Pluronic P85 Increases Permeability of a Broad Spectrum of Drugs in Polarized BBMEC and Caco-2 Cell Monolayers

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Purpose. Previous studies demonstrated that inhibition of P glycoprotein (P-gp) by Pluronic P85 (P85) block copolymer increases apical (AP) to basolateral (BL) transport of rhodamine 123 (R123) in the polarized monolayers of bovine brain microvessel endothelial cells (BBMEC) and Caco-2 cells. The present work examines the effects of P85 on the transport of fluorescein (Flu), doxorubicin (Dox), etoposide (Et), taxol (Tax), 3′-azido-3′-deoxythymidine (AZT), valproic acid (VPA) and loperamide (Lo) using BBMEC and Caco-2 monolayers as *in vitro* models of the blood brain barrier and intestinal epithelium respectively.

Methods. Drug permeability studies were performed on the confluent BBMEC and Caco-2 cell monolayers mounted in Side-Bi-Side diffusion cells.

Results. Exposure of the cells to P85 significantly enhanced AP to BL permeability coefficients of Flu, Tax, Dox and AZT in both cell models. Further, P85 enhanced AP to BL transport of Et, VPA and Lo in Caco-2 monolayers. No changes in the permeability coefficients of the paracellular marker mannitol were observed in the presence of the copolymer.

Conclusions. P85 increases AP to BL permeability in BBMEC and Caco-2 monolayers with respect to a broad panel of structurally diverse compounds, that were previously shown to be affected by P-gp and/or multidrug resistance associated protein (MRP) efflux systems. Broad specificity of the block copolymer effects with respect to drugs and efflux systems appears to be a valuable property in view of developing pharmaceutical formulations to increase drug accumulation in selected organs and overcome both acquired and intrinsic drug resistance that limits the effectiveness of many chemotherapeutic agents.

KEY WORDS: blood brain barrier; intestinal barrier; MRP; P-gp; Pluronic block copolymer.

INTRODUCTION

Multidrug resistance (MDR) has been associated with the overexpression of membrane transport proteins belonging to the superfamily of the ATP-binding cassette (ABC) proteins (1–3). For example, P-glycoprotein (P-gp) and multidrug resistance associated protein (MRP) have been implicated in MDR in cancer cells. The expression of these same 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 (BBB) have an important protective and detoxification function (4–6). Both P-gp and MRP drug efflux systems are present in the brain microvessel endothelial cells (7,8). The functional role of the P-gp pump in the intestinal epithelium cells, Caco-2 is also well documented (9,10). The identification of methods for circumventing these drug efflux transport proteins has attracted significant attention as potential ways to improve the absorption and alter the distribution of therapeutic agents in the body.

Recent studies suggested that Pluronic block copolymers (poly(ethylene oxide)-block-poly(propylene oxide)-block-poly(ethylene oxide), $EO_m/2\text{-}PO_n\text{-}EO_m/2$) are potent nonionic surfactant inhibitors of both P-gp and MRP efflux systems in cells (11–15). In MDR cancer cells, Pluronic block copolymers enhanced accumulation of drugs and increased their cytotoxic effects by ca. 3 orders of magnitude (11,12). More recently, increased cellular accumulation of fluorescein (Flu) was observed following treatment with Pluronic block copolymer in a human pancreatic adenocarcinoma cell line (Panc-1), that express MRP. In these studies, the Pluronic copolymers displayed a similar pattern of inhibition as described previously in the P-gp expressing cells (13). Given the possible role of the drug efflux pumps in controlling permeability in BBB and intestinal epithelium (7–10), we studied the effects of Pluronic copolymers on drug transport in polarized monolayers of bovine brain microvessel endothelial cells (BBMEC) (14) and human intestinal epithelial cells, Caco-2 (15). Using rhodamine 123 (R123) as a P-gp-dependent probe, these studies displayed significant enhancement of R123 accumulation and permeability in both cell models following treatment with Pluronic block copolymers (14–16). In this work we extend permeability studies with Pluronic P85 (P85) using a broader drug panel presented in Fig. 1. This panel includes both well-known substrates for P-gp and/or MRP, Flu, doxorubicin (Dox), etoposide (Et), taxol (Tax), as well as compounds with less studied specificity for the efflux pumps, 3′-azido-3′-deoxythymidine (AZT), valproic acid (VPA) and loperamide (Lo). The results of these studies suggest that formulation with Pluronic block copolymers may be useful in increasing brain and/or oral absorption of broad spectrum of drugs. On the other hand the results of this study might be important in view of the use of Pluronic formulations in chemotherapy, particularly in the cases where the effects of the membrane efflux proteins contribute to drug resistance.

