Biochemical and Biophysical Research Communications **279,** 124–130 (2000)
doi:10.1006/bbrc.2000.3916, available online at http://www.idealibrary.com on IDEAL®

Carrier-Mediated Uptake of Rhodamine 123: Implications on Its Use for MDR Research

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Received October 30, 2000

We have examined the effects of verapamil and PSC 833 on cellular uptake and release of rhodamine 123 (R123) in two human cancer cell lines. Both verapamil and PSC 833 were able to increase R123 accumulation in the multidrug resistant (MDR) MV522/Q6 and KB-8-5 lines in the release study. However, the effects of these drugs on R123 accumulation during accumulation study were quite different. Incubation with PSC 833 increased R123 accumulation in both MDR lines. By contrast, incubation with verapamil only increased R123 accumulation in the KB-8-5 line. The failure of verapamil to increase R123 accumulation in the MV522/Q6 cells can be attributed to the presence of a carrier system in the parent MV522 cells that recognizes both R123 and verapamil, but not PSC 833, as substrates. These results imply that performing R123 accumulation study without first ascertaining possible role of a carrier system for cellular uptake of R123 and putative P-gp modulators might inadvertently lead one to draw improper conclusions on P-gp activity.

***Key Words:* P-glycoprotein; rhodamine 123; multidrug resistance; MDR; efflux; uptake; cancer; lung carcinoma.**

Multidrug resistance (MDR) represents a major obstacle to successful chemotherapy of metastatic diseases (1). The nature of the drug resistance is complex, but it is generally believed that cells become multidrug-resistant by either developing mechanisms which interfere with programmed cell death or by expression of energy-dependent pump systems which exclude or extrude anti-cancer drugs from cells (1). Of these pumps, the MDR1 gene-encoded multidrug transporter or *P*-glycoprotein (P-gp) (2, 3) and the MRP gene-encoded MDR-associated proteins (4) have been most extensively studied. Both transporters are ATP-dependent transporters, and belong to a larger family of ATP-binding cassette (ABC) proteins (5).

The MDR1-encoded P-gp is known to recognize and transport many structurally and functionally unrelated anticancer drugs, including vinca alkaloids (6), anthracyclines (7), epipodophyllotoxins (8), and taxanes (9, 10). In addition to anticancer drugs, a variety of other chemicals have been found to be substrates of P-gp. One such chemical is a lipophilic cationic fluorescent dye, rhodamine 123 (R123). R123, which selectively locates in mitochondria (11), has been found to be relatively non-toxic (11, 12) and effluxed more efficiently by MDR cells (13, 14). Moreover, such efflux has also been found to be inhibited by established modulators of MDR (14, 15). Thus, by following cellular R123 accumulation or retention in the presence or absence of P-gp modulators, valuable information on P-gp activity as well as efficiency of these modulators in reversing P-gp activity can be obtained.

Since R123 is considered a permeant cationic fluorescent probe (12), its uptake into cells has generally been assumed to occur via a passive diffusion process (16). Here, utilizing the parent and P-gp overexpressing MDR human metastatic lung carcinoma (MV522) and epidermoid carcinoma (KB) cell lines, we have information to suggest that cellular uptake of R123 in the MV522 cells might occur via a carrier system. In addition, verapamil, which is an established P-gp modulator, might serve as competitive inhibitor for such a system. These results have important implications on how one should use R123 for P-gp evaluation in that performing R123 accumulation analysis without first knowing whether the P-gp modulators use the same carrier system as R123 for uptake into cells might inappropriately rule out the presence of P-gp activity and thereby underestimates the effectiveness of modulators in reversing MDR activity. R123

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0006-291X/00 $35.00

efflux studies, however, would not be subject to this constraint.

MATERIALS AND METHODS

Materials. Cell culture medium and fetal calf serum (FCS) were obtained from Gibco (Grand Island, NY). Rhodamine 123 (R123), vinblastine, verapamil, fentanyl, and imipramine were purchased from Sigma Chemical Co. (St. Louis, MO). ^{3}H-verapamil (85 Ci/mmol) was purchased from American Radiolabeled Chemicals (St. Louis, MO). PSC 833 was purchased from Norvartis (Basel, Switzerland). All other reagents, unless specifically stated otherwise, were purchased from Sigma Chemical Co.

