MRP8, ATP-binding Cassette C11 (ABCC11), Is a Cyclic Nucleotide Efflux Pump and a Resistance Factor for Fluoropyrimidines 2’,3’-Dideoxycytidine and 9’-(2’-Phosphorylmethoxyethyl)adenine*

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MRP8 (ABCC11) is a recently identified cDNA that has been assigned to the multidrug resistance-associated protein (MRP) family of ATP-binding cassette transporters, but its functional characteristics have not been determined. Here we examine the functional properties of the protein using transfected LLC-PK1 cells. It is shown that ectopic expression of MRP8 reduces basal intracellular levels of cAMP and cGMP and enhances cellular extrusion of cyclic nucleotides in the presence or absence of stimulation with forskolin or SIN-1A. Analysis of the sensitivity of MRP8-overexpressing cells revealed that they are resistant to a range of clinically relevant nucleotide analogs, including the anticancer fluoropyrimidines 5’-fluorouracil (−3-fold), 5’-fluoro-2’-deoxycytidine (−5-fold), and 5’-fluoro-5’-deoxyuridine (−3-fold), the anti-human immunodeficiency virus agent 2,3’-dideoxycytidine (−6-fold) and the anti-hepatitis B agent 9’-(2’-phosphorylmethoxyethyl)adenine (PMEA) (−5-fold). By contrast, increased resistance was not observed for several natural product chemotherapeutic agents. In accord with the notion that MRP8 functions as a drug efflux pump for nucleotide analogs, MRP8-transfected cells exhibited reduced accumulation and increased efflux of radiolabeled PMEA. In addition, it is shown by the use of in vitro transport assays that MRP8 is able to confer resistance to fluoropyrimidines by mediating the MgATP-dependent transport of 5’-fluoro-2’-deoxyuridine monophosphate, the cytotoxic intracellular metabolite of this class of agents, but not of 5’-fluorouracil or 5’-fluoro-2’-deoxyuridine. We conclude that MRP8 is an amphipathic anion transporter that is able to efflux cAMP and cGMP and to function as a resistance factor for commonly employed purine and pyrimidine nucleotide analogs.

Cellular extrusion of cyclic nucleotides has been described in prokaryotic and eukaryotic cells (1–4). This process provides extracellular cAMP involved in intercellular signaling, as determined for Dictyostelium discoideum, in which cAMP effluxed by solitary amoebae under low nutrient conditions mediated cellular aggregation and differentiation, and has also been proposed as a potential mechanism that may contribute to the attenuation of intracellular signaling mediated by these second messengers (5). Investigations employing cultured cells and membrane vesicle preparations have established that cyclic nucleotide efflux is energy-dependent, and the susceptibility of this process to inhibition by antagonists of organic anion pumps indicates that it is mediated by amphilipic anion transporters (2, 3, 6–16). Recently, insights into the identities of the cellular components that mediate cyclic nucleotide efflux have come from studies of the MRP4 family of ABC transporters. MRP4 and MRP5, two members of this extended family of amphilipic anion transporters (17), have been determined to be competent in the transport of cyclic nucleotides (18–20). By contrast, other characterized MRP family members are able to transport a variety of lipophilic anions, such as glutathione and glucuronic acid conjugates, but not cyclic nucleotides (17). In addition to its ability to efflux cyclic nucleotides, MRP4 is also able to mediate the transport of methotrexate, reduced folates, estradiol 17β-o-glucuronide and DHEAS (19, 21–23), and both MRP4 and MRP5 have the facility for conferring resistance to certain purine nucleotide analogs, such as PMEA, an amphilipic antiviral agent used in the treatment of hepatitis B infections, and 6-mercaptopurine, an anticancer nucleobase analog (21, 24, 25).

