ABSTRACT

Drug delivery across the blood-brain barrier is limited by several mechanisms. One important mechanism is drug efflux, mediated by several transport proteins, including P-glycoprotein. The goal of this work was to examine the effect of a novel drug delivery system, Pluronic block copolymer P85, on P-glycoprotein-mediated efflux from the brain using in vitro and in vivo methods. The hypothesis was that specific Pluronic copolymer systems enhance drug delivery to the central nervous system through the inhibition of P-glycoprotein. The effect of P85 on the cellular accumulation and transport of digoxin, a model P-glycoprotein substrate, was examined in porcine kidney epithelial cells (LLC-PK1) transfected with the human MDR1 gene. The effect of P85 on the directional flux across an in vitro BBB was also characterized. In vivo brain distribution studies were accomplished using wild-type and P-glycoprotein knockout mice. Pluronic increased the cellular accumulation of digoxin 3-fold in LLC-PK1 cells and 5-fold in the LLC-PK1-MDR1-transfected cells. Similar effects were observed for a prototypical P-glycoprotein substrate rhodamine-123. P85 treatment decreased the basolateral-to-apical and increased the apical-to-basolateral digoxin flux across LLC-PK1-MDR1 cell monolayers, and analogous results were observed with the in vitro BBB monolayers. The coadministration of 1% P85 with radio-labeled digoxin in wild-type mice increased the brain penetration of digoxin 3-fold and the digoxin level in the P85-treated wild-type mice was similar to that observed in the P-glycoprotein-deficient animals. These data indicate that Pluronic P85 can enhance the delivery of digoxin to the brain through the inhibition of the P-glycoprotein-mediated efflux mechanism.

It is well known that the permeability of the blood-brain barrier (BBB) to drugs is limited by the anatomical features of the BBB, such as tight intracellular junctions and reduced pinocytotic activity (Betz, 1992). Recently, it has become apparent that another important factor that limits central nervous system (CNS) drug distribution is the drug efflux pump, P-glycoprotein, which is located on the luminal side of the brain capillary endothelial cell. An emerging strategy to enhance drug delivery to the CNS is the coadministration of a pharmacological modulator or a formulation component that may inhibit P-glycoprotein-mediated efflux of a desired therapeutic agent out of the brain.

Novel Pluronic block copolymer drug delivery systems have recently attracted attention and one of these formulations is undergoing phase I clinical trials as a tool to overcome drug efflux systems to treat multidrug-resistant cancers (Venne et al., 1996; Alakhov et al., 1999). These drug delivery systems also have been shown to enhance drug transport across in vitro models of the BBB (Batrakova et al., 1999). The mechanism of this increased transport is related to the inhibition of P-glycoprotein-mediated efflux. Upon treatment with a particular Pluronic block copolymer, several P-glycoprotein substrates showed an increased apical-to-basolateral transport, indicating that the polymer may have potential as a CNS-targeted delivery system for drugs that are substrates for the P-glycoprotein efflux pump (Batrakova et al., 1998, 1999).

The objective of this study was to examine the effects of a Pluronic drug delivery system (Pluronic P85; Fig. 1) on the transport of a model P-glycoprotein substrate, digoxin, to the CNS. Digoxin was chosen as the model substrate for this study because of 1) its affinity for P-glycoprotein (Mayer et al., 1996; Kawahara et al., 1999); 2) its relative lack of metabolism in the mouse (Mayer et al., 1996; Kawahara et al., 1999); and 3) its history as a substrate in other in vivo studies that examine the effect of pharmacological inhibition of P-glycoprotein on drug absorption, distribution, and elimination (Mayer et al., 1997; Fromm et al., 1999).

This study was supported by National Institutes of Health grants RO1 NS366229-01-A1 (to A.V.K.), R15 NS536401 (to D.W.M.), and R15 CA71012-01 (to W.F.E.), and grants from the Nebraska Research Initiative Drug Delivery Program.
HO-\[
\begin{array}{c}
\text{CH}_2\text{CH}_2\text{O} \n/2 \\
\text{CH}_2\text{CHO} \\
\text{CH}_2\text{CH}_2\text{O} \n/2
\end{array}
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\text{CH}_3

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\text{m}

\text{Fig. 1.} Structure of Pluronic P85. \text{n} = 52, \text{m} = 40.