Cell cultures. MV522 (human metastatic lung carcinoma) and its MDR variant MV522/Q6 cells (a transfectoma expressing high levels of the MDR1 gene-encoded 170-kd *P*-glycoprotein) were provided by Dr. Michael J. Kelner (University of California, San Diego) (17, 18). KB-3-1 (human epidermoid carcinoma) and its MDR variant KB-8-5 cells were provided by Dr. Michael Gottesman (NCI, NIH) (19). All cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in minimal essential medium (MEM) supplemented with 10% FCS and 2 mM glutamine. In order to maintain the MDR characteristics; cell culture media for MDR sublines MV522/Q6 and KB-8-5 were supplemented with 40 ng/ml of vinblastine and 10 ng/ml of colchicine, respectively.

Intracellular accumulation of R123. Parent or MDR variants of MV522 and KB cells were seeded in 48-well plates at 1×10^5 cells/well in vinblastine-free or colchicine-free MEM one day before experiments. Prior to experiments, cells were washed twice with serum-free MEM. Subsequently, cells were exposed to 0.25 ml serum-free MEM alone or 0.25 ml serum-free MEM containing 4 μM of R123 in the presence or absence of various concentrations of verapamil or PSC 833 for 60 min at 37°C. After incubation, cellular accumulation studies were terminated by removing the assay solutions and washing the cells three times with 1.0 ml of ice-cold phosphate buffered saline (PBS) solution. The cells were then solubilized by incubation with 1 ml of 0.2 N NaOH overnight. Aliquots (500 μl and 50 μl) of the cell lysate solution were removed for analysis of R123 and protein content, respectively. The amount of protein in each sample was determined by the Pierce BCA method (Pierce Chemical, Rockford, IL). The concentration of R123 in each sample was determined quantitatively by fluorescence spectrophotometry (Shimadzu RF 1501; λ_{ex} = 492 nm, λ_{em} = 535 nm) as described previously (20) and standardized by the protein content of each sample. All experiments were carried out in triplicate.

Intracellular accumulation of ^{3}H-verapamil. MV522 and KB-3-1 cells were seeded in 48-well plates at 1×10^5 cells/well in MEM one day before experiments. Prior to experiments, cells were washed twice with serum-free MEM. Subsequently, cells were exposed to 0.25 ml serum-free MEM alone or 0.25 ml serum-free MEM containing 0.1 μCi (5 nM) ^{3}H-verapamil in the presence or absence of various concentrations of R123 for 60 min at 37°C. After incubation, cellular accumulation studies were terminated by removing the assay solutions and washing the cells three times with 1.0 ml of ice-cold phosphate buffered saline (PBS) solution. The cells were then solubilized by incubation with 1 ml of 0.2 N NaOH overnight. Aliquots (500 μl and 50 μl) of the cell lysate solution were removed for analysis of ^{3}H-verapamil and protein content, respectively. The level of radioactivity taken up into MV522 and KB-3-1 cells was determined using a Beckman LS6000 IC liquid scintillation counter and standardized with the amount of protein in each sample. The amount of protein in each sample was determined as described above.

Cellular efflux of R123. P-gp expressing cells (MV522/Q6 or KB-8-5) were seeded in 48-well plates at 1×10^5 cells/well in vinblastine-free or colchicine-free MEM one day before experiments. Prior to the experiments, cells were washed twice with serum-free MEM. Subsequently, the cells were incubated with 0.25 ml serum-free MEM alone or serum-free MEM containing 4 μM R123 for 60 min at 37°C. After incubation was complete, the culture media was aspirated gently, and cells were washed three times with 1 ml of ice-cold serum-free MEM to remove any extracellular R123. After the washing was complete, cells were restored to either fresh serum-free MEM or serum-free MEM containing no additional drugs, 10 μM verapamil or 1 μM PSC 833 at 37°C. Sixty minutes thereafter, culture medium was removed and cells were washed three times with 1.0 ml of ice-cold PBS. The cells were then solubilized and assayed for R123 and protein contents as described above.

Evaluation of drug effects on cell viability. Parent cells (MV522 or KB-3-1) were seeded at 2×10^4 cells/well in 96-well plates one day before experiments. Prior to experiments, cells were washed twice with serum-free MEM. Subsequently, cells were exposed to 0.25 ml serum-free MEM alone or 0.25 ml serum-free MEM containing 4 μM R123 in the presence or absence of various concentrations of verapamil or PSC 833 for 60 min at 37°C.

Following exposure of the cells to R123 alone or in combination with various concentrations of verapamil or PSC 833, cell viability was measured by MTS assay (Promega, Madison, WI).

Statistical analysis. All data are presented as mean ± SD. Mean values were calculated from at least three experiments. Treatment groups were compared with control for significance by Student unpaired *t* tests. $P < 0.05$ was considered significant.

