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Modulation of the Function of Human MDR1 P-Glycoprotein by the Antimalarial Drug Mefloquine

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ABSTRACT. MDR1 P-glycoprotein in membranes of human tumor cells of the CEM/VBL_{100} line was selectively labelled using photoreactive analogs of verapamil, *N*-(*p*-azido-3-[^{125}I]salicyl)amino-verapamil ([^{125}I]ASA-V) and prazosin, 2-[4-(4-azido-3-[^{125}I]iodobenzoyl)piperazin-1-yl]4-amino-6,7-dimethoxyyquinazoline ([^{125}I]ASA-P). Mefloquine, a quinolinemethanol antimalarial drug, was shown to inhibit the labelling of P-glycoprotein with an efficiency similar to that for verapamil, a known chemosensitizer. By contrast, chloroquine competed poorly for the binding site on P-glycoprotein. Mefloquine also inhibited the functional activity of P-glycoprotein. It decreased the rates of extrusion of [^{3}H]vinblastine and the fluorescent dyes, fluo-3 acetomethoxy ester and rhodamine 123, from drug-resistant cells and decreased the level of resistance of these cells to vinblastine. The ability of mefloquine to inhibit P-glycoprotein function may be involved in the neurotoxic side-effects occasionally associated with the use of mefloquine as an antimalarial drug. BIOCHEM PHARMACOL 52;10:1545–1552, 1996. *Copyright © 1996 Elsevier Science Inc.*

KEY WORDS. P-glycoprotein; multidrug resistance; mefloquine; quinoline antimalarials

MDR¶ is a major problem in the chemotherapeutic treatment of cancers. Drug-resistant tumor cell lines, displaying the MDR phenotype, are characterized by their ability to extrude a range of structurally unrelated drugs (see Ref. 1 for review). These cells overexpress a 170-kDa membrane glycoprotein, referred to as MDR1 P-glycoprotein, which is the product of the *mdr1* gene [2]. MDR1 P-glycoprotein is postulated to confer multidrug resistance by acting as an ATP-dependent drug efflux protein. Studies using the technique of photoaffinity labelling have demonstrated that a range of cytotoxic drugs can interact with the P-glycoprotein substrate-binding sites that reside within transmembrane domains 6 and 12, adjacent to the ATP-binding domains [3, 4].

A number of chemosensitizers, including verapamil and cyclosporin A, are capable of reducing the levels of drug resistance of tumor cell lines [5–7]. These compounds appear to inhibit the binding of cytotoxic drugs by binding to an allosteric site on the MDR1 P-glycoprotein [8, 9]. It has been suggested that these chemosensitizing agents might be used in patients to reduce the P-glycoprotein-mediated resistance of clinical tumors [10, 11].

One group of compounds receiving increasing interest as potential chemosensitizers is the quinine-related compounds. Quinine, quinacrine, and chloroquine have each been shown to bind to P-glycoprotein and partially reverse drug resistance *in vitro* [12–14]. Indeed, quinine has been used in preliminary trials, in combination with mitoxantrone and aracytine, for the treatment of acute leukemias [15]. More recently, another quinine analogue, cinchonine, also has been shown to be an efficient chemosensitizer both *in vitro* and *in vivo* [16, 17]. These studies have suggested that quinine-related compounds of low toxicity may be useful in the clinical treatment of drug-resistant tumors.

Mefloquine, another quinine-related compound, is widely used to treat chloroquine-resistant strains of the malaria parasite *Plasmodium falciparum*. Mefloquine is transported in the circulation bound to serum lipoproteins (Desneves *et al.*),‖ and partitions into erythrocytes and other tissue compartments [18], leading to a long half-life in the body [19, 20]. In this study, we have used the technique of photoaffinity labelling to examine the ability of mefloquine to interact with the substrate-binding site on human MDR1 P-glycoprotein. We have found that mefloquine is a potent

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¶ *Abbreviations:* MDR, multidrug resistance; [^{125}I]ASA-V, *N*-(*p*-azido-3-[^{125}I]salicyl)amino-verapamil; [^{125}I]ASA-P, 2-[4-(4-azido-3-[^{125}I]iodobenzoyl)piperazin-1-yl]4-amino-6,7-dimethoxyquinazoline; FBS, fetal bovine serum; fluo-3, fluo-3 acetomethoxy ester; and R123, rhodamine 123.

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inhibitor of the photoaffinity labelling of P-glycoprotein. Moreover, mefloquine is shown to be an efficient modulator of the transport activity of P-glycoprotein.

MATERIALS AND METHODS

Cells

The parental human leukemia cell line CCRF-CEM was derived from lymphoblasts from a patient with acute T-cell leukemia [21]. A moderate level vinblastine-resistant cell line, CEM/A7 [22], and a strongly resistant line, CEM/VBL_{100} [23], were derived from this original isolate. CEM/A7 shows over-expression of the *mdr1* gene without amplification of the gene [22], while CEM/VBL_{100} has undergone amplification of the *mdr1* gene [24]. The two resistant cell lines were maintained in RPMI 1640 (Gibco, Gaithersburg, MD, U.S.A.), containing 10% FBS (Gibco), at 37°, in a humidified CO_2 incubator, in the presence of 70 ng/mL doxorubicin (CEM/A7) or 100 ng/mL vinblastine (CEM/VBL_{100}) (both from Sigma, St. Louis, MO, U.S.A.), as described by Zalcberg *et al.* [25]. The plasma membrane fraction of the CEM/VBL_{100} cells was prepared by differential centrifugation of cell homogenates, as described by Peterson *et al.* [26]. The degree of enrichment of the membrane preparation was monitored by western blot analysis of the level of P-glycoprotein. The membranes were resuspended in PBS, pH 7.5, and stored at −80° until required.