Several model systems have been used to examine the role that P-glycoprotein has in drug delivery to the brain. These include in vitro models of the BBB (Fenart et al., 1998; Shah et al., 1989), in vivo models using pharmacological inhibition of the pump (Choo et al., 2000), and transgenic mouse models in which the gene encoding the P-glycoprotein has been deleted (mdr1 knockout mice) (Schinkel et al., 1997; de Lange et al., 1998). In the current study, in vitro and in vivo models were used to examine the effect of a Pluronic block copolymer on the mechanism and degree of enhancement of digoxin transport across the BBB.

**Materials and Methods**

**Drugs and Chemicals**

The present study used P85 block copolymer (lot number WPOP-587A) provided by BASF Corp. (Parsippany, NJ). The molecular mass of the polypropylene-oxide segment in this sample was approximately 2500 Da and the content of the polyethylene-oxide chains was approximately 50% (w/w). The physicochemical characteristics of Pluronic copolymers have been previously reported (Kabanov et al., 1995). [\( ^{3}H \)]Digoxin was obtained from New England Nuclear Life Science Products (Boston, MA) and \([^{14}C\)]mannitol was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO). The rhodamine-123 fluorescent dye was purchased from Acros (Fairlawn, NJ).

**In Vitro Studies**

**Cell Culture.** A porcine kidney epithelial cell line, transfected with human MDR1 cDNA (L-MDR1), and the parental line (LLC-PK1), were obtained from the Netherlands National Cancer Institute (Piet Borst). To maintain a high expression of P-gp in the L-MDR1 cells, they were cultured in Medium 199 containing 10% heat-inactivated fetal bovine serum and supplemented with 640 mM vincristine. The corresponding parental (wild-type) cells were maintained in similar conditions without supplemental vincristine. All tissue culture media were obtained from Life Technologies (Grand Island, NY). The epithelial cells were seeded at a density of 25,000 cells/cm\(^2\) and handled according to institutional guidelines. Food and water were given ad libitum.

**Accumulation Studies.** The effect of P85 on the cellular accumulation of various solutes (digoxin, rhodamine-123, and mannitol) was studied in the wild-type and MDR1-transfected epithelial cells. Confluent monolayers had media removed and were pretreated with assay buffer for 30 min at 37°C, and then this buffer was replaced with a solution containing the solute and various concentrations of the Pluronic copolymer. The solutions of P85 were prepared in assay buffer containing 122 mM sodium chloride, 25 mM sodium bicarbonate, 10 mM glucose, 10 mM HEPES, 3 mM potassium chloride, 1.2 mM magnesium sulfate, 1.4 mM calcium chloride, and 0.4 mM potassium phosphate dibasic (pH 7.4). The compound of interest (i.e., digoxin, rhodamine-123, or mannitol) was added to the copolymer solutions and incubated at 37°C for at least 1 h before their use in the experiments. Cells were incubated with the corresponding solution for 2 h and then the solution was removed and cells were washed three times with ice-cold PBS. Cells were solubilized in 1% Triton X-100, and aliquots were obtained for subsequent determination of fluorescense (Shimadzu RF-5000; Shimadzu, Columbia, MD) or radioactivity (Tricarb 4000; Packard, Meriden, CT). All experiments were conducted in quadruplicate. Values for cellular accumulation of the solutes were normalized for cellular protein content. Proteins were determined using the Pierce (Rockford, IL) bicinchoninic acid method.

**Permeability Studies.** Polycarbonate membrane inserts with confluent L-MDR1 monolayers were placed in Side-Bi-Side diffusion cells from Crown Bio Scientific, Inc. (Somerville, NJ) maintained at 37°C. Cell monolayers were preincubated for 30 min at 37°C with the assay buffer (3 ml) added to both donor and receiver chambers. Following the preincubation period, fresh assay buffer was added to the receiver chamber and the assay buffer in the donor chamber was replaced with digoxin in either assay buffer alone or P85 containing solution [P85 was 0.01% for wild-type and MDR1-transfected LLC-PK1 cells, and 0.01, 0.1, or 1% in the bovine brain microvessel endothelial cell (BBMEC) permeability studies]. In apical (AP)-to-basolateral (BL) transport studies, the AP side of the monolayers was exposed to the donor chamber, whereas in BL-to-AP studies the BL side of the membranes was exposed to the donor chamber. At 0-, 15-, 30-, 60-, and 90-min time points, the solutions in the receiver chamber and aliquots (20 \( \mu \)l) from the donor chamber were removed for the determination of the drug concentration. Three milliliters of fresh assay buffer was immediately added to the receiver chamber. The amounts of \([^{3}H\)]digoxin were determined using Beckman LS 6000 IC liquid scintillation counter. All transport experiments were conducted in triplicate.

