# Inhibition of Multidrug Resistance-Associated Protein (MRP) Functional Activity with Pluronic Block Copolymers

Donald W. Miller,[1] Elena V. Batrakova,[1] and Alexander V. Kabanov[1,2]

Received September 11, 1998; accepted December 7, 1998

**Purpose.** Using monolayers of human pancreatic adenocarcinoma cells (Panc-1) that express multidrug resistance-associated protein (MRP), the present work investigates the effects of Pluronic block copolymers on the functional activity of MRP.
**Methods.** The studies examined the accumulation and efflux of the MRP selective probe fluorescein (FLU) in Panc-1 cell monolayers with and without Pluronic P85 (P85), Pluronic L81 (L81) and Pluronic F108 (F108).
**Results.** Treatment of Panc-1 cells with P85 resulted in concentration-dependent increases in FLU accumulation and elimination of FLU sequestration in vesicular compartments in these cells. The effects of P85 were selective for FLU in the Panc-1 cell monolayers. Inhibition of MRP-mediated transport was dependent on the composition of Pluronic block copolymer: the more hydrophobic copolymer had the greater effect on FLU uptake in Panc-1 monolayers (L81 > P85 > F108).
**Conclusions.** This paper demonstrates for the first time that Pluronic block copolymers inhibit multidrug resistance-associated protein (MRP). The similarities in the effects of Pluronic block copolymers on MRP and P-glycoprotein drug efflux systems suggest that a single unifying mechanism may explain the inhibition observed.

**KEY WORDS:** block copolymer; cancer; multidrug resistance; Pluronic.

## INTRODUCTION

The presence of drug efflux transport proteins that function to pump selected agents out of a cell have an important role in both normal and pathophysiological processes. These transporters which are part of a superfamily of ATP binding cassette (ABC) proteins have been implicated in multidrug resistance in cancer cells (1,2), as well as bacterial and parasitic drug resistance (2,3). Furthermore, the selective expression of various drug efflux transport proteins in normal tissues such as the epithelial cells of the intestine, liver and kidney, and endothelial cells that form the blood-brain barrier have an important protective and detoxification function. The identification of methods for circumventing these drug efflux transport proteins could have a significant impact on the treatment of various diseases and the distribution of therapeutic agents in the body.

One of the more extensively studied ABC transport proteins is P-glycoprotein (P-gp). The importance of P-gp in drug resistance and tissue distribution is well known (4,5). Previous studies have shown that Pluronic block copolymers (poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), $EO_{m/2}$-$PO_n$-$EO_{m/2}$) can inhibit P-gp function (6–8). While the exact mechanisms are unclear, the ability of Pluronic to block P-gp activity appears to involve the interaction of the individual block copolymer chains with P-gp or the lipid microenvironment surrounding P-gp (6).

Recently, another drug efflux transport protein of 190-210 kDa, multidrug resistance associated protein (MRP), has been identified (9–11). Like P-gp, MRP is also an ATP-dependent efflux pump that actively transports selected chemotherapeutic agents. However, MRP has different substrate characteristics and different inhibitor specificity from P-gp. MRP was shown to have a broad specificity for amphiphilic anions as well as glutathione and glucuronide conjugates of many drugs (9–11). Histological studies suggest MRP may be intrinsically expressed in many solid tumors and normal tissues (12). The results of the present studies demonstrate for the first time, that Pluronic block copolymers can inhibit MRP-mediated drug efflux transport. Furthermore, the observation that the Pluronic block copolymer compositions required to inhibit MRP-mediated efflux transporters are similar to those required for inhibiting P-gp transport, suggests that a similar or uniform mechanism may explain the inhibition of drug efflux proteins by Pluronic block copolymers.

## MATERIALS AND METHODS

### Preparation of Block Copolymer Solutions

The present study used Pluronic block copolymers (Table 1): Pluronic F68 (F68) (lot # WPOP-590B), Pluronic P85 (P85) (lot # WPOP-587A) and Pluronic L81 (L81) (lot # WSOO-83457 25087) that were provided by BASF Corp. (Parispany, NJ). The solutions of Pluronic block copolymers and polyoxyethylated detergents were prepared in assay buffer containing 122 mM sodium chloride, 25 mM sodium bicarbonate, 10 mM glucose, 10 mM HEPES, 3mM potassium chloride, 1.2 mM magnesium sulfate, calcium chloride (1.4 mM) and potassium phosphate dibasic (0.4 mM). Fluorescein (FLU) and rhodamine 123 (R123) were added to the copolymer solutions and incubated at 37°C one hour prior to their use in the experiments.