RESULTS

We first examined the effects of verapamil and PSC 833 on R123 accumulation in the parent (MV522 and KB-3-1) cell lines. In both lines, incubation with verapamil resulted in a dose-dependent decrease in R123 accumulation (Fig. 1). In contrast, PSC 833 exerted no effect on R123 accumulation in MV522 cells, but it caused a dose-dependent decrease in R123 accumulation in KB-3-1 cells (Fig. 1). R123 primarily binds to intact mitochondria and, accordingly, processes that influence cell viability may affect R123 retention by mitochondria and, hence, cellular accumulation of R123. To examine if the concentration-dependent reduction in R123 accumulation was related to cytotoxic actions of either verapamil or PSC 833, we measured their effects on cell viability using MTS assay. Both verapamil and PSC 833 had no effect on MV522 cells, whereas they exerted a concentration-dependent cytotoxicity to KB-3-1 cells (Fig. 2). These observations imply that KB-3-1 cells are more sensitive to the cytotoxic effects of these drugs than MV522 cells and that the probable cause for concentration-dependent reduction in R123 accumulation in KB-3-1 cells is most likely related to drug-induced cytotoxicity.

Inhibition of R123 accumulation by verapamil but not by PSC 833 may be explained by a carrier system being present in MV522 cells for R123 and verapamil competes for this carrier. To examine this possibility, we determined cellular uptake of radiolabeled verapamil in the presence of increasing concentrations of

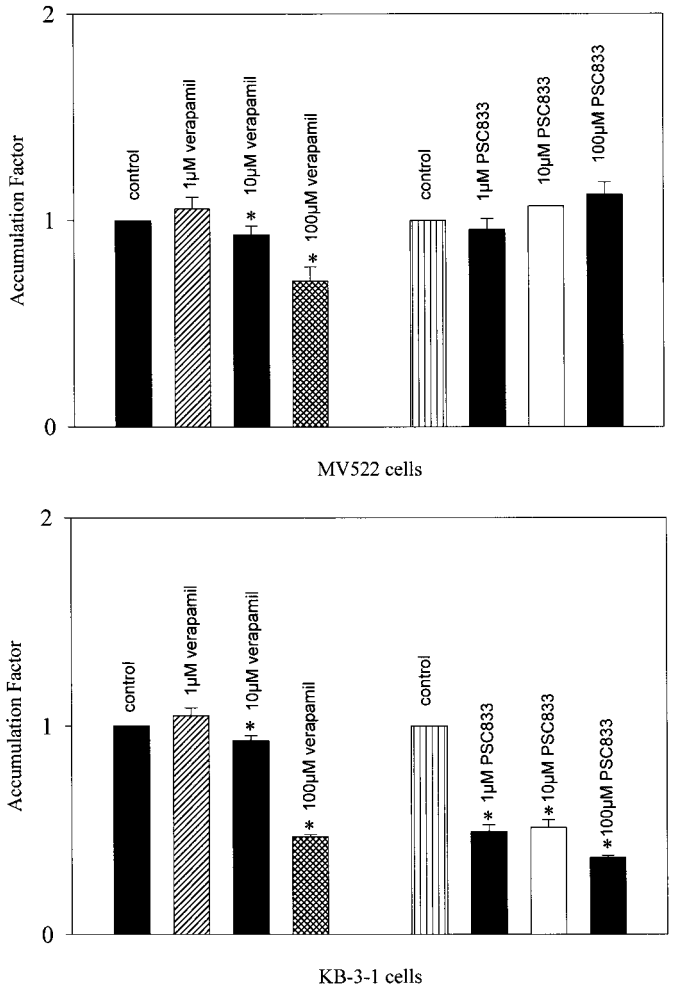

FIG. 1. Effect of verapamil or PSC 833 on cellular accumulation of R123 by the parent MV522 and KB-3-1 cells. R123 accumulation normalized to protein content was expressed as accumulation factor. Accumulation factor for control or untreated cells is defined as equal to 1. Accumulation factor for treated cells is defined as ratio of cellular R123 accumulation in the presence of P-gp modulators to cellular R123 accumulation in the absence of P-gp modulators.

R123. The notion is that if verapamil uses the same uptake mechanism as R123, then R123 should inhibit verapamil uptake in a concentration-dependent manner. As proposed, cellular uptake of radiolabeled verapamil in MV522 cells was inhibited in a concentration-dependent fashion by R123 (Fig. 3). These results confirm our notion that a carrier system is present in MV522 cells for uptake of both R123 and verapamil. To eliminate the possibility that the same mechanism might be responsible for decrease in R123 accumulation in KB-3-1 cells, we studied cellular uptake of radiolabeled verapamil in the presence of increasing concentrations of R123 in KB-3-1 cells. Addition of increasing amounts of R123 did not inhibit uptake of radiolabeled verapamil (Fig. 3). These results are consistent with the reduction in R123 accumulation in KB-3-1 cells being primarily related to concentration-related cytotoxicity.