**In Vivo Studies**

**Animals.** All experiments were performed with female FVB mdr1b/\( +/- \) or wild-type mice between 12 and 14 weeks of age (Taconic Laboratories, Germantown, NY). The animals were housed and handled according to institutional guidelines. Food and water were given ad libitum.

**Digoxin Pharmacokinetic Studies.** A tracer dose of \([^{3}H\)]digoxin (4 \( \mu \)Ci; 7.8 \( \mu \)g/kg) in PBS control or 1% Pluronic P85 solution (100 \( \mu \)l) was administered to each mouse in the tail vein. At each sampling time (1, 3, 5, 7, and 10 h postdose), animals were sacrificed by CO\(_2\) euthanasia and whole blood sampling from the abdominal vein was taken into a heparin-coated syringe. Blood samples were immediately centrifuged at 12,000 rpm for 5 min to obtain plasma. The brain was removed, washed in ice-cold saline, blotted, and then weighed. An equivalent volume of a 4% of bovine serum albumin solution in PBS was added to brain samples and they were homogenized in glass tissue grinder. Then, 100 \( \mu \)l of serum or 200 \( \mu \)l of brain homogenate was placed into 4 ml of liquid scintillation cocktail, and the quantity of radioactivity was calculated using a Packard Tricarb 4000 liquid scintillation counter. All experiments were conducted in quadruplicate. Digoxin transport across BBB was expressed using the ratio of the digoxin area under the curve (AUC) in the brain normalized for the area under the curve of digoxin in the blood. Digoxin concentrations in the plasma and brain were expressed as cpm/100 \( \mu \)l of plasma and cpm/200 \( \mu \)l of brain homogeneate, respectively, and the AUC was determined using the linear trapezoidal rule (Gibaldi and Perrier, 1982). Therefore, AUC\(_{\text{plasma}}\) to AUC\(_{\text{brain}}\) ratios were expressed using an equivalent brain weight (100 mg) to plasma volume (100 \( \mu \)l). The same procedure was used for examining the effect of P85 on mannitol distribution to the brain, with the intravenous administration of 4 \( \mu \)Ci of \([^{14}C\)]mannitol in the tail vein of each mouse.

**Statistical Methods.** Tests for significant differences between groups were done using one-way ANOVA with multiple comparisons (Fisher’s pairwise comparisons) using Minitab.
Results

Cell Culture Studies

Effect of Pluronic P85 on Digoxin Accumulation. The effect of Pluronic on the accumulation of digoxin in the wild-type and P-gp-transfected cells is shown in Fig. 2A. Digoxin cellular accumulation is significantly reduced (2.7-fold less) in the MDR1-transfected LLC-PK1 epithelial cells compared with the wild-type cells in the control experiment (no Pluronic). When digoxin was incubated with different concentrations of Pluronic, the cellular accumulation of digoxin was significantly enhanced over the control in both the wild-type and P-gp-transfected cells; however, at 0.1% P85, there was a 3-fold increase in the wild-type cells and a 5-fold increase in the P-gp-transfected cells. This indicates that there is an endogenous expression of a digoxin efflux transport system in the wild-type cells (such as P-glycoprotein), but the relative magnitude of inhibition by Pluronic was greater in the transfected cells. The highest level of enhanced accumulation was seen at the 0.1% Pluronic concentration, and at higher concentrations there was a decrease in accumulation. This observation may be due to the formation of micelles, which can trap the solute, in this case digoxin, and decrease the concentration of solute available for transport into the cell. The effects of P85 on digoxin accumulation in these cell lines are comparable to those seen with a standard P-glycoprotein substrate rhodamine-123 (R-123). Figure 2B shows the effect of P85 on R-123 accumulation in wild-type and transfected cells, again with the maximum effect at 0.1% Pluronic. A comparison of the digoxin and R-123 results suggests that the mechanism limiting digoxin accumulation in these cells is P-glycoprotein-mediated, which can be inhibited by P85. It is important to note that there is a different relative effect of P85 on the accumulation of digoxin versus rhodamine-123. The enhancement of rhodamine-123 was significantly greater than that of digoxin. To ensure that the P85-induced changes in P-glycoprotein substrate (i.e., R-123 and digoxin) accumulation are not due to nonspecific changes in membrane permeability, a control experiment examining [14C]mannitol accumulation was performed. No enhancement of [14C]mannitol accumulation was seen in either wild-type or P-gp-transfected cells using the same concentration range of P85 as was used in the R-123 and digoxin experiments (data not shown).