### Cell Culture

Panc-1 cells, a human pancreatic adenocarcinoma cell line that expresses MRP (13,14), were purchased from ATCC (Baltimore, MD). Panc-1 cell monolayers were grown on 24-well culture plates using DMEM supplemented with 10% fetal bovine serum. The Panc-1 cells (passages from 50 to 60) were seeded at a density of 25,000 cells/cm$^2$ and were used after reaching confluency (typically within 4–6 days). As a control, the effects of Pluronic block copolymers were also evaluated in multidrug resistant human oral epidermoid carcinoma cells (KBv) that were generously provided by Dr. J. Pezzuto (University Illinois). The KBv cells are a drug resistant cancer cell line that over express P-gp but have little if any MRP drug efflux transport systems (14). The KBv cells were grown in DMEM supplemented with 10% fetal bovine serum and 1 μg/ml vinblastine and were used in the drug accumulation studies after reaching confluency.

[1] Department of Pharmaceutical Sciences, University of Nebraska Medical Center, 600 S. 42nd St., Omaha, Nebraska 68198-6025.
[2] To whom correspondence should be addressed. (e-mail: akabanov@mail.unmc.edu)

0724-8741/99/0300-0396$16.00/0 © 1999 Plenum Publishing Corporation

**Table 1.** Concentration-Dependent Effects of P85 on the Accumulation of FLU in Panc-1 Cell Monolayers

| System studied | P85 concentration, μM | FLU accumulation, nmol/mg protein[a] |
|---|---|---|
| FLU in assay buffer | 0 | 0.084 ± 0.003 |
| FLU in P85 solution | 0.022 | 0.087 ± 0.004[b] |
| | 0.22 | 0.097 ± 0.005* |
| | 2.2 | 0.117 ± 0.010* |
| | 22 | 0.341 ± 0.018** |
| | 220 | 0.359 ± 0.003** |
| | 2200 | 0.457 ± 0.018** |

[a] Cells were incubated with FLU (100 μM) with or without P85 as described in materials and methods. Values represent the mean ± SEM of four monolayers per the treatment group. Statistical significance of the P85 compared to the P85-free control is shown below.
[b] n.s., not significant.
* $p < 0.05$.
** $p < 0.005$.

### MRP Functional Assay

Cells were washed and pre-incubated for 30 minutes at 37°C in either assay buffer or assay buffer containing corresponding Pluronic block copolymer (0.00001–1.0% wt) or indomethacin (IND) (10 μM). Following the pre-incubation period, cell monolayers were incubated with FLU (100 μM) in either assay buffer or solutions of Pluronic block copolymers for up to 90 min. at 37°C. Functional studies were terminated by removing the FLU solutions, washing the monolayers three times in ice-cold PBS to remove any unbound FLU, and then solubilizing the cells using 1.0% Triton X-100. The cellular accumulation of FLU was determined by measuring fluorescence of the cell lysates with a Shimadzu RF5000 fluorescent spectrophotometer (488 nm excitation; 510 nm emission). Samples were taken for protein assay using the Pierce BCA method. The accumulation of FLU under control conditions was compared to the cellular accumulation observed in the presence of Pluronic copolymers or the MRP inhibitor, IND.

### P-gp Functional Assay

The cellular accumulation of R123 was used to examine effects of the Pluronic block copolymers on P-gp. Cell monolayers were preincubated for 30 min. at 37°C with assay buffer. After this the assay buffer was removed and the cells were exposed to 3.2 μM R123 in either assay buffer or solutions of Pluronic block copolymers. The cells were incubated with the dye solutions for up to 90 min. at 37°C. After that the dye solutions were removed and cell monolayers were washed three times with ice-cold PBS. The cells were then solubilized in 1.0% Triton X-100. The cellular accumulation of R123 was determined using Shimadzu RF5000 fluorescent spectrophotometer (505 nm excitation; 540 nm emission). Samples were taken for protein assay using the Pierce BCA method.

### Efflux of FLU

For the FLU efflux studies Panc-1 monolayers were preincubated for 30 min. at 37°C with assay buffer and loaded with FLU using two different treatment protocols (A, B) for 60 min at 37°C. The A-protocol used 100 μM FLU in assay buffer and B-protocol used 100 μM FLU in 22 μM (0.01%) P85. Then all cell monolayers were washed three times with ice-cold PBS. Four monolayers in each treatment protocol were left for determining total FLU accumulation in Panc-1 monolayers. The remaining monolayers were incubated at 37°C in FLU-free assay buffer (A-protocol) or 22 μM P85 (B-protocol) respectively. The residual cellular levels of FLU ("FLU retention") were determined after 5, 10, 15, and 25 min. incubation using four monolayers for each time point. At these time points the incubation solutions were removed from the monolayers and the cells were solubilized in 1.0% Triton X-100. Cellular FLU was determined by measuring fluorescence of the cell lysates as described above.