MATERIALS AND METHODS

Cell Culture

BBMEC were isolated from fresh cow brains using a combination of enzymatic digestion and density centrifugation as described previously (17). The BBMEC cells were maintained in MEM:F12 culture medium supplemented with 10% horse serum, heparin sulfate (100 μg/mL), amphotericin B (2.5 μg/mL), and gentamicin (50 μg/mL). Caco-2 cells originally from a human colorectal carcinoma were cultured in Dulbecco's Modified Eagle's Medium (DMEM), containing 10% heat-inactivated fetal bovine serum (FBS), 1% non-essential amino acids, benzylpenicilin (100 U/ml) and streptomycin (10 μg/ml) as described previously (18). In Caco-2 experiments the cells of passage numbers 40–60 were used. All tissue culture media were obtained from Gibco Life Technologies, Inc. (Grand Island, NY). Cell monolayers were grown on collagen coated

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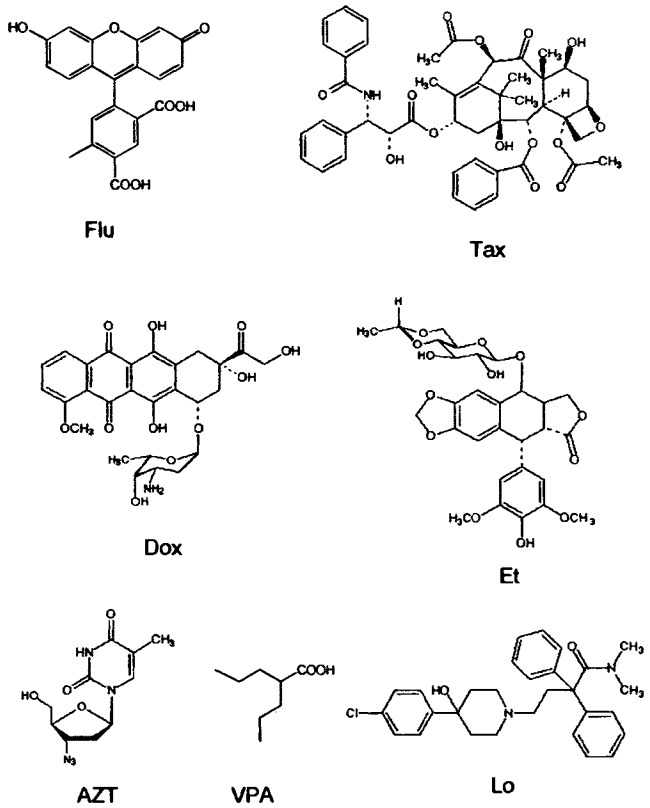

Fig. 1. Fluorescent probes and drugs used in the current permeability study.

(Caco-2) or fibronectin and collagen coated (BBMEC) polycarbonate membrane inserts (Transwell, Costar Brand Tissue Culture Products, Contd.; pore size 0.4 μm; diameter 24.0 mm). Cells were seeded at a density of 250,000 cells per insert and were allowed to grow and differentiate for up to 14 days till complete maturation of the monolayers.

Preparation of Pluronic Block Copolymer and Drug Solutions

The present study uses P85 block copolymer (lot # WPOP-587A) that was provided by BASF Corp. (Parispany, NJ). The molecular mass of the PO segment in this sample was approximately 2,500 and the content of EO chains was approximately 50% (w/w). Dox, R123, Flu, Lo and Et were purchased from Sigma Chemical Corp. (St. Louis, MO). [H^3]-AZT (3′-azido-3′-deoxythymidine-methyl-^{3}H), [H^3]-Tax and [H^3]-VPA were obtained from Moravek Biochemicals, Inc. (Brea, CA). The solutions of P85 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). The drugs and fluorescent dyes were added to the copolymer solutions and incubated at 37°C for at least one hour prior to their use in the experiments.

