ (nucleotides 3176-3157) producing a 156 bp product. Following an initial denaturation at 91°C for 5 min and then at 59°C for 5 min each amplification cycle was performed sequentially at 72°C for 2 min, at 9°C for 30 s, and at 5°C for 1 min (16). Human MDR3 primers were forward 5′-AGG GCG ACT TTG AAC TGG GC-3′ (nucleotides 85-104) and reverse 5′-TTT GCC TGG ATT TAG CAG CG-3′ (nucleotides 334°C for 6 min and then at 60°C for 2 min each amplification was performed sequentially at 72°C for 2 min, at 94°C for 1 min, and at 60°C for 1 min. Primers for human GAPDH were forward: 5′-ACC ACA GTC CAT GCC ATC AC-3′ (nucleotides 586-605); and reverse: 5′-TCC ACC ACC CTG TTG CTG TA-3′ (nucleotides 1018-1037) producing a 451 bp product (GenBank accession sequence M33197) (17). The thermal cycling parameters consisted of 1 min at 94°C, 30 s at 55°C, 30 s at 72°C (18). 15 μl of the PCR products were electrophoresed in a 2% agarose gel and stained with a solution of ethidium bromide (0.5 μg/ml). Negative controls consisted of omitting the reverse transcription reaction or the cDNA product (data not shown). Positive control for human MDR-3 was performed using cDNA prepared from human liver tissue.

Preparation of liposomal C12-NBD-PC and functional assay using FACS. 2 mg of NBD-PC (2 ml of 1 mg/ml in chloroform) was added to 3 mg of egg phosphatidylcholine (150 μl of 20 mg/ml in chloroform) and a lipid film produced. Following rehydration with water and extrusion ($\times$10 through 0.1 μ pore polycarbonate membranes at 250–350 psi) uniform unilamellar liposomes (100 nm diameter) were produced containing NBD-PC tracer. Liposomes were then evaporated to dryness under nitrogen and used in transport studies on the day of preparation. The PC liposomes were then reconstituted in 500 μl of ethanol of which 35 μl was added to 10 ml of DMEM (without FBS) while stirring. Cells (5×10^5) grown on plastic were disaggregated, washed in PBS, pelleted and resuspended in 1 ml DMEM (without FBS). Cells were then incubated in the dark at 37°C for 1 h in the presence of C12-NBD-PC liposomes (C12-NBD-PC concentration of 3 μM). Following this, the cells were pelleted ($\times$600 g centrifugation for 5 min), washed in PBS and 50% of the cells then aliquoted for flow cytometric analysis representative of the C12-NBD-PC accumulation phase ([A]). For the study of C12-NBD-PC cellular retention ([R]), the remaining cells were resuspended in DMEM free of C12-NBD-PC, and its cellular efflux conducted over 2 h at 37°C, after which the cells were pelleted, washed in ice-cold PBS and analysed by flow cytometry. The effect of the P-gp modulator, verapamil (40 μM) upon both accumulation and retention phases was also studied. To eliminate any extracellular membrane associated C12-NBD-PC fluorescence, dithionite (20 mM) was added to the cell incubate at the end of either accumulation or retention phases just prior (1 min) to FACS analysis. Dithionite reduces the 7-nitro-2,1,3,-benzooxadiazol-4,yl (NBD) moiety eliminating (within 30 s) 98% of any extracellular membrane associated NBD fluorescence (22). Flow cytometry was performed with the collection of a minimum of 4000 events for each sample, with gated populations (excluding cellular debris and large cellular aggregates) analysed for cell associated fluorescence (FL-1 channel). Each treatment was represented by at least 5 replicates, and each experiment repeated at least twice.

P-gp mediated transport of rhodamine-123 and doxorubicin. The transport kinetics of well established P-gp substrates, rhodamine-123 and doxorubicin (19), were characterized for comparison to C12-NBD-PC kinetics and to confirm functional MDR1 P-gp expression.