### Statistical Analysis

All statistical tests were performed using Microsoft Excel 97 SR-1 program using the two-tailed heteroscedastic t-tests. A minimum p value of 0.05 was used as the significance level for all tests. The results of accumulation and efflux studies are presented as means ± SEM.

### Fluorescence Microscopy

In separate studies, Panc-1 cells were grown on chamber slides (Fisher, St. Louis, MO) for examining FLU cellular accumulation using fluorescent microscopy. The Panc-1 calls were preincubated for 30 min. with either assay buffer or 22 μM P85 and then exposed to the FLU in either assay buffer or 22 μM P85 solution for 90 minutes at 37°C. After this incubation period, the loading solutions were removed and the Panc-1 cells were washed a total of three times with ice-cold PBS containg 1% bovine serum albumin and examined using Nikon microphot FXA microscope with a 60 × 1.4 n.a. oil plan apochromat objective and FITC filter set (Omega Optical). Digital images were obtained using the same Nikon microscope coupled to a cooled CCD camera (Photometrics, Tucson AZ).

## RESULTS AND DISCUSSION

To access MRP functional activity and the effects that Pluronic block copolymers had on MRP activity, FLU accumulation was examined in Panc-1 monolayers. The use of FLU and FLU analogs as probes for MRP functional activity has been reported previously (14,15). Furthermore, the Panc-1 cells are known to express MRP (13,14). Compared to control monolayers receiving only FLU, there was a significant enhancement of FLU accumulation in Panc-1 cells following exposure to P85 (Table 1). Furthermore, the effects of P85 were concentration-dependent (Table 1). At low concentrations of P85 ranging from 0.022 to 22 μM, treatment with the increasing doses of the block copolymer caused a greater uptake of FLU in Panc-1 monolayers. At concentrations of P85 above 22 μM the FLU accumulation practically leveled off. Since the critical micelle concentration (CMC) of P85 is 67 μM (0.03% w/v) (8) it appears that the effects of this block copolymer on FLU accumulation increase at sub-micellar concentrations and level off in the vicinity of CMC. This is indicative of the effect of the block copolymer single chains ("unimers") resulting in increased accumulation of FLU in Panc-1 monolayers.

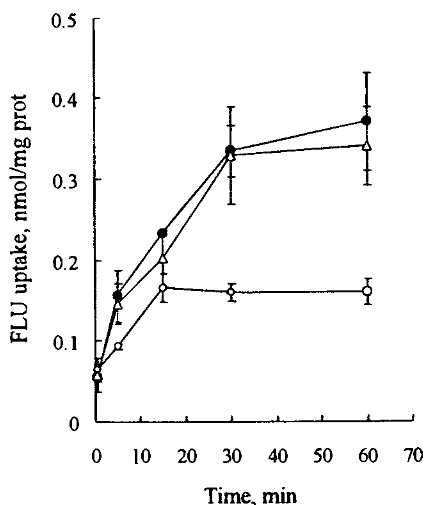



**Fig. 1.** Time-dependent accumulation of FLU in Panc-1 cell monolayers using assay buffer (open circles), 22 μM P85 (filled circles) and 10 μM IND (open triangles). Values represent the mean ± SEM of four cell monolayers.

The increased accumulation of FLU in P85 treatment groups is consistent with the blockade of MRP-efflux pump in Panc-1 cells. Further evidence supporting inhibition of MRP with P85 unimers is found in Fig. 1. In these experiments, FLU accumulation in Panc-1 cell monolayers examined under normal conditions and following treatment with either P85 (22 μM) or the MRP inhibitor, IND. Under normal conditions, FLU accumulation in the Panc-1 cell monolayers was limited, reaching a maximum of approximately 0.15 nmol/mg protein within the first 15 minutes of incubation (Fig. 1). However, following exposure to P85, FLU accumulation in the Panc-1 cell monolayers steadily increased to over 0.3 nmol/mg protein. The enhanced accumulation of FLU following treatment of Panc-1 monolayers with P85 resembled in both magnitude and scope, the increase in FLU observed with IND, a known inhibitor of MRP (10,15).

