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The Quinoline-Based Drug, *N*-{4-[1-hydroxy-2-(dibutylamino)ethyl] quinolin-8-yl}-4-azidosalicylamide, Photoaffinity Labels the Multidrug Resistance Protein (MRP) at a Biologically Relevant Site[1]

Marko Vezmar,* Leslie W. Deady,† Leann Tilley,‡ and Elias Georges*,[2]
**Institute of Parasitology, McGill University, Macdonald Campus, Ste-Anne de Bellevue, Quebec, Canada; and †The School of Chemistry and ‡The School of Biochemistry, La Trobe University, Bundoora, Victoria, Australia*

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MRP is a member of the ABC trafficking proteins thought to mediate the transport of glutathione S-conjugates and amphiphilic natural products. However, unlike P-glycoprotein, the biochemical mechanism by which MRP mediates the resistance to cytotoxic drugs is not clear. In this report, we describe the interactions of a quinoline-based drug, N-{4-[1-hydroxy-2-(dibutylamino)ethyl] quinolin-8-yl}-4-azidosalicylamide (IAAQ), with MRP. Our results demonstrate the ability of IAAQ to photoaffinity label a 190 kDa protein in resistant Small Cell Lung Cancer cells (H69/AR) but not in the parental H69 cells. The photoaffinity labeling of the 190 kDa protein with IAAQ was both saturable and specific. The identity of the 190 kDa protein, as MRP, was confirmed by immunoprecipitation with the monoclonal antibody, QCRL-1. Furthermore, a molar excess of LTC_4, MK 571 or vinblastine inhibited the photoaffinity labeling of MRP with IAAQ in intact cells and plasma membranes. Cell growth and drug transport studies showed H69/AR cells to be less sensitive to and to accumulate less IAAQ than the parental H69 cells. In addition, MK 571 and doxorubicin increased the sensitivity to and the accumulation of IAAQ in H69/AR cells. Together, the results of this study show for the first time the direct binding of unaltered cytotoxic drug to MRP. Moreover, given the structural similarities between IAAQ and MK 571, we suggest that MK 571 modulates MRP-mediated resistance by direct binding to MRP.

Key Words: **Multidrug resistance; P-glycoprotein; Multidrug resistance protein; Photoaffinity labeling; Quinoline; LTC_4; MK 571.**

Selection of tumor cell lines with lipophilic anticancer drugs often leads to the over-expression of two plasma membrane proteins, the P-glycoprotein (P-gp1; (1, 2)) and the 190 kDa multidrug resistance protein (MRP; (3)). P-gp1 and MRP mediate the transport of amphiphilic drugs in an energy-dependent fashion (4-6). Although, the normal substrates for P-gp1 and MRP transporters remain unknown, tissue distribution studies have predicted the transport of normal cell metabolites and natural toxins (7, 8). In support of the latter, disruption of *mdr1* gene from the mouse genome was shown to increase the accumulation of drugs in normal tissues that express high levels of P-gp1 (9). Similarly, disruption of *MRP* gene from embryonic stem cells was shown to increase the toxicity of cells to several lipophilic anticancer drugs and to sodium arensite (10).

P-gp1 and MRP are members of a large family of ABC trafficking proteins with substrate specificity ranging from ions to large polypeptides (11, 12). Although, P-gp1 and MRP share certain structural and functional homologies, important differences exist between these two proteins (3). Among these differences are MRP and P-gp membrane topologies (13, 14) and substrate specificity (5). Regarding the latter, conjugation of amphiphilic drugs with glutathione has been shown to be predict their transport by MRP (15, 16). Furthermore, the recent findings that both LTC_4 and the photoactive glutathione conjugate, S-(p-azidophenylacyl)-glutathi-

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[2] Corresponding author: Elias Georges, Institute of Parasitology, McGill University, 21,111 Lakeshore Road, Ste Anne de Bellevue, Quebec, Canada, H9X 3V9. Fax: 514 398 7857. E-mail: Elias_Georges@maclan.McGill.CA.

Abbreviations: MDR, multidrug resistance; P-gp, P-glycoprotein; MRP, multidrug resistance protein; SCLC, small cell lung cancer; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; ABC, ATP binding cassette.

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one, interact directly with MRP suggests the presence of GSH recognition site (17, 18). By contrast, attempts to identify common structural moieties that predict drug binding to, and transport by P-gp1 have thus far been unsuccessful. Some common physical-chemical properties of drugs that interact with P-gp1 have been proposed and include planar benzene rings and a cationic charge, in addition to lipophilicity (19-21).

Besides glutathione S-conjugated compounds, MRP has been shown to mediate the resistance to other cytotoxic drugs (5, 6, 15, 22, 23). However, unlike P-gp1, which has been shown to interact directly with cytotoxic drugs; it is not clear if MRP binds directly to unaltered cytotoxic drugs. In this report, we demonstrate a direct and specific binding of a photoactive quinoline-based drug (IAAQ) to MRP in MDR SCLC Cells (H69/AR). Moreover, we show that the photoaffinity labeling of MRP with IAAQ occurs at a biologically relevant site as determined by drug transport studies and inhibition of photoaffinity labeling with known substrates of MRP.