Effect of Pluronic P85 on Digoxin Transport across Cell Monolayers. Treatment of MDR1-transfected LLC-PK1 epithelial cell monolayers with P85 had an effect on the directional flux of digoxin across the monolayer. As seen in Fig. 3A, there was a significant directionality in the transport of digoxin across the monolayer of P-gp-transfected cells, with the apical-to-basolateral (AP-to-BL) flux much lower than the basolateral-to-apical (BL-to-AP) flux. This directionality is in agreement with the known localization of P-gp on the apical side of these cells (Tanigawara et al., 1992). Upon cotreatment with digoxin and 0.01% Pluronic on the apical side, the directional flux was significantly increased in the AP-to-BL direction and significantly decreased from the BL-to-AP direction (Fig. 3A). This indicates that Pluronic inhibited the P-gp-mediated efflux of digoxin through the apical membrane. It is interesting to note that in a separate experiment where 0.01% Pluronic was added to the basolateral side, there was no decrease in the BL-to-AP transport of digoxin (Fig. 3B). In this same experiment, when the Pluronic was added to the apical side, a similar increase in AP-to-BL transport was observed as seen in Fig. 3A. These results suggest that the accessibility of the transport protein for the Pluronic is limited when the Pluronic is administered to the opposite side of the cell monolayer.

The effect of Pluronic on digoxin transport was also examined in an in vitro model of the blood-brain barrier, the BBMECs. Figure 4 shows the effects of different concentrations of apically administered P85 on the apical-to-basolateral flux of digoxin across the BBMEC monolayers. The greatest effect was seen with the 0.01% concentration, with slightly diminished effects at higher concentrations of Pluronic. This decreased flux at higher Pluronic concentrations may be due to an increased formation of Pluronic micelles, and subsequent drug-trapping, which would in turn decrease the digoxin flux, similar to the accumulation studies above (Fig. 2A). Moreover, in a separate experiment using [14C]mannitol as a paracellular marker, it was shown that these same doses of P85 administered apically do not change the apical-to-basolateral flux of mannitol (Batrakova et al.,...
The in vitro data suggest that Pluronic can increase the delivery of digoxin across the blood-brain barrier, and they agree with the in vitro results obtained using the P-gp-transfected monolayers.

**In Vivo Studies in Wild-Type and P-gp Knockout Mice**

**Effect of Pluronic on Digoxin Distribution in Wild-Type Mice.** The effect of Pluronic on the distribution of radiolabeled digoxin into the mouse brain was examined in FVB mice. The brain and plasma concentrations of digoxin equivalents are shown in Fig. 5. The plasma concentrations slightly increased with the coadministration of 1% P85, i.e., there was a 1.14-fold increase in the mean area-under-the-concentration versus time curve (AUC\textsubscript{plasma}). However, the brain concentrations of digoxin significantly increased with P85 treatment (3.4-fold increase in AUC\textsubscript{brain}). Therefore, the distribution enhancement of digoxin to the brain by P85 that was due to effects at the blood-brain barrier in the wild-type (control mice) was approximately 3-fold. The effects of P85 on the brain distribution of \([^{14}\text{C}]\text{mannitol}\) were also examined in FVB mice to ensure that an enhancement in the brain distribution of digoxin was not due to nonspecific effects of the Pluronic on the blood-brain barrier (i.e., increasing passive diffusion). There were no differences between Pluronic treatment and control in the mannitol brain/plasma concentration ratio at 0.5, 5, and 10 h postinjection (data not shown). This indicates that the Pluronic is not altering blood-brain barrier permeability through nonspecific effects on the membrane that might influence transport by passive diffusion.

