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Multidrug Resistance Protein 1 (MRP1, ABCC1) Mediates Resistance to Mitoxantrone via Glutathione-Dependent Drug Efflux

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ABSTRACT

Based upon several previous reports, no consistent relationship between multidrug resistance protein 1 (MRP1, ABCC1) expression and cellular sensitivity to mitoxantrone (MX) toxicity can be ascertained; thus, the role of MRP1 in MX resistance remains controversial. The present study, using paired parental, MRP1-poor, and transduced MRP1-overexpressing MCF7 cells, unequivocally demonstrates that MRP1 confers resistance to MX cytotoxicity and that resistance is associated with reduced cellular accumulation of MX. This MRP1-associated reduced accumulation of MX was partially reversed by treatment of cells with 50 μM MK571 [3-[[3-[2-(7-chloroquinolin-2-yl)vinyl]phenyl]-(2-dimethylcarbamoylethylsulfanyl)methylsulfanyl] propionic acid]—an MRP inhibitor that increased MX accumulation in MRP1-expressing MCF7 cells but had no effect on MRP-poor MCF7 cells. Moreover, in vitro experiments using inside-out membrane vesicles show that MRP1 supports ATP-dependent, osmotically sensitive uptake of MX. Unlike ABCG2 (breast cancer resistance protein, mitoxantrone-resistant protein), MRP1-mediated MX transport is dependent upon the presence of glutathione or its *S*-methyl analog. In addition, MX stimulates transport of [^{3}H]glutathione. Together, these data are consistent with the interpretation that MX efflux by MRP1 involves cotransport of MX and glutathione. The results suggest that MRP1—like the alternative MX transporters ABCG2 and ABCB1 (MDR1, P-glycoprotein)—can significantly influence tumor cell sensitivity to and pharmacological disposition of MX.

Mitoxantrone (MX)—a DNA intercalator and topoisomerase II poison—has been used in the treatment of breast, hematological, prostate, and other malignancies (DiPaola et al., 2001; Kufe et al., 2003). Multiple mechanisms of cancer cell resistance to MX have been described, including altered topoisomerase II and increased efflux (Schurr et al., 1989; Harker et al., 1991, 1995a,b; Consoli et al., 1997; Errington et al., 1999; Zhou et al., 1999). Regarding increased efflux, an association between P-glycoprotein (MDR1, ABCB1) and MX resistance has long been appreciated (Dalton et al., 1986; Schurr et al., 1989; Consoli et al., 1997; Litman et al., 2000), and more recently, an association between high-level MX resistance and overexpression of ABCG2 (breast cancer resistance protein, mitoxantrone-resistant protein) has been reported (Doyle et al., 1998; Allen et al., 1999; Brangi et al., 1999; Maliepaard et al., 1999; Ross et al., 1999; Litman et al., 2000). Although ABCG2 clearly mediates MX transport and thereby confers robust cellular resistance, the role of MRP1 (ABCC1) in MX sensitivity has remained controversial; some groups have found no relationship between MRP1 overexpression and MX sensitivity, whereas others have reported low-level resistance associated with increased MRP1 (Mirski et al., 1987; Cole et al., 1994; Schneider et al., 1994; Breuninger et al., 1995; Borst et al., 2000).

Previous work in our laboratory examined an etoposide-selected cell line, MCF7/VP, that is multidrug-resistant on the basis of MRP1 amplification and overexpression (Schneider et al., 1994; Diah et al., 2001). MCF7/VP cells are cross-resistant to MX (Schneider et al., 1994) and have reduced MX accumulation because of an ATP-dependent efflux mechanism (Diah et al., 2001). However, because other genetic and phenotypic changes may have occurred during drug selection, including altered expression of other ABC transporters, we could not unequivocally attribute MX resistance to MRP1.

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ABBREVIATIONS: MX, mitoxantrone; MRP, multidrug resistance (or resistance-associated) protein; GSH, glutathione; MK571, (*E*)-3-((3-(2-(7-chloroquinolin-2-yl)vinyl)phenyl) (3-dimethylamino)-3-oxopropylthio)propanoic acid; FITC, fluorescein isothiocyanate; BSO, L-buthionine-(*S,R*)-sulfoximine; PBS, phosphate-buffered saline; BSA, bovine serum albumin; me-SG, *S*-methyl analog of glutathione.