MATERIALS AND METHODS

Materials. Iodine-125 (100.7 mCi/ml) and [^{125}I]-iodoarylazidoprazosin (2200 Ci/mmol) were purchased from Amersham Biochemical Inc., (Mississauga, Ontario, Canada). Protein-A coupled Sepharose was purchased from Pharmacia Inc., (Montreal, Quebec, Canada). The LTD_4 receptor antagonist MK 571 was kindly provided by Dr. A.W. Ford-Hutchinson (Merck-Frosst Centre for Therapeutic Research, Point Claire-Dorval, Quebec, Canada; (24)). Leukotriene C_4 (LTC_4) was purchased from Cayman Chemical Co., (Ann Arbor, MI). The Small Cell Lung Cancer cells (H69 and H69/AR) and the MRP-specific monoclonal antibody (QCRL-1) were kind gifts from Dr. Susan P. C. Cole (Cancer Research Laboratories, Queen's University, Kingston, Ontario, Canada). The monoclonal antibody C494 was a generous gift from Dr. Victor Ling at British Columbia Cancer Center (Vancouver, Canada). All other chemicals were of the highest commercial grade available.

Cell culture and plasma membrane preparations. Drug sensitive (H69) and resistant (H69/AR) cells were grown in RPMI 1640 media containing 4 mM glutamine and 5% fetal calf serum (Hyclone). Resistant cells were cultured continuously in the presence of 0.8 μM of doxorubicin; however, cells used for drug transport studies were grown in drug-free media for ten days prior to the date of the experiment. Plasma membranes from H69 and H69/AR cells were prepared essentially as described by Lin *et al.*, (25). In brief, cells were collected by low speed centrifugation and washed three times with ice-cold phosphate buffered saline (PBS), pH 7.4. Cells were homogenized in 50 mM mannitol, 5 mM Hepes and 10 mM Tris-HCl, pH 7.4 (containing 2 mM PMSF and 3 μg/ml leupeptin) in a Dounce glass homogenizer. Calcium chloride solution was then added to the homogenate to a final concentration of 10 mM and mixed by stirring to ensure even distribution of the cation. The slightly turbid supernatant solution that contains plasmalemma vesicles was precipitated by high speed centrifugation at 100,000 $\times$g for 1 hour at 4°C using Beckman SW28 rotor. The enriched plasma membrane pellet was washed with 10 mM Tris-HCl, pH 7.4 and resuspended in the same buffer containing 250 mM sucrose. Membrane fractions were stored at -80°C if not immediately used. Protein concentrations were determined by the Lowry method (26).

Radioiodination of IAAQ and photoaffinity labeling. The synthesis of photoreactive drug (IAAQ) has been previously described elsewhere (27). Iodination of IAAQ was carried out in the dark. Briefly, IAAQ (10 nM) was dissolved in 20 μl of dimethylsulfoxide (DMSO) and mixed with 10 μl of carrier-free $Na^{125}I$ (1 mCi, 0.5 nmol) and 10 μl of chloramine T (10 nmole) in 1 M K_2HPO_4, pH 7.4. The reaction was allowed to continue for 5 minutes and was stopped by the addition of sodium metabisulphite (50 μl of 5% (w/v) solution). The reaction mixture was loaded onto a C_{18} cartridge (Sep-Pak, Waters-Millipore) prewashed with 10 mM K_2HPO_4, pH 7.4. The column was washed with 5 ml aliquots of 10 mM K_2HPO_4, pH 7.4 containing 10% (v/v) methanol until no significant radiolabel was detected. IAAQ was eluted with 2.5 ml methanol and vacuum-dried in the dark. The dried residue was resuspended in DMSO and the concentration of the radioactive, photoactive drug was determined by HPLC.

For photoaffinity labeling of cells, H69 or H69/AR cells were washed with PBS and preincubated for 20 minutes at 37°C in the presence of a molar excess of vinblastine, LTC_4 or MK 571 before the addition of IAAQ (0.25 μM). Cells were incubated at room temperature in the dark for 30 minutes and then transferred to ice for 10 minutes. Following the latter incubation, cells were irradiated for 10 minutes on ice with a *UV* source at 254 nm (Stratagene *UV* crosslinker, Stratagene, La Jolla, CA). The free photoactive drug was removed by low speed centrifugation and cells were lysed in 20 μl of 50 mM Tris (pH 7.4) containing 1% Nonidet P-40 (NP40), 5 mM $MgCl_2$ and protease inhibitors (3 μg/ml of leupeptin and 2 mM PMSF). IAAQ labeled proteins were isolated by brief centrifugation at 4°C and resolved on SDS-PAGE. For photoaffinity labeling of plasma membranes, 15 μg aliquots of membrane proteins prepared from H69 and H69/AR cells were photoaffinity labeled as described above. It should be mentioned that incubation of cell or plasma membranes with IAAQ but without *UV* irradiation did not result in the photoaffinity labeling of proteins (data not shown).

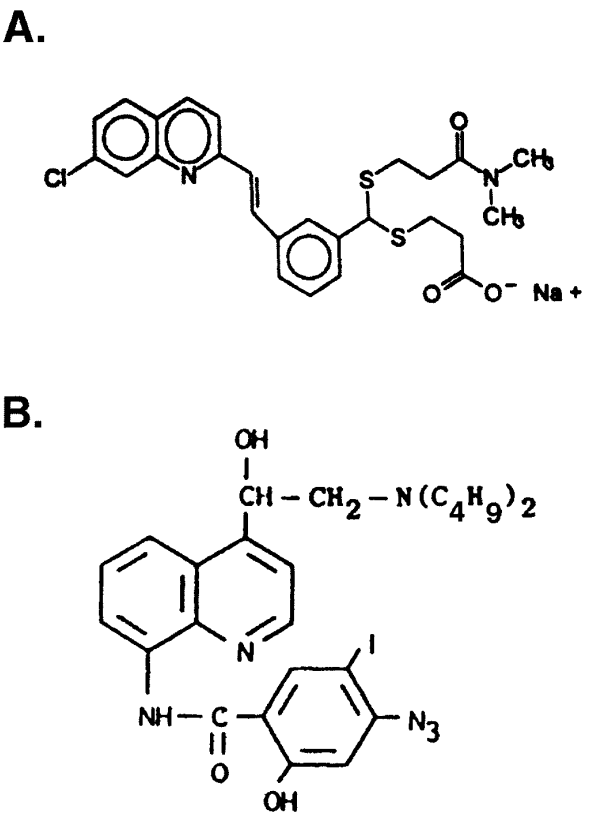

FIG. 1. Structures of MK 571(A) and IAAQ (N-{4-[1-hydroxy-2-(dibutylamino) ethyl] quinolin-8-yl}-4-azidosalicylamide) (B).