MRP8, a newly identified cDNA, was recently assigned to the MRP family based upon analyses of its predicted protein (26–28). MRP8 resembles MRP4 and MRP5 in that it lacks a third (N-terminal) membrane-spanning domain that is present in MRP1, MRP2, MRP3, MRP6, and MRP7. In addition, sequence comparisons with MRP family members indicate that it most closely resembles MRP5 (26, 27). These features suggest the possibility that MRP8 might represent a component of the efflux system for cyclic nucleotides. However, the functional properties of MRP8 have not been determined with respect to either its substrate selectivity or drug resistance capabilities. Here we examine the functional characteristics of MRP8 in transfected LLC-PK1 cells. It is demonstrated that MRP8 is an efflux pump for cAMP and cGMP and that it not only is capable of conferring resistance to the purine nucleotide analog PMEA but also has the ability to function as a resistance factor for fluoropyrimidines, a widely employed class of antineoplastic agents, and the anti-AIDS drug 2’,3’-dideoxycytidine.

* This work was supported in part by NCI, National Institutes of Health, Grants CA73728 (to G. D. K.) and CA6927 and by an appropriation from the Commonwealth of Pennsylvania. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Recipient of a W. J. Avery Fellowship from the Fox Chase Cancer Center and a Japan Research Foundation Award for Clinical Pharmacology.

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1 The abbreviations used are: MRP, multidrug resistance-associated protein; ABC, ATP-binding cassette; PMEA, 9-(2’-phosphorylmethoxyethyl)adenine; bis-POM-PMEA, bis(pivaloyloxymethyl)-PMEA; ddC, 2’,3’-dideoxycytidine; 5-dFDUd, 5’-deoxy-5’-fluorouridine; 5-FdUMP, 5’-fluorouracil; 5-FUra, 5’-fluorouracil; 5’-fluoro-2’-deoxyuridine; 5-FdUr, 5’-fluorouracil; 5-FdUr, 5’-fluoro-2’-deoxyuridine.
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EXPERIMENTAL PROCEDURES

Materials—[3H]Bis-OM-PMEA (2 Ci/mmol), [3H]-fluorouracil (5-FUra) (17.5 Ci/mmol), [3H]-fluoro-2-deoxyuridine (5-FdUrd) (13.9 Ci/mmol), [3H]-fluoro-2-deoxyuridine 5'-monophosphate (5-FdUMP) (10.7 Ci/mmol) were purchased from Moravek (Brea, CA). Unlabeled 2',3'-dideoxyctydine (ddc), 3'-azido-3'-deoxythymidine, 5-FUra, 5-FdUrd, 5'-fluoro-5'-deoxyuridine (5-FdUrd), 6-thioguanine, 2'-chloro-2'-deoxyadenosine, doxorubicin, paclitaxel, and vincristine were obtained from Sigma. Etoposide was obtained from Bristol-Myers Squibb (NY). GenBank PEPA was obtained from Gilead (Forest City, CA). 2',3'-Dideoxy-3'-thial-cytidine was provided by the National Institutes of Health AIDS program. Forskolin and SIN-1A were obtained from the Sigma and Cayman Chemical (Ann Arbor, MI, respectively).

Expression Vector Construction, Transfection, and Cell Culture—Two overlapping cDNA fragments that encode the MMP8 open reading frame were prepared by PCR using rapid amplification of cDNA ends (RACE) ready prostate library (Stratagene, La Jolla, CA) and oligonucleotide primers derived from the reported sequence (26) and were assembled into Bluescript SK(−) (Stratagene). The predicted coding sequence was identical to that reported by Berra et al. (26). The MMP8 cDNA was inserted into pcDNA3.1 (Invitrogen) to create pcDNA-MMP8. LLC-PK1 cells grown in M199 medium supplemented with 10% fetal bovine serum were electroporated with 10 μg of pcDNA-MRP8 or parental plasmid, and G418-resistant clones were isolated. For expression in insect cells the MMP8 coding sequence was inserted into PVL1392, and production of baculovirus and infection of insect cells were accomplished according to the manufacturer’s directions (Pharminigen).