Together, our data point to the presence of a carrier system for cellular uptake of R123 and verapamil in MV522 cells but not in KB-3-1 cells. These observations imply that the modulatory effects of verapamil on R123 accumulation in the MDR MV522/Q6 and KB-8-5 cells may be quite different from those associated with

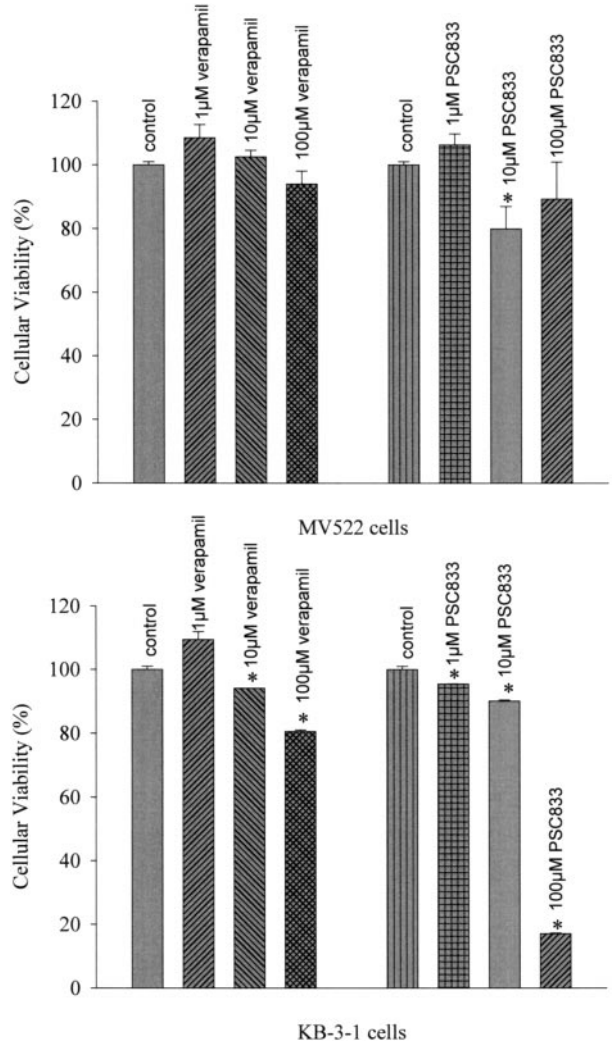

FIG. 2. Effect of verapamil or PSC 833 on the cellular viability of the parent cells. MV522 and KB-3-1 cells were incubated for 60 min in MEM alone (control) or MEM containing 4 μM R123 plus various concentrations of verapamil or 4 μM R123 plus various concentrations of PSC 833 (treatment groups). After incubation, MTS assay was used to quantitate viable cells. Data are expressed as % cellular viability calculated by the following formula: % cellular viability = (optical absorbance of cells treated with various combinations of R123 and P-gp modulators)/optical absorbance of control or untreated cells) $\times$ 100. % cellular viability for control cells is defined as equal to 100.

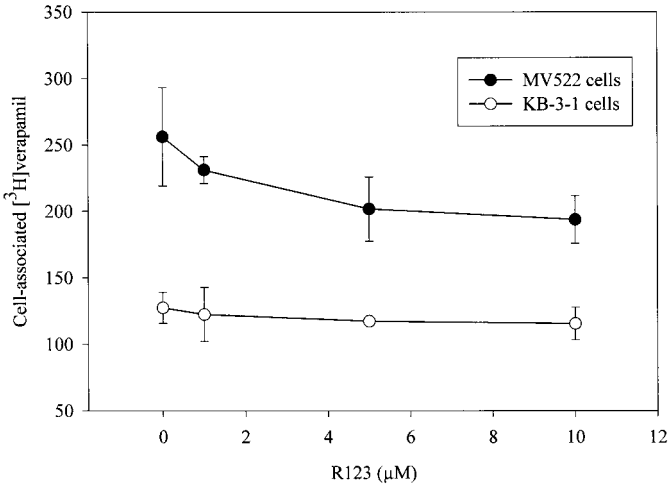

FIG. 3. Effect of R123 on cellular accumulation of [^{3}H]verapamil by parent MV522 or KB cells.