Western Blot Analysis

Proteins were transferred electrophoretically from SDS–polyacrylamide gels to PVDF-plus membranes (Micron Separation Inc., Westboro, MA, U.S.A.). The membranes were probed with the JBS-1 murine monoclonal antibody to human P-glycoprotein (Stanbio, USA), and reactive zones were visualized using ^{125}I-labelled protein A.

Synthesis of N-ASA-V

N-ASA-V was prepared using modifications of the procedures described by Safa [7] and Desneves *et al.*** This method minimizes the number of manipulations involving radiolabelled reagents. A solution of 4-azidosalicylic acid (1.12 mmol) and 1,1′-carbonyldiimidazole (1.12 mmol) in dry dioxan (4 mL) was refluxed for 10 min and left at room temperature for 1 hr. A solution of 5-[(3,4-dimethoxyphenethyl)methyl-amino]-2-(3,4-dimethoxyphenyl)-2-isopropylpentylamine (0.56 mmol) in dry dioxan (4 mL) was added, the mixture was heated under reflux for 10 min, and then it was stirred overnight at room temperature. The solvent was removed at reduced pressure, the residue was dissolved in chloroform (8 mL), washed successively with 10% sodium bicarbonate (2 × 6 mL), water (2 × 6 mL), and dried ($MgSO_4$), and the solvent was removed at reduced pressure. A yellow solid was obtained in close to quantitative yield. The product was purified by silica gel chromatography using chloroform:methanol:ammonium hydroxide (92.5:2.5:5.0, by vol.). ESMS: m/z 620.4 (M + 1). FTIR:neat (NaCl) 2160 cm^{-1}, indicating the azide group. TLC (on silica gel G, Merck, Darmstadt, Germany), developed with a mixture of chloroform:methanol:water (80:20:3, by vol.), gave an R_f value of 0.76, in agreement with previous data [7].

Preparation of Radioiodinated Photoaffinity Reagents

ASA-V was radioiodinated under reduced light. ASA-V (0.65 nmol) was dissolved in 10 μL of dimethyl formamide and mixed with 10 μL of carrier-free $Na^{125}I$ (1 mCi, 0.5 nmol, Amersham, Buckinghamshire, England) and 10 μL of chloramine T (2 nmol, Sigma) in acetonitrile/dimethyl formamide (9:1, v/v). After 10 min at room temperature, the reaction was quenched with 10 μL of 5% aqueous sodium metabisulfite solution. The reaction mixture was applied to a TLC plate (silica G, Merck) and separated using chloroform:methanol:water (90:10:0.3, by vol.). In this solvent system, the radioiodinated ASA-V migrated with an R_f value of 0.72. [^{125}I]ASA-V was visualized by autoradiography and the silica gel band containing the product was collected and extracted twice with 30 mL of chloroform:methanol (8:2, v/v). The extract was dried under nitrogen in siliconized glass tubes and redissolved in DMSO. The specific activity of the preparation of [^{125}I]ASA-V used in this study was estimated to be 750 mCi/μmol. [^{125}I]ASA-V was also synthesized according to Safa [7]. Similar photoaffinity labelling results were obtained using either preparation of [^{125}I]ASA-V.

[^{125}I]ASA-P was purchased from Du Pont/NEN (Boston, MA, U.S.A.). The preparation had a specific activity of 2200 mCi/μmol.

Photoaffinity Labelling of MDR1 P-Glycoprotein

Plasma membrane preparations from CEM/VBL_{100} cells (50 μL, equivalent to approximately 10^7 cells) in PBS were mixed with 2 μL (0.5 μCi) of the iodinated photoreactive ligand in DMSO and incubated under reduced light for 20 min at 37°. The samples were photoactivated by irradiation with long wavelength ultraviolet light (365 nm, 20°), using a Mineralight UVGL-58 held 10 cm above the samples. Incorporation of radiolabel was found to reach a plateau after approximately 10 min of exposure to ultraviolet light; thus, a 10-min illumination period was used in all experiments. Following photoactivation, the samples were subjected to SDS-PAGE, and radiolabelled proteins were visualized by phosphorimage analysis (Molecular Dynamics, Sunnyvale, CA, U.S.A.). Image analysis was performed using software contained within the ImageQuant package (Molecular Dynamics). For competition experiments, mefloquine (donated by Hoffmann-La Roche, Switzerland),

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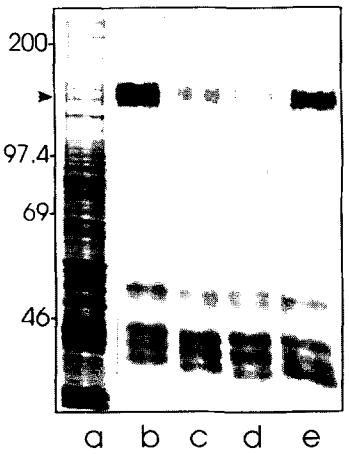

FIG. 1. Inhibition of photoaffinity labelling of MDR1 P-glycoprotein with the prazosin analogue [^{125}I]ASA-P, by verapamil, mefloquine, and chloroquine. A plasma membrane sample (10 μg protein, 50 μL), prepared from the multidrug-resistant human tumor cell line CEM/VBL$_{100}$, was incubated with [^{125}I]ASA-P (0.5 μCi, 5 nM) and exposed to UV illumination either alone (b) or in the presence of 50 μM verapamil (c), 50 μM mefloquine (d), or 50 μM chloroquine (e). Samples were subjected to SDS-PAGE (7.5% acrylamide) and visualized by phosphorimage analysis. The position of the 170-kDa P-glycoprotein is marked with an arrow head. Lane (a) shows the silver-stained profile of lane (b). Typical data are presented from experiments performed on three separate occasions.

chloroquine, prazosin, and verapamil (all for Sigma) were added from concentrated stocks in DMSO and incubated with the membrane samples for 15 min at 37°. An equivalent volume of DMSO was added to the control samples. The photoreactive ligand was added, and the samples were incubated for a further 20 min at 37° prior to exposure to ultraviolet light.

