A Human Lymphocyte Based *Ex Vivo* Assay to Study the Effect of Drugs on P-Glycoprotein (P-gp) Function[1]

Dolly A. Parasrampuria,[2,3] Marianne V. Lantz,[4] and Leslie Z. Benet[2,5]

Received October 4, 2000; accepted October 12, 2000

Purpose. The effect of drugs on P-glycoprotein (P-gp) is normally studied in transfected or overexpressing cell lines derived from tumor cells or animal tissue. We wanted to develop an assay using normal healthy human tissue to study and characterize the drug-transporter interaction.

Methods. Lymphocytes were isolated from healthy human blood. The effect of inhibitors of P-gp (cyclosporine, tacrolimus, verapamil, quinidine, vinblastine) and of other transporters (indomethacin, probenecid, sulfinpyrazone) on intracellular accumulation of rhodamine 123 was evaluated by flow cytometry.

Results. The efflux of rhodamine 123 was inhibited by P-gp inhibitors in a saturable, concentration-dependent manner. The potency of inhibition of P-gp was cyclosporine > tacrolimus > quinidine > verapamil > vinblastine. Vinblastine inhibited P-gp at lower concentrations, whereas at high concentrations, there was an activation of rhodamine 123 efflux from lymphocytes. The multidrug resistance associated protein (MRP) inhibitors, sulfinpyrazone and probenecid, did not have any significant effect on intracellular accumulation of rhodamine 123, but indomethacin caused a concentration-dependent increase in retention of rhodamine 123, indicating the involvement of other uncharacterized transporters.

Conclusions. Lymphocytes can serve as a model tissue for studying modulation of P-gp activity by drugs. Both inhibitors and inducers of P-gp activity can be evaluated.

KEY WORDS: P-glycoprotein (P-gp); multidrug resistance associated protein (MRP); rhodamine 123; flow cytometer; cyclosporine; tacrolimus; vinblastine; verapamil; quinidine.

INTRODUCTION

Transporters that are similar in structure and function are present in diverse organisms such as yeast, bacteria, and humans. ATP-binding cassette (ABC) transporters are one such class of energy dependent transporters (1). The multidrug resistance (MDR) and multidrug resistance associated protein (MRP) groups of transporters belong to this class (2,3). The MDR genes encode P-glycoprotein (P-gp) that has a wide tissue distribution in humans (4). P-gp transporters are also present in human pathogens such as *Plasmodium falciparum, Leishmania donovani,* and *Candida albicans* (5). In humans, P-gp is predominantly involved in xenobiotic efflux, thereby protecting host tissue from toxic side effects.

Most P-gp substrates are hydrophobic molecules with a basic nitrogen atom and two planar aromatic rings, but there are exceptions (6). The substrates include such structurally dissimilar drugs as peptides, alkaloids, steroids, immunosuppressive drugs, and calcium channel blockers (7,8). The molecular weights of most substrates range from 300 to 2000 Da (8).

P-gp is widely expressed in several tissues (4) and is overexpressed in several tumors, making them refractory to drug therapy. In tissues such as the intestines, P-gp limits the bioavailability of orally delivered drugs such as paclitaxel, by pumping the drugs back into the gut lumen (9). The widespread expression of P-gp, not only in malignant cells but normal human tissues, has important implications in drug therapy. The drugs can be substrates of P-gp where their intracellular concentrations could be altered because of efflux; the drugs could themselves modulate the expression and/or function of P-gp, thereby altering not only their intracellular concentrations but also those of other drugs that may be substrates of P-gp. This could result in drug-drug interactions. Defining the specific P-gp modulators and their kinetics is thus essential in order to fully characterize and understand pharmacokinetic and pharmacodynamic interactions of these drugs.

Most substrates and inhibitors of P-gp have been characterized *in vitro* using tumor-derived cell lines such as Caco-2, or transfected animal tissue-derived cell lines such as MDCK-MDR1, which overexpress P-gp following exposure to chemotherapeutic drugs (10–12). *In vivo* experiments have been performed in normal and knockout mice (13). The information generated by both methods is extremely valuable, but it is possible that there may be biochemical differences in the expressed P-gp in both kinds of models (14). Thus, it is of interest to study P-gp function in normal human tissues.