Moreover, in these studies, it was apparent that the behavior of MX in MCF7/VP cells was in some ways atypical for an MRP1 substrate. First, unlike many classic MRP1 substrates, MX failed to form detectable conjugates of glutathione (GSH) or glucuronide. Second, in contrast to resistance associated with other weakly basic lipophilic MRP1 substrates, cellular accumulation of and resistance to MX was insensitive to depletion of intracellular glutathione (Diah et al., 2001).

Lacking in all of these studies was direct evidence that demonstrated whether or not MRP1 could mediate ATP-dependent MX efflux. Hence, the current studies, using materials derived from parental MCF7 cells stably transduced with MRP1, were undertaken to determine whether selective overexpression of MRP1 can confer resistance to MX by mediating its efflux and, if so, to characterize MX transport and its glutathione dependence. The results—indeed showing MRP1-dependent MX resistance and transport—are discussed in terms of the role of MRP1 in tumor cell sensitivity to MX and the roles of MRP family proteins in the pharmacological disposition of MX.

Materials and Methods

Materials. [Glycine-2-^{3}H]GSH (50 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA), and [^{3}H]MX (3.2 Ci/mmol) was purchased from Moravek Biochemicals (Brea, CA). Unlabeled MX and herring sperm DNA were obtained from Sigma-Aldrich (St. Louis, MO). Mouse monoclonal antibodies against ABCG2 (BXP-21), MRP1 (QCRL-1), and MK571 were from Alexis Biochemicals (San Diego, CA). Fluorescein isothiocyanate (FITC)-conjugated anti-MRP1 (QCRL-3) was obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG) was obtained from BD Transduction Laboratories (Lexington, KY).

Cell Lines and Culture. MRP1-overexpressing MCF7 cells, MCF7/MRP1-10, were derived from MRP-poor parental MCF7 cells (MCF7/WT) by stable transduction with an MRP1-expression vector as described previously (Smitherman et al., 2004). Additional transgenic MRP1-expressing clones (MCF7/MRP1-M6 and MCF7/MRP1-M24) were similarly obtained by independent retroviral transductions. The levels of MRP1 expression in the three transgenic cell lines were similar by Western blot analysis (not shown). Drug-selected MCF7 derivatives expressing MRP1 (MCF7/VP) or ABCG2 (MCF7/MX) have been reported elsewhere (Nakagawa et al., 1992; Schneider et al., 1994; Ross et al., 1999). Cell culture, cytotoxicity assays, and [^{3}H]MX accumulation studies were accomplished exactly as described previously (Diah et al., 2001). For some [^{3}H]MX accumulation experiments, cells were coincubated with 50 μM MK571, an MRP inhibitor. For some cytotoxicity experiments, intracellular GSH was depleted by 48-h treatment with 50 μM BSO before a 1-h exposure to MX (Diah et al., 2001). Total intracellular GSH concentrations were determined and calculated as described previously (Diah et al., 1999).

Transport Studies. ATP-dependent MRP1- or ABCG2-mediated transport was done using inside-out plasma membrane vesicles derived from MCF7/WT (MRP1-negative/ABCG2-negative controls), MCF7/MRP1-10 (MRP1-positive), and MCF7/MX (ABCG2-positive) cells as described previously (Paumi et al., 2001; Smitherman et al., 2004) with the inclusion of the creatine kinase/creatine phosphate ATP-regenerating system (Peklak-Scott et al., 2005). Transport reactions contained radiolabeled substrate (5 μM [^{3}H]MX or 100 μM [^{3}H]GSH) and were supplemented with reduced GSH, *S*-methyl glutathione, and/or L-glutamate as indicated. Reactions were initiated at 37°C by the addition of vesicles (15–30 μg of protein/50 μl of reaction). Reactions (10-μl aliquots) were terminated at indicated times in 1 ml of ice-cold Tris (10 mM), pH 7.5, plus 250 mM sucrose containing 10 mg/ml herring sperm DNA and processed by the rapid filtration method using hydrophilic membrane filters (GVWP; Millipore, Billerica, MA) (Paumi et al., 2001). ATP-dependent radiolabeled substrate uptake was calculated by subtracting uptake observed in ATP-negative controls containing a 4 mM concentration of the nonhydrolyzable analog β,γ-methylene ATP from uptake observed in the presence of 4 mM ATP.