Kinetic Studies

The accumulation of [^{3}H]vinblastine (Amersham, 12 Ci/mmol) into CEM/VBL$_{100}$ cells was determined as described by Zalcberg *et al.* [25]. Approximately 5 × 10^6 cells were incubated, at 37°, in RPMI 1640 and 10% FBS, with [^{3}H]vinblastine (0.25 μCi/mL, 20 nM), in the presence of increasing concentrations of verapamil or mefloquine. Aliquots were removed at intervals and washed twice with PBS, and the cell-associated radioactivity was determined by scintillation counting. The accumulation of [^{3}H]vinblastine by cells incubated in the presence of 5 mM deoxycholate and 10 mM sodium azide in PBS was used to determine the level of non-specific binding of the drug.

The accumulation of the fluorescent dye fluo-3 (Molecular Probes, Eugene, OR, U.S.A.) was performed as described by Wall *et al.* [27, 28]. Briefly, a suspension of CEM/A7 cells (approximately 5 × 10^6 cells) was incubated, at 37°, in RPMI 1640 and 10% FBS. Fluo-3 was added to a final concentration of 6 μM from a concentrated stock in DMSO, and data were collected continuously, using a FACscan flow cytometer (Becton Dickinson, Franklin Lake, NJ, U.S.A.) that had been modified to allow continuous stirring and thermoregulation at 37°. The fluorescence of single viable cells was measured on a linear scale at 530 nm (bandwidth 30 nm) at a rate of 200–1000 cells/sec for 2 min. Verapamil and mefloquine were added from concentrated stocks in ethanol.

The efflux of R123 (Sigma) was assessed using methods described previously [29, 30]. Briefly, a suspension of CEM/A7 cells (approximately 5 × 10^6 cells) was incubated for 30 min, at 37°, in RPMI 1640 and 10% FBS, in the presence of 4.2 μM R123. Accumulation was confirmed by flow cytometry, and the cells were washed three times and resuspended in RPMI 1640, 10% FBS, containing various concentrations of verapamil, mefloquine, or diluent controls. The cells were incubated with stirring at 37° for 1 hr prior to cytometric analysis using a logarithmic scale at 530 nm (30 nm bandwidth). The mean channel fluorescence was analyzed using the Lysis II program (Becton Dickinson).

Toxicity Studies

The sensitivity of the CEM/VBL$_{100}$ cell line to vinblastine, in the presence and absence of mefloquine or verapamil, was determined as described by Hu *et al.* [31]. Briefly, approximately 10^5 cells (2 mL) were suspended in 24-well plates (Flow Laboratories, Switzerland). Verapamil and mefloquine were added from concentrated stocks in ethanol to give final concentrations of 0–8 μM. Following incubation for 24 hr, at 37°, increasing concentrations of vinblastine were added to the wells. The cells were incubated for a further 44 hr, and the number of viable cells was determined using the CellTitre 96 cell proliferation assay (Promega, Madison, WI, U.S.A.).

RESULTS AND DISCUSSION

In this study, we have examined the ability of mefloquine, an antimalarial drug, to interact with the substrate-binding site on human MDR1 P-glycoprotein. To do this, we have made use of the observation that P-glycoprotein can be selectively labelled using a photoreactive analogue of prazosin, [^{125}I]ASA-P [4]. We confirmed that [^{125}I]ASA-P was incorporated into a 170-kDa polypeptide in membranes of CEM/VBL$_{100}$ cells (Fig. 1, lanes a and b). The 170-kDa band co-migrated with a polypeptide identified as MDR1 P-glycoprotein by western blotting (data not shown). Mefloquine was shown to compete efficiently for the ligand-binding site on P-glycoprotein (Fig. 1, lane d). The level of competition in the presence of mefloquine was similar to that observed for the chemosensitizing agent verapamil (Fig. 1, lane c). By contrast, chloroquine induced only a small decrease in the level of labelling (Fig. 1, lane e). In the presence of verapamil, mefloquine, or chloroquine (at 50 μM), the level of incorporation of [^{125}I]ASA-P was decreased by 75 ± 3, 89 ± 3, and 20 ± 10%, respectively. It

should be noted that [^{125}I]ASA-P was also incorporated into a number of lower molecular weight proteins; however, this labelling was not decreased in the presence of the competing drugs, and is presumably non-specific in nature. A more extensive analysis of the abilities of mefloquine and verapamil to compete with [^{125}I]ASA-P for binding to P-glycoprotein is shown in Fig. 2A. Under the conditions of this experiment, the concentrations of verapamil and mefloquine required to achieve 50% inhibition of labelling were estimated to be 13 and 2 μM, respectively (Fig. 2B).

A photoreactive analogue of verapamil ([^{125}I]ASA-V) has also been used previously to label MDR1 P-glycoprotein [7]. We used a modified procedure for the synthesis of [^{125}I]ASA-V, and confirmed that this reagent labels P-glycoprotein (Fig. 3, lane a). Mefloquine and verapamil competed efficiently with [^{125}]ASA-V for binding to P-glycoprotein (Fig. 3, lanes b and c), indicating identical or overlapping binding sites for [^{125}]ASA-V and [^{125}I]ASA-P. Photoaffinity-labelling studies do not allow the determination of precise association constants; however, taken together, the above data suggest that, under the conditions of this experiment, mefloquine interacts with P-glycoprotein with an affinity similar to, or slightly higher than, that for verapamil.