Our goal was to develop and characterize an assay method using normal human tissue to study the effect of drugs on P-gp-mediated transport of a model substrate. Lymphocytes represent such a tissue because they inherently express P-gp (15) and are relatively easy to procure. The functional activity of P-gp can be investigated by measuring rhodamine 123 (Rh 123) retention/efflux in the presence of P-gp modulators. Rhodamine 123 is a cationic, fluorescent dye that is readily taken up by cells and actively pumped out of the cells by P-gp (16). The ability of lymphocytes to efflux Rh 123 in the presence of a P-gp inhibitor is decreased (15,17,18), leading to increased intracellular accumulation of Rh 123. This increase in intracellular retention of Rh 123 is reflected in increased intensity of Rh 123 fluorescence. By measuring the intensity of Rh 123 in the presence and absence of inhibitors, the inhibition constants for different drugs can be determined. In a clinical study, Robey and co-workers (19) used Rh 123 to study the effects of *in vivo* inhibition of P-gp in patients dosed with PSC 833 along with vinblastine or paclitaxel. We investigated the effect of known P-gp inhibitors (7):

[1] Presented in part at the 14th Annual Meeting of the American Association of Pharmaceutical Scientists, New Orleans, Louisiana, 1999.

[2] Department of Biopharmaceutical Sciences, School of Pharmacy, University of California, San Francisco, California 94143-0446.

[3] Present address: Clinical Drug Metabolism, The R.W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08869.

[4] Department of Surgery, School of Medicine, University of California, San Francisco, California 94143-0446.

[5] To whom correspondence should be addressed. (e-mail: benet@itsa.ucsf.edu)

0724-8741/01/0100-0039$19.50/0

cyclosporine, tacrolimus (FK506), quinidine, verapamil, and vinblastine. We also studied the effect of drugs that are not inhibitors of P-gp but of other drug efflux transporters (10,20), such as MRP. These drugs included probenecid, sulfinpyrazone, and indomethacin.

MATERIALS AND METHODS

Chemicals and Specimens

Cyclosporine, quinidine, verapamil HCl, vinblastine sulfate, indomethacin, sulfinpyrazone, probenecid, sodium azide, and rhodamine 123 were purchased from Sigma Chemical Co. (St. Louis, MO). Fujisawa Pharmaceutical Company (Deerfield, IL) kindly provided Tacrolimus (FK 506). Mycophenolate Mofetil (MMF) was a kind gift from Roche Biosciences (Palo Alto, CA). Optilyse C was purchased from Immunotech (Marseille, France). Dye-free Ca^{2+}/Mg^{2+}-free Hanks BaLanced Salt Solution (BSS), RPMI 1640 with 25 mM *N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid (HEPES), fetal calf serum (FCS), phosphate buffered saline (PBS), bovine serum albumin (BSA), and cell preservation medium (10% FCS, 10% dimethylsulfoxide [DMSO] in minimum Eagle's essential medium [MEM] EarLe's BSS (EBSS), pH 7.2) were all obtained from the UCSF cell culture facility (San Francisco, CA). Acridine orange, ethidium bromide, and propidium iodide were procured from Molecular Probes Inc. (Eugene, OR). Ficoll-Paque® was obtained from Pharmacia Biotech AB (Uppsala, Sweden). Flow-Check™ fluorospheres were purchased from Coulter Corporation (Miami, FL). Custom-ordered unconjugated P-gp antibody, 15D3, second-step goat anti-mouse Ig fluorescein isothiocyanate (FITC) and mouse Ig fluorescence control antibodies were purchased from Becton-Dickinson (San Jose, CA). All other solvents were obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated by density centrifugation from buffy coats purchased from the UCSF blood bank (San Francisco, CA). Briefly, blood was diluted with dye-free Ca^{2+}/Mg^{2+} free Hanks BSS at the ratio of 1:2 (buffy coat/Hanks). The diluted blood was layered according to manufacturer's instructions on top of Ficoll-Hypaque (Ficoll-Paque) and centrifuged at 1500 rpm for 30 min. The PBMC-rich layer was withdrawn and washed twice with Hanks BSS (dye-free, Ca^{2+}/Mg^{2+}-free). Cells were resuspendend in cell preservation medium at 40 million cells/ml in each vial and cryo-frozen in liquid nitrogen for later use.