Immunochemical Analyses. Western blotting was done as described previously using mouse monoclonal antibodies directed against MRP1 (QCRL-1) or ABCG2 (BXP-21) (Morrow et al., 1998). For flow cytometry, trypsinized cells were washed and fixed with 3.7% formaldehyde in phosphate-buffered saline (PBS) plus 1% bovine serum albumin (BSA) solution. Cells were permeabilized with 0.1% saponin in PBS-BSA solution. Permeabilized cells were incubated with FITC-conjugated QCRL-3 (20 μg/ml) for 2 h at room temperature, washed, and postfixed in 3.7% formaldehyde/PBS-BSA solution before analysis on a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA).

Sequence Analysis of MRP1 Expressed in MCF7/VP Cells. The entire coding region of MRP1 expressed in the drug-selected MCF7/VP cells was amplified by reverse transcription-polymerase chain reaction and sequenced by the DNA sequencing core laboratory of Wake Forest University School of Medicine.

Results

MX Resistance in MCF7/VP Cells Is Not Attributable to ABCG2. It was previously shown that etoposide-selected, MRP1-overexpressing MCF7/VP cells are cross-resistant to MX cytotoxicity and accumulate less intracellular MX than parental MCF7/WT cells (Schneider et al., 1994; Diah et al., 2001). Western analysis showed that ABCG2 expression was quite low in both MCF7/WT and MCF7/VP compared with MX-selected MCF7/MX cells (Fig. 1). Moreover, as suggested earlier (Ross et al., 1999; Yang et al., 2000), the levels of ABCG2 expressed in MCF7/WT and MCF7/VP cells were similar, indicating that ABCG2 cannot account for MX cross-resistance in MCF7/VP cells.

MRP1 Confers Resistance to MX by Reducing Intracellular Drug Accumulation. MCF7 cells stably transduced with MRP1 were used to determine whether selective overexpression of MRP1 could confer resistance to MX. Flow cytometry and Western blot analyses (Fig. 2, A and B) demonstrate that the level of MRP1 expression in the transgenic MCF7/MRP1-10 cell line was similar to that of the drug-selected MCF7/VP cell line. In addition, DNA sequence analysis revealed that the mRNA expressed in MCF7/VP cells encoded the common MRP1 variant (Cole et al., 1992), which

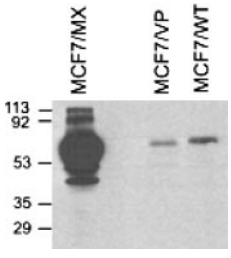

Fig. 1. Expression of ABCG2 in derivatives of MCF7 cells. Shown is a Western blot analyzed for ABCG2 expression as described under *Materials and Methods*. Each lane contained 30 μg of membrane-associated protein derived from parental (MCF7/WT), etoposide-selected MRP1-overexpressing (MCF7/VP), and MX-selected ABCG2-overexpressing (MCF7/MX) cells. Positions of molecular mass markers (kDa) are shown on the left.

is also identical to that encoded by the cDNA used to generate the transduced MCF7 cell lines (Zaman et al., 1994). Overexpression of MRP1 in three independently derived stably transduced MCF7 clones conferred 2.4-fold (MCF7/MRP1-M6 and MCF7/MRP1-M24) and 3.1-fold (MCF7/MRP1-10) greater resistance compared with control cells stably transduced with empty vector (Fig. 3). Moreover, MRP1 expression was associated with reduced MX accumulation in both transduced MCF7/MRP1-10 (~45%) and drug-selected MCF7/VP (~60%) cells (Fig. 4A). This MRP1-dependent reduction in MX accumulation was partially reversed by the MRP inhibitor MK571; as shown in Fig. 4B, 50 μM MK571 increased MX accumulation in MCF7/MRP1-10 but not MRP-poor MCF7/WT cells.

MRP1 Mediates ATP- and GSH-Dependent Transport of MX. Drug accumulation studies suggested MRP1 supports MX efflux. To verify the ability of MRP1 to mediate MX transport and to characterize its GSH dependence, inside-out membrane vesicles were used for in vitro drug transport studies. As shown in Fig. 5A, MRP1-mediated ATP-dependent transport of MX, but only in the presence of GSH (5 mM). In contrast, vesicles derived from MRP1-negative parental cells did not support ATP-dependent MX transport, even in the presence of GSH. MX uptake into inside-out vesicles derived from MCF7/MRP1-10 cells was osmotically sensitive [transport was progressively reduced with increasing sucrose concentrations (Fig. 5B)], indicating that the retained [^{3}H]MX measured represented true uptake into the intravesicular space. In contrast to MRP1-mediated uptake, transport of MX by ABCG2 was independent of GSH (Fig. 6).