To eliminate the possible contributions of non-specific effects of Pluronic block copolymers on membrane permeability, the cellular accumulation of R123 was examined in Panc-1 monolayers. R123 is a fluorescent probe used to assess P-gp activity (16). Since the Panc-1 have little, if any, P-gp (13), any increases in R123 accumulation following exposure to the block copolymer would most likely reflect non-specific alterations in membrane permeability. As shown in Table 2, the accumulation of R123 in Panc-1 cell monolayers was similar in both the control and P85 treatment groups. The lack of effect with P85 on the accumulation of the non-MRP probe, R123, in Panc-1 monolayers indicates that the response observed with the MRP probe, FLU, is attributable to inhibition of MRP-related efflux transport systems rather than non-specific altering the membrane permeability with respect to the drug passive diffusion.

For comparison, Table 2 also presents the effects of P85 on R123 accumulation in P-gp expressing KBv cells, where the treatment with the block copolymer unimers resulted in considerable increases in R123 accumulation. It was previously shown that this effect on the P-gp expressing cell lines is due to inhibition of the P-gp efflux function by Pluronic block copolymers (6–8,17). To evaluate whether P85 unimers have inhibitory effect on MRP efflux system in Panc-1 cell monolayers, the FLU efflux studies were carried out with these cells. As it is shown in Fig. 2, treatment of the Panc-1 monolayers with P85 (22 μM) results in a considerable decrease of FLU efflux compared to the control groups (the rate of efflux, i.e. the slope of the efflux curve after 5 minutes, is decreased by 40% in the presence of P85). This provides further evidence that P85 is blocking MRP efflux system in Panc-1 monolayers, which is similar to the effects of P85 unimers on the P-gp efflux pump in KBv monolayers.

Previous studies using P-gp expressing cells demonstrated that effects of Pluronic block copolymers on P-gp efflux system are strongly dependent on their molecular structure, particularly, the lengths of EO (hydrophilic) and PO (hydrophobic) chain blocks (17). To determine if changes in Pluronic block copolymer composition could be used to confer selectivity for one drug efflux transport system over the other, inhibition of P-gp

**Table 2.** Time-Dependent Accumulation of R123 in Assay Buffer and 22 μM P85 Solution in Panc-1 and KBv Cell Monolayers

| Incubation time, min | R123 accumulation, nmol/mg protein[a] | | | |
|---|---|---|---|---|
| | Panc-1 | | KBv | |
| | Assay buffer | 22 μM P85 | Assay buffer | 22 μM P85 |
| 5 | 0.16 ± 0.03 | 0.17 ± 0.05 | 0.09 ± 0.01 | 0.09 ± 0.01[b] |
| 15 | 0.71 ± 0.11 | 0.65 ± 0.01 | 0.17 ± 0.03 | 0.17 ± 0.01[b] |
| 30 | 0.96 ± 0.15 | 1.60 ± 0.02 | 0.17 ± 0.03 | 0.28 ± 0.01** |
| 60 | 3.30 ± 0.18 | 3.53 ± 0.34 | 0.18 ± 0.04 | 0.48 ± 0.01** |
| 90 | 4.59 ± 0.10 | 4.43 ± 0.25 | 0.18 ± 0.03 | 0.58 ± 0.06* |

[a] Cells were incubated with R123 (3.2 μM) with or without P85 as described in materials and methods. Values represent the mean ± SEM of four monolayers per the treatment group. Statistical significance of the P85 effects in KBv cells compared to the P85-free controls at the same time point is shown below. The R123 accumulation in Panc-1 cells with and without P85 is the same at each time point with the significance levels of 0.05 or better.

[b] n.s., not significant.

* $p < 0.05$.

** $p < 0.005$.

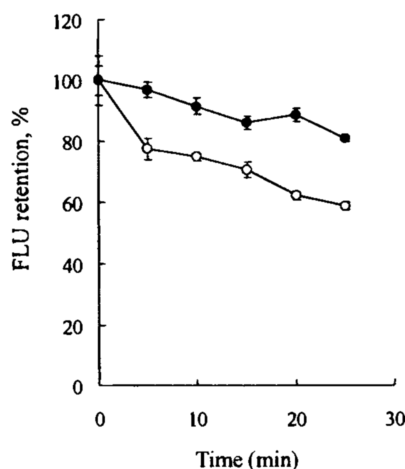



**Fig. 2.** Effect of P85 on R123 efflux in Panc-1 cell monolayers. Efflux was examined using assay buffer (open circles) and 22 μM P85 (filled circles).