Rhodamine 123 Accumulation/Efflux

PBMCs were thawed by rapid increase in temperature to 37°C. The cells were washed twice with RPMI 1640 (with 10% FCS). Cells were allowed to equilibrate in RPMI 1640 (with 10% FCS) for 1 h at 37°C. After 1 h, cell viability and cell count was assessed by ethidium bromide/acridine orange uptake. If greater than 10% cells took up ethidium bromide, the dead cells were removed from the viable by Ficoll-Hypaque density centrifugation as described above. RPMI 1640 was washed off and cells were resuspended in Hanks BSS (dye-free, Ca^{2+}/Mg^{2+}-free) at 1 million cells/ml. Cells were incubated with or without inhibitors for 15 min at 37°C. After incubation with inhibitors, without washing, PBMCs were incubated with 20 ng/ml rhodamine 123 for 30 min at 37°C. After this incubation period, cells were placed on ice and incubated with 300 μl Optilyse C for 5 min to lyse contaminating erythrocytes. Following incubation, the PBMCs were washed twice with ice-cold Hanks BSS (dye-free, Ca^{2+}/Mg^{2+}-free) and resuspended in ice-cold PBS (with 0.5% BSA and 0.1% sodium azide). An EPICS XL flow cytometer (Coulter) with a 488-nm argon laser was used to analyze the samples. Rhodamine 123 fluorescence was collected after a 525-nm bandpass filter and propidium iodide fluorescence was collected after a 575-nm bandpass filter. Data were collected for a minimum of 5000 events per sample, and the samples were gated on forward scatter versus side scatter to exclude nonlymphocyte populations, clumps, and debris. Propidium iodide staining was used for excluding dead cells from the analysis. The instrument was calibrated each time before analysis using Flow-Check fluorospheres for day-to-day and within-day stability, sensitivity, and resolution.

Data Analysis

The data were collected as mean intensity for all samples. The intensity of rhodamine fluorescence was plotted against increasing concentration of inhibitor. Data from net intensity versus inhibitor concentration were fitted to the Michaelis-Menten equation using Kaliedagraph™ 3.0.2 (Abelbeck Software) to derive inhibition constants and maximum effect.

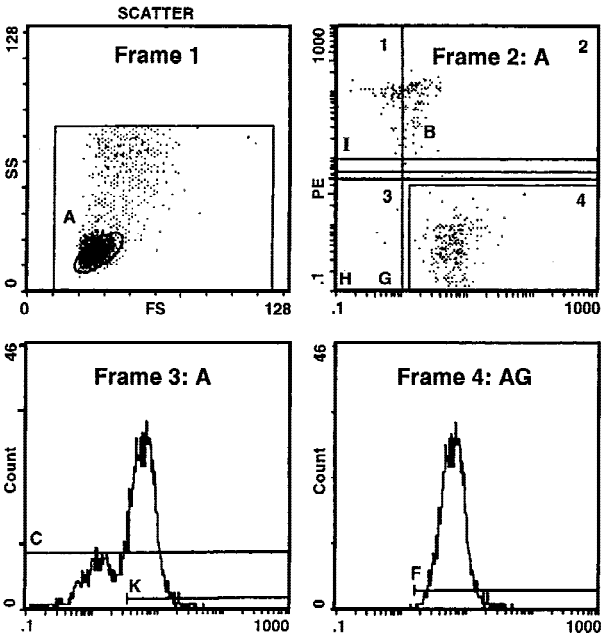

Fig. 1. Representative flow chromatogram for rhodamine 123 uptake. Frame 1: Gate E consists of all cells sampled from the tube. Gate A is based on size and shape and represents mostly lymphocytes. Frame 2: Cells in gate A from frame 1 are further subdivided into live (gate G,quadrant B4) and dead (gate I, quadrants B1 and 2) based on propidium iodide uptake by dead cells. Frame 3: Represents all cells in gate A from frame 1 which take up rhodamine 123 and segregate into two populations based on intensity at channel FL1 (505–545 nm). Frame 4: Live cells in gate A due to rhodamine 123 uptake (FL1).