To further characterize the role of GSH in MX transport, we examined whether MX would reciprocally stimulate

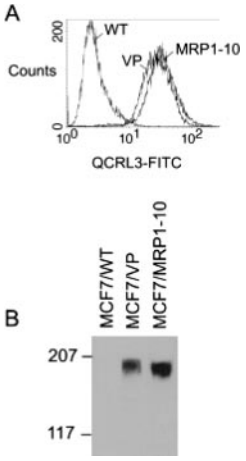

Fig. 2. Expression of MRP1 (ABCC1) in MCF7 cell derivatives. Flow cytometry (A) and Western blot (B) analyses demonstrate comparable high levels of MRP1 overexpression in stably transduced (MRP1-10) and etoposide-selected (VP) but not parental (WT) MCF7 cells. A, cell number counted (counts) and relative fluorescence (QCRL3-FITC) are shown. B, positions of molecular mass markers (kDa) are shown on the left.

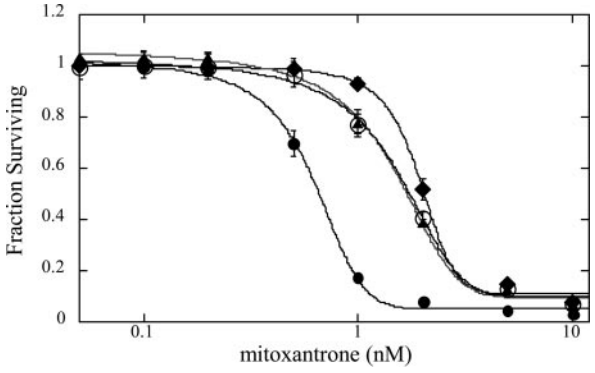

Fig. 3. Selective expression of MRP1 in stably transduced MCF7 cells confers resistance to MX cytotoxicity. Cells were treated with the indicated concentrations of MX or vehicle by continuous exposure. Cell lines used were control MRP1-negative MCF7 cells stably transduced with empty vector (●) and MRP1-positive MCF7 cells stably transduced with an MRP1 retroviral expression vector (♦, MCF7/MRP1-10; ▲, MCF7/MRP1-M6; ○, MCF7/MRP1-M24). Points represent mean values ± 1 S.D. from eight replicate determinations. IC_{50} values for the four cell lines were as follows: MCF7/WT, 0.62 nM; MCF7/MRP1-10, 1.9 nM; MCF7/MRP1-M6, 1.5 nM; and MCF7/MRP1-M24, 1.5 nM.