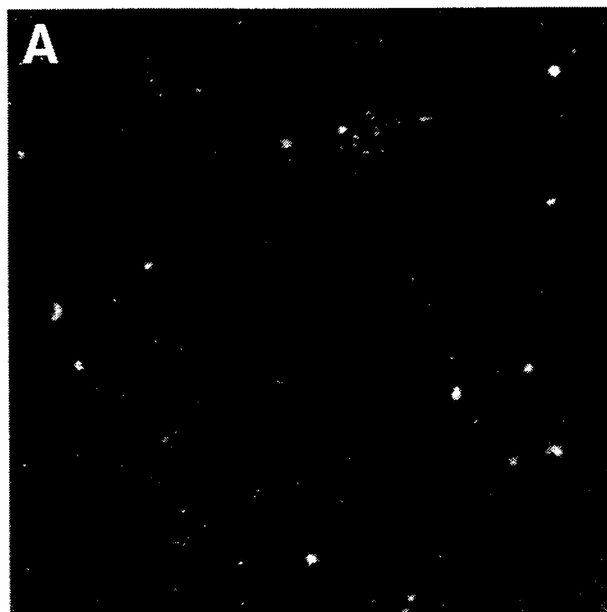
and MRP by P85 was examined along with the more hydrophobic (L81) and less hydrophobic (F108) Pluronic copolymer compositions (Table 3). All the Pluronic block copolymers examined significantly enhanced FLU accumulation in Panc-1 cell monolayers, and R123 accumulation in KBv cell monolayers. However of the three compositions examined, L81 was the most effective, while F108 was the least effective, at enhancing FLU accumulation in the PANC-1 cell monolayers and R123 accumulation in KBv monolayers (Table 3). These studies suggest that although composition of the copolymer will influence the extent of inhibition observed, it is unlikely that altering the hydrophobicity or size of the block copolymer will produce selectivity for the MRP or P-gp efflux transport systems.

Previous studies show that the effects of Pluronic copolymers in P-gp expressing multidrug resistant cells are not limited

**Table 3.** Effects of Various Pluronic Block Copolymer Compositions on MRP and P-gp Functional Activity

| System studied[a] | R123 enhancement factor in KBv monolayers[b] | FLU enhancement factor in Panc-1 monolayers[b] |
|---|---|---|
| F108 | 1.68 ± 0.18* | 1.32 ± 0.19* |
| P85 | 2.63 ± 0.33** | 2.19 ± 0.08** |
| L81 | 5.67 ± 0.23** | 3.29 ± 0.21** |

[a] All Pluronic block copolymer compositions were examined at 100 μM concentrations which corresponded to their maximal effective doses.
[b] R123 enhancement factor is defined as the increase in accumulation of R123 (3.2 μM) in KBv cell monolayers over a one hour incubation period in the presence of Pluronic block copolymer, with accumulation of R123 in the control group being 1.0. FLU enhancement factor is defined as the increase in accumulation of FLU (100 μM) in Panc-1 cell monolayers over a one hour incubation period in the presence of Pluronic block copolymer, with accumulation of FLU in the control group being 1.0. Statistical significance of the P85 effects compared to the P85-free controls at the same time point is shown below.
* $p < 0.05$.
** $p < 0.005$.



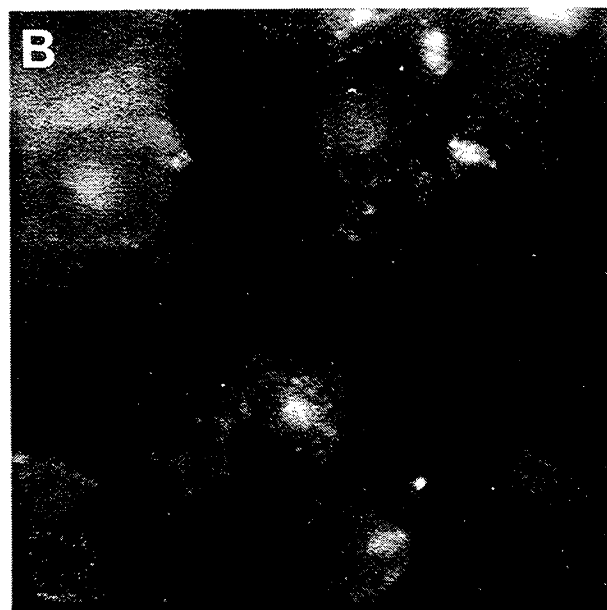




**Fig. 3.** Fluorescence microscopy of FLU accumulation in Panc-1 cell monolayers following exposure with 1 mM FLU in (A) assay buffer and (B) 22 μM P85.