RESULTS

Figure 1 shows the flow chromatogram for rhodamine 123 accumulation by lymphocytes. In these experiments, the cells were gated (selected for analysis) on the basis of shape and size determined from initial experiments using specific antibodies for T-cells. Live cells as well as apoptosing cells took up rhodamine 123. For accurate analysis of functional P-gp, it is essential to exclude the dead and apoptosing cells from the live cell population using propidium iodide (PI) staining. The live cells did not stain with PI and appeared as a distinct population in gate G, frame 2 (Fig. 1). The intensity of this peak represents true accumulation of Rh 123 by viable cells and was quantified in the presence and absence of an inhibitor to represent the functional characteristics of P-gp. In the presence of an inhibitor such as tacrolimus, the peak intensity of Rh 123 increases (Fig. 2).

P-glycoprotein is an energy-dependent efflux pump with maximum activity at physiological temperature (37°C). To determine if Rh 123 efflux was an active process, the accumulation/efflux was studied at two different temperatures, 4 and 37°C, in the presence and absence of tacrolimus (Fig. 3). The accumulation of Rh 123 as measured by mean intensity was significantly higher at 37°C both with and without the inhibitor. The mean intensity of Rh 123 was sevenfold higher at 37°C than at 4°C without the inhibitor. In the presence of tacrolimus (P-gp inhibitor), there was a 13-fold increase in intensity of Rh 123 at 37°C compared to 4°C. At 4°C, there was no difference in the accumulation of rhodamine when an inhibitor (tacrolimus) was present.

Lymphocytes were incubated with different escalating concentrations of cyclosporine, tacrolimus, quinidine, or verapamil, resulting in a corresponding increase in Rh 123 intensity of viable cells with respect to the Rh123 intensity in the presence of solvent alone (Figs. 2 and 4). These analyses were performed over a wide concentration range determined by the boundary conditions of "no effect" to "extensive cell death" due to toxicity of test compound *in vitro*. On the basis of these studies, the net increase in intensity of Rh 123 was determined and plotted against concentration of inhibitor to determine the inhibition constants and maximum inhibitory effect (Table 1). When lymphocytes were incubated with increasing concentrations of MMF, there was no significant effect on efflux of Rh 123 (data not shown).

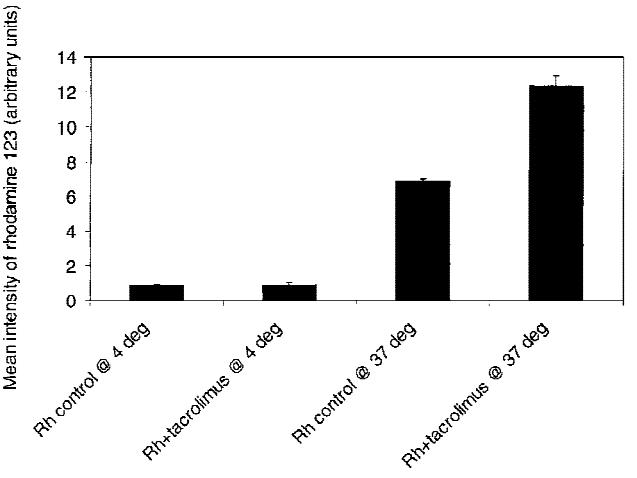

Fig. 3. Effect of temperature on uptake and efflux of rhodamine 123 in the presence and absence of an inhibitor (tacrolimus). $N = 3$ (mean ± SD).

To investigate if the increase in Rh 123 intensity was due to inhibition of P-gp or other transporters, we investigated the effect of other compounds that are not P-gp inhibitors such as indomethacin, probenecid, and sulfinpyrazone. Draper and co-workers (19) showed that indomethacin does not modulate P-gp-mediated efflux of Rh 123 in P-gp expressing murine and human cell lines (PC-V160 and HL60/Vinc). Here, however, indomethacin resulted in a concentration-dependent, saturable increase in Rh 123 accumulation (Fig. 5). Over the concentration range tested, probenecid and sulfinpyrazone did not result in a significant increase in accumulation of Rh 123 with respect to solvent control (data not shown).