Partitioning of R123, Dox, and Flu in P85 Micelles

The partitioning coefficient (P) of the fluorescent probes (R123, Dox, and Flu) in P85 micelles were evaluated by measuring the fluorescence in probe solutions (0.1 μM) containing various concentrations of P85. The fluorescence measurements were performed at λex = 512 nm and λem = 540 nm for R123, λex = 471 nm, λem = 560 nm for Dox, and λex = 488 nm and λem = 510 nm for Flu using a Shimadzu RF5000 fluorescent spectrophotometer with a thermostatic unit to maintain samples at 37°C. The partitioning coefficients (P) were obtained as previously described (11) using the dependence of the probe fluorescence on the P85 concentration:

$$\frac{I_{max} - I}{I - I_o} = \frac{119}{P([P85] - CMC)} - \frac{1}{P} \quad (1)$$

where I_o is the fluorescence intensity in the absence of P85, I is the fluorescence at the given P85 concentration [P85] (% wt), I_{max} is the fluorescence at "saturating" concentration of P85 (when fluorescence reaches the maximal value), and CMC is the critical micelle concentration of P85 (= 0.03%) (11).

The portions of the drug incorporated into the micelles (α) were determined from the following equation (11):

$$\alpha = \frac{P([P85] - CMC)}{199 + (P - 1)([P85] - CMC)} \cdot 100\% \quad (2)$$

Permeability Studies

Polycarbonate membrane inserts with confluent BBMEC or Caco-2 monolayers were placed in Side-Bi-Side diffusion cells from Crown Bio Scientific, Inc. (Somerville, NJ) maintained at 37°C. The transport of the drugs across cell monolayers was determined as described earlier (19). Cell monolayers were pre-incubated for 30 min. at 37°C with the assay buffer (3 ml) added to both donor and receiver chambers (control groups) or P85 solutions (3 ml) added to donor chamber and assay buffer (3 ml) added to receiver chamber (treated groups). Following the preincubation period, fresh assay buffer was added to the receiver chamber and the assay buffer in the donor chamber was replaced with the drug in either assay buffer alone or P85 solution (0.001% to 5%). In apical (AP) to basolateral (BL) transport studies, the AP side of the monolayers was exposed to the donor chamber, while in BL to AP studies the BL side of the membranes was exposed to the donor chamber. In all the permeability experiments, except those using radioactively labeled drugs ([H^3]-Tax, [H^3]-AZT, and [H^3]-VPA) the solutions in the donor chamber also contained 1 nM [H^3]-mannitol, as a paracellular marker (DuPont Corp., Boston, NA). At 0, 15, 30, 60 and 90 min. time points the solutions in the receiver chamber and aliquots (20 μl) from the donor chamber were removed for the determination of the drug concentration. 3 ml of the fresh assay buffer was immediately added to the receiver chamber. The amounts of Dox and Flu in the donor and receiver compartments were determined using a Shimadzu RF5000 fluorescent spectrophotometer as described above. Lo and Et were assayed using a Shimadzu UV 160U spectrophotometer at the specific absorbency wavelengths 259 nm (Lo) and 229 (Et). [H^3]-AZT, [H^3]-Tax, [H^3]-VPA and [H^3]-mannitol concentrations were determined using Beckman LS 6000 IC liquid scintillation counter. All experiments were conducted in triplicate.

Apparent Permeability Coefficients

Apparent permeability coefficients (P_{app}) of the drugs were calculated using the following equation (20):

$$P_{app} = \frac{1}{AC_0} \cdot \frac{dQ}{dt} \quad (3)$$

where dQ/dt is the flux across the cell monolayers, A is the surface area of the membrane and C_0 is the initial concentration of the drug.