Statistical analysis. Results are presented as mean $\pm$ SD. Student t-test comparisons were utilized for statistical analysis and significance was accepted if the two-sided P value was <0.05. The non-parametric Kolomogorov-Smirnov test (21) was used to compare flow cytometric histograms.

RESULTS AND DISCUSSION

Two types of P-gp are recognised in humans, MDR1 P-gp, which extrudes a variety of drugs across the plasma membrane and confers multidrug resistance in tumor cells, and MDR3 P-gp which is unable to transport cytotoxic drugs. Recently, in cells expressing recombinant MDR1, the MDR1 P-gp has been shown to transport phosopholipids, including phosphatidylcholine, phosphatidylethanolamine and sphingomyelin, while MDR3 P-gp is reported to transport only phosphatidylcholine (13). Here we examined phosphatidylcholine transport by MDR1 P-gp arising in epithelial cells expressing the multidrug resistant phenotype following prolonged exposure to cytotoxics.

Figure 1 shows the flow cytometric distributions of immunofluorescence in resistant, KBV1, and sensitive, KB3-1, cell lines when probed with the anti-P-gp monoclonal antibody, MRK16, which is specific for MDR1 and does not bind to MDR3 (20). KB3-1 was negative for MDR1 P-gp, displaying equivalent immunofluorescence distributions for the MRK16 and isotypic IgG_{2a} control antibodies. In the KBV1 cells, expression of MDR1 P-gp was confirmed with a 5.3 fold greater immunofluorescence for MRK16 antibody compared to isotypic control. Similar patterns of immunofluorescence were observed for MCFadr cells (2.3 fold greater immunofluorescence with MRK16 antibody) and its sensitive parental cell line, MCF7 (immunofluorescence distributions not shown). The Kolomogorov-Smirnov test verified statistical difference between the immunofluorescence histograms for the resistant and the sensitive cell lines; the calculated D values were 0.76 and 0.06 for KBV1 and KB3-1, respectively, whereas the D value for MCFadr and MCF7 were 0.65 and 0.02, respectively. Illustrated in Fig. 2a is an agarose gel image of the RT-PCR products obtained with the use of MDR1 primers. For MCFadr and KBV1 cells an MDR1 specific 156 bp amplified product is evident, while for MCF7 and KB3-1 cells no signal at the appropriate molecular weight is seen. In Fig. 2b can be seen the RT-PCR 451 bp product for the house-keeping gene, GAPDH, observed in all the cell lines, and liver sample, at equivalent levels implicating the reproducible harvesting of intact RNA from the cell preparations. MDR3 mRNA was not detected at any level in the resistant (MCFadr and KBV1) or the sensitive

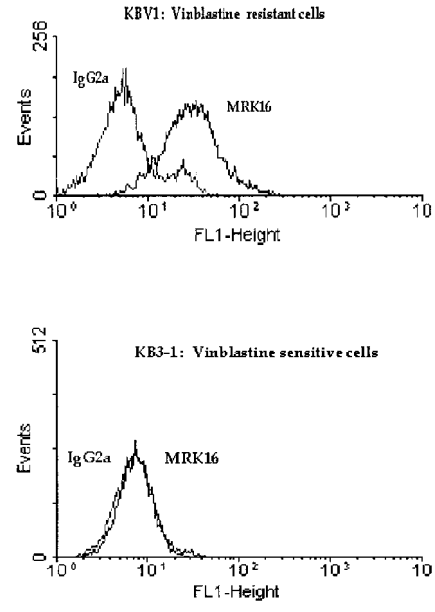

FIG. 1. Overlay plots showing the flow cytometric distribution of immunofluorescence in the MDR1-positive cell line, KBV1, and in the MDR1-negative cell line, KB3-1, when probed with the P-gp antibody, MRK16, versus an isotypic (IgG_{2a}) control antibody. Each plot represents 10,000 events. For KBV1 a significant shift in fluorescence compared to isotypic control antibody is seen with the MRK16 antibody.