Immunoprecipitation and SDS gel electrophoresis. IAAQ photoaffinity labeled cells were lysed in 50 mM Tris-HCl, pH 7.4, containing 0.5% CHAPS, 0.5% sodium deoxycholate, 150 mM NaCl and protease inhibitors (3 μg/ml of leupeptin and 2 mM PMSF). The cell lysates were clarified by centrifugation at 12,000 $\times$g at 4°C. Equal amounts of cell lysate proteins were separately incubated overnight at 4°C with 10 μg of C494 and QCRL-1 monoclonal antibodies or an irrelevant IgG_{2a}. Protein-A coupled sepharose was added to the cell lysates and allowed to incubate for one hour at room temperature. After several washes in lysis buffer, proteins were released from the sepharose beads with buffer I (10 mM Tris-HCl, pH 8.0 containing 2% SDS, 50 mM dithiothreitol (DTT), 1 mM ethylene diaminetetraacetate (EDTA)) and II (2$\times$ buffer I and 9 M urea). The eluted proteins were then resolved by SDS PAGE using the Fairbanks gel system with some modifications (28). Gel slabs containing the immunoprecipitated proteins were fixed in 50% methanol, dried and exposed to XAR Kodak film at -70°C.

Cytotoxicity and drug transport assays. Cells were harvested and plated into 96-well plates at 0.5-1.0$\times 10^4$ cells/well. Following a 24 hour recovery period, increasing concentrations of IAAQ was added to cells in the absence or presence of non-toxic concentrations of MK 571. Cells were allowed to grow in the dark for four days at 37°C before the addition of the MTT dye (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide). The plates were processed in the dark as previously described (29).

For drug transport, cells (1×10^6) were incubated for 45 minutes in PBS/glucose containing 0.25 μM IAAQ without or with an excess of vinblastine, MK 571 or doxorubicin. Drug accumulation was stopped with the addition of 1 ml of ice-cold PBS and cells were washed several times with the same buffer. Cells were lysed in 100 μl of 1M NaOH followed by neutralization with an equal volume of 1M HCl and the accumulated radiolabel was determined by fluorometry using 1219 Racbeta model counters (LKB, Wallace). It is emphasized that incubations with IAAQ were done under a safety light to prevent the photodestruction of the photoreactive group. Furthermore, the integrity of the photoreactive moiety was monitored by the absorbance at 254 nm and controls to check for non-specific photolabeling of proteins were included in each experiment (data not shown).

RESULTS

Photoaffinity Labeling of MRP with IAAQ

MRP has been shown to bind directly to the cysteinyl leukotriene, LTC_4, and other GSH conjugated drugs ((5, 6, 15, 22, 23). However, direct binding between MRP and unaltered cytotoxic drugs has not been demonstrated. In this study, we have used a photoactive quinoline-based drug (IAAQ) to study MRP drug interactions. To determine if IAAQ binds directly to MRP, drug sensitive (H69) and resistant (H69/AR) SCLC cells were incubated in the presence of 0.25 μM of IAAQ and *UV* irradiated (see experimental procedures). The results in Figure 2A show a 190 kDa protein photolabeled with IAAQ in H69/AR, but not in H69 cells. When the same cells were incubated in the presence of 20 nM of IAAP ([^{125}I]-iodoarylazidoprazosin), shown previously to photoaffinity label P-glycoprotein (30), no 190 kDa protein was photoaffinity labeled (figure 2A). The latter results suggest that IAAP is not a substrate for MRP.

To determine the binding specificity of IAAQ towards the 190 kDa protein, H69/AR cells were photoaffinity labeled with increasing concentrations (0.25 μM to 2.5 μM) of IAAQ. Figure 2B, *inset*, shows the photoaffinity