Vinblastine is a known P-gp inhibitor. To determine the kinetics of P-gp inhibition, different concentrations of vinblastine were tested. With increasing concentrations of vinblastine, there was an increase in accumulation of Rh 123, but at higher concentrations of vinblastine, there was a decrease in intensity of Rh 123 (Fig. 6). This was a curious phenomenon because the lymphocytes are treated with vinblastine for

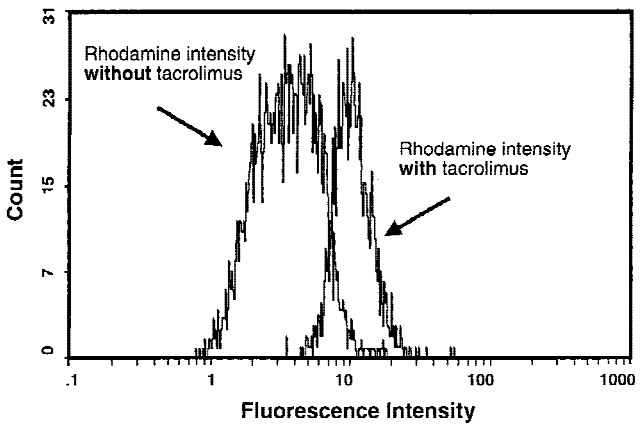

Fig. 2. Representative chromatogram for rhodamine 123 uptake in the presence (right) and absence (left) of an inhibitor (tacrolimus) of P-glycoprotein.

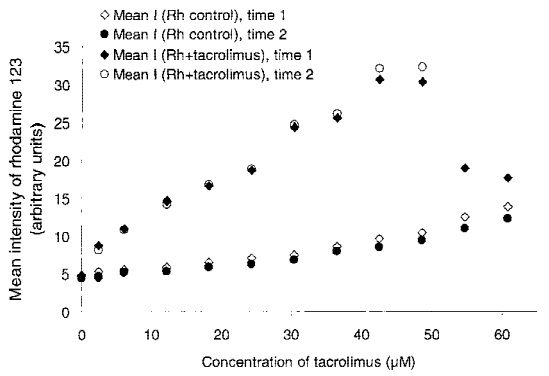

Fig. 4. Representative plot for P-gp inhibition by test compounds (tacrolimus). The fluorescence intensity due to Rh 123 uptake is plotted in the presence of solvent control and in the presence of test compound at two different times (immediately afterward and 2 h later).

Table 1. Kinetic Parameters for Inhibition of Rhodamine 123 Efflux by Lymphocytes

Inhibitor	N (no. of blood sample sources)	n (data points for kinetic parameters)	K_i (μM)	E_{max} (arbitrary units)
Cyclosporine	3	8	0.3 ± 0.2	3.9 ± 1.1
Tacrolimus	3	5	8.8 ± 4.2	15.1 ± 5.4
Quinidine	3	4	24.6 ± 17.4	3.8 ± 1.6
Verapamil	3	8	40.1 ± 12.7	7.0 ± 1.9
Vinblastine	1	3	82.4	21.9
Indomethacin[a]	1	3	199.2	30.1

[a] Indomethacin is not a P-gp inhibitor; it inhibits the efflux of rhodamine 123 from lymphocytes via an unknown mechanism.

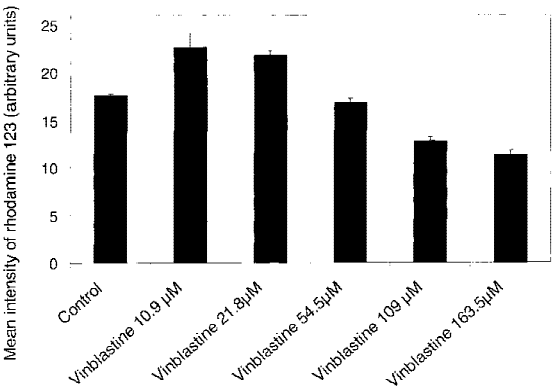

Fig. 6. Effect of vinblastine on rhodamine 123 accumulation by lymphocytes. $N = 3$ (mean ± SD).

less than an hour during the experiment, and no other reference has been found in the literature for a similar effect. To investigate if this effect was due to increased expression of P-gp, lymphocytes were incubated with vinblastine and anti-P-gp antibody, 15D3, or appropriate isotype control. There did not appear to be a significant change in the expression of P-gp as detected by antibody binding.