PSC 833. In the case of PSC 833, its expected competition with R123 for P-gp mediated efflux process would be expected to increase R123 accumulation in both MV522/Q6 and KB-8-5 cells. However, because of the presence of a carrier system in the MV522 cells, the effects of verapamil on R123 accumulation in MV522/Q6 cells should be different from that in the KB-8-5 cells. In KB-8-5 cells that lack the carrier system, verapamil would be expected to increase R123 accumulation through its P-gp modulating effects. By contrast, in MV522/Q6 cells, verapamil can increase, decrease or cause no change to R123 accumulation depending on how R123 and verapamil interact with the two transporter proteins, i.e., the inward carrier and the P-gp. To examine these possibilities, we studied the effects of verapamil and PSC 833 on cellular accumulation of R123 in MV522/Q6 and KB-8-5 cells. PSC 833 caused a dose-dependent increase in R123 accumulation in both MDR cell lines (Fig. 4). In contrast, verapamil failed to cause any change in R123 accumulation in the MV522/Q6 cells, but did increase R123 accumulation in KB-8-5 cells. However, it is interesting to note that while all concentrations of verapamil increased R123 accumulation relative to control in KB-8-5 cells, the relative effects of verapamil for modulating P-gp activity decreased as its concentration was increased. It is possible that at high concentration, verapamil might have reached cytotoxic levels in KB-8-5 cells, thereby, nullifying its supposedly P-gp modulating effect. When verapamil and PSC 833 were later examined for their ability to increase R123 accumulation in the R123 retention study, a very different result was observed in that both verapamil and PSC 833 were effective in increasing R123 accumulation in the two MDR cell lines (Fig. 5).

DISCUSSION

Rhodamine 123 (R123) is a lipophilic cationic fluorescent dye that has often been used for studying the functional activity of P-glycoprotein (P-gp). When used as a molecular probe for study of P-gp activity, it is customary to either follow the accumulation (21–24) or release (23, 25, 26) of R123 in the presence or absence of P-gp modulators. If the P-gp modulators were to increase R123 accumulation, a conclusion for the presence of P-gp activity is usually drawn. Conversely, by comparing the activity of different P-gp modulators in increasing R123 accumulation, information on the potency of these modulators in reversing P-gp activity can also be obtained.

In the present communication, the effects of two widely used P-gp modulators, verapamil and PSC 833 (27), on R123 accumulation in two cancer cell culture systems were presented. Both verapamil and PSC 833 were able to increase R123 accumulation in the MDR

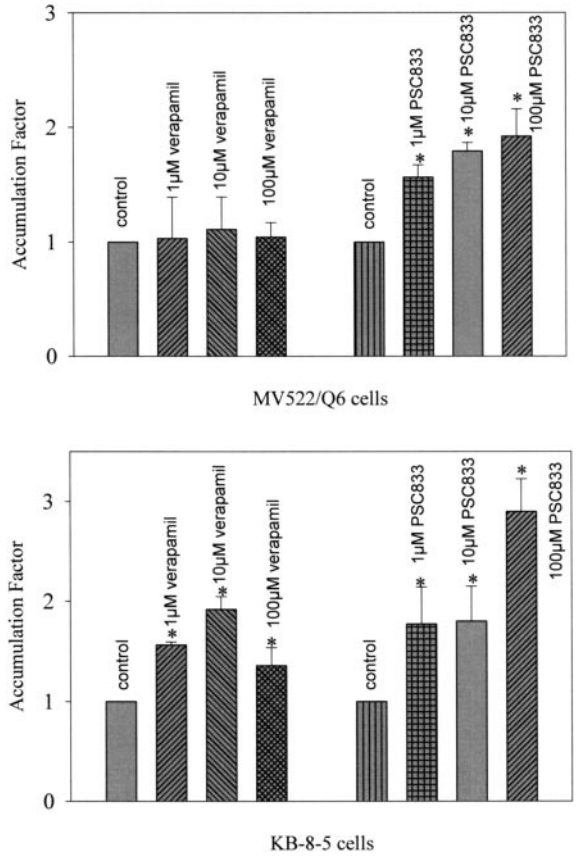

FIG. 4. Effects of verapamil or PSC 833 on cellular accumulation of R123 by the MDR MV522/Q6 and KB-8-5 cells. R123 accumulation normalized to protein content was expressed as accumulation factor. Accumulation factor for control cells is defined as equal to 1. Accumulation factor for treated cells is defined as ratio of cellular R123 accumulation in the presence of P-gp modulators to cellular R123 accumulation in the absence of P-gp modulators.