Statistical Analysis

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

RESULTS

Effects of P85 on Flu Transport

Flu was previously described as a specific probe for the MRP efflux pump in the BBMEC monolayers and human pancreatic adenocarcinoma cells, Panc-1 (8,13). Specificity of the known MRP inhibitors (probenecid, indomethacin) on the transport of Flu in BBMEC and Panc-1 cells has been previously demonstrated (8,13). Further, the earlier work demonstrated that P85 increases accumulation of Flu in Panc-1 monolayers due to blockage of the MRP efflux system (13). Therefore, we used Flu as a probe to evaluate possible effects of the block copolymer on the drug efflux transport systems in the cell monolayers. As is seen in Table 1, exposure to the P85 block copolymer caused concentration-dependent effects on the AP to BL permeability in both BBMEC and Caco-2 monolayers. In the BBMEC monolayers there was a significant increase in the permeability coefficient of Flu in 0.01% P85 compared to that in the assay buffer (Table 1). At higher concentrations of P85 (0.1% and 1%) the permeability coefficients were sharply decreased below the level observed in the absence of the copolymer. The concentration dependency was different in Caco-2 monolayers. In this case the permeability coefficients were increased significantly in all P85 treatment groups studied (Table 1). Both in BBMEC and Caco-2 monolayers, there were no changes in the flux of the paracellular marker, mannitol in any of the P85 treatment groups examined (data not shown). The apparent mannitol permeability coefficients were 5.7 (±0.4) × 10^{-6} cm/s for BBMEC and 0.21 (±0.004) × 10^{-6} cm/s for Caco-2 monolayers. Therefore, the observed effects of the Pluronic block copolymer on the permeability coefficients of Flu are due to the changes in the transcellular transport of this probe rather changes in paracellular diffusion.

To evaluate possible directionality effects in the flux of Flu in BBMEC and Caco-2 monolayers, we studied the transport of this probe in AP to BL and BL to AP directions. As is seen in Fig. 2, in both cell monolayers there is a significant difference in the directionality of the transport of the probe in the assay buffer: the AP to BL flux is much less than the BL to AP flux. This result is consistent with the presence of the Flu efflux systems in these cells acting in BL to AP direction. The effects of the P85 in the BBMEC and Caco-2 monolayers were also direction-dependent (Fig. 2). The transport of Flu in AP to BL direction was increased in the presence of the block copolymer. This suggests that the block copolymer inhibited Flu efflux system at the AP side of the BBMEC and Caco-2 monolayers. When added at the BL side of these cell monolayers the block copolymer did not affect transport of Flu in BL to AP direction. This result is similar to the previously reported effect of P85 on the transport of P-gp substrate, R123, in the same cell models (16). It suggests that the block copolymer added at the BL side does not affect drug efflux transporters, P-gp and MRP, located at the AP side of the cell monolayers.

Table 1. Effects of P85 on the Drug Permeability in BBMEC and Caco-2 Monolayers in AP to BL Direction

Drug[a]	P85 concentration (% wt)	$P_{app} \times 10^6$ (cm/s)[b] BBMEC	Caco-2
Flu	0	16.50 ± 0.57	3.82 ± 0.44
	0.01	24.37 ± 1.65 (**)	7.26 ± 1.40 (**)
	0.1	6.81 ± 0.29 (*)	9.33 ± 0.50 (**)
	1	7.12 ± 0.51 (*)	13.92 ± 0.50 (**)
Dox	0	13.22 ± 0.17	14.50 ± 1.17
	0.01	n.d.	15.00 ± 0.37 (n.s.)
	0.1	11.20 ± 0.23 (n.s.)	35.17 ± 0.65 (**)
	1	31.45 ± 7.27 (**)	33.33 ± 3.22 (**)
[^{3}H]-Tax	0	1.47 ± 0.13	0.36 ± 0.01
	0.01	16.47 ± 2.13 (*)	2.34 ± 0.12 (**)
	0.1	10.74 ± 1.94 (*)	4.73 ± 0.35 (**)
	1	6.01 ± 0.77 (*)	1.26 ± 0.11 (*)
Et	0	n.d.	4.02 ± 0.57
	0.01	n.d.	6.53 ± 0.70 (*)
	0.1	n.d.	5.07 ± 0.23 (n.s.)
	1	n.d.	10.62 ± 0.77 (**)
[^{3}H]-AZT	0	16.50 ± 1.83	2.85 ± 0.07
	0.01	23.98 ± 4.70 (n.s.)	3.87 ± 0.07 (**)
	0.1	26.67 ± 1.32 (**)	11.85 ± 0.23 (**)
	1	31.13 ± 9.13 (**)	14.53 ± 0.40 (**)
[^{3}H]-VPA	0	25.81 ± 1.37	10.38 ± 0.54
	0.01	22.85 ± 0.31 (n.s.)	10.96 ± 0.50 (n.s.)
	0.1	26.61 ± 0.40 (n.s.)	12.82 ± 0.32 (*)
	1	26.71 ± 0.58 (n.s.)	14.25 ± 0.52 (**)
Lo	0	25.23 ± 2.82	1.62 ± 0.08
	0.01	24.47 ± 1.15 (n.s.)	2.00 ± 0.03 (*)
	0.1	26.78 ± 2.42 (n.s.)	3.25 ± 0.18 (*)
	1	25.23 ± 2.82 (n.s.)	2.62 ± 0.05 (**)