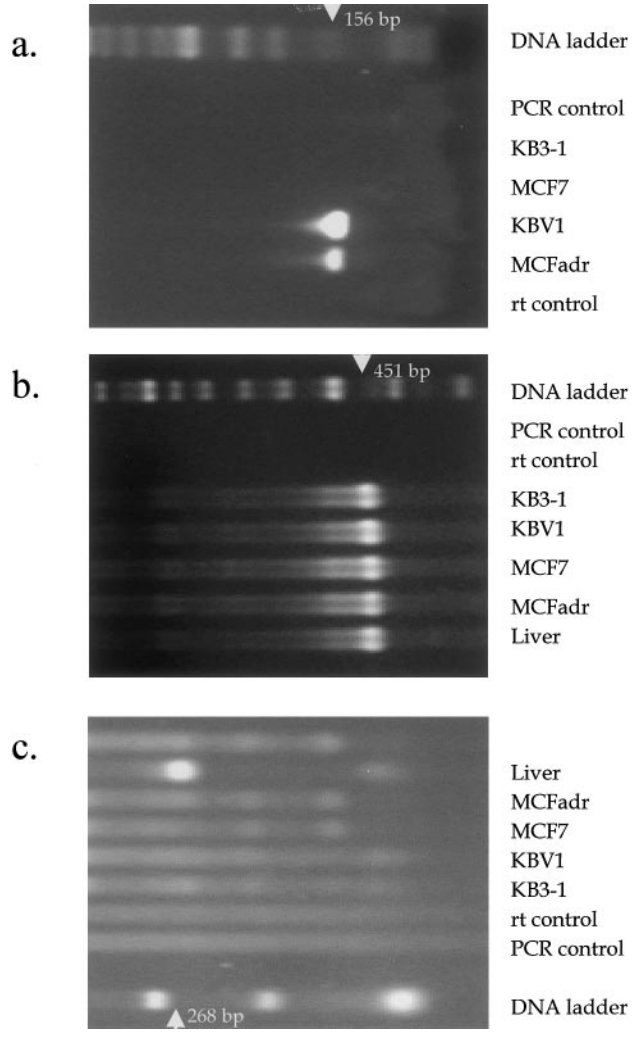

FIG. 2. Agarose gel of the RT-PCR-derived cDNA from sensitive (KB3-1, MCF7) and resistant (KBV1, MCFadr) cell lines, amplified using primers specific for: a, MDR1; b, GAPDH; and c, MDR3. cDNA was electrophoresed on agarose gels and post-stained with ethidium bromide. PCR control refers to omission of cDNA in the PCR reaction, while rt control refers to omission of the reverse transcription step.

(MCF7 and KB3-1) cell lines, while the positive control human liver produced an intense band at 268bp (Fig. 2c).