**Comparison of Pluronic Administration with P-gp Knockout on the Digoxin Brain Distribution.** To evaluate the magnitude of inhibition of P-glycoprotein by P85, we examined the digoxin brain-to-plasma ratio in mdr1a/b \((-/-)\) mice at 5 h postinjection (\(n = 4\) at each time point). Figure 6 shows the brain/plasma ratio of digoxin in control wild-type mice, Pluronic-treated wild-type mice, and mdr1a/b knockout mice. There was a significant increase (4-fold) in brain penetration in Pluronic-treated animals compared with control for wild-type mice (\(p < 0.001\)). An important observation is that the digoxin brain/plasma ratio in the Pluronic-treated animals was not significantly different from the ratio in the knockout mice, an animal model that is deficient in both mdr1a and mdr1b isoforms of P-glycoprotein. This suggests that, at this dose of Pluronic, close to a complete inhibition of P-glycoprotein in the blood-brain barrier is achieved.

**Discussion**

**In Vitro.** To determine whether P85 effects on digoxin cellular accumulation are P-glycoprotein-mediated, we used a comparison between the LLC-PK1 parental and MDR1-transfected cells, where the only difference between these cells should be the level of P-glycoprotein expression (van Helvoort et al., 1996; Smit et al., 1998). The digoxin accumulation studies in wild-type and transfected LLC-PK1 cells show that there was a greater enhancement of digoxin accumulation by Pluronic P85 in the P-glycoprotein-transfected cells than in the parental cell line. The enhancement of digoxin accumulation by P85 in the wild-type cells may be indicative of the baseline expression of P-glycoprotein in these cells, or, conversely, the expression of another trans-
porter that can transport digoxin and can also be inhibited by P85. This suggests that the mechanism by which P85 is increasing cellular accumulation is through an inhibition of P-glycoprotein-mediated efflux. Similar results have been reported for other P-glycoprotein substrates (rhodamine-123, etc.) in Caco-2 cells and BBMECs (Miller et al., 1997; Batrakova et al., 1998, 1999), all these cell types are known to contain the P-glycoprotein efflux transport system. Since P85 treatment had no effect on the cellular accumulation of a marker of passive transport, mannitol, there is no evidence that P85 increased digoxin accumulation through a nonspecific mechanism, and this further implicates P-glycoprotein-mediated efflux. The maximum effect was seen at a P85 concentration of 0.1%, with a subsequent decrease in digoxin and rhodamine-123 accumulation when the P85 concentration was 1%. This decrease may be related to the formation of Pluronic micelles at the higher concentration (P85 critical micellar concentration is 0.03%), where the drug can partition into the micelle, making it unavailable for distribution into the cell (Miller et al., 1997). This would lead to a decreased accumulation of total drug compared with the lower, yet still P-glycoprotein-inhibitory, concentration of P85.

Therefore, a balance is achieved between increasing the cellular accumulation of a P-glycoprotein substrate through efflux inhibition, and limiting its cellular accumulation through decreasing the free substrate in solution available to enter the cell.

The results of the digoxin transport studies across the LLC-PK1 MDR1-transfected cell monolayers support conclusions drawn from the accumulation studies. When the P85 is coadministered with digoxin on the apical side of the monolayer, there was a significant increase in the apical-to-basolateral transport. Moreover, when the P85 was again placed on the apical side, and the digoxin was administered on the basolateral side, there was a significant reduction in the transport from the basolateral-to-apical direction. Given the localization and directionality of P-glycoprotein in these cells (Tanigawara et al., 1992), i.e., an efflux pump on the apical membrane, these data strongly indicate that the Pluronic P85 is inhibiting P-glycoprotein-mediated digoxin transport. Furthermore, when P85 was administered on the basolateral side of the monolayer, the side that has no P-glycoprotein, there was no effect on digoxin transport. Using the Caco-2 model, it was previously shown that the difference in directional transport was completely abolished when P85 was added to the apical side of the monolayer; however, like the current digoxin results, when P85 was added to the basolateral side, there was no effect on R-123 flux across the Caco-2 cells (Batrakova et al., 1998). This suggests that, in the time frame of this experiment (90 min), the access of the Pluronic to P-glycoprotein is limited, and may be due to the ability of the Pluronic itself to cross the monolayer. Taken together, these directional flux data also support the view that P85 is not affecting digoxin transport through a nonspecific mechanism (i.e., general effects on membrane permeability that would influence transport in both directions).