[a] Concentration of drugs in the donor chamber were: 3.2 μM (R123); 10 μM (Flu, BBMEC); 100 μM (Flu, Caco-2); 10 μM (Dox); 6.5 nM ([^{3}H]-Tax); 10 μM (Et); 1.7 nM ([^{3}H]-AZT); 55 nM ([^{3}H]-VPA); 66 μM (Lo).

[b] Statistical significance of P85 effects compared to the P85-free controls is shown in the brackets: (n.s.)-non-significant, (*) $p < 0.05$, (**) $p < 0.005$; $n = 3$.

Effects of P85 on Dox Transport

Dox is a substrate of both P-gp and MRP efflux pumps (21,22). Pluronic block copolymers have been shown to increase Dox absorption in P-gp expressing cancer tumor cells (12). Present permeability studies revealed significant increases in the Dox transport in AP to BL direction in BBMEC and Caco-2 monolayers treated with P85 compared to the control groups

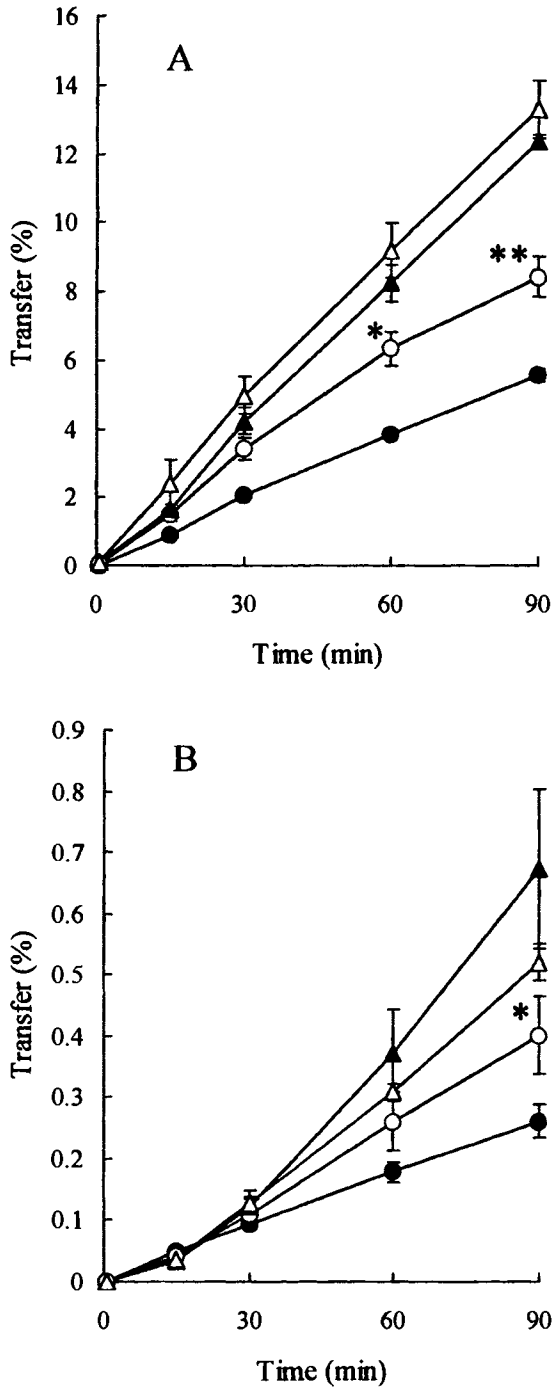

Fig. 2. Flux of Flu in BBMEC (panel A) and Caco-2 (panel B) monolayers. Transport of the probe in AP to BL direction was examined using assay buffer (filled circles) and 0.01% P85 at the AP side of the cell monolayers (empty circles). Transport of the probe in BL to AP direction was examined using assay buffer (filled triangles) and 0.01% P85 at the BL side (empty triangles). Flu concentration in the donor chamber was 100μM (BBMEC) or 10 μM (Caco-2). Values represent the mean ± SEM of three monolayers per the treatment group. Statistical significance of P85 effects compared to the P85-free controls in AP to BL transport studies is shown: * $p < 0.05$, ** $p < 0.005$. Effects of P85 in BL to AP transport studies are not significant.

(Table 1). P85 effects were observed at 1% with BBMEC monolayers and at 0.1%–1% in Caco-2 monolayers. No changes in mannitol permeability were observed with either the BBMEC or Caco-2 monolayers following treatment with Dox and/or P85 at all doses examined.

Effects of P85 on [^{3}H]-Tax Transport

Tax is a well-known substrate of the P-gp transporter (23). The effects of MRP on Tax transport are either insignificant or appear to be much less substantial than those of P-gp (23). The flux of Tax in AP to BL direction in both BBMEC and Caco-2 monolayers was dramatically increased at 0.01% and 0.1% P85 compared to the assay buffer controls (Table 1). In both the BBMEC and Caco-2 monolayers, the Tax permeability coefficients were decreased at high concentration of P85, 1%. However, even in the 1% P85 treatment groups the permeability coefficients were still significantly higher than those in the assay buffer controls.

Effects of P85 on Et Transport