To determine whether the binding of mefloquine alters the transport function of P-glycoprotein, we examined the ability of mefloquine to modulate the rate of extrusion of small molecular weight compounds from drug-resistant tumor cells. A number of chemosensitizers have been shown previously to modulate drug uptake in the P-glycoprotein-overexpressing line CEM-VBL$_{100}$, but not in the parent cell line CCRF-CEM [22]. We found that CEM/VBL$_{100}$ cells efficiently extruded [^{3}H]vinblastine, so that, during a 160-min incubation period, the drug was accumulated only to very low levels (Fig. 4). In the presence of 8 μM verapamil, [^{3}H]vinblastine was accumulated much more extensively (Fig. 4), in agreement with previous studies [31, 32]. Mefloquine, at a concentration of 8 μM, appeared to be an even more potent inhibitor of P-glycoprotein efflux activity and allowed the rapid accumulation of [^{3}H]vinblastine (Fig. 4).

The ability of mefloquine to modulate P-glycoprotein transport activity was also examined in the tumor cell line, CEM/A7, which displays an increased level of P-glycoprotein expression without amplification of the *mdr1* gene. This cell line is thought to more closely represent the phenotype of clinical MDR [22]. In the absence of any modulating agent, the fluorescent dye fluo-3, which is a

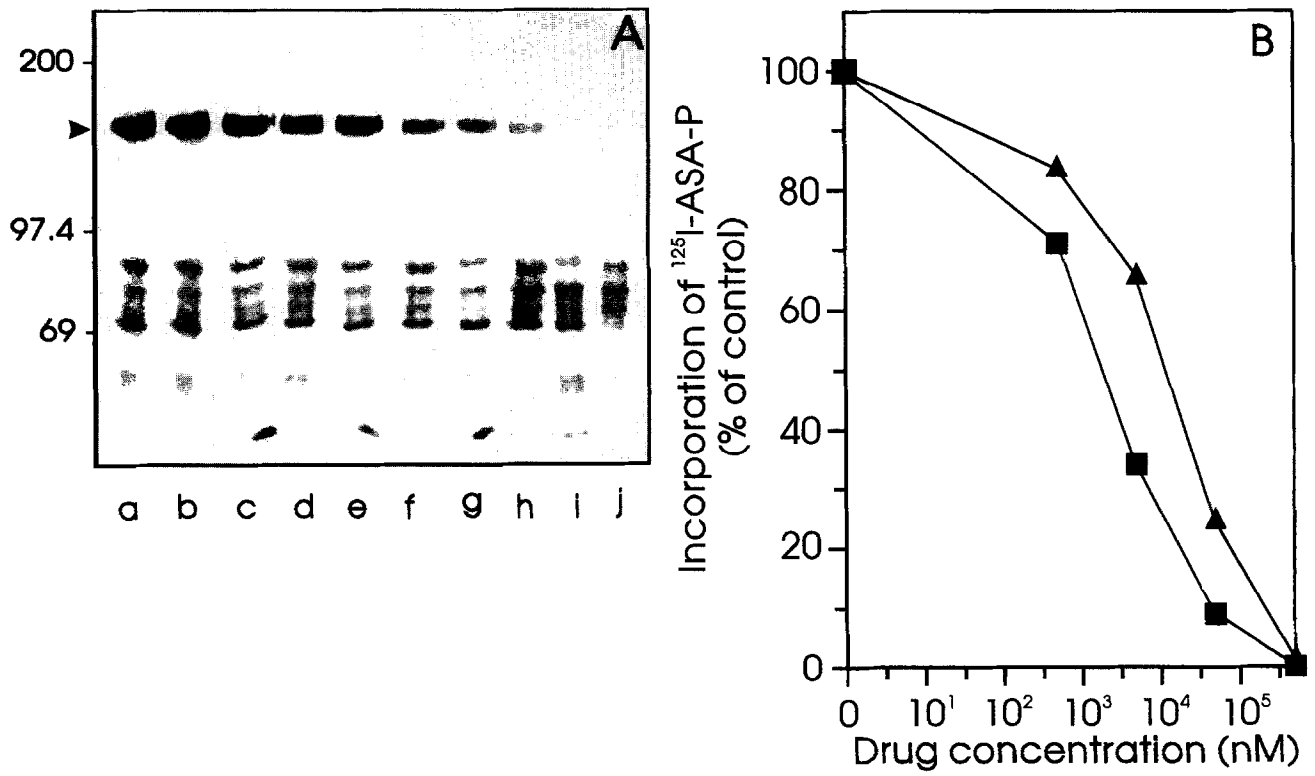

FIG. 2. Quantitative analysis of the inhibition of [^{125}I]ASA-P photoaffinity labelling of MDR1 P-glycoprotein by mefloquine and verapamil. (A) A plasma membrane sample (10 μg protein, 50 μL), prepared from CEM/VBL$_{100}$ cells, was incubated with [^{125}I]ASA-P (0.5 μCi, 5 nM) and exposed to UV illumination either alone (a and b) or in the presence of verapamil at a final concentration of 0.5 μM (c), 5 μM (e), 50 μM (g), or 500 μM (i) or mefloquine at a final concentration of 0.5 μM (d), 5 μM (f), 50 μM (h), or 500 μM (j). Samples were subjected to SDS–PAGE (7.5% acrylamide) and visualized by phosphorimage analysis. The position of the 170-kDa P-glycoprotein is marked with an arrow head. (B) The level of incorporation of [^{125}I]ASA-P into P-glycoprotein in the presence of verapamil (▲) or mefloquine (■) was analysed using ImageQuant software. The data presented are typical of experiments performed on three separate occasions.