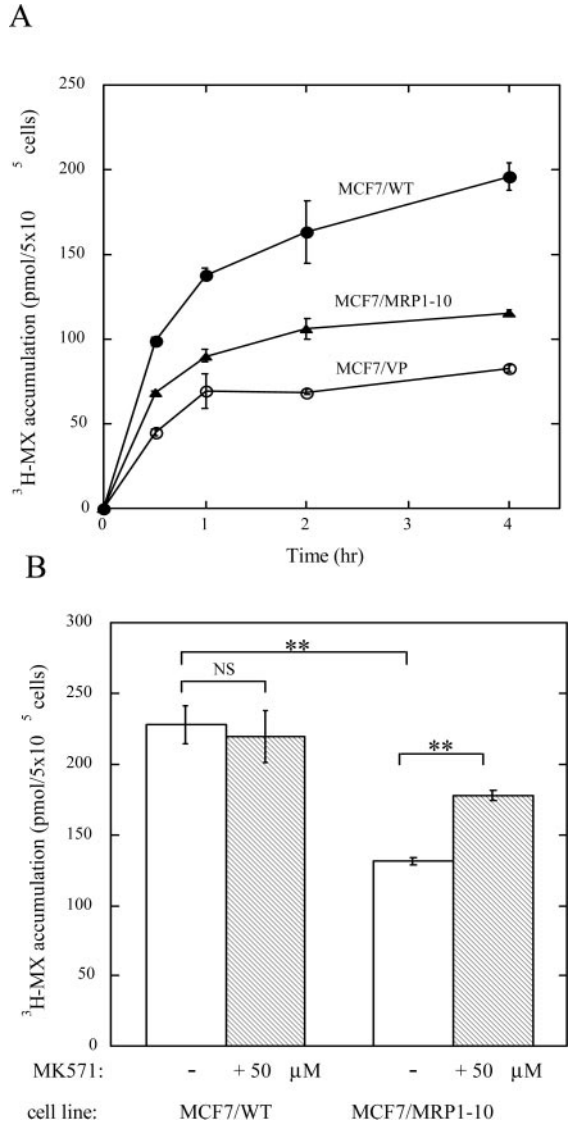

Fig. 4. Overexpression of MRP1 reduces MX accumulation in both transgenic (MCF7/MRP1-10) and drug-selected (MCF7/VP) cell lines. A, accumulation of [^{3}H]MX (1 μM) examined in parental, MRP1-poor (MCF7/WT), and MRP1-overexpressing (MCF7/MRP1-10 and MCF7/VP) cells as described previously (Diah et al., 2001). Points are the averages ± the range of duplicate determinations. B, accumulation of [^{3}H]MX (1 μM) over 4 h examined in MCF7/WT and MCF7/MRP1-10 cells in the presence or absence (−) of the MRP inhibitor MK571 (50 μM). Exposure to MK571 or vehicle control began 15 min before the addition of [^{3}H]MX and continued throughout the subsequent 4-h incubation. Bars represent the mean values ± 1 S.D. from three to six independent determinations. N.S., not significant; **, $P < 0.0001$ (two-tailed unpaired t test).

[^{3}H]GSH uptake into MRP1-containing vesicles. Indeed, as shown in Fig. 7A, unlabeled MX stimulated [^{3}H]GSH uptake 1.4-fold ($P < 0.024$). Additional experiments showed that the *S*-methyl analog of GSH (me-SG), like GSH, would support MRP1-mediated [^{3}H]MX uptake, demonstrating that the free thiol of GSH was dispensable for this transport function. However, stimulation of MX uptake was selective in that another organic anion, L-glutamate, would not substitute for GSH or me-SG (Fig. 7B). Last, the concentration dependence of GSH-stimulated MX uptake was examined. As shown in Fig. 7C, a progressive decrease in MRP1-mediated MX transport was observed as the GSH concentration was lowered below 5 mM, although a significant level of MX uptake was seen with GSH concentrations as low as 300 μM.

Having demonstrated the GSH dependence of MRP1-mediated MX transport in membrane vesicles, we next examined the role of GSH in MRP1-mediated resistance of transduced MCF7/MRP1-10 cells. Basal intracellular levels of GSH in MCF7/WT and MCF7/MRP1-10 cells were 11.7 ± 1.5 and 6.5 ± 1.3 mM (mean values ± 1 S.D., $n = 3$), respectively. MCF7/MRP1-10 cells were highly susceptible to GSH depletion by BSO; treatment of these cells with 50 μM BSO for 48 h reduced intracellular GSH to <20 μM (<0.3% of basal concentration). Such GSH depletion resulted in selective sensitization to MX cytotoxicity (1.6-fold) of MCF7/MRP1-10 but not MCF7/WT cells (Table 1). These data suggest that MRP1-mediated resistance to MX in MCF7/MRP1-10 cells at least partially depends upon GSH.