Similarly to Dox, Et was described as a substrate of both P-gp and MRP (21,22). The studies on Et permeability in BBMEC monolayers were not successful, because, free Et and its block copolymer formulations affected the confluence of these monolayers. This problem could not be avoided by decreasing the working concentration of Et (10 μM) because of the sensitivity limitation of the UV spectroscopy method used to determine Et concentration. The same concentrations of free Et and its block copolymer solutions were not toxic with respect to Caco-2 cells, as no changes in the mannitol permeability were observed in Caco-2 monolayers upon treatment with Et in the assay buffer or P85 solutions. At the same time, there was a significant enhancement in the Et flux across the Caco-2 monolayers in the groups treated with 1% P85 compared to the control groups. It is likely that the increase in the Et permeability observed in this case is due to the inhibition of the drug transporters by the block copolymer.

Effects of P85 on [^{3}H]-AZT Transport

Although the specificity of AZT to efflux pumps is yet unknown the existence of an efflux system in the BBB and blood-cerebral fluid barrier transporting AZT from brain to blood has been reported (24–26). Given the apparent probenecid-sensitivity of the efflux of AZT in the BBB (24,26) such effects may be due to MRP system. There were significant increases in the permeability of [^{3}H]-AZT at 0.1 and 1 % P85 in the BBMEC monolayers (Table 1). At 0.01% P85 the permeability coefficient of [^{3}H]-AZT was ca. 1.5 times higher that that in the control group although it was statistically not significant. In Caco-2 monolayers, significant increases in permeability coefficients were observed with all P85 doses examined (Table 1).

Effects of P85 on VPA Transport

The role of the drug carrier systems in VPA transport is much less studied than in the cases of Dox, Tax and Et. However, recent work indicates that VPA efflux in the rabbit brain was inhibited by probenecid, which might be indicative of the effects

of MRP system in the brain capillary endothelium (27). As is seen in Table 1 no effect of the block copolymer on the VPA permeability was observed in the BBMEC monolayers. At the same time significant increase in VPA flux was seen in Caco-2 monolayers at 0.1% and 1% (Table 1). In this case, however, the permeability increase was ca. 1.4 times, which is less substantial than those observed in the Caco-2 monolayers with Flu and drugs (Dox, Et, AZT, Tax) described above.