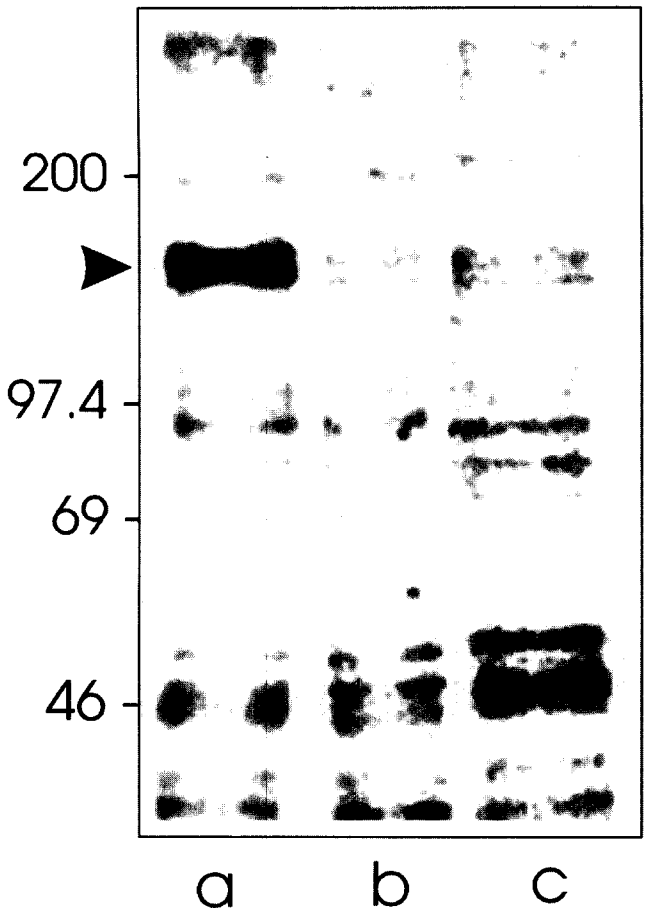

FIG. 3. Photoaffinity labelling of MDR1 P-glycoprotein with the verapamil analogue [^{125}I]ASA-V. A plasma membrane preparation (10 μg protein, 50 μL) of CEM/VBL$_{100}$ cells was incubated with [^{125}I]ASA-V (0.5 μCi, 13 nM) and exposed to UV illumination either alone (a) or in the presence of 130 μM mefloquine (b) or 130 μM verapamil (c). Samples were subjected to SDS–PAGE (7.5% acrylamide) and visualized by phosphorimage analysis. The position of the 170-kDa P-glycoprotein is marked with an arrow head.

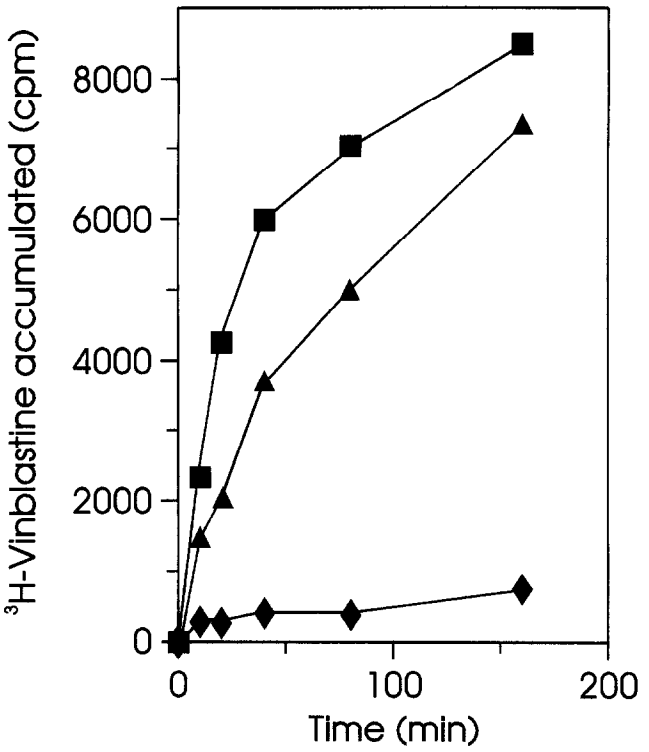

FIG. 4. Effects of mefloquine and verapamil on accumulation of [^{3}H]vinblastine by CEM/VBL$_{100}$ cells. A suspension of cells (approximately 5 × 10^6 cells) was incubated, at 37°, with [^{3}H]vinblastine (0.25 μCi/mL) either alone (♦) or in the presence of 8 μM verapamil (▲) or 8 μM mefloquine (■). Aliquots were removed at intervals and washed twice with PBS, and the cell-associated radioactivity was determined by scintillation counting. The assays were performed in triplicate, and the data presented are typical results from experiments performed on three separate occasions.

substrate for P-glycoprotein, was largely excluded from these cells (Fig. 5A). In the presence of increasing concentrations of verapamil, the linear rate of accumulation of fluo-3 was enhanced (Fig. 5A). Mefloquine had an even more dramatic effect on the rate of accumulation of the fluorescent dye (Fig. 5A). Similarly, when the CEM/A7 cells were loaded with another fluorescent dye, R123, subsequent incubation in the presence of increasing concentrations of mefloquine or verapamil inhibited the efflux of R123 (Fig. 5B).