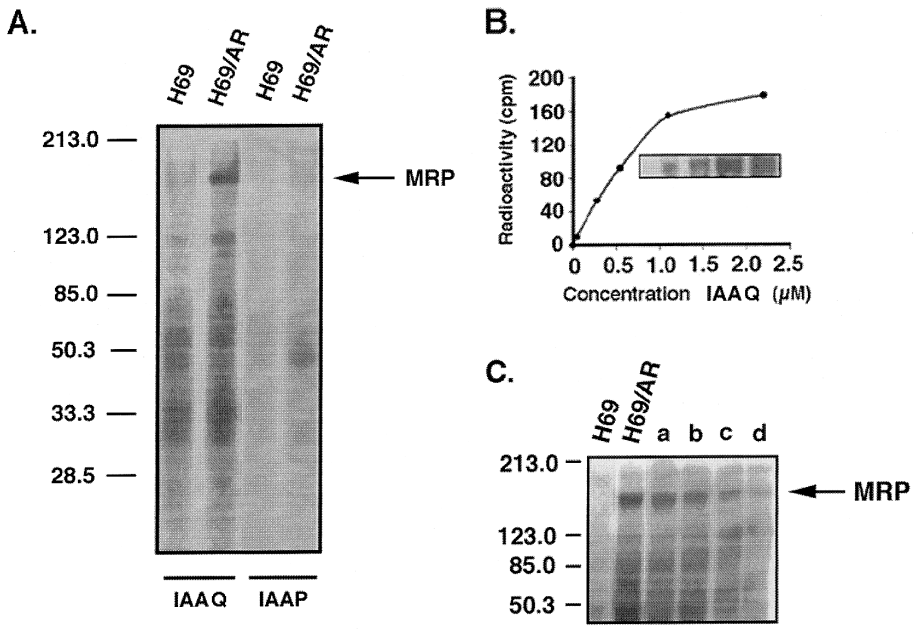

FIG. 2. Photoaffinity labeling of H69 and H69/AR cells with IAAQ. Drug sensitive (H69) and resistant (H69/AR) cells were photoaffinity labeled with 0.25 μM IAAQ or 20 nM IAAP. Figure 2B shows photoaffinity labeling of H69/AR cells in the presence of increasing concentrations of IAAQ (0-2.5 μM). The inset in figure 2B shows the increase in the intensity of 190 kDa photolabeled protein which was excised and the radiolabel quantified. Figure 2C shows the photoaffinity labeled proteins from H69 or H69/AR cells incubated in the absence or presence of excess (40-500 μM) non-iodinated IAAQ (lanes a-d).

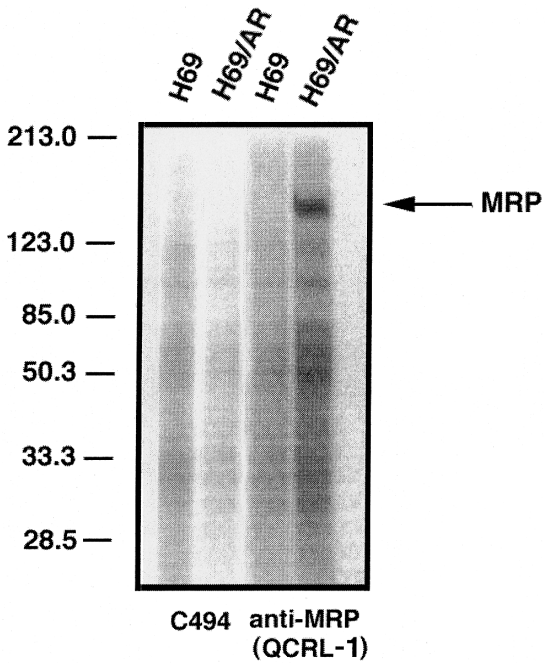

FIG. 3. Immunoprecipitation of IAAQ photoaffinity labeled MRP. H69 and H69/AR cells were photolabeled with 0.25 μM IAAQ and immunoprecipitated with P-glycoprotein-specific mAb (C494) or MRP-specific mAb (QCRL-1). The position of MRP protein is marked with an arrow head.

labeling of the 190 kDa protein to be saturable in the range 0.1-2.5 μM of the drug. Furthermore, the specificity of IAAQ towards the 190 kDa protein was confirmed by photolabeling of cells in the presence of molar excess (150-2000-fold) of non-iodinated IAAQ. Figure 2C, *lanes a-d*, shows a marked decrease in the photolabeling of the 190 kDa protein in the presence of molar excess of IAAQ. The non-specific labeling, which is evident in some lower molecular mass proteins (mostly in the 120 kDa protein), was not significantly affected with excess non-iodinated IAAQ. The identity of the 190 kDa protein as MRP was further confirmed by immunoprecipitation of IAAQ photolabeled cell lyzate from H69/AR cells with the MRP or P-gp-specific mAbs. Figure 3 shows a photolabeled 190 kDa protein specifically immunoprecipitated with mAb QCRL-1 from H69/AR, but not from H69 cells. Moreover, no IAAQ photoaffinity labeled 190 kDa protein was immunoprecipitated with a P-glycoprotein-specific mAb, C494 (Figure 3).

Inhibition of Photoaffinity Labeling of MRP

To demonstrate that IAAQ binds to MRP at a biologically relevant site(s), H69/AR cells were photolabeled with IAAQ in the presence of molar excess of LTC_4, MK 571 and vinblastine. The photoaffinity labeling of MRP was completely inhibited at 75-fold molar excess of LTC_4 (Figure 4A). Similarly, molar excess of MK 571 also led to a dramatic decrease in the photolabeling of MRP with IAAQ. Vinblastine at 1000-folds molar excess was much weaker than LTC_4 and MK 571 at inhibiting the photolabeling of MRP with IAAQ (Figure 4A). Together, these results confirm the specificity of IAAQ towards MRP and suggest that IAAQ binding to MRP occurs at the same or overlapping site(s) as MK 571 and LTC_4.

Several reports have now demonstrated that MRP mediates the transport of glutathione conjugated and glucunorinated or sulfated drugs (5, 6, 15, 22, 23); however it remains unclear if modification of amphiphilic drugs is required for binding to, and transport by MRP (6, 31). To determine if unaltered IAAQ binds directly to MRP, membrane fractions from H69/AR cells were labeled with IAAQ in the absence and presence of molar excess of vinblastine and MK 571. Figure 4b shows the photolabeling of MRP in membranes from H69/AR but not H69 cells. Moreover, molar excess of MK 571 and to a less extend vinblastine inhibited the photoaffinity labeling of MRP with IAAQ (figure 4B). Furthermore, the presence of exogenously added GSH (up to 1.5 mM) did not inhibit the photoaffinity labeling of MRP with IAAQ (data not shown).