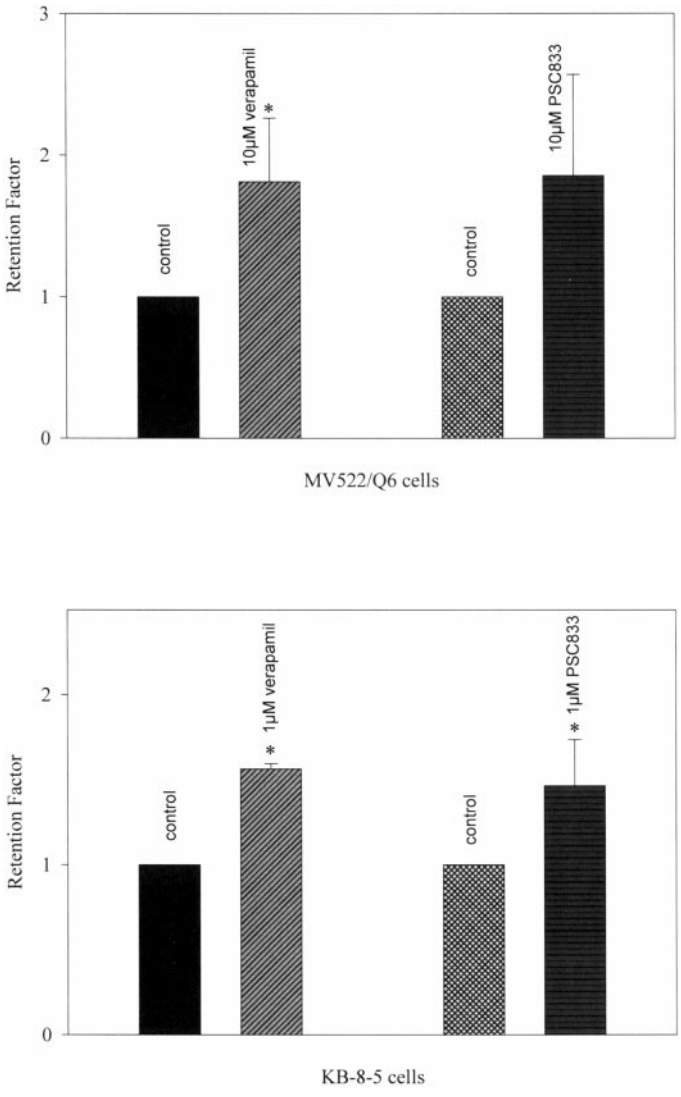

FIG. 5. Effects of verapamil or PSC 833 on cellular retention of R123 in the MDR MV522/Q6 cells and KB-8-5 cells. For these studies, cells were incubated with R123 for 60 min. After washing, cells were then incubated for another 60 min in R123-free MEM with and without 1 μM of PSC 833 or 10 μM verapamil. Thereafter, cells were measured for R123 contents. R123 retention normalized to protein content was expressed as retention factor. Retention factor for control cells is defined as equal to 1, whereas retention factor for treated cells is defined as ratio of cellular R123 retention in the presence of P-gp modulators to cellular R123 retention in the absence of P-gp modulators.

cells (MV522/Q6 and KB-8-5) in the release study. These confirm what others have previously shown regarding the action of these drugs in reversing P-gp activity. However, the effects of these two modulators on R123 accumulation in the MDR cell lines are quite different during accumulation study. Specifically, incubation with PSC 833 resulted in an increase in R123 accumulation in both MV522/Q6 and KB-8-5 cells. In contrast, incubation with verapamil did not result in any change in R123 accumulation in MV522/Q6 cells, but it caused an increase in R123 accumulation in the KB-8-5 cells. This discrepancy between the effects of these two drugs on R123 accumulation in MV522/Q6 cells can be explained if R123 uptake into MV522 cells is assumed to be a carrier-mediated event, and that verapamil, but not PSC 833, competes for the carrier. While there is no direct evidence that this carrier exists, the findings that R123 can inhibit ^{3}H-verapamil accumulation in MV522 cells in a concentration-dependent fashion provide strong evidence for such a carrier. In light of this information, it is interesting to comment on the significance of this carrier as it relates to P-gp in the MDR studies. It is expected that when both the drugs (in this case R123) and P-gp modulators are substrates of the inward carrier and P-gp, incubating cells with P-gp modulators might not necessarily lead to increase of drug uptake. This is because the affinity and/or capacity of these transporters for the substrates might vary. Therefore, depending on the specificity for the substrates and the overall capacity of these transporters, there might either be uptake or no net cellular uptake of these substrates. Based on our findings that verapamil neither increases nor decreases R123 accumulation in MV522/Q6 cells, it seems that these two transporters in MV522/Q6 cells might possess similar affinity and capacity for the two substrates. Currently, work is being undertaken to identify and characterize this carrier. Our preliminary data so far indicate that other lipophilic cationic drugs, such as imipramine and fentanyl, are also effective inhibitors for R123 uptake into MV522/Q6 cells (unpublished observations).