In addition to these studies on the modulation of uptake of P-glycoprotein substrates, we examined the ability of mefloquine to modulate the sensitivity of CEM/VBL$_{100}$ cells to cytotoxic agents. At concentrations up to 16 μM, the growth of CEM/VBL$_{100}$ cells was not inhibited by either verapamil or mefloquine alone. However, both mefloquine and verapamil (at concentrations of 2–8 μM) greatly increased the sensitivity of CEM/VBL$_{100}$ cells to vinblastine (Fig. 6). In the absence of the sensitizing agents, the CEM/VBL$_{100}$ cells were resistant to vinblastine at concentrations up to 160 nM. In the presence of 8 μM mefloquine or verapamil, cell growth was inhibited 50% at vinblastine concentrations of 3.2 and 14 nM, respectively. The precise concentration at which the drugs modulate P-glycoprotein function is likely to depend on the conditions of the experiments; however, the data indicate that, under the conditions of the present conditions, mefloquine is a very potent modulator of drug sensitivity.

It has been proposed that quinine and cinchonine, quinoline-containing compounds, could be used in combination with cytotoxic drugs in the treatment of tumors, in a bid to prevent or reverse clinical multidrug resistance [16]. The data from this study indicate that mefloquine could also potentially be administered in conjunction with an anti-cancer drug in an effort to increase the potency of the cytotoxic agent. The side-effects of mefloquine administration are relatively mild, even at serum concentrations of 1–10 μM [19, 20]. However, extreme caution needs to be exercised in the development of this type of strategy. MDR1 P-glycoprotein is expressed in a number of locations, including the endothelial cells of the blood–brain barrier [33] and the blood–testes barrier [34]. The P-glycoprotein

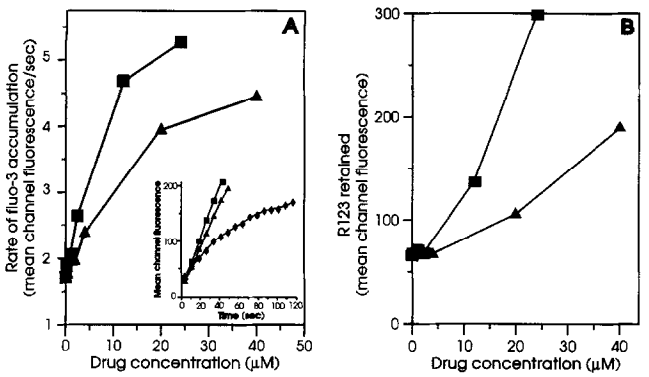

FIG. 5. Effects of mefloquine and verapamil on the rate of extrusion of fluorescent dyes by CEM/A7 cells. (A) A suspension of cells (approximately 5 × 10^6 cells) was incubated at 37° with 6 μM fluo-3 in the presence of increasing concentrations of verapamil (▲) or mefloquine (■). The rate of fluo-3 accumulation (mean channel fluorescence per second) was determined using the kinetic software Chronys on a FACscan flow cytometer. Inset: Representative data used for the determination of the rate of fluo-3 accumulation. The mean channel fluorescence was monitored as a function of time in the absence (♦) of any modulator or in the presence of 20 μM verapamil (▲) or 12 μM mefloquine (■). (B) A suspension of cells (approximately 5 × 10^6 cells) was incubated at 37° for 30 min with 4.2 μM R123, then washed, and incubated for 1 hr in the presence of increasing concentrations of verapamil (▲) or mefloquine (■). The level of R123 that remained associated with the cells (mean channel fluorescence) was assessed by flow cytometry. The data presented are typical results from experiments performed on two separate occasions.

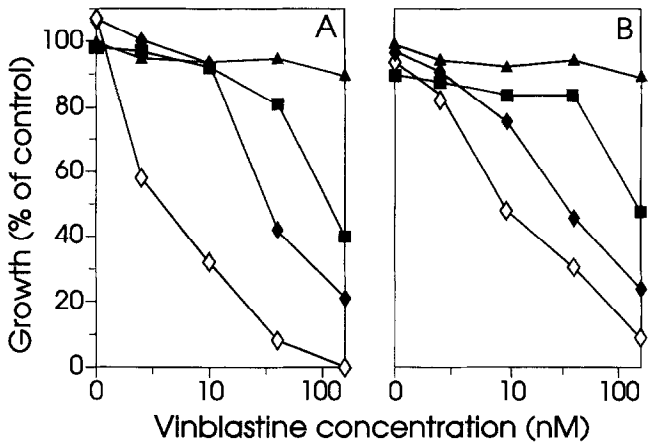

FIG. 6. Effects of mefloquine and verapamil on vinblastine toxicity in CEM/VBL_{100} cells. Cells (2 mL, approximately 10^5 cells) were cultured for 24 hr in the presence of mefloquine (A) or verapamil (B) at concentrations of 0 μM (▲), 2 μM (■), 4 μM (♦), or 8 μM (◇). Vinblastine was added and the cells were incubated for a further 44 hr. The number of viable cells was determined using a dye exclusion assay (see Materials and Methods). The assays were performed in triplicate, and the data presented are typical results from experiments performed on three separate occasions.

transport function may play an important physiological role in protecting these organs against the accumulation of toxic compounds [33, 35]. Inhibition of P-glycoprotein function *in vivo* by chemosensitizers, such as verapamil and cyclosporin, has been shown to increase the toxic side-effects of drugs such as daunorubicin and doxorubicin (see Ref. 10 for review) and to allow P-glycoprotein substrates to cross the blood–brain barrier [36]. Furthermore, recent "knockout" experiments produced mice that were homozygous for a defective gene for the brain-associated murine P-glycoprotein, *mdr1a* (−/−). These mice were extremely susceptible to the neurotoxic drug ivermectin [35]. Thus, major complications could arise due to inhibition of the physiological detoxification role of MDR1 P-glycoprotein at the blood–tissue barriers.