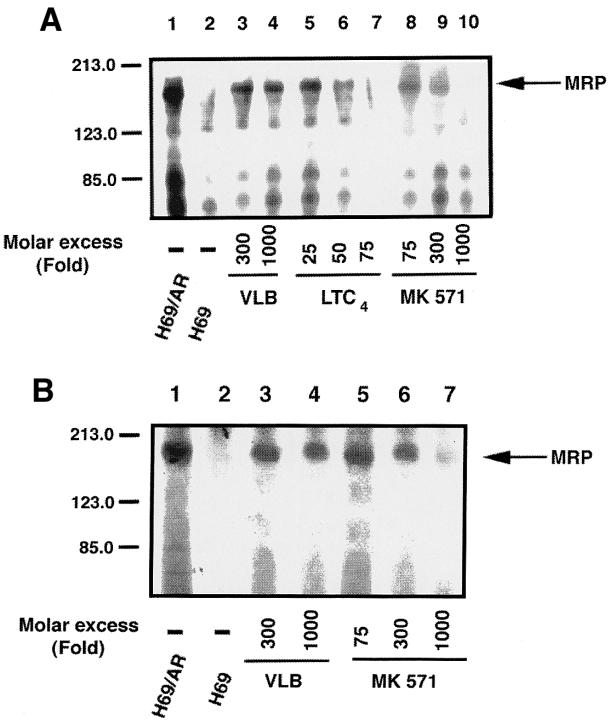

FIG. 4. Effects of vinblastine, MK 571 and LTC_4 on photoaffinity labeling of MRP. H69 or H69/AR cells (A) were photoaffinity labeled with IAAQ in the absence (lane 1) or presence of molar excess of vinblastine (VLB) (lanes 3 and 4), LTC_4 (lanes 5-7) and MK 571 (lanes 8-10). Figure 4B shows the photoaffinity labeling of plasma membranes of H69 (lane 2) and H69/AR in the absence (lane 1) or in the presence of molar excess of vinblastine (VLB) (lanes 3 and 4) and MK 571 (lanes 5-7).

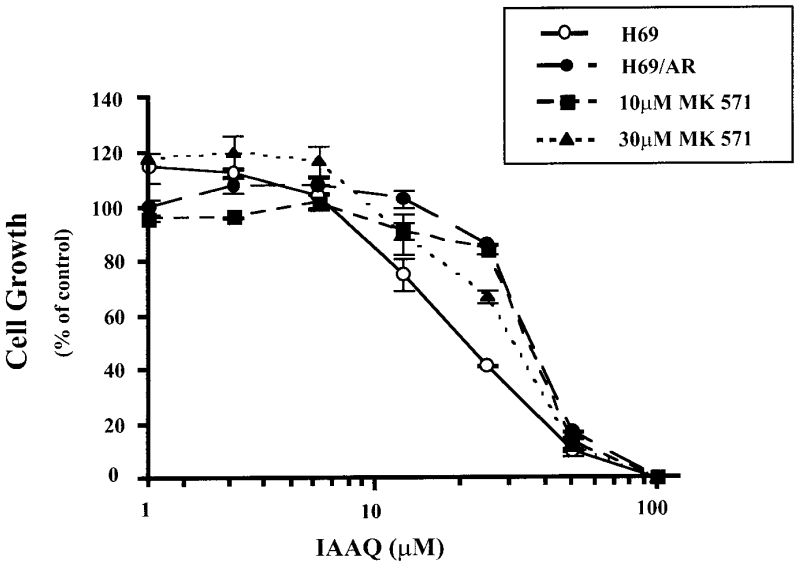

FIG. 5. Effects of IAAQ on the growth of H69 and H69/AR cells. H69 and H69/AR cells were incubated with increasing concentrations of IAAQ alone or together with 10 μM and 30 μM of MK 571. The level of cell growth was determined using the MTT assay (see Materials and Methods). Each value is mean +/− SD of the two experiments in which triplicates were assayed.

Although, our membrane extraction method is likely to remove most of the cellular GSH from H69/AR cells (which normally contain low levels of GSH; (32)), the possibility that IAAQ is altered in intact cells was examined. H69/AR cells were incubated with IAAQ and the mobility of the extracted radiolabeled material was compared to the unaltered drug by HPLC using C18 column as previously described (33). Analysis of the HPLC results showed identical elution times for IAAQ without and with incubation with H69/AR cells (data not shown).

IAAQ Is a Substrate for MRP in H69/AR Cells

Given the above results, it was of interest to determine if IAAQ is a substrate for MRP in intact cells. Figure 5 shows the effects of increasing concentrations of IAAQ on the growth of H69 and H69/AR cells. The results in figure 5 show H69/AR cells to be less sensitive to IAAQ than the parental H69 cells (IC_{50} of 20 μM and 38 μM, respectively). Furthermore, MK 571, previously shown to reverse MRP-mediated MDR (34), potentiated the toxicity of IAAQ in H69/AR (figure 5).

To determine if the decreased sensitivity of H69/AR cells to IAAQ was due to reduced drug accumulation, figure 6 shows the accumulation of IAAQ in H69 and H69/AR cells in the absence and in the presence of increasing concentrations of vinblastine, doxorubicin or MK 571. H69/AR cells show lower steady-state drug accumulation than H69 cells (figure 6B versus 6A). Moreover, a molar excess (1-25 μM) of MK 571 or doxorubicin but not VLB potentiated the accumulation of IAAQ in H69/AR cells (Figure 6b).