In Fig. 3 is shown the intracellular accumulation and retention of C12-NBD-PC fluorescence in sensitive and resistant cell lines, where background cell autofluorescence was less than 1.8 for any of the experimental variables. Consistant with a role for MDR1 P-gp in the cell efflux of phosphatidylcholine, the sensitive MDR1 P-gp negative cells lines, KB3-1 and MCF7, accumulated significantly greater ($P < 0.05$) C12-NBD-PC compared to their respective MDR1 P-gp positive, KBV1 and MCFadr, resistant cell lines. At the end of the accumulation phase C12-NBD-PC levels in KBV1 were 56%, and in MCFadr 60%, of the levels observed in the respective KB3-1 and MCF7 cell lines. Further, at the end of the efflux period both KBV1 and MCFadr cell lines showed significantly ($P < 0.05$) less cellular retention of C12-NBD-PC compared to their respective MDR1 P-gp negative parental cell lines. In particular a greater rate of cell efflux of C12-NBD-PC during the retention phase was evident in the KBV1 cells as revealed in Table 1. with KB3-1 accumulating 1.77 fold, and retaining 1.81 fold, greater C12-NBD-PC than in the KBV1 cell line. Table 1 also highlights how treatment with the MDR1 P-gp inhibitor, verapamil (40 μM), modulates the transport kinetics of C12-NBD-PC. Verapamil afforded increased accumulation of C12-NBD-PC in both MCFadr and KBV-1 cells, evidenced by a reduction in the accumulation ratio [Sensitive cells]/[Resistant cells]. Similarly, in the retention phase the continued incubation of verapamil afforded the transport kinetics of C12-NBD-PC in the resistant cells to more closely parallel that in the sensitive cells, e.g. retention ratio [MCF7]/[MCFadr] decreased from 1.35 to 1.05. The trend in KBV1 cells was similar, i.e., verapamil decreasing the retention ratio [Sensitive cells]/[Resistant cells], although the effects were not as profound. In Table 1 can also be seen comparison of C12-NBD-PC kinetics to that of the model high affinity MDR1 P-gp substrate, rhodamine-123, and to the cytotoxic agent, doxorubicin, another recognised MDR1 P-gp substrate. As expected the accumulation and retention of rhodamine-123 in the MDR1 P-gp negative cell lines was considerably greater than in the corresponding MDR1 P-gp positive cells, e.g. the retention of rhodamine-123 was 97-fold greater in MCF7 than in MCFadr, and 134-fold greater in KB3-1 than in KBV1. The transport differences between MDR1 P-gp positive and negative cells considerably diminished by treatment with verapamil. Comparison of C12-NBD-PC to

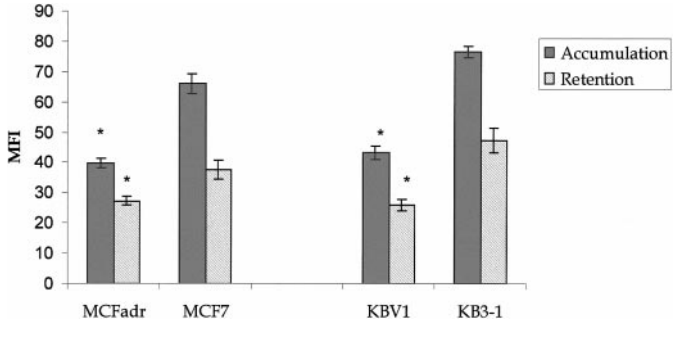

FIG. 3. Intracellular accumulation and retention of C12-NBD-PC in the parental-sensitive cells (KB3-1 and MCF7) and their respective cytotoxic selected resistant sublines (KBV1 and MCFadr), as described in Materials and Methods. Accumulation—Cells were incubated for 60 min at 37°C in media containing 3 μM C12-NBD-PC. Retention—Cells were then washed and C12-NBD-PC efflux carried out over 120 min at 37°C in C12-NBD-PC-free media. Data are expressed as Mean ± s.d. ($n = 5$) and each experiment repeated at least twice. An asterisk indicates a statistically significant ($P < 0.05$) difference between resistant and sensitive cells in the respective accumulation or retention phases.

TABLE 1

Intracellular Accumulation and Retention of C12-NBD-PC (3 μM), Rhodamine-123 (0.2 μg/ml) and Doxorubicin (1.3 μg/ml) in the Resistant and Sensitive Cells, Conducted in the Absence or Presence of Verapamil (40 μM)

	Accumulation		Retention	
	MCF7/MCFadr	KB3-1/KBV1	MCF7/MCFadr	KB3-1/KBV1
C12-NBD-PC	1.66 ± 0.04	1.77 ± 0.02	1.37 ± 0.05	1.81 ± 0.06
C12-NBD-PC + verapamil	0.73 ± 0.03	0.91 ± 0.06	1.05 ± 0.12	1.52 ± 0.06
Rhodamine 123	7.38 ± 0.15	12.43 ± 0.05	96.90 ± 0.24	134.00 ± 0.10
Rhodamine 123 + verapamil	1.16 ± 0.23	1.54 ± 0.08	1.30 ± 0.21	4.20 ± 0.11
Doxorubicin	1.43 ± 0.05	1.67 ± 0.03	2.61 ± 0.08	2.31 ± 0.07
Doxorubicin + verapamil	0.89 ± 0.02	1.16 ± 0.05	0.99 ± 0.04	1.09 ± 0.04