There were some interesting observations in these experiments. In experiments involving verapamil, quinidine, and cyclosporine, there appear to be at least two different transporters that are involved in Rh 123 efflux and are inhibited by these compounds. One of these was a high-affinity (P-gp) transporter (in the micromolar range) which was saturated in the test inhibitor concentration range, and another low-affinity transporter (probably in the millimolar range) which could not be saturated at concentrations that could be tested (because of decreased cell viability at higher concentrations). Tacrolimus does not appear to have any effect on this other unknown transporter. The maximum effect for accumulation of Rh 123 was observed with tacrolimus, which was at least twofold higher than the other P-gp inhibitors, cyclosporine, quinidine, and verapamil. The other interesting observation was with regard to cell viability in the presence of an inhibitor. In general, increasing concentrations of drugs and solvents (ethanol, acetonitrile, and DMSO) were toxic to cells. Cyclosporine however, appeared to have a protective effect on cells at higher (above 10 μM) concentrations (Fig. 7).

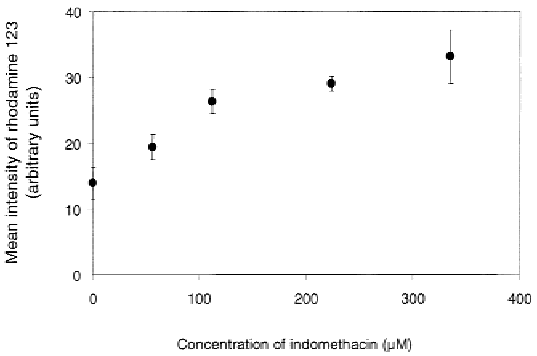

Fig. 5. Effect of indomethacin on rhodamine 123 accumulation by lymphoyctes. $N = 3$ (mean ± SD).

DISCUSSION

These results show that lymphocytes have functional P-gp, which is inhibited by various drugs. The activity of P-gp is temperature dependent both in the presence and absence of an inhibitor. At 4°C, the P-gp was not functional and there was no effect when inhibitor was added, whereas at 37°C, Rh 123 accumulation was higher (Fig. 3). The uptake of Rh 123 at lower temperature was also markedly lower than at 37°C. This implies that the uptake of Rh 123 may involve active or facilitated transport processes.

Verapamil, quinidine, tacrolimus, and cyclosporine inhibit the P-gp-mediated efflux of Rh 123 in the following order: cyclosporine>tacrolimus>quinidine>verapamil. The maximum increase in Rh 123 accumulation did not correspond to the inhibitory potential of these drugs. The maximum effect on rhodamine accumulation was in the order: tacrolimus>verapamil>cyclosporine>quinidine. The difference in maximum activity and potency may indicate the presence of other transporters. The inhibitory effect of all these drugs was observed up to 2 h 30 min after the inhibitor was washed off. The high variability in the K_i and E_{max} values (Table 1) reflect in part the inherent variability in P-gp from different blood sources. Similar results are obtained when cytochrome P450 activity is compared in human liver microsome samples from different individuals. Inhibition constants (K_i) were somewhat higher at later time points, but the increase was not significantly different. The maximum effect on

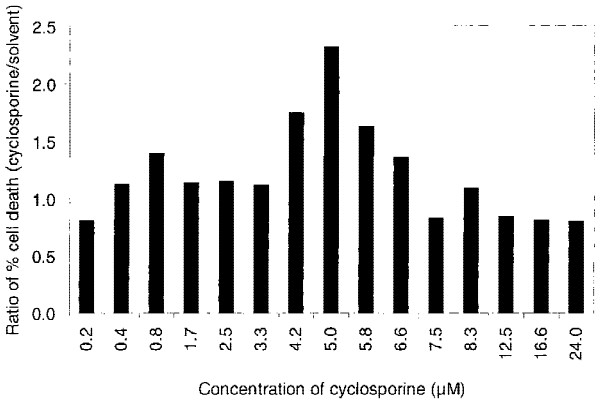

Fig. 7. Effect of cyclosporine on cell viability in rhodamine 123 accumulation/efflux studies.

rhodamine accumulation was the same at different times. At higher concentrations, verapamil, quinidine, and cyclosporine appear to inhibit another low-affinity transporter. In Rh 123 uptake studies, it is important to measure Rh 123 intensity in live cells because including data from apoptosing/dying cells can skew the analysis.

The activation of Rh 123 transport by P-gp at high concentrations of vinblastine was an interesting observation because it did not involve an increase in expression of P-gp. Most studies reported in the literature have used lower concentrations (usually lower than 50 μM) of vinblastine where it shows inhibitory effects on P-gp (21,22). The molecular mechanism underlying this phenomenon is not yet understood, but could involve allosteric conformational changes in P-gp, thus affecting the transport of rhodamine 123.