Generation of MRP8 Polyclonal Antibody and Immunoblot Analysis—A cDNA fragment encoding amino acids 746–804 of MMP8 was inserted downstream of the glutathione S-transferase coding sequence in PGEX2T, and the fusion protein was purified by the use of glutathione-Sepharose beads (Amersham Biosciences). Rabbits were immunized with the fusion protein, and the specificity of the resulting antiserum was confirmed by immunoblot analysis of cellular lysates prepared from insect cells infected with MMP8 baculovirus. Proteins were separated by 8% SDS-PAGE and electrotransferred to nitrocellulose filters using a wet transfer system as described previously (29, 30). MMP8 was detected using polyclonal MRP8 antibody (1:500) and horseradish peroxidase-conjugated antibody (Amersham Biosciences).

Measurement of Cyclic AMP and Cyclic GMP—To determine whether MRP8 is capable of extruding cyclic nucleotides from cells, intracellular cAMP levels in LLC-PK1-MRP8-1 and LLC-PK1-MRP8-2 cells were determined by subtracting the values obtained in the presence of 4 mM AMP from those obtained in the absence of AMP.

To characterize the functional properties of MMP8, a cellular model was generated by transfecting LLC-PK1 cells with MMP8 expression vector. Immunoblot analysis indicated that MMP8 protein was expressed in several of the resulting G418-selected clones, as indicated by the intensely immunoreactive bands present in three clones transfected with MMP8 vector, but not in the parental vector-transfected control cells (Fig. 1). The apparent molecular weight of MMP8 (170,000–190,000) was higher than its calculated molecular mass (154 kDa) and the apparent molecular weight of the protein expressed in insect cells (155,000, data not shown), as would be expected for a glycosylated transmembrane protein. Two of these clones were selected for functional studies on MMP8.

Cellular Efflux of Cyclic Nucleotides by MMP8—To determine whether MMP8 is capable of extruding cyclic nucleotides from cells, intracellular cAMP levels were analyzed before and after stimulation with forskolin. Basal cAMP levels in the MMP8-transfected cells were consistently lower than those of the parental vector control cells (Table I). The intracellular levels in LLC-PK1-MRP8-1 and LLC-PK1-MRP8-2 cells were 4.7 ± 2.1 pmol/10⁶ cells and 4.3 ± 2.7 pmol/10⁶ cells, respectively, in comparison with the control cells in which the level was 6.6 ± 3.1 pmol/10⁶ cells. These values corresponded to

![Fig. 1. Immunoblot detection of MRP8 in transfected LLC-PK1 cells.](http://www.jbc.org/content/183/2/29510/F1.expansion.html)
reductions of 29 and 35% for LLC-PK1-MRP8-1 and LLC PK1-MRP8-2, respectively. Reduced cAMP levels were also observed for the MRP8-transfected cells after stimulation with forskolin. After 30 min of stimulation with 50 μM forskolin, the cAMP levels in LLC-PK1-MRP8-1 and LLC-PK1-MRP8-2 were 34 and 18% lower than the levels in control cells (Table I).

Separate experiments were performed to analyze the appearance of cAMP in the medium following forskolin stimulation. Cellular efflux was significantly greater for the MRP8-transfected cells after stimulation with forskolin. At the 15-min time point, the cAMP levels in the medium of LLC-PK1-MRP8-1 cells were 35% lower than the concentrations in LLC-PK1-MRP8-2 cells. Thus the intracellular and extracellular cGMP levels were measured as described under “Experimental Procedures.” Values are means ± S.E. of a representative experiment performed in triplicate.

Table II

| Treatment        | LLC-PK1-pcDNA | LLC-PK1-MRP8-1 |
|------------------|---------------|----------------|
|                  | Intracellular | Extracellular  | Intracellular | Extracellular |
| None             | 2.0 ± 0.8     | 4.3 ± 1.8      | 1.3 ± 0.6       | 5.0 ± 1.2     |
| SIN-1A           | 9.0 ± 4.5     | 26.1 ± 3.2     | 5.1 ± 1.8       | 32.4 ± 5.1    |

a Values are significantly different (p < 0.05) from the corresponding control transfectant values as determined by the two-tailed nonparametric Wilcoxon test.