DISCUSSION

MRP has been shown to mediate the transport of cytotoxic natural products, in addition to glutathione S-conjugates and glucuronidated steroids (5, 6, 15, 22, 23, 35). In this report, we extend MRP substrate specificity by demonstrating its direct binding and transport of a quinoline-based drug (IAAQ). Moreover, we demonstrate for the first time a direct and specific binding between MRP and unaltered cytotoxic drug. In addition, we show that molar excess of LTC_4, MK 571 and to a lesser extend vinblastine inhibit the photoaffinity labeling of MRP with IAAQ. Interestingly, LTC_4 was more potent than MK 571 at inhibiting MRP photoaffinity labeling with IAAQ. Although these results were unexpected, considering the structural similarities between MK 571 and IAAQ (figure 1), LTC_4 is the highest affinity substrate of MRP and is more lipophilic than MK 571 (17). The reason for the weak inhibitory effect of vinblastine on IAAQ photolabeling of MRP and transport from H69/AR cells is not clear. However, it is consistent with earlier results which showed vinblastine to be a poor substrate for MRP (36, 37). Together, these results demonstrate a direct and specific binding of IAAQ to MRP.

The normal physiological substrate for MRP is presently not know. However, MRP has been shown to transport the cysteinyl leukotriene, LTC_4, and other glutathione conjugates (5, 6, 15, 22, 23). The inhibition of MRP photolabeling by IAAQ with molar excess of LTC_4 indicates that IAAQ binding to MRP occurs at a biologically relevant site. Although little is known about MRP drug binding domain(s), we speculate that

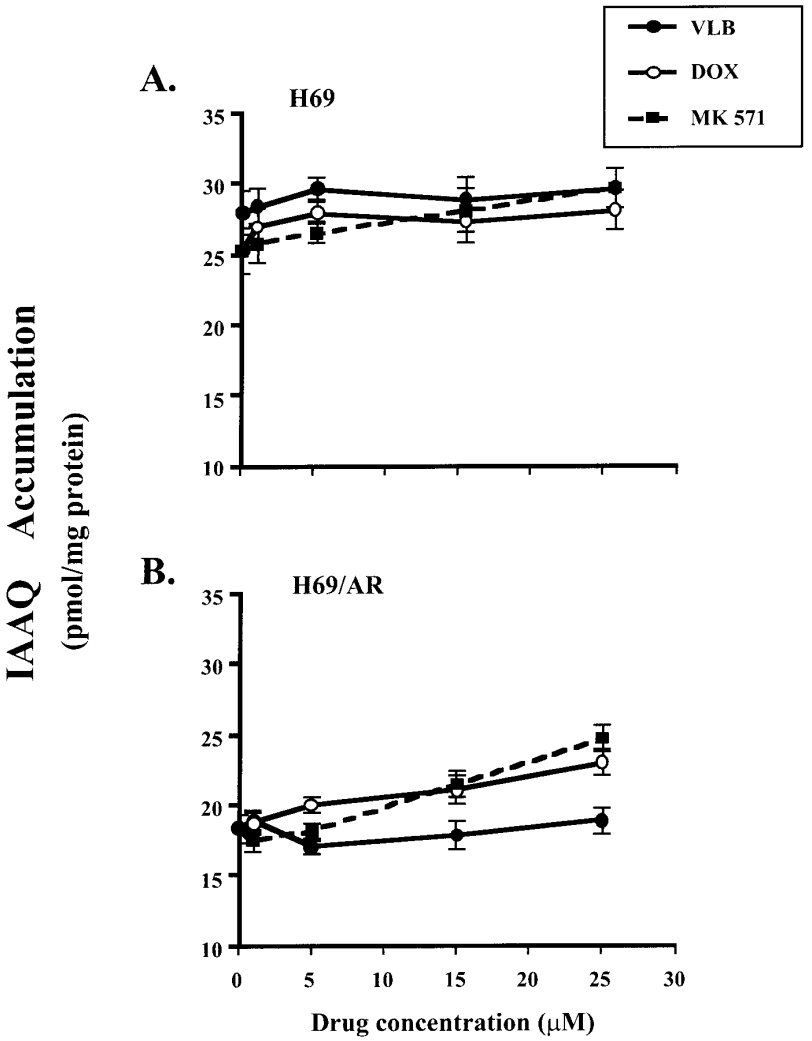

FIG. 6. IAAQ drug uptake in H69 and H69/AR cells. H69 (A) and H69/AR (B) cells were preincubated in the presence of molar excess (100-500-fold) of vinblastine (VLB), doxorubicin (Dox) and MK 571 for 30 minutes at 37°C. IAAQ (50 nM) was added to each sample and the incubation of cells was continued for another 45 minutes at 37°C. Cell were lysed and the amounts of accumulated radiolabel were determined by fluorography. Each value is mean +/− SD of the two experiments in which triplicates were assayed.

IAAQ binds to the same or an overlapping domain as that of MK 571 or LTC_4. Alternatively, IAAQ may bind at another site that is allosterically linked to LTC_4 binding domain. Indeed, glutathione S-conjugation of drugs has been shown to confer binding to MRP (16, 15). Thus, it is likely that MRP contains several allosterically linked or overlapping domains for different classes of drugs. In addition, evidence from another broad spectrum transporter (P-gp1) supports the presence of multiple drug binding sites (38-40).

Consistent with the photoaffinity labeling results, IAAQ is a substrate for MRP as it accumulates to lower levels in H69/AR than in the parental H69 cells. Furthermore, both MK 571 and doxorubicin but not vinblastine increase the accumulation of IAAQ in H69/AR cells. In addition, we show that no modification of IAAQ is required for binding to, or transport by MRP. Although the latter results are consistent with the ability of MRP to mediate the transport of unaltered natural products (41), other studies have shown that GSH alone can increase MRP binding to, and transport of natural products (31). In this study, the presence of GSH (up to 1.5 mM) did not modulate the photoaffinity labeling of MRP with IAAQ in plasma membranes. However, more careful analysis of drug binding and transport kinetics is required to rule out the effect of GSH on the binding of IAAQ to, and transport by MRP.