In our experiments, known MRP inhibitors such as sulfinpyrazone and probenecid did not result in an increase in the accumulation of Rh 123 over a range of concentrations tested. However, indomethacin, which is reported in the literature as an inhibitor of MRP but not of P-gp, resulted in an increase in the accumulation of Rh 123 in lymphocytes from different sources (inhibition curve presented in Fig. 5 is from one source). We speculate that this increase probably results from the effect of indomethacin on some other transporter also capable of transporting Rh 123. These results are also supported by other unpublished studies from our laboratory in which indomethacin appears to inhibit an unknown transporter distinct from MRP and P-gp. Hatse and co-workers (23) also reported the existence of a highly specific indomethacin-sensitive efflux pump in an erythroleukemia cell-line. They report that this indomethacin-sensitive pump is not susceptible to verapamil and cyclosporine or MRP inhibitors such as genistein. It is involved in the transport of Rh 123. Annaert and co-workers (24) reported that a distinct anion-specific transporter that is inhibited by indomethacin is present in Caco-2 monolayers.

Cyclosporine at micromolar concentrations appeared to exert a protective effect on lymphocytes. This effect could be due to mitochondrial protection by inhibition of P-gp or other transporters, thereby protecting the cell from toxic effects of solvent or rhodamine. The other drugs did not have any such effects on cell viability. For lymphocyte-based rhodamine 123 uptake studies, dye-free $Ca^{2+/}Mg^{2+}$-free Hanks BSS buffer was the preferred medium as compared to PBS, which compromised cell viability.

The results suggest that lymphocytes can be used for studying the effect of drugs on P-gp function and expression. The advantage of using lymphocytes instead of overexpressed cell-lines is that the lymphocytes are normal human tissue and the parameters obtained using such a tissue are likely to be a better representation of true effects of drugs. This assay method is fast and can be used in high-throughput screening of compounds. Lymphocytes can be used for studying both induction and inhibition effects of drugs on transporters. However, there are particular limitations. The level of P-gp expression in lymphocytes is low and requires the use of sensitive instruments such as a flow cytometer for appropriate analysis. Compared to overexpressed or transfected cell-lines, lymphocytes are fragile and difficult to use. Because of the presence of several transporters, the results can be difficult to assign to a particular transporter.

ACKNOWLEDGMENTS

We are grateful to Dr. Nancy Ascher (Departments of Medicine and Surgery) for the use of flow cytometer and laboratory equipment.