The ability of mefloquine to inhibit the transport activity of MDR1 P-glycoprotein may also have important implications in the use of this drug as an antimalarial agent. The use of mefloquine as a prophylactic or curative antimalarial drug has been associated with relatively frequent reports of mild central nervous system toxicity. Administration of mefloquine can result in symptoms of confusion, dizziness, and dysphoria, and occasionally in severe neuropsychiatric effects [37–41]. While these occasional side-effects are considered acceptable during the treatment of life-threatening severe malaria, they have compromised the usefulness of mefloquine as a prophylactic drug. For example, mefloquine is contraindicated for patients with a history of convulsions or psychiatric disorders [42]. The data from our studies indicate that serum levels of mefloquine of 1–10 μM would be sufficient to modulate MDR1 P-glycoprotein function. As a consequence, mefloquine could cross the blood–brain barrier and exert a mild neurotoxic effect itself, or exacerbate the toxicity of other compounds encountered concurrently. For example, the insect repellent compound, *N,N*-diethyl-*m*-toluamide (DEET), which has been shown to be neurotoxic in some individuals [43], is likely to be used in conjunction with mefloquine.

In conclusion, our studies indicate that mefloquine is a potent inhibitor of the function of human MDR1 P-glycoprotein. Accordingly, mefloquine may deserve further attention as a candidate for use in the reversal of clinical multidrug resistance. Given that chloroquine, which binds only poorly to P-glycoprotein, has minimal neurologic side-effects, the ability of mefloquine to inhibit P-glycoprotein function may be involved in the neurotoxic side-effects occasionally associated with the use of mefloquine as an antimalarial drug.

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References

1. Gottesman MM and Pastan I, Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* **62:** 385–427, 1993.
2. Riordan JR, Deuchars K, Kartner N, Alon N, Trent J and Ling

V, Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature* **316:** 817–819, 1985.
3. Safa AR, Glover CJ, Sewell JL, Meyers MB, Biedler JL and Felsted RL, Identification of the multidrug resistance-related membrane glycoprotein as an acceptor for calcium channel blockers. *J Biol Chem* **262:** 7884–7888, 1987.
4. Greenberger LM, Major photoaffinity drug labelling sites for iodoaryl azidoprazosin in P-glycoprotein are within, or immediately C-terminal to, transmembrane domains 6 and 12. *J Biol Chem* **268:** 11417–11425, 1993.
5. Ford JM and Hait WN, Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev* **42:** 155–199, 1990.
6. Wigler PW and Patterson FK, Inhibition of the multidrug resistance efflux pump. *Biochim Biophys Acta* **1154:** 173–181, 1993.
7. Safa AR, Photoaffinity labelling of the multidrug-resistance-related P-glycoprotein with photoactive analogs of verapamil. *Proc Natl Acad Sci USA* **85:** 7187–7191, 1988.
8. Boer R, Dichtl M, Borchers C, Ulrich WR, Marecek JF, Prestwich GD, Glossmann H and Striessnig J, Reversible labeling of a chemosensitizer binding domain of P-glycoprotein with a novel 1,4-dihydropyridine drug transport inhibitor. *Biochemistry* **35:** 1387–1396, 1996.
9. Ferry DR, Russell MA and Cullen MH, P-Glycoprotein possesses a 1,4-dihydropyridine-selective drug acceptor site which is allosterically coupled to a vinca-alkaloid-selective binding site. *Biochim Biophys Res Commun* **188:** 440–445, 1992.
10. Sikic BI, Modulation of multidrug resistance: At the threshold. *J Clin Oncol* **11:** 1629–1635, 1993.
11. Lum BL, Fisher GA, Brophy NA, Yahanda AM, Adler KM, Kaubisch S, Halsey J and Sikic BI, Clinical trials of modulation of multidrug resistance. *Cancer* **72** (Suppl): 3502–3514, 1993.
12. Akiyama S-I, Cornwell MM, Kuwano M, Pastan I and Gottesman MM, Most drugs that reverse multidrug resistance also inhibit photoaffinity labelling of P-glycoprotein by a vinblastine analog. *Mol Pharmacol* **33:** 144–147, 1988.
13. Solary E, Velay I, Chauffert B, Bidan JM, Caillot D, Dumas M and Guy H, Sufficient levels of quinine in serum circumvent the multidrug resistance of the leukemic cell line K562/ADM. *Cancer* **68:** 1714–1718, 1991.
14. Beck WT, Cirtain MC, Glover CJ, Felsted RL and Safa AR, Effects of indole alkaloids on multidrug resistance and labelling of P-glycoprotein by a photoaffinity analog of vinblastine. *Biochem Biophys Res Commun* **153:** 959–966, 1988.
15. Solary E, Caillot D, Chauffert B, Casasnovas R-O, Dumas M, Maynadie M and Guy H, Feasibility of using quinine, a potential multidrug resistance-reversing agent, in combination with mitoxantrone and cytarabine for the treatment of acute leukemia. *J Clin Oncol* **10:** 1730–1736, 1992.
16. Genne P, Duchamp O, Solary E, Pinard D, Belon JP, Dimanche-Boitrel MT and Chauffert B, Comparative effects of quinine and cinchonine in reversing multidrug resistance on human leukemic cell line K562/ADM. *Leukemia* **8:** 160–164, 1994.
17. Genne P, Duchamp O, Solary E, Magnette J, Belon JP and Chauffert B, Cinchonine *per os*: Efficient circumvention of P-glycoprotein-mediated multidrug resistance. *Anticancer Drug Des* **10:** 103–118, 1995.
18. San George RC, Nagel RL and Fabry ME, On the mechanism of the red cell accumulation of mefloquine, an antimalarial drug. *Biochim Biophys Acta* **803:** 174–181, 1984.
19. Schwartz DE, Eckert G, Hartmann D, Weber B, Richard-Lenoble D, Ekue JMK and Gentilini M, Single dose kinetics of mefloquine in man. *Chemotherapy* **28:** 70–84, 1982.
20. ter Kuile FO, Nosten F, Thieren M, Luxemburger C, Edstein MD, Chongsuphajaisiddhi T, Phaipun L, Webster HK and White NJ, High-dose mefloquine in the treatment of multidrug-resistant falciparum malaria. *J Infect Dis* **166:** 1393–1400, 1992.
21. Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA and McCarthy RE, Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18:** 522–529, 1965.
22. Zalcberg JR, Hu XF, Wall DM, Mirski S, Cole S, Nadalin G, De Luise M, Parkin JD, Vrazas V, Cambell L and Kantharidis P, Cellular and karyotypic characterization of two doxorubicin-resistant cell lines isolated from the same parental human leukemic cell line. *Int J Cancer* **57:** 522–528, 1994.
23. Beck WT, Mueller TJ and Tanzer LR, Altered surface membrane glycoproteins in *Vinca* alkaloid-resistant human leukemic lymphoblasts. *Cancer Res* **39:** 2070–2076, 1979.
24. Beck WT, *Vinca* alkaloid-resistant phenotype in cultured human leukemic lymphoblasts. *Cancer Treat Rep* **67:** 875–882, 1983.
25. Zalcberg JR, Hu XF, Ching M, Wakeling A, Wall DM, Marschner IC and De Luise M, Differential effects of estrogen, tamoxifen and the pure antiestrogen ICI 182,780 in human drug-resistant leukemia cell lines. *Cancer Chemother Pharmacol* **33:** 123–129, 1993.
26. Peterson RHF, Meyers MB, Spengler BA and Biedler JL, Alteration of plasma membrane glycopeptides and gangliosides of Chinese hamster ovary cells accompanying development of resistance to daunorubicin. *Cancer Res* **43:** 222–228, 1983.
27. Wall DM, Hu XF, Zalcberg JR and Parkin JD, Rapid functional assay for multidrug resistance in human tumor cell lines using the fluorescent indicator fluo-3. *J Natl Cancer Inst* **3:** 206–207, 1991.
28. Wall DM, Sparrow R, Hu XF, Nadalin G, Zalcberg JR, Marschner IC, Van Der Weyden M and Parkin JD, Clinical application of a rapid, functional assay for multidrug resistance based on accumulation of the fluorescent dye, fluo-3. *Eur J Cancer* **29A:** 1024–1027, 1993.
29. Coon JS, Wang Y and Bines SD, Multidrug resistance activity in human lymphocytes. *Hum Immunol* **32:** 134–140, 1991.
30. Kessel D, Beck WT, Kukuruga D and Schulz V, Characterization of multidrug resistance by fluorescent dyes. *Cancer Res* **51:** 4665–4670, 1991.
31. Hu XF, Martin TJ, Bell DR, De Luise M and Zalcberg JR, Combined use of cyclosporin A and verapamil in modulating multidrug resistance in human leukemia cell lines. *Cancer Res* **50:** 2953–2957, 1990.
32. Hu XF, De Luise M, Martin TJ and Zalcberg JR, Effect of cyclosporin and verapamil on the cellular kinetics of daunorubicin. *Eur J Cancer* **26:** 814–817, 1990.
33. Cordon-Cardo C, O'Brien JP, Casals D, Rittman-Grauer L, Biedler JL, Melamed MR and Bertino JR, Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood–brain barrier sites. *Proc Natl Acad Sci USA* **86:** 695–698, 1989.
34. Holash JA, Harik SI, Perry G and Stewart PA, Barrier properties of testis microvessels. *Proc Natl Acad Sci USA* **90:** 11069–11073, 1993.
35. Schinkel AH, Smit JJM, van Tellingen O, Beijnen JH, Wagenaar E, van Deemter L, Mol CAAM, van der Valk MA, Robanus-Maandag EC, te Riele HPJ, Berns AJM and Borst P, 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.
36. Wang Q, Yang H, Miller DW, and Elmquist WF, Effect of the

P-glycoprotein inhibitor, cyclosporin A, on the distribution of rhodamine-123 to the brain: An *in vivo* microdialysis study in freely moving rats. *Biochem Biophys Res Commun* **211:** 719–726, 1995.
37. Luxemburger C, Nosten F, ter Kuile F, Frejacques L, Chongsuphajaisiddhi T and White NJ, Mefloquine for multidrug-resistant malaria. *Lancet* **338:** 1268, 1991.
38. Weinke T, Trautmann M, Held T, Weber G, Eichenlaub D, Fleischer K, Kern W and Phole HD, Neuropsychiatric side effects after the use of mefloquine. *Am J Trop Med Hyg* **45:** 86–91, 1991.
39. Bem JL, Kerr L and Stuerchler D, Mefloquine prophylaxis: An overview of spontaneous reports of severe psychiatric reactions and convulsions. *J Trop Med Hyg* **95:** 167–179, 1992.
40. Hennequin C, Bourée P, Bazin N, Bisaro F and Feline A, Severe psychiatric side effects observed during prophylaxis and treatment with mefloquine. *Arch Intern Med* **154:** 2360–2362, 1994.
41. Wittes RC and Saginur R, Adverse reaction to mefloquine associated with ethanol ingestion. *Can Med Assoc J* **152:** 515–517, 1995.
42. White NJ, Mefloquine. In the prophylaxis and treatment of falciparum malaria. *Br Med J* **308:** 286–287, 1994.
43. Osimitz TG and Grothaus RH, The present safety assessment of DEET. *J Am Mosq Control Assoc* **11:** 274–278, 1995.