Fundamental Relationships Between the Composition of Pluronic Block Copolymers and Their Hypersensitization Effect in MDR Cancer Cells

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Purpose. Previous studies have demonstrated that Pluronic block copolymers hypersensitize multiple drug resistant (MDR) cancer cells, drastically increasing the cytotoxic effects of anthracyclines and other anticancer cytotoxics in these cells. This work evaluates the dose dependent effects of these polymers on (i) doxorubicin (Dox) cytotoxicity and (ii) cellular accumulation of P-glycoprotein probe, rhodamine 123 (R123) in MDR cancer cells.

Methods. Dox cytotoxicity and R123 accumulation studies are performed on monolayers of drug-sensitive (KB, MCF-7, Aux-B1) and MDR (KBv, MCF-7/ADR, CH^{r}C5) cells.

Results. Both tests reveal strong effects of Pluronic copolymers observed at concentrations below the critical micelle concentration (CMC) and suggest that these effects are due to the copolymer single chains ("unimers"). Using block copolymers with various lengths of hydrophobic propylene oxide (PO) and hydrophilic ethylene oxide (EO) segments these studies suggest that the potency of Pluronic unimers in MDR cells increases with elevation of the hydrophobicity of their molecule. Optimization of Pluronic composition in R123 accumulation and Dox cytotoxicity studies reveals that Pluronic copolymers with intermediate lengths of PO chains and relatively short EO segments have the highest net efficacy in MDR cells.

Conclusions. The relationship between the structure of Pluronic block copolymers and their biological response modifying effects in MDR cells is useful for determining formulations with maximal efficacy with respect to MDR tumors.

KEY WORDS: doxorubicin; rhodamine; multidrug resistance; Pluronic; block copolymers; hypersensitization.

INTRODUCTION

The studies on the polymer-based drug delivery and drug targeting systems have a tremendous impact on development of novel drug therapies (1). Amphiphilic block copolymers have attracted significant attention in these studies (2,3). Initial work using Pluronic block copolymers (poly(ethylene oxide)-block-poly(propylene oxide)-block-poly (ethylene oxide), $EO_{m/2}$-PO_n-$EO_{m/2}$) focused on their ability to self-assemble into micelles, incorporate drug molecules and transport drug within the body (4). However, the recent discovery of the biological response modifying activity of these compounds with respect to multiple drug resistant (MDR) tumors has important implications in drug delivery, which were not initially recognized (3). These studies demonstrated that Pluronic block copolymers "hypersensitize" MDR cells resulting in the increase of the cytotoxic activity of antineoplastic agents with respect to these cells by 2 to 3 orders of magnitude (5,6). MDR cells overexpress efflux proteins belonging to a superfamily of ATP binding cassette (ABC) that pump drugs out of a cell (7,8). Pluronic block copolymers were shown to inhibit glycoprotein P (P-gp) mediated drug efflux, which appears to be an important factor in the hypersensitizing activity of these compounds in MDR tumors (6).

These findings place Pluronic block copolymers among the most potent sensitizers of drug resistant cancer cells, a unique property for a drug delivery system. However, no systematic study of the effects of the concentration and composition of Pluronic block copolymers on their activity in MDR cells has yet been reported. This paper provides first study of the effects of these compounds in MDR cancer cells using a wide range of Pluronic block copolymers differing in the lengths of ethylene oxide (EO) and propylene oxide (PO) chain segments. The concentration dependency and structural-functional relationships, which are decisive for optimization of the performance of Pluronic formulations with MDR drugs, are established.

MATERIALS AND METHODS

Cell Culture

The human oral epidermoid carcinoma cells KB and their MDR subline KBv were kindly provided by Dr. D. W. Miller (University of Nebraska Medical Center, Omaha, NE). Human breast carcinoma MCF-7 cells (ATCC HTB22) and their MDR cell subline MCF-7/ADR, derived from the parental cells by selection with Dox, were kindly presented by Y. L. Lee (Willian Beaumont Hospital, Royal Oak, MI). The Aux-B1 Chinese hamster ovary cells and their MDR subline CH^{r}C5 were