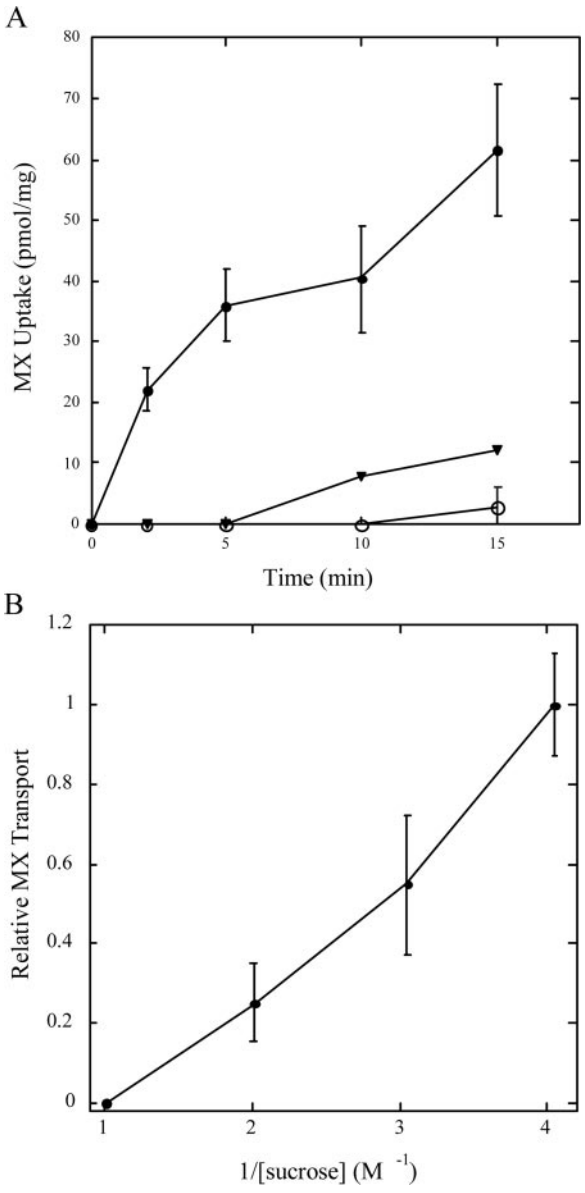

Fig. 5. MRP1 mediates osmotically sensitive ATP- and GSH-dependent transport of MX. A, ATP-dependent uptake of [^{3}H]MX (5 μM) into inside-out membrane vesicles derived from control, MRP1-negative (MCF7/WT), and MRP1-expressing (MCF7/MRP1-10) cells examined as described under *Materials and Methods*. Uptake was determined in MCF7/MRP1-10-derived vesicles in the presence (●) or absence (○) of 5 mM GSH. In the latter (GSH-negative; ○), 5 mM L-glutamate was substituted for GSH. Also shown are results from studies using control, MCF7/WT vesicles done in the presence of 5 mM GSH (▲). Points represent the mean ATP-dependent uptake ± 1 S.E.M. of triplicate determinations. B, ATP-dependent uptake of [^{3}H]MX (5 μM) by vesicles derived from MCF7/MRP1-10 cells determined over 15 min in reactions containing 5 mM GSH and varying concentrations (0.25, 0.33, 0.5, and 1 M) of sucrose as indicated. Data were normalized to uptake at standard sucrose concentration (0.25 M) to obtain relative MX transport. Values represent the means ± 1 S.E.M. of six independent determinations.

Discussion

The work described herein demonstrates that MRP1 mediates MX resistance via supporting ATP-dependent drug efflux, thus resolving issues raised by conflicting data on the role of MRP1 in MX resistance as well as uncertainties raised by our own studies using a drug-selected cell line (Mirski et al., 1987; Cole et al., 1994; Schneider et al., 1994; Breuninger et al., 1995; Borst et al., 2000; Diah et al., 2001). Moreover, transport of MX by MRP1—like transport of other neutral or cationic MRP1-substrates (Loe et al., 1996, 1997, 1998; Renes et al., 1999)—is GSH-dependent, whereas transport of MX by the alternative efflux protein ABCG2 is not. In addition, MX reciprocally stimulates GSH transport by MRP1. These data are consistent with the interpretation that for MX, as for some other MRP1 substrates such as vincristine (Loe et al., 1996, 1998; Rappa et al., 1997), the requirement for GSH involves drug-GSH cotransport. However, this cotransport

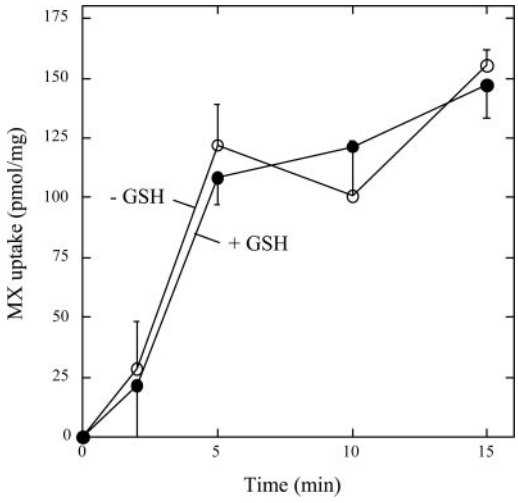

Fig. 6. Transport of MX by ABCG2 is independent of GSH. ATP-dependent uptake of [^{3}H]MX (5 μM) into ABCG2-containing membrane vesicles derived from MCF7/MX cells was examined in the presence (●) or absence (○) of 5 mM GSH. For the latter (GSH-negative; ○), 5 mM L-glutamate was substituted for GSH. Shown are the mean values ± 1 S.E.M. from triplicate determinations.