Table III

| Agent            | IC50 μM | LLC-PK1-pcDNA | LLC-PK1-MRP8-1 | Fold resistance |
|------------------|---------|---------------|----------------|----------------|
| PMEA             | 457 ± 79 | 2463 ± 386    | 5.4a           |
| ddC              | 546 ± 11  | 546 ± 190     | 1.1            |
| CdA              | 8.1 ± 2.7 | 8.2 ± 4.6     | 1.0            |
| DCF              | 256 ± 52  | 263 ± 50      | 1.0            |
| 3TC              | 869 ± 56  | 1078 ± 46     | 1.4            |
| AZT              | 507 ± 86  | 639 ± 41      | 1.3            |
| ddC              | 384 ± 52  | 2430 ± 155    | 6.1a           |
| 5-FUra           | 8.0 ± 3.0 | 26.2 ± 12     | 2.9            |
| 5-FdUrd          | 20.3 ± 9.0 | 105 ± 39     | 5.2a           |
| 5-dFdUrd         | 29.0 ± 8.6 | 98.7 ± 35    | 3.4a           |
| VCR              | 0.09 ± 0.01 | 0.10 ± 0.02  | 1.1            |
| Taxol            | 0.35 ± 0.07 | 0.37 ± 0.05  | 1.1            |
| DOX              | 0.42 ± 0.03 | 0.45 ± 0.1   | 1.1            |
| ETOP             | 0.92 ± 0.04 | 1.21 ± 0.10 | 1.2            |

a Values are significantly different from the control transfectant (p < 0.05) as determined by the nonparametric two-tailed Wilcoxon test.

Figure 2: Time course of cAMP efflux following forskolin stimulation. Parental vector-transfected (open symbols) and MRP8-transfected LLC-PK1 cells (closed symbols, LLC-PK1-MRP8-1) were stimulated with 50 μM forskolin, and the appearance of cAMP in the medium was measured as described under “Experimental Procedures.” Values are means ± S.E. of a representative experiment performed in triplicate.

The drug sensitivities of LLC-PK1 cells transfected with parental vector or MRP8 expression vector were measured in 3-day growth assays as described under “Experimental Procedures.” The IC50 values are the drug concentrations that inhibited growth by 50% and represent means ± S.E. of at least four independent experiments, each performed in triplicate. Fold resistance is enumerated at the IC50 of LLC-PK1-MRP8-1 divided by the IC50 of the parental vector transfected control line. 6-TG, 6-thioguanine; CdA, 2′-deoxy-2′-deoxyadenosine; DCF, deoxycorofomycin; 3TC, 2′,3′-dideoxy-3′-thial-cytidine; AZT, 3′-azido-3′-deoxythymidine; VCR, vincristine; DOX, doxorubicin, ETOP, etoposide.

Table III

| Agent            | IC50 μM | LLC-PK1-pcDNA | LLC-PK1-MRP8-1 | Fold resistance |
|------------------|---------|---------------|----------------|----------------|
| PMEA             | 457 ± 79 | 2463 ± 386    | 5.4a           |
| ddC              | 546 ± 11  | 546 ± 190     | 1.1            |
| CdA              | 8.1 ± 2.7 | 8.2 ± 4.6     | 1.0            |
| DCF              | 256 ± 52  | 263 ± 50      | 1.0            |
| 3TC              | 869 ± 56  | 1078 ± 46     | 1.4            |
| AZT              | 507 ± 86  | 639 ± 41      | 1.3            |
| ddC              | 384 ± 52  | 2430 ± 155    | 6.1a           |
| 5-FUra           | 8.0 ± 3.0 | 26.2 ± 12     | 2.9            |
| 5-FdUrd          | 20.3 ± 9.0 | 105 ± 39     | 5.2a           |
| 5-dFdUrd         | 29.0 ± 8.6 | 98.7 ± 35    | 3.4a           |
| VCR              | 0.09 ± 0.01 | 0.10 ± 0.02  | 1.1            |
| Taxol            | 0.35 ± 0.07 | 0.37 ± 0.05  | 1.1            |
| DOX              | 0.42 ± 0.03 | 0.45 ± 0.1   | 1.1            |
| ETOP             | 0.92 ± 0.04 | 1.21 ± 0.10 | 1.2            |

a Values are significantly different from the control transfectant (p < 0.05) as determined by the nonparametric two-tailed Wilcoxon test.