Our observations that verapamil causes a decrease in R123 accumulation in MV522 cells during accumulation study are in agreement with studies performed in human lymphoblastoid cells (22) and suggest that a similar carrier system might be present in other cancer cells for cellular uptake of R123 and verapamil. However, since R123 uptake has always been assumed to occur via a passive diffusion process (12, 16), the existence of a carrier system for R123 uptake into cells has not received much experimental attention. Given the significant role of R123 in P-gp studies and the current effort to develop new classes of P-gp modulators, many of which are considered derivatives of verapamil (27), our findings have important implications on the use of R123 in P-gp related studies. It is expected that carrying out R123 accumulation study without first determining whether a carrier system for cellular uptake of R123 exists and, if it is affected by P-gp modulator under investigation, might lead one to underestimate P-gp modulatory activity. For example, the failure of a P-gp modulator to increase R123 accumulation will often be interpreted as either (i) the cells do not express

P-gp proteins, or (ii) the modulators are not effective in reversing P-gp activity. Based on the results of the present study, this negative result might be the direct consequence of competition between R123 and P-gp modulator for uptake into the cells. These issues, however, will not be a concern if one follows R123 accumulation in the format of a release study. This is because, under this scenario, one evaluates the P-gp modulator by its ability to reduce cellular release of R123 previously loaded inside cells.

In conclusion, when information such as whether a P-gp modulator might compete with R123 for uptake into cells is not readily available, R123 release studies should be conducted rather than R123 accumulation studies. Furthermore, administration of drugs that are known to inhibit P-gp, such as verapamil, may unexpectedly reduce, rather than increase the uptake of some P-gp substrates in some tissues if inhibition of an inward transporter overshadows inhibition of the P-gp transporter.

ACKNOWLEDGMENTS

We thank David Thompson for proofreading our manuscript. This work was supported by National Cancer Institute Grant No. CA79788 and National Institute of General Medicine Grant No. GM47502.