to the increased cell accumulation of the drug as a result of inhibition of the P-gp efflux pump, but also include significant changes in the intracellular localization of the drug (7). Therefore, we compared localization of FLU in Panc-1 cells treated with the assay buffer and P85 solutions (Fig. 3). It is seen in panel A that FLU in assay buffer appears to be sequestered in intracellular vesicular compartments. In contrast, in P85 treated cells, FLU spreads throughout the cells cytoplasm and nucleus (panel B). Sequestration of drugs in both P-gp and MRP overexpressing cells has been well-documented (18–22). It is currently believed that sequestration occurs in intracellular vesicles containing drug transporters, in which the transporter is oriented so that drugs are pumped into and concentrated in the interior

of the vesicle (21,22). Therefore, current observation that P85 copolymer abolishes FLU sequestration in MRP expressing Panc-1 cells appears to be consistent with the previous report that Pluronic block copolymers decrease sequestration and allow nuclear transport in P-gp expressing CHrC5 Chinese hamster ovary cells (7).

The present study is the first to demonstrate that Pluronic copolymers can inhibit MRP-mediated efflux transport systems. While the mechanism(s) by which Pluronic copolymers inhibit MRP activity is unclear, there are many similarities to the inhibition of P-gp. The actual site at which the Pluronic unimers are interacting with MRP is currently under investigation. Given the comparable effects of the different Pluronic compositions on MRP and P-gp functional activity, it is tempting to suggest that there is a singular mechanism to explain the inhibitory effects of Pluronic on various drug efflux transport systems. Such mechanism may involve the copolymer-induced changes in the plasma membrane that affect function of both proteins (6).

There are also indications that the molecules of the Pluronic block copolymers must first undergo endocytic transport into the cell to reach the site of action with the membrane to inhibit P-gp (8). The effects of P85 on P-gp were completely abolished, when the endocytosis of the block copolymer was inhibited. Also, there is a lag period of ca. 15-min. before the blockade of the P-gp could be observed (8,17). In the case of the MRP inhibition the lag period for the P85 effect is somewhat longer—from 30 to 60 min. Therefore, in the experiments involving the transport of the MRP substrates the cells were pretreated with Pluronic solutions for 30-min. The difference in the kinetics of P85 effects on P-gp and MRP may be indicative of different sites of the block copolymer interactions with these drug efflux systems. Conversely, the differences in the kinetics of P85 effects on P-gp and MRP may be related to the differences in the expression levels of P-gp and MRP, or the substrates selected for examining functional activity of the drug efflux transporters.

The ability of Pluronic block copolymers to inhibit both P-gp and MRP functional activity has several clinical implications. In the treatment of cancer, both acquired and intrinsic drug resistance limits the effectiveness of many chemotherapeutic agents. While P-gp is thought to have a major role in acquired drug resistance (23), the widespread expression of MRP in many solid organ, and untreated tumor cells (24,25), suggests MRP may have a more important role in intrinsic drug resistance. Therefore, formulation of chemotherapeutic agents with Pluronic block copolymers (6,7) may improve treatment outcomes in both solid organ tumors, which display high intrinsic resistance, as well as other types of cancer such as leukemia's which display acquired drug resistance. Since there is significant overlap in the chemotherapeutic agents that are substrates for P-gp and MRP, formulation of these agents with Pluronic block copolymers may both enhance the initial cytotoxicity and extend the effective treatment time for the chemotherapeutic agent. *In vivo* evaluation studies revealed major anti-tumor effects of Pluronic-formulated anthracycline antibiotics on a broad panel of tumors (26,27).

High levels of MRP mRNA have been detected in normal tissue, such as the kidney, heart and bronchiolar epithelium (25,28). Therefore, Pluronic-based formulations may increase drug accumulation in selected organs as well as increase plasma concentrations of the drug through prevention of renal excretion. It has recently been demonstrated that Pluronic block copolymers effectively inhibit P-gp efflux system in normal tissues resulting in enhanced permeability of the P-gp-dependent drugs in the monolayers of the intestinal epithelial cells and brain microvessel endothelial cells (29). Low levels of mRNA for MRP have been found in the organs such as the brain and intestine (28). Recent studies demonstrating MRP expression in the endothelial cells that form the blood-brain barrier (14) suggest that the brain expression of MRP may be localized to those areas that control the passage of compounds into the central nervous system. Given the impact that P-gp has on the intestinal absorption and blood-brain barrier permeability of selected agents (5,30), it is likely that MRP has a similar role.