Effects of P85 on Lo Transport

A recent work indicated that P-gp is implicated in the decreased accumulation of Lo in the central nervous system (28). Therefore we studied the effects of P85 on the permeability coefficients of this drug in BBMEC and Caco-2 monolayers. No effects on the permeability in the BBMEC monolayers were observed with all P85 doses examined (Table 1). There was a significant increase in the permeability coefficient of Lo in Caco-2 monolayers exposed to 1% P85. However, like in the case of VPA the increase in the permeability coefficient of Lo in Caco-2 monolayers was relatively small (ca. 1.6 times) compared to those observed in the cases of other drugs.

DISCUSSION

Previous studies using R123 as a P-gp-dependent probe, demonstrated that Pluronic block copolymers can increase permeability in BBMEC and Caco-2 monolayers by inhibiting P-gp efflux system (16). This work (i) characterized the effects of Pluronic P85 on the permeability of BBMEC and Caco-2 monolayers using Flu as MRP-specific probe and (ii) investigated effects of this copolymer on the transport of a broad panel of therapeutic agents including anticancer (Dox, Tax, Et), antiviral (AZT), antiepileptic (VPA) and analgetic (Lo) drugs.

Both functional and biochemical data demonstrating the presence of MRP in BBMEC and Caco-2 monolayers have recently been presented in the literature (8,10). While the studies of Makhey *et al.* (10) suggested that the MRP efflux transporter reduced the AP to BL permeability of MRP substrates in Caco-2, the directionality of transport has not been reported in the brain endothelial cells forming the blood-brain barrier. In the current study, Flu permeability under control conditions was lower in the AP to BL direction compared to the BL to AP direction in both the BBMEC and Caco-2 monolayers. Furthermore, P85 was able to increase the AP to BL permeability of Flu in the BBMEC and Caco-2 monolayers, despite having no effect on the permeability of the diffusion marker ^{3}H-mannitol. Together, these data would support the presence of MRP drug efflux transporters on the AP side of the BBMEC and Caco-2 monolayers. However, a certain amount of caution must be taken when attributing the effects of P85 on Flu permeability solely to the inhibition of MRP drug efflux transporters. First, it is clear that multiple drug transporters are expressed in brain microvessel endothelial and intestinal epithelial cells, and it is quite possible that the range and specificity of these transporters is broader than is presently known. Second, given the similar effects of the Pluronic block copolymers on P-gp and MRP transporters, it is possible that the inhibitory effects of P85 may extend to other transporter systems.

In addition to examining the effects of P85 on the permeability of the model MRP probe, Flu, in BBMEC and Caco-2 monolayers, the permeability of several structurally diverse drugs (Fig. 1) in the presence and absence of P85 block copolymer were also examined. The agents evaluated in the present study included well-documented P-gp and MRP substrates (Dox, Tax, Et), as well as agents with less studied interactions for the drug efflux transporters (AZT, VPA, Lo). The greatest effects on drug permeability with P85 were observed in both BBMEC and Caco-2 monolayers with Tax (approximately 10-fold permeability enhancement). The permeability of AZT was also significantly enhanced in the BBMEC and Caco-2 in the presence of P85 (approximately 2 and 4-fold, respectively). The effects of P85 on the permeability of Dox, Et, VPA, and Lo were substantially less in BBMEC and Caco-2 monolayers (2-fold or less).

Of the current drugs examined, Tax and Lo are known to interact with the P-gp transporter (23,28), while Dox and Et have interactions with both P-gp and MRP (21,22). Furthermore, drug efflux transport systems have been described previously for both AZT (24–26) and VPA (27) in the blood-brain barrier. The current study demonstrated significant increases in the permeability of the various drugs in BBMEC and Caco-2 monolayers following treatment with the Pluronic block copolymer, P85. Given the interactions of P85 with P-gp and MRP (11–15), and the expression of multiple efflux transport systems in the BBMEC and Caco-2 monolayers (7–10), it is tempting to suggest that the increased drug permeability observed in the present study is attributable, in part, to inhibition of efflux transport systems with P85. The only drugs, which did not display an increased permeability following exposure to P85 were Lo and VPA in the BBMEC monolayers. This was somewhat surprising given the previous studies examining the interactions of Lo and VPA with drug efflux transporters (27,28). However, it should be noted that the lack of effect of P85 on Lo and VPA permeability in the BBMEC monolayers may be attributable to a combination of the leakiness of the BBMEC monolayers and the sensitivity of the analytical assay.