REFERENCES

1. Stavrovskaya, A. A. (2000) Cellular mechanisms of multidrug resistance of tumor cells. *Biochemistry* (Moscow) **65,** 95–106.
2. Endicott, J. A., and Ling, V. (1989) The biochemistry of P-glycoprotein-mediated multidrug resistance. *Ann. Rev. Biochem.* **58,** 137–171.
3. Gottesman, M. M., Pastan, I., and Ambudkar, S. V. (1996) P-glycoprotein and multidrug resistance. *Curr. Opin. Genet. Dev.* **6,** 610–617.
4. Cole, S. P., Bhardwaj, G., Gerlach, J. H., Mackie, J. E., Grant, C. E., Almquist, K. C., Stewart, A. J., Kurz, E. U., Duncan, A. M., and Deeley, R. G. (1992) Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. *Science* **258,** 1650–1654.
5. Higgins, C. F. (1995) The ABC of channel regulation. *Cell* **82,** 693–696.
6. Fojo, A., Akiyama, S., Gottesman, M. M., and Pastan, I. (1985) Reduced drug accumulation in multiply drug-resistant human KB carcinoma cell lines. *Cancer Res.* **45,** 3002–3007.
7. Inaba, M., and Johnson, R. K. (1978) Uptake and retention of adriamycin and daunorubicin by sensitive and anthracycline-resistant sublines of P388 leukemia. *Biochem. Pharmacol.* **27,** 2123–2130.
8. Dano, K. (1973) Active outward transport of daunomycin in resistant Ehrlich ascites tumor cells. *Biochim. Biophys. Acta* **323,** 466–483.
9. Tsuruo, T., Iida-Saito, H., Kawabata, H., Oh-hara, T., Hamada, H., and Utakoji, T. (1986) Characteristics of resistance to adriamycin in human myelogenous leukemia K562 resistant to adriamycin and in isolated clones. *Jap. J. Cancer Res.* **77,** 682–692.
10. Tatsuta, T., Naito, M., Oh-hara, T., Sugawara, I., and Tsuruo, T. (1992) Functional involvement of P-glycoprotein in blood-brain barrier. *J. Biol. Chem.* **267,** 20383–20391.
11. Johnson, L. V., Walsh, M. L., and Chen, L. B. (1980) Localization of mitochondria in living cells with rhodamine 123. *Proc. Natl. Acad. Sci. USA* **77,** 990–994.
12. Johnson, L. V., Walsh, M. L., Bockus, B. J., and Chen, L. B. (1981) Monitoring of relative mitochondrial membrane potential in living cells by fluorescence microscopy. *J. Cell Biol.* **88,** 526–535.
13. Tapiero, H., Munck, J. N., Fourcade, A., and Lampidis, T. J. (1984) Cross-resistance to rhodamine 123 in adriamycin- and daunorubicin-resistant Friend leukemia cell variants. *Cancer Res.* **44,** 5544–5549.
14. Neyfakh, A. A. (1988) Use of fluorescent dyes as molecular probes for the study of multidrug resistance. *Exp. Cell Res.* **174,** 168–176.
15. Lampidis, T. J., Munck, J. N., Krishan, A., and Tapiero, H. (1985) Reversal of resistance to rhodamine 123 in adriamycin-resistant Friend leukemia cells. *Cancer Res.* **45,** 2626–2631.
16. Altenberg, G. A., Vanoye, C. G., Horton, J. K., and Reuss, L. (1994) Unidirectional fluxes of rhodamine 123 in multidrug-resistant cells: Evidence against direct drug extrusion from the plasma membrane. *Proc. Nat. Acad. Sci. USA* **91,** 4654–4657.
17. Kelner, M. J., McMorris, T. C., Estes, L., Starr, R., Samson, K., Varki, N., and Taetle, R. (1995) Nonresponsiveness of the metastatic human lung carcinoma MV522 xenograft to conventional anticancer agents. *Anticancer Res.* **15,** 867–871.
18. Kelner, M. J., McMorris, T. C., Estes, L., Samson, K. M., Bagnell, R. D., and Taetle, R. (1998) Efficacy of MGI 114 (6-hydroxymethylacylfulvene, HMAF) against the mdr1/gp170 metastatic MV522 lung carcinoma xenograft. *Eur. J. Cancer* **34,** 908–913.
19. Akiyama, S., Fojo, A., Hanover, J. A., Pastan, I., and Gottesman, M. M. (1985) Isolation and genetic characterization of human KB cell lines resistant to multiple drugs. *Somatic Cell Mol. Genet.* **11,** 117–126.
20. Henthorn, T. K., Liu, Y., Mahapatro, M., and Ng, K. Y. (1999) Active transport of fentanyl by the blood-brain barrier. *J. Pharmacol. Exp. Therap.* **289,** 1084–1089.
21. Boer, R., Haas, S., and Schodl, A. (1994) Influence of dexniguldipine-HC1 on rhodamine-123 accumulation in a multidrug-resistant leukaemia cell line: Comparison with other chemosensitisers. *Eur. J. Cancer* **30,** 1117–1123.
22. Lautier, D., Canitrot, Y., and Salmon, J. M. (1994) Effects of vinblastine, colchicine, and verapamil on rhodamine 123 accumulation in human *P*-glycoprotein-positive leukemia cells. *Anticancer Res.* **14,** 2589–2595.
23. Budworth, J., Davies, R., Malkhandi, J., Gant, T. W., Ferry, D. R., and Gescher, A. (1996) Comparison of staurosporine and four analogues: Their effects on growth, rhodamine 123 retention and binding to *P*-glycoprotein in multidrug-resistant MCF-7/Adr cells. *Br. J. Cancer* **73,** 1063–1068.
24. Maia, R. C., Vasconcelos, F. C., Harab, R. C., Coelho, A. M., Dobbin, J. A., and Rumjanek, V. M. (1998) Comparison between anthracyclines and rhodamine-123 accumulation in chronic lymphoid leukemia: Effect of cyclosporin A and verapamil. *Tumour Biol.* **19,** 41–51.
25. Delville, J. P., Pradier, O., Pauwels, O., Van Onderbergen, A., Kiss, R., Feremans, W., and Capel, P. (1995) Comparative study of multidrug resistance evaluated by means of the quantitative

immunohistochemical detection of *P*-glycoprotein and the functional release of rhodamine 123. *Am. J. Hematol.* **49,** 183–193.

26. Ludescher, C., Eisterer, W., Hilbe, W., Hofmann, J., and Thaler, J. (1995) Decreased potency of MDR-modulators under serum conditions determined by a functional assay. *Br. J. Haematol.* **91,** 652–657.
27. Sikic, B. I. (1997) Pharmacologic approaches to reversing multidrug resistance. [Review] [53 refs]. *Sem. Hematol.* **34,** 40–47.