## ACKNOWLEDGMENTS

The studies were funded in part by grants from the National Institutes of Health (R15-NSOD35364 awarded to DWM and R01 NS36229-01A1 awarded to AVK) and by the Nebraska Research Initiative. The authors thank Dr. Valery Alakhov (Supratek Pharma, Inc.) for very useful discussions of the effects of amphiphilic block copolymers in cancer and normal cells. We also thank Huai-Yun Han and Shu Li for technical assistance during cell experiments, and Cathy Talmadge for help during fluorescence microscopy studies.

## REFERENCES

1. A. Krishan, C. M. Fitz, and I. Andritsch. Drug retention, efflux, and resistance in tumor cells. *Cytometry* **29**:279–285 (1997).
2. H. W. Van Veen and W. N. Konings. Multidrug transport from bacteria to man: similarities in structure and function. *Semin. Cancer Biol.* **8**:183–191 (1997).
3. P. G. Bray and S. A. Ward. A comparison of the phenomenology and genetics of multidrug resistance in cancer cells and quinoline resistance in plasmodium falciparum. *Pharmacol. Ther.* **77**:1–28 (1998).
4. U. A. Germann, I. Pastan, and M. M. Gottesman. p-Glycoproteins: mediators of multidrug resistance. *Semin. Cell. Biol.* **4**:63–76 (1993).
5. A. H. Schinkel, J. J. M. Smit, O. van Tellingen, J. H. Beijnen, E. Wagenaar, L. van Deemter, C. A. A. M. Mol, M. A. van der Valk, E. C. Robanus-Maandag, H. P. J. te Riele, A. J. M. Berns, and P. Borst. Disruption of the mouse mdr-1 a P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell* **77**:491–502 (1994).
6. V. Y. Alakhov, E. Y. Moskaleva, E. V. Batrakova, and A. V. Kabanov. Hypersensitization of multidrug resistant human ovarian carcinoma cells by pluronic P85 block copolymer. *Bioconjugate Chem.* **7**:209–216 (1996).
7. A. Venne, S. Li, R. Mandeville, A. V. Kabanov, and V. Y. Alakhov. Hypersensitizing effect of pluronic L61 on cytotoxic activity, transport and subcellular distribution of doxorubicin in multiple drug-resistant cells. *Cancer Res.* **56**:3626–3629 (1996).
8. D. W. Miller, E. V. Batrakova, T. O. Waltner, V. Y. Alakhov, and A. V. Kabanov. Interactions of Pluronic block copolymers with brain microvessel endothelial cells: evidence of two potential pathways for drug absorption. *Bioconjugate Chem.* **8**:649–657 (1997).
9. M. A. Barrand, T. Bagrij, and S. Y. Neo. Multidrug resistance-associated protein: a protein disinct from P-glycoprotein involved in cytotoxic drug expulsion. *Gen. Pharmacol.* **28**:639–645 (1997).