Note. Study design followed that described in Fig. 3 legend and in Materials and Methods. Data are expressed as the accumulation or retention ratios in [Sensitive]/[Resistant] cells. The sensitive cells (MDR1 P-gp negative) will accumulate and retain the fluorescent probes to a greater extent than the resistant (MDR1 P-gp positive) cells. In the presence of the MDR1 P-gp inhibitor, verapamil, the ratio [Sensitive]/[Resistant] will decrease toward 1. Mean ± s.d. (n = 5) and each experiment repeated at least twice.

rhodamine-123 indicates phosphatidylcholine turnover kinetics to be relatively low. Doxorubicin kinetics more closely resembled that of C12-NBD-PC, with accumulation and retention of doxorubicin in the sensitive cells between 1.4-fold and 2.6-fold greater than in resistant cells, a differential effect considerably diminished by verapamil treatment.

The physiological role of MDR3 P-gp in humans and mdr2 in rodents as a phospholipid transporter has been revealed by studies in mice with disrupted mdr2 gene failing to secrete phospholipid into the bile and eventually suffering severe liver disease (23). Indeed, mutations in the human MDR3 gene cause progressive familial intrahepatic cholestasis (24). In this current study we show in two MDR3 negative epithelial cell lines induced to express the MDR1 P-gp phenotype by exposure to cytotoxics a role for MDR1 P-gp phenotype in phosphatidylcholine transport. The transport by MDR1 P-gp of phosphatidylcholine from inner to outer membrane leaflet may well function to regulate the access or binding of substrates to P-gp, influence directly P-gp efflux pump activity, and indeed fulfill a function in the MDR1 multidrug resistant phenotype. By transporting phospholipids across the lipid bilayer, P-gp drug binding affinity may be adjusted to give optimum efflux pump activity. Nevertheless, the binding affinity of phospholipids for MDR1 should be low, giving susbtrate binding priority to xenobiotics whose efflux from the cell via the P-gp pump would be essential to maintain cell viability. MDR1 P-gp functions as a "hydrophobic vacuum cleaner" interacting with its substrates from within the membrane itself rather than within the cytoplasm (25). Trafficking of phospholipids between membrane leaflets could determine the differential partitioning of substrates within the membrane and their access to P-gp binding sites. Although little is known about the movement of P-gp substrates across the membrane bilayer, there is now an overwhelming amount of evidence indicating that changing the lipid environment will modulate P-gp function. P-gp ATPase function is highly dependent on the surrounding lipid environment (26). There are 55 different phospholipids, essential for ATPase activity, that remain tightly associated with the P-gp molecule after purification using CHAPS (27). Notably, phosphatidylcholine is the most effective phospholipid restoring P-gp ATPase activity after inactivation by delipidation with triton X-100 (28). Romsicki and Sharom (29) reported that binding affinity of vinblastine to purified P-gp was critically dependent on the lipid environment surrounding the protein, and when the choline lipid head group was changed to ethanolamine (with similar acyl chains), a 17 fold increase in binding affinity was observed. Even increasing saturation of the acyl chain will result in an increase in drug binding affinity. Further, several P-gp modulators can inhibit P-gp function by modifying membrane properties, including the non ionic surfactants Solutol HS15 and Cremophor EL (30), and the membrane fluidizer benzyl alcohol (31).

In summary we report a role for MDR1 P-gp in the transport of phosphatidylcholine in MDR3- negative epithelial cells that have been induced to express the MDR1 P-gp multidrug resistant phenotype by prolonged exposure to cytotoxic agents. The transport of phospholipids by MDR1 P-gp may fulfill a regulatory function in the multidrug resistant phenotype.

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