The LTD_4 receptor antagonist, MK 571 (24), was recently shown to inhibit LTC_4 and S-(p-azidophenylacyl)-glutathione labeling of MRP (17, 18) and to reverse MRP-mediated MDR (34). Our results show MK 571 inhibit IAAQ binding to MRP in a dose dependent fash-

ion. We speculate that the inhibition of IAAQ photolabeling of MRP with MK 571 is due to the quinoline moiety which is found in both IAAQ and MK 571. Furthermore, we have recently demonstrated a direct binding between MRP and other quinoline-based drugs (Vezmar et al., unpublished results). Consistent with these results, H69/AR cells which overexpress MRP were previously shown to be cross-resistant to chloroquine, also a quinoline-based drug (42). Collectively, our results support the notion that MK 571 interacts directly with MRP through its quinoline moiety.

The quinoline moiety is found in many therapeutically important drugs that include several antimalarials and anti-inflammatory drugs (43). Concerning the former class of compounds, the results in this report are important to our understanding of antimalarial drug resistance in *Plasmodium falciparum*. In fact, a P-gp homologue (*pfmdr1*) has been previously implicated in the resistance to antimalarial drugs in *P. falciparum* (44). However, the role of *pfmdr1* in the resistance to antimalarial drugs in the parasite remains controversial (45, 46). Considering the findings in this study and the overlap in MRP and P-gp1 functions and substrate specificity, we speculate that an MRP homologue could mediate the resistance to quinoline based drugs in *P. falciparum*. Recent studies have implicated MRP homologues in the resistance to heavy metal oxyanions and antimony in *Leishmania tarentolae*, in yeast and in *Caenorhabditis elegans* (47-49).

In conclusion, we have used a photoactive drug (IAAQ) to demonstrate a direct and specific binding to MRP in intact cells and plasma membranes from H69/AR SCLC cells. We speculate that IAAQ binds to the same or an overlapping domain(s) as that of LTC_4 or MK 571. It would be of interest in future studies to determine if IAAQ photoaffinity labels the same or different sequences as MK 571 and LTC_4. Furthermore, the availability of photoactive radio-iodinated drug that binds specifically to MRP should facilitate future analysis of MRP drug interactions.