10. Z. Hollo, L. Homolya, T. Hegedus, and B. Sarkadi. Transport properties of the multidrug resistance-associated protein (MRP) in human tumor cells. *FEBS Lett.* **383**, 99–104 (1996).
11. G. Jedlitschky, I. Leier, U. Buchholz, K. Barnouin, G. Kurz, and D. Keppler. Transport of glutatione, glucuronate, and sulfate conjugates by the MRP gene-encoded export pump. *Cancer Res.* **56**:988–994 (1996).
12. M. J. Flens, G. J. Zaman, P. van-der-Valk, M. A. Izquierdo, A. B. Schroeijers, G. L. Scheffer, P. van-der-Groep, M. de-Haas, C. J. Meijer, R. J. Scheper. Tissue distribution of the multidrug resistance protein. *Am. J. Pathol.* **148**:1237–1247 (1996).
13. D. W. Miller, M. Fontaine, C. Kolar, T. Lawson. The expression of multidrug resistance-associated protein (MRP) in pancreatic adenocarcinoma cell lines. *Cancer Lett.* **107**:301–306 (1996).
14. H. Huai-Yun, D. T. Secrest, K. S. Mark, D. Carney, C. Brandquist, W. F. Elmquist, and D. W. Miller. Expression of multidrug resistance-associated protein (MRP) in brain microvessel endothelial cells. *Biochem. Biophys. Res. Com.* **243**: 816–820 (1998).
15. M. P. Draper, R. L. Martell, and S. B. Levy. Indomethacin-mediated reversal of multidrug resistance and drug efflux in human and murine cell lines overexpressing MRP, but not P-glycoprotein. *Br. J. Cancer* **75**:810–815 (1997).
16. M. Fontaine, W. F. Elmquist, and D. W. Miller. Use of rhodamine 123 to examine the functional activity of P-glycoprotein in primary cultured brain microvessel endothelial cell monolayers. *Life Sci.* **59**:1521–1531 (1996).
17. E. V. Batrakova, H.-Y. Han, V. Yu. Alakhov, D. W. Miller, and A. V. Kabanov. Effects of Pluronic block copolymers on drug absorption in Caco-2 cell monolayers. *Pharm. Res.* **15**:850–855 (1998).
18. K. Nooter and G. Stoter. Molecular mechanisms of multidrug resistance in cancer chemotherapy. *Pathol. Res. Pract.* **192**:768–780 (1996).
19. L. M. Breuninger, S. Paul, K. Gaughan, T. Miki, A. Chan, S. A. Aaronson, and G. D. Kruh. Expression of multidrug resistance-associated protein in NIH/3T3 cells confers multidrug resistance associated with increased drug efflux and altered intracellular drug distribution. *Cancer. Res.* **55**:5342–5347 (1995).
20. I. Cleary, G. Doherty, E. Moran, and M. Clynes. The multidrug-resistant human lung tumour cell line, DLKP-A10, expresses novel drug accumulation and sequestration systems. *Biochem. Pharmacol.* **53**:1493–1502 (1997).
21. P. R. Twentyman. Transport proteins in drug resistance: biology and approaches to circumvention. *J. Intern. Med. Suppl.* **740**:133–137 (1997).
22. A. B. Shapiro, K. Fox, P. Lee, Y. D. Yang, and V. F. Ling. Functional intracellular P-glycoprotein. *Int. J. Cancer* **76**:857–864 (1998).
23. Y. Abe, Y. Ohnishi, M. Yoshimura, E. Ota, Y. Ozeki, Y. Oshika, T. Tokunaga, H. Yamazaki, Y. Ueyema, T. Ogata, N. Tamaoki, and M. Nakamura. P-Glycoprotein-mediated acquired multidrug resistance of human lung cancer cells *in vivo*. *Br. J. Cancer* **74**:1929–1934 (1996).
24. G. D. Kruh, K. T. Gaughan, A. Godwin, and A. Chan. Expression pattern of MRP in human tissues and adult solid tumor cell lines. *J. Natl. Cancer Inst.* **87**:1256–1258 (1995).
25. M. J. Flens, G. J. Zaman, P. van der Valk, M. A. Izquierdo, A. B. Schroeijers, G. L. Scheffer, P. van der Groep, M. de Haas, C. J. Meijer, and R. J. Scheper. Tissue distribution of the multidrug resistance protein. *Am. J. Phathol.* **148**:1237–1247 (1996).
26. B. D. Stride, G. Valdimarsson, J. H. Gerlach, G. M. Wilson, S. P. Cole, and R. G. Deeley. Structure and expression of the messenger RNA encoding the murine multidrug resistance protein, an ATP-binding cassette transporter. *Mol. Pharmacol.* **49**:962–971 (1996).
27. E. V. Batrakova, T. Yu. Dorodnych, E. Yu. Klinskii, E. N. Kliushnenkova, O. B. Shemchukova, S. A. Arjakov, V. Yu. Alakhov, A. V. Kabanov. Anthracycline antibiotics non-covalently incorporated into the block-copolymer micelles: *in vivo* evaluation of anti-cancer activity. *Br. J. Cancer* **74**:1545–1552 (1996).
28. V. Yu. Alakhov, A. V. Kabanov. Block copolymeric biotransport carriers as versatile vehicles for drug delivery. *Expert Op. Invest. Drugs* **7**:1453–1473 (1998).
29. E. V. Batrakova, H -Y. Han, D. W. Miller, A. V. Kabanov. Effects of Pluronic P85 unimers and micelles on drug permeability in polarized BBMEC and Caco-2 cells. *Pharm. Res.* **15**:1525–1532 (1998).
30. R. B. Kim, M. F. Fromm, C. Wandel, B. Leake, A. J. J. Wood, D. M. Roden, and G. R. Wilkinson. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J. Clin. Invest.* **101**:289–294 (1998).