Analysis of the Drug Sensitivity of MRP8-transfected LLC-PK1 Cells—MRP8-transfected cells exhibited increased resistance toward clinically relevant purine and pyrimidine nucleotide analogs (Table III). LLC-PK1-MRP8-1 cells were 5.4-fold resistant to the purine nucleotide analog PMEA but did not exhibit increased resistance toward three other purine nucleotide analogs, 6-thioguanine, 2′-chloro-2′-deoxyadenosine, and deoxycoformycin. In addition, LLC-PK1-MRP8-1 cells were 6.1-fold resistant to the antiviral pyrimidine analog ddC and 2.9-fold resistant to the anticancer pyrimidine analog 5-FUra. Increased resistance was also observed for two other fluoropyrimidines of clinical significance, 5-FdUrd (5.2-fold), a ribosylated intracellular metabolite of 5-FUra, which like 5-FUra is employed as an intravenous agent, and 5-dFdUrd (3.4-fold), a metabolite of the oral fluoropyrimidine capcitabine, which is further metabolized in the cell to 5-FUra. Small decreases in the sensitivity of LLC-PK1-MRP8-1 cells were observed for the pyrimidine nucleotide analogs 2′,3′-dideoxy-3′-thial-cytidine and 3′-azido-3′-deoxythymidine, but these differences did not reach statistical significance. Representative growth curves for PMEA, ddC, and 5-FUra are shown in Fig. 3. Increased resistance was not detected for several natural product anticancer agents, including vincristine, paclitaxel, doxorubicin, and etoposide (Table III). A similar drug resistance phenotype was observed for LLC-PK1-MRP8-2, which exhibited 5.6-, 7.1-, 4.1-, 3.2-, and 2.5-fold resistance toward PMEA, ddC, 5-FUra, 5-FdUrd, and 5-dFdUrd, respectively (p values < 0.05; data not shown).
Analysis of PMEA Accumulation and Efflux—The effect of MRP8 on the cellular kinetics of a representative agent was analyzed. To this end we employed bis-POM-PMEA, an uncharged bis-ester prodrug of PMEA that is more effective than the parent compound in crossing the plasma membrane (33). Once inside the cell the pivaloyloxymethyl moiety of bis-POM-PMEA is cleaved to release free PMEA. LLC-PK1-MRP8-1 exhibited reduced accumulation of [3H]bis-POM-PMEA compared with parental vector-transfected cells (Fig. 4A). After 15 min of incubation in growth medium containing 1 μM [3H]bis-POM-PMEA, drug accumulation in LLC-PK1-MRP8-1 cells was 60% of the control cells. This difference in accumulation was maintained throughout the time course of the assay. Separate efflux experiments were performed under conditions in which initial intracellular drug levels were comparable in the two cell lines, by first allowing accumulation of 1 μM [3H]bis-POM-PMEA to proceed under energy-depletion conditions. Following a 2-h incubation period, the growth medium was replaced with complete medium lacking drug, and efflux of radiolabeled drug into the medium was measured. As shown in Fig. 4B, LLC-PK1-MRP8-1 exhibited enhanced drug efflux by comparison with the control cells. At the 2-h time point, efflux by the MRP8-transfected cells was 40% greater than the control cells.