REFERENCES

1. C. F. Higgins. ABC transporters: from microorganisms to multidrug efflux systems. *Microbiol. Rev.* **60**:575–608 (1992).
2. S. P. C. Cole, G. Bhardwaj, J. H. Gerlach, J. E. Mackie, C. E. Grant, K. C. Almquist, A. J. Stewart, E. U. Kurz, A. M. V. Duncan, and R. G. Deeley. Overexpression of a transporter gene in a multidrug-resistnat human lung cancer cell line. *Science* **258**: 1650–1654 (1992).
3. M. M. Gottesman and I. Pastan. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu. Rev. Biochem.* **62**:385–427 (1993).
4. J. P. O'Brien and C. Cordon-Cardo. P-glycoprotein expression in normal human tissues. In S. Gupta and T. Tsuruo (eds.), *Multidrug Resistance in Cancer Cells,* John Wiley, New York, 1996, pp. 285–291.
5. H. W. van Veen and W. N. Konigs. Multidrug transporters from bacteria to man: similarities in structure and function. *Sem. Cancer Bio.* **8**:183–191 (1997).
6. J. M. Zamora, H. L. Pearce, and W. T. Beck. Physical-chemical properties shared by compounds that modulate multidrug resistance in human leukemic ells. *Mol. Pharmacol.* **33**:454–462 (1988).
7. V. J. Wacher, C-Y. Wu, and L. Z. Benet. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Mol. Carcinogen.* **13**:129–134 (1995).
8. K. Ueda, Y. Taguchi, and M. Morishima. How does P-glycoprotein recognize its substrates? *Sem. Cancer Biol.* **8**:151–159 (1997).
9. A. Sparreboom, J. van Asperen, U. Mayer, A. H. Schinkel, J. W. Smit, D. K. F. Meijer, P. Borst, W. J. Nooijen, J. H. Beijnen, and O. van Tellingen. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc. Natl. Acad. Sci. USA* **94**:2031–2035 (1997).
10. Z. Hollo, L. Homolya, T. Hegedus, and B. Sarkadi. Transport properties of the multidrug resistance-associated protein (MRP) in human tumour cells. *FEBS Lett.* **383**:99–104 (1996).
11. F. Tiberghien and F. Loor. Ranking of P-glycoprotein substrates and inhibitors by a calcein-AM fluorometry screening assay. *Anti-Cancer Drugs* **7**:568–578 (1996).
12. S. D. Flanagan and L. Z. Benet. Net secretion of furosemide is subject to indomethacin inhibition, as observed in Caco-2 monolayers and excised rat jejunum. *Pharm. Res.* **16**:221–224 (1999).
13. A. H. Schinkel, J. J. M. Smit, O. van Tellingen, J. H. Beijnen, E. Wagenaar, L. van Deemter, C. A. A. M. Mol, M. A. van der Valk, E. C. Robanus-Maandag, H. P. J. te Riele, A. J. M. Berns, and P. Borst. Disruption of the mouse *mdr1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**:491–502 (1994).
14. M. M. Hoffman and P. D. Roepe. Analysis of ion transport perturbations caused by hu MDR 1 protein overexpression. *Biochemistry* **36**:11153–11168 (1997).
15. S. Aggarwal, T. Tsuruo, and S. Gupta. Altered expression and function of P-glycoprotein (170 kDa), encoded by the MDR 1 gene, in T cell subsets from aging humans. *J. Clin. Immunol.* **17**:448–454 (1997).
16. A. B. Shapiro and V. Ling. The mechanism of ATP-dependent multidrug transport by P-glycoprotein. *Acta Physiol. Scand. Suppl.* **643**:227–234 (1998).
17. A. Andreana, S. Aggarwal, S. Gollapudi, D. Wien, T. Tsuruo, and S. Gupta. Abnormal expression of a 170-kilodalton P-glycoprotein encoded by MDR1 gene, a metabolically active efflux pump, in CD4+ and CD8+ T cells from patients with human

immunodeficiency virus type1 infection. *AIDS Res. Hum. Retrovir.* **12**:1457–1462 (1996).
18. J. Drach, A. Gsur, G. Hamilton, S. Zhao, J. Angerier, M. Fiegi, N. Zojer, M. Raderer, I. Haberl, M. Andreef, and H. Huber. Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4, and interferon-γ in normal human T lymphocytes. *Blood* **88**:1747–1754 (1996).
19. R. Robey, S. Bakke, W. Stein, B. Meadows, T. Litman, S. Patil, T. Smith, T. Fojo, and S. Bates. Efflux of rhodamine from $CD56^+$ cells as a surrogate marker for reversal of P-glycoprotein-mediated drug efflux by PSC 833. *Blood* **93**:306–314 (1999).
20. M. P. Draper, R. L. Martell, and S. B. Levy. Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. *Br. J. Cancer* **75**:810–815 (1997).
21. Z. Hollo, L. Homolya, C. W. Davis, and B. Sarkadi. Calcein accumulation as a flurometric functional assay of the multidrug transporter. *Biochim. Biophys. Acta* **1191**:384–388 (1994).
22. A. Soldner, U. Christians, M. Susanto, V. J. Wacher, J. A. Silverman, and L. Z. Benet. Grapefruit juice activates P-glycoprotein-mediated drug transport. *Pharm. Res.* **16**:478–485 (1999).
23. S. Hatse, E. De Clercq, and J. Balzarini. Enhanced 9-(2-phosphonylmethoxyethyl) adeninyl) adenine-esistant human erythroleukemia K562 cell line. *Mol. Pharmacol.* **54**:907–917 (1998).
24. P. Annaert, J. Van Gelder, L. Naesens, E. De Clercq, G. Van den Mooter, R. Kinget, and P. Augustijns. Carrier mechanisms involved in the transepithelial transport of bis(POM)-PMEA and its metabolites across Caco-2 monolayers. *Pharm. Res.* **15**:1168–1173 (1998).