Consistent with the previous P-gp and MRP functional studies using P85 (13–16), the effects of the block copolymer on Flu and drug permeability were apparent at concentrations below the CMC (= 0.03%), suggesting it is the individual polymer units ("unimers") that inhibit the drug efflux transport systems. The only exceptions were VPA in Caco-2 cells and Dox in BBMEC and Caco-2 cell where permeability increases were observed only at P85 concentrations above CMC. It needs to be said that the response with P85 micelles in cells could be quite complex. Indeed, a biphasic concentration response has been reported previously with the effects of P85 on R123 accumulation in BBMEC and Caco-2 monolayers (i.e. enhanced accumulation below CMC, decreased accumulation above CMC) (14–16). A similar biphasic concentration response was observed in the present study with regard to the effects of P85 on Flu transport in the BBMEC. Also the concentration dependency of the block copolymer effect on the transport of Tax in both cell models is very similar to the previously reported concentration dependency of P85 effect on R123 permeability (16). At the same time Flu, Dox, Et, VPA and Lo in Caco-2 cells or Dox in BBMEC revealed no decreased accumulation above CMC. Previous studies demonstrated that the concentration-dependent effects of Pluronic on drug accumulation and permeability are due to multiple mechanisms of drug transport with the P85 block copolymer in BBMEC (14,16) and Caco-2 monolayers (15,16). Particularly, decreased transport of R123 across these cell monolayers observed at high concentrations

Table 2. Partitioning Coefficients of R123, Dox and Flu in P85 Micelles and Portions of the Drug Incorporated in the Micelles (α) at 1% P85

	Partitioning coefficient, P		α at 1% P85 (%)	
Drug	pH7.4	pH5.0	pH7.4	pH5.0
R123	112	36	49	23
Dox	13	18	9	13
Flu	n.s.*	179	0	34

* No changes in Flu fluorescence were observed at all concentrations of P85 suggesting that there was no solubilization under these conditions.

of P85 is due to incorporation of this probe into the micelles (16). To evaluate possible differences in the drug interaction with the micelles we studied solubilization of R123, Flu and Dox in P85 solutions. The interaction of a drug with the block copolymer micelle can be quantitatively characterized in terms of the partitioning coefficient, *P* (11). Table 2 presents the values of *P* for these compounds. It also presents for comparison the portions of the drugs incorporated in the micelles at 1% P85, α. To account for possible effects of the changes in ionization of the drugs in the culture medium and intracellular vesicles the values of *P* and α were determined at pH 7.4 and pH 5.0. It is seen that at both pH's the retention of Dox in the P85 micelles is relatively low. In contrast, much stronger retention was observed with R123, the probe revealing the strongest effects of the micelles in the accumulation and permeability studies (14–16). Interestingly, Flu did not bind to the P85 micelles at pH 7.4. However, Flu revealed significant retention in the micelles at acidic pH, which may result in enhanced binding of this probe with the micelles in the endosomes. Therefore, we believe that the effects of the micelles on the transport of various drugs in cell monolayers that were also reported for some nonionic detergents (29) could be a complex function of the drug partitioning, drug ionization, and type of cells studied.

In conclusion, the results of this work suggest that that Pluronic block copolymers increase permeability in BBMEC and Caco-2 monolayers not only with respect to R123 (as has previously been reported (16)) but also with respect to a broad panel of pharmaceutically significant drugs. The broad specificity of the block copolymer effects with respect to the drugs and efflux mechanisms have important clinical implications. The doses of P85 exhibiting significant effects on drug transport systems can be achieved in the body. For example, 10 to 100-fold dilution of P85 that is expected following the systemic administration of its 1% solution (30) will still result in the doses sufficient to enhance drug permeability based on the results of current study. Therefore the effects of P85 on drug efflux transporters may be valuable characteristics in view of developing pharmaceutical formulations to increase drug accumulation in selected organs and overcome both acquired and intrinsic drug resistance that limits the effectiveness of many chemotherapeutic agents.

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