Transport of 5′-Fluoro-2′-deoxyuridine Monophosphate—By contrast with cyclic nucleotides and PMEA, both of which are amphipathic anions, 5-FUra is an uncharged pyrimidine analog. The possibility that MRP8 confers resistance to this agent, as well as to 5-FdUrd and 5-dFUr, by transporting 5-FdUMP, the anionic cytotoxic metabolite of these compounds as opposed to the parent compounds, was therefore considered. This was examined by analyzing the ability of the pump to transport 5-FUra, 5-FdR, and 5-FdUMP into inside-out membrane vesicles. As shown in Fig. 5, although membrane vesicles prepared from parental vector transfected cells were able to catalyze the MgATP-dependent transport of [3H]5-FdUMP, an increment attributable to MRP8 was consistently observed. MgATP-de-
MgATP-dependent uptake of $[{}^{3}H]5'$-fluorouracil, $[{}^{3}H]5'$-fluorodeoxyuridine, and $[{}^{3}H]5'$-fluorodeoxyuridine monophosphate into inside-out membrane vesicles. Membrane vesicles (10 µg) prepared from parental vector-transfected LLC-PK1 cells or LLC-PK1-MRP8-1 and LLC-PK1-MRP8-2 cells were incubated for 10 min at 37 °C in uptake medium containing 4 mM ATP or 4 mM AMP and the indicated radiolabeled compounds. MgATP-dependent uptake for 1 µM $[{}^{3}H]5'$-FdUMP, 1 µM $[{}^{3}H]5'$-FUra, or 1 µM $[{}^{3}H]5'$-FUr was calculated by subtracting the values obtained in transport medium containing MgAMP from the values obtained in medium containing MgATP. Values shown are means ± S.E. of a representative experiment. This experiment was repeated at least five times with similar results.

DISCUSSION

In the present study the functional properties of MRP8 were analyzed by the use of transfected LLC-PK1 cells. In combination, the results showing that MRP8 is able to depress intracellular levels of cAMP and cGMP by enhancing cellular efflux, confer resistance to PMEA, and transport 5-FdUMP provide the first evidence that this protein functions as a lipophilic anion pump. These results indicate that the cellular efflux of cyclic nucleotides, a phenomenon that has been well documented in numerous types of mammalian cells, is accomplished by a plasma membrane system that is composed of at least three pumps. Although its substrates have yet be determined, the high degree of structural resemblance between MRP9 and MRP8 (27, 28) suggests that it may also participate in this process.

Although cellular efflux of cyclic nucleotides from mammalian cells is a well established phenomenon, it has not been considered a major factor in attenuating the elevation of these second messengers consequent to the activation of cyclases. This process is thought to be mediated primarily by the action of phosphodiesterases, a view based upon the notion that an extremely rapid, high capacity system is required for the precise time-sensitive signaling mediated by cyclic nucleotides. By comparison with the enzymatic breakdown of cyclic nucleotides by phosphodiesterases, cellular efflux is thought to be low capacity, and more importantly, relatively slow. The identification of efflux pumps capable of mediating this process (this study and Refs. 18 and 19) has provided the molecular tools to investigate directly their involvement in these and related processes. The results, showing that intracellular levels of cAMP and cGMP under basal or stimulated conditions were only modestly depressed (1.2–1.8-fold) by ectopic expression of MRP8, tend to support the view that efflux pumps are not potent attenuation factors, as do previous reports showing that ectopic expression of MRP4 and MRP5 is similarly associated with modest effects on intracellular cyclic nucleotide levels (34, 35). These studies, in combination with measurements showing that the latter two pumps have reasonably high affinities for cGMP, and in the case of MRP4, for cAMP (18, 19), suggest that it is the high efficiency of the phosphodiesterase system that limits the impact of efflux pumps as opposed to the inability of pumps to function at physiological concentrations of these second messengers. Further studies should help to determine the circumstances and extent to which efflux pumps participate in intracellular cyclic nucleotide homeostasis and also to define their involvement in the physiological processes in which extruded cyclic nucleotides have been proposed as primary messengers.