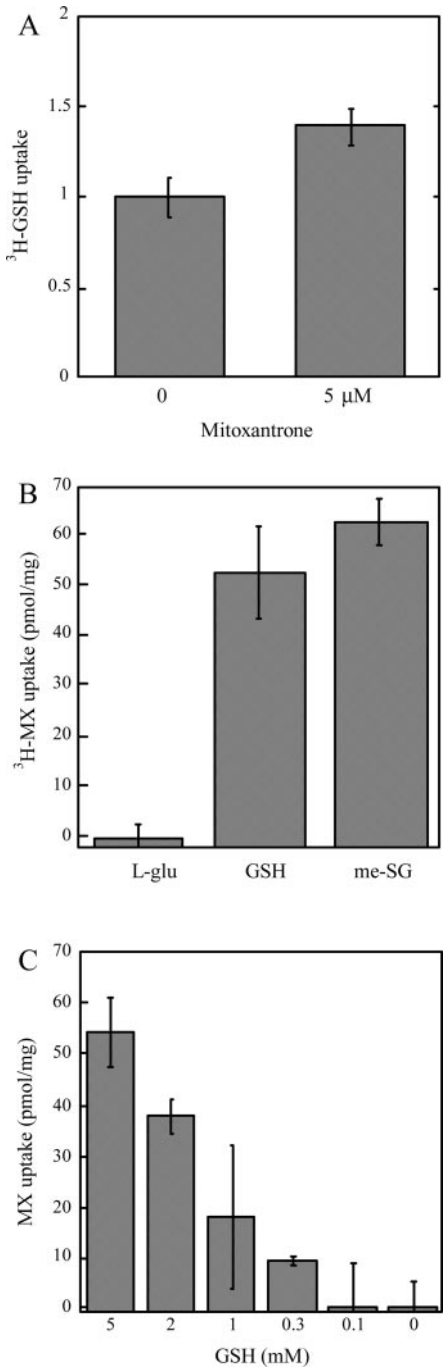

Fig. 7. Role of GSH in MRP1 transport of MX. A, unlabeled MX (5 μM) stimulates the uptake of [^{3}H]GSH (100 μM). Shown are the relative ATP-dependent uptakes of [^{3}H]GSH by MRP1-containing MCF7/MRP1-10-derived vesicles in the presence or absence of 5 μM MX. All reactions were for 15 min. Data were normalized to zero mitoxantrone, and the bars represent mean values ± 1 S.E.M. from 13 independent determinations ($P < 0.024$; two-tailed unpaired t test). B, *S*-methyl analog of glutathione, but not L-glutamate (L-glu), substitutes for glutathione in supporting MRP1-mediated transport of MX. ATP-dependent uptake of [^{3}H]MX (5 μM) into MRP1-containing vesicles (derived from MCF7/MRP1-10 cells) was determined over 15 min in the presence of 5 mM L-Glu, GSH, or *S*-methyl glutathione. Bars indicate the mean values ± 1 S.E.M. of eight (L-glu and GSH) or three (me-SG) independent determinations. C, ATP-dependent uptake of [^{3}H]MX (5 μM) by MRP1-containing vesicles derived from MCF7/MRP1-10 cells determined over 15 min in the presence of 0 to 5 mM GSH as indicated. L-glutamate was added to reactions to achieve a total GSH + L-glutamate concentration equal to 5 mM. Bars represent mean values ± 1 S.E.M. from three independent determinations.

mechanism does not exclude the possibility that GSH may also serve as a transport regulator by interacting at a functionally distinct, nontransporting site on MRP1 (Zelcer et al., 2003; Chu et al., 2004; Peklak-Scott et al., 2005). Indeed, for some other substrates, GSH does not seem to be cotransported, yet it still serves to modulate transport activity of MRP1 (Leslie et al., 2001; Qian et al., 2001; Peklak-Scott et al., 2005).