REFERENCES

1. Endicott, J. A., and Ling, V. (1989) *Ann. Rev. Biochem.* **58,** 137–171.
2. Gottesman, M. M., and Pastan, I. (1993) *Ann. Rev. Biochem.* **62,** 385–427.
3. Cole, S. P., and Deeley, R. G. (1996) *Cancer Treat. Res.* **87,** 39–62.
4. Shapiro, A. B., and Ling, V. (1995) *J. Biol. Chem.* **270,** 16167–16175.
5. Almquist, K. C., Loe, D. W., Hipfner, D. R., Mackie, J. E., Cole, S. P., and Deeley, R. G. (1995) *Cancer Res.* **55,** 102–110.
6. Zaman, G. J. R., Flens, M. J., Vanleusden, M. R., Dehaas, M., Mulder, H. S., Lankelma, J., Pinedo, H. M., Scheper, R. J., Baas, F., Broxterman, H. J., and Borst, P. (1994) *Proc. Nati. Acad. Sci. USA* **91,** 8822–8826.
7. Pavelic, Z. P., Reisin, J., Pavelic, L., Kelley, D. J., Stambrook, P. J., and Gluckman, J. L. (1993) *Arch. Otolaryngol. Head Neck Surg.* **119,** 753–757.
8. Kruh, G. D., Gaughan, K. T., Godwin, A., and Chan, A. (1995) *J. Natl. Cancer Inst.* **87,** 1256–1268.
9. Schinkel, A. H., Smit, J. J. M., van Tellingen, O., Beijnen, J. H., Wagenaar, E., van Deemter, L., Mol, C. A. A. M., var der Valk, M. A., Robanus-Maandag, E. C., te Riele, H. P. J., Berns, A. J. M., and Borst, P. (1994) *Cell* **77,** 491–502.
10. Lorico, A., Rappa, G., Flavell, R. A., and Sartorelli, A. C. (1996) *Cancer Res.* **56,** 5351–5355.
11. Higgins, C. F. (1992) *Ann. Rev. Cell Biol.* **8,** 67–113.
12. Hyde, S. C., Emsley, P., Hartshorn, M. J., Mimmack, M. M., Gileadi, U., Pearce, S. R., Gallagher, M. P., Gill, D. R., Hubbard, R. E., and Higgins, C. F. (1990) *Nature* **346,** 362–365.
13. Bakos, E., Hegedus, T., Hollo, Z., Welker, E., Tusnady, G. E., Zaman, G. J., Flens, M. J., Varadi, A., and Sarkadi, B. (1996) *J. Biol. Chem.* **271,** 12322–12326.
14. Tusnady, G. E., Bakos, E., Varadi, A., and Sarkadi, B. (1997) *FEBS Letters.* **402,** 1–3.
15. Shen, H., Paul, S., Breuninger, L. M., Ciaccio, P. J., Laing, N. M., Helt, M., Tew, K. D., and Kruh, G. D. (1996) *Biochemistry* **35,** 5719–5726.
16. Jedlitschky, G., Leier, I., Buchholz, U., Center, M., and Keppler, D. (1994) *Cancer Res.* **54,** 4833–4836.
17. Leier, I., Jedlitschky, G., Buchholz, U., Cole, S. P., Deeley, R. G., and Keppler, D. (1994) *J. Biol. Chem.* **269,** 27807–27810.
18. Leier, I., Jedlitschky, G., Buchholz, U., and Keppler, D. (1994) *Eur. J. Biochem.* **220,** 599–606.
19. Pearce, H. L., Safa, A. R., Bach, N. J., Winter, M. A., Cirtain, M. C., and Beck, W. T. (1989) *Proc. Nati. Acad. Sci. USA* **86,** 5128–5132.
20. Zamora, J. M., Pearce, H. L., and Beck, W. T. (1988) *Mol. Pharmacol.* **33,** 454–462.
21. Pawagi, A. B., Wang, J., Silverman, M., Reithmeier, R. A., and Deber, C. M. (1994) *J. Mol. Biol.* **235,** 554–564.
22. Loe, D. W., Almquist, K. C., Cole, S. P., and Deeley, R. G. (1996) *J. Biol. Chem.* **271,** 9683–9689.
23. Paul, S., Breuninger, L. M., Tew, K. D., Shen, H., and Kruh, G. D. (1996) *Proc. Nati. Acad. Sci. USA* **93,** 6929–6934.
24. Jones, T. R., Zamboni, R., Belley, M., Champion, E., Charette, L., Ford-Hutchison, A. W., Frenette, R., Gauthier, J.-Y., Leger, S., Masson, P., McFarlane, C. S., Piechuta, H., Rokach, J., Williams, H., Young, R. N., DeHaven, R. N., and Pong, S. S. (1989) *Can. J. Phys. Pharmacol.* **67,** 17–28.
25. Lin, P. H., Selinfreund, R., Wakshull, E., and Wharton, W. (1987) *Biochemistry* **26,** 731–736.
26. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193.**
27. Desneves, J., Thorn, G., Berman, A., Galatis, D., La Greca, N., Sinding, J., Foley, M., Deady, L. W., Cowman, A. F., and Tilley, L. (1996) *Mol. Biochem. Parasit.* **82,** 181–194.
28. Fairbanks, G., Steck, T. L., and Wallach, D. F. H. (1971) *Biochemistry* **10.**
29. Nare, B., Prichard, R. K., and Georges, E. (1994) *Mol. Pharmacol.* **45,** 1145–1152.
30. Safa, A. R., Agresti, M., Tamai, I., Mehta, N. D., and Vahabi, S. (1990) *Biochem. Biophys. Res. Commun.* **166,** 259–266.
31. Loe, D. W., Almquist, K. C., Deeley, R. G., and Cole, S. P. (1996) *J. Biol. Chem.* **271,** 9675–9682.
32. Campling, B. G., Baer, K., Baker, H. M., Lam, Y.-M., and Cole, S. P. C. (1993) *Br. J. Cancer* **68,** 327–335.
33. Nare, B., Liu, Z., Prichard, R. K., and Georges, E. (1994) *Biochem. Pharmacol.* **48,** 2215–2222.

34. Gekeler, V., Ise, W., Sanders, K. H., Ulrich, W. R., and Beck, J. (1995) *Biochem. Biophys. Res. Commun.* **208,** 345–352.
35. Jedlitschky, G., Leier, I., Buchholz, U., Barnouin, K., Kurz, G., and Keppler, D. (1996) *Cancer Res.* **56,** 988–994.
36. Cole, S. P., Sparks, K. E., Fraser, K., Loe, D. W., Grant, C. E., Wilson, G. M., and Deeley, R. G. (1994) *Cancer Res.* **54,** 5902–5910.
37. Breuninger, L. M., Paul, S., Gaughan, K., Miki, T., Chan, A., Aaronson, S. A., and Kruh, G. D. (1995) *Cancer Res.* **55,** 5342–5347.
38. Loo, T. W., and Clarke, D. M. (1993) *J. Biol. Chem.* **268,** 19965–19972.
39. Loo, T. W., and Clarke, D. M. (1994) *Biochemistry* **33,** 14049–14057.
40. Tamai, I., and Safa, A. R. (1991) *J. Biol. Chem.* **266,** 16796–16800.
41. Tew, D. T. (1994) *Cancer Research* **54,** 4313–4320.
42. Cole, S. P. C., Downes, H. F., and Slovak, M. L. (1989) *Br. J. Cancer* **59,** 42–46.
43. Foley, M., and Tilley, L. (1997) *Intl. J. Parasitology* **27,** 231–240.
44. Foote, S. J., Thompson, J. K., Cowman, A. F., and Kemp, D. J. (1989) *Cell* **57,** 921–930.
45. Lim, A. S., Galatis, D., and Cowman, A. F. (1996) *Exp. Parasitology* **83,** 295–303.
46. Wellems, T. E., Panton, L. J., Gluzman, I. Y., do Rosario, V. E., Gwadz, R. W., Walker-Jonah, A., and Krogstad, D. J. (1990) *Nature* **345,** 202–203.
47. Papadopoulou, B., Roy, G., Dey, S., Rosen, B. P., and Ouellette, M. (1994) *J. Biol. Chem.* **269,** 11980–11986.
48. Szcypka, M. S., Wemmie, I. A., Moye-Rowley, W. S., and Thiele, D. J. (1994) *J. Biol. Chem.* **269,** 22853–22857.
49. Broeks, A., Gerrard, B., Allikmets, R., Dean, M., and Plasterk, R. H. A. (1996) *EMBO J.* **15,** 6132–6143.