Analysis of the drug sensitivity of MRP8-transfected LLC-PK1 cells showed that MRP8 is able to confer resistance to fluoropyrimidines, ddC, and PMEA. However, resistance to 6-thioguanine, an agent that is part of the resistance profiles of MRP4 and MRP5, was not detected (19, 25). Fluoropyrimidines, which are a mainstay in the treatment of colon cancer and are also active in breast and head and neck cancer, are among the most widely employed anticancer agents. The cytotoxicity of 5-FUra is mediated predominately by its intracellular metabolite, 5-FdUMP, which in combination with 5,10-methylene tetrahydrofolate forms a stable inhibitory complex with thymidylate synthase. The biochemical consequences of this block, depletion of thymidine nucleotides and build-up of dUTP, engender impaired DNA synthesis and misincorporation of uracil into DNA, respectively. Cellular resistance factors for fluoropyrimidines include increased expression of thymidylate synthase, decreased expression of enzymes involved in metabolic activation, and increased expression of dUTPase (36–41). Our results indicate that MRP8 is a potential clinical resistance factor for fluoropyrimidines and that it confers resistance to this class of agents by mediating the efflux of 5-FdUMP, the intracellular cytotoxic metabolite of 5-FUra,

![Fig. 5](image_url)

**Fig. 5.** MgATP-dependent uptake of $[{}^{3}H]5'$-fluorouracil, $[{}^{3}H]5'$-fluorodeoxyuridine, and $[{}^{3}H]5'$-fluorodeoxyuridine monophosphate into inside-out membrane vesicles. Membrane vesicles (10 µg) prepared from parental vector-transfected LLC-PK1 cells or LLC-PK1-MRP8-1 and LLC-PK1-MRP8-2 cells were incubated for 10 min at 37 °C in uptake medium containing 4 mM ATP or 4 mM AMP and the indicated radiolabeled compounds. MgATP-dependent uptake for 1 µM $[{}^{3}H]5'$-FdUMP, 1 µM $[{}^{3}H]5'$-FUra, or 1 µM $[{}^{3}H]5'$-FUr was calculated by subtracting the values obtained in transport medium containing MgAMP from the values obtained in medium containing MgATP. Values shown are means ± S.E. of a representative experiment. This experiment was repeated at least five times with similar results.

![Fig. 6](image_url)

**Fig. 6.** Schematic diagram depicting the role played by MRP8 in conferring cellular resistance to fluoropyrimidines. 5-FUra, 5-FdUrd, and 5-dFUr are converted to the cytotoxic intracellular metabolite 5-FdUMP. 5-FdUMP, in the presence of the reduced folate cofactor 5,10-methylene tetrahydrofolate, forms an inhibitory complex with thymidylate synthase (TS), which blocks the de novo synthesis of thymidine monophosphate. This block leads to depletion of thymidine pools required for DNA synthesis, and misincorporation of dUTP into DNA consequent to elevated levels of this nucleotide. MRP8 is able to mediate the transport of 5-FdUMP. TP, thymidine phosphorylase; TK, thymidine kinase.
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5-FdR, and the oral fluoropyrimidine capecitabine (Fig. 6). The presence of significant levels of 5-FdUMP transport by membranes prepared from control LLC-PK1 cells (Fig. 5), in which MRP8 is barely detectable (Fig. 1), suggests that pumps other than MRP8 may also be involved in this process. Although, to the best of our knowledge, increased efflux of fluoropyrimidine metabolites has not been described in drug-resistant cell lines, this potential mechanism warrants more detailed analysis in view of our findings. The ability of MRP8 to confer resistance to the anti-AIDS nucleotide analog ddC is also noteworthy. We infer that this activity is consequent to the efflux of the intracellular nucleotide metabolites of this agent, by analogy with the mechanisms by which MRP8 confers resistance to fluoropyrimidines, and MRP4 and MRP5 confer resistance to and 6-mercaptapurine (42). Investigations of clinical resistance to ddC have focused primarily on mutations in the human immunodeficiency virus reverse transcriptase and alterations in the levels of cellular metabolizing enzymes (43). Our results suggest that cellular efflux is a potential clinical resistance for this agent. A recent study showing that a drug-resistant cell line in which ABCG2 (breast cancer resistance protein (BCRP)) is over-expressed is cross-resistant to 3’-azido-3’-deoxythymidine suggests that ABC transporters that are not MRP family members may also be involved in resistance to this class of agents (44), although this remains to be confirmed in experiments using recombinant ABCG2. Analysis of the expression of MRP8 in clinical samples should help to determine its importance in clinical resistance to fluoropyrimidines, ddC, and the anti-hepatitis B agent PMEA.

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