It is remarkable that a consistent relationship between MRP1 overexpression and MX resistance has not previously been forthcoming. This may be because the level of MX resistance conferred by MRP1, although significant, is considerably less than that observed with other MRP1 substrates such as anthracyclines and etoposide (Schneider et al., 1994; Borst et al., 2000). When drug-selected or poorly matched cell lines are compared, other mechanisms of MX resistance may confound analysis. Indeed, the transgenic MCF7/MRP1-10 cells are ~3-fold resistant to MX (compared with NCF7/WT), whereas drug-selected MCF7/VP cells are 6- to 10-fold resistant—a difference observed even though the levels of MRP1 expressed (Fig. 2) and intracellular MX accumulated are comparable (Fig. 4). Here, the difference in drug sensitivity is probably due to a second mechanism of MX resistance, decreased topoisomerase II, that emerged during etoposide selection of MCF7/VP cells (Schneider et al., 1994). Thus, the use of carefully matched parental and transgenic cell lines was required to reliably establish the role of MRP1 in conferring these relatively more modest levels of MX resistance. Other explanations for the failure of some other groups to detect MRP1-mediated MX resistance, even using matched transgenic cell lines, may include the following. The cell lines may differ with respect to GSH levels or other modulators of MRP1 transport activity. On the other hand, some cell lines may coexpress high levels of other MX transporters (e.g., P-glycoprotein or ABCG2) that may have masked the contribution of MRP1 to overall MX efflux and cellular resistance. Last, some cell lines may have expressed other allelic variants, or mutations, of MRP1 with altered activities toward MX. With regard to this last possibility, it is notable that both groups of MRP1-overexpressing cell lines used in our studies, the drug-selected MCF7/VP and the transduced MCF7/MRP1-series cells, express the most common, or wild-type variant of MRP1.

Another significant finding was the absolute dependence upon GSH (or its *S*-methyl analog) of MRP1-mediated MX transport in vitro (Figs. 5 and 7). In contrast, our previous study showed no effect of intracellular GSH depletion by BSO on either MX resistance or accumulation in MCF7/VP cells (Diah et al., 2001). The reason for this apparent discrepancy

TABLE 1

Glutathione depletion by BSO treatment selectively sensitizes MRP1-expressing MCF7/MRP1-10 but not MCF7/WT cells to mitoxantrone cytotoxicity

Cells were treated with 50 μM BSO or vehicle for 48 h before 1-h exposure to MX. Dose-dependent MX cytotoxicities were accomplished as described under *Materials and Methods*. IC_{50} values were determined from three independent experiments. -Fold sensitization is defined as IC_{50} without GSH depletion (−BSO)/IC_{50} with GSH depletion (+BSO). Values are expressed as the means of triplicate determinations ± 1 S.D.

Cell Line	-Fold Sensitization
MCF7/WT	0.81 ± 0.03
MCF7/MRP1-10	1.6 ± 0.1

regarding the role of GSH in cell-free transport versus intact cells may be explained as follows. In our previous studies, treatment of MCF7 cells with BSO resulted in 90 to 95% depletion of intracellular GSH (Morrow et al., 1998; Diah et al., 2001). Thus, in MCF7 cells, which contain approximately 6 to 12 mM GSH (Diah et al., 1999; present study), such BSO treatment would leave as much as 0.35 to 1.2 mM residual intracellular GSH. At these GSH concentrations, the level of MRP1-mediated MX transport estimated in vesicle studies (Fig. 7C), although reduced, would remain significant. Thus, cellular depletion of GSH, even at 90 to 95%, may have been insufficient to achieve discernible differences in MX sensitivity. In the present study, we found that MCF7/MRP1-10 cells are particularly sensitive to BSO-mediated GSH depletion. Indeed, 48-h BSO treatment reduced intracellular GSH to <20 μM and resulted in significant, selective sensitization of MCF7/MRP1-10 cells to MX cytotoxicity (Table 1). Thus, we conclude that MRP1-mediated MX resistance is at least partially GSH-dependent and that the failure to show this dependence in the earlier study was most likely to be due to insufficient depletion of intracellular GSH in the MCF7/VP cells.

In conclusion, like the other known MX transporters P-glycoprotein and ABCG2, MRP1 may have a significant impact on tumor cell sensitivity to MX cytotoxicity, with MRP1-overexpressing tumors being relatively refractory. Moreover, MRP1, which is ubiquitously expressed but at variable levels in normal cells and tissues, also probably plays a significant role in the pharmacological disposition of MX. Other MRP family proteins, with more restricted tissue distributions, may also be important (Leslie et al., 2005). In particular, MRP2, which has significant substrate overlap with MRP1, is strategically located at the apical, excretory surfaces of cells within the liver, gut, and kidney as well as at the blood-brain barrier; thus, MRP2, along with P-glycoprotein and ABCG2, may have a significant impact on the clearance and pharmacodynamics of MX.

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