In vitro studies in a model of the blood-brain barrier further support this idea. The apical-to-basolateral transport of digoxin in the bovine brain microvessel endothelial cell monolayers was significantly increased with the coadministration of P85 on the apical side. However, it is interesting to note that there was a reduction in this increased transport with increasing concentrations of P85. This is in accordance
with the observations seen in the digoxin accumulation experiments discussed above, and may be again due to micellar trapping of digoxin, reducing the available concentration for transcellular flux.

The overall conclusion from the digoxin accumulation and transport studies in both the epithelial cells and the in vitro model of the BBB is that Pluronic P85 inhibits the P-glycoprotein-mediated transport of digoxin. Therefore, this combination would be useful to examine the effects of P85 on enhancing drug delivery of P-glycoprotein substrates to the brain in an in vivo setting.

**In Vivo.** The efficacious use P85 as a drug delivery system to the brain is suggested by the in vitro experiments. However, many variables that may affect the use of a drug delivery system cannot be simulated during in vitro experiments. Therefore, we examined the initial in vivo feasibility of P85 as a CNS drug delivery system using radiolabeled digoxin and the wild-type and P-glycoprotein-deficient mouse model. This was considered a useful model since digoxin is metabolically stable in the mouse and previous studies have shown that P-glycoprotein plays an important role in limiting the distribution of digoxin to the brain (Mayer et al., 1996; Kawahara et al., 1999).

The kinetic profiles of radiolabeled digoxin in the plasma and the brain show that treatment with P85 prolongs the residence time of digoxin in the plasma and dramatically increased the residence time and concentrations of digoxin in the brain. In the untreated wild-type animals, the brain levels fell rapidly from a peak at 1 h (the first measurement time) to low levels at 10-h postdose. The plasma levels in the P85-treated mice declined over the experimental time from the measured maximum concentration at 1 h. Conversely, the brain digoxin levels in the P85-treated mice increased throughout the 10-h experiment, indicating a significantly decreased efflux out of the brain. These results show that P85 can enhance the delivery of a P-glycoprotein substrate to the CNS.

The relative contribution of P-glycoprotein inhibition to this CNS delivery enhancement can be evaluated by comparing P85-treated wild-type mice with nontreated P-glycoprotein-deficient mice. In a comparison of the brain-to-blood ratio of digoxin 5-h postdose, there was no statistical difference between the P85-treated wild-type mice and the mdr1a/b (-/-) mice. It is important to note that the brain-to-plasma ratio of digoxin in these knockout mice was substantially lower (0.17) than previously reported (1.54; Schinkel et al., 1995). These experiments were carried out in mdr1a male knockout mice. It is not clear how this may affect the brain-to-plasma ratio of digoxin, and may be worth pursuing in the future.

These studies show that the delivery to the CNS of a prototypical P-glycoprotein substrate, in this case digoxin, can be significantly enhanced by the coadministration of a novel formulation component, the Pluronic block copolymer P85. The in vitro results clearly indicate that the mechanism by which Pluronic P85 enhances the transport of digoxin across the BBB is related to the inhibition of P-glycoprotein-mediated efflux. Moreover, the fact that the Pluronic treatment increased digoxin brain penetration to a comparable level as seen in the mdr1a/b (-/-) knockout mouse, at nontoxic doses, strongly indicates that this may be a promising approach to enhance the delivery to the brain of those compounds whose brain penetration is significantly diminished by the efflux action of P-glycoprotein at the BBB. The use of Pluronic copolymers as drug delivery systems has been previously discussed (Kabanov et al., 1995). One issue that is always of concern when considering the inhibition of P-glycoprotein as a means to increase drug bioavailability or distribution to a selected site, is the toxicity of the coadministered inhibitor. The cytotoxicity of P85 on the BBM ECs was tested in a 2-h exposure, and it was found that the Pluronic was nontoxic to the cells up to a 5% w/v solution (Miller et al., 1997). The toxicity of some of the copolymer formulations has been tested before its use in clinical trials as a formulation for doxorubicin, and it was found that the Pluronic formulation did not change the toxicity profile of doxorubicin (Alakhov et al., 1999). These toxicity results, coupled with the efficacy by which P85 can enhance the distribution of a prototypical P-gp substrate to the brain, suggest that the Pluronic formulations may be feasible to use as targeted drug delivery systems to the CNS for agents that are P-glycoprotein substrates, and therefore these results warrant further investigation regarding the use of these formulations as CNS delivery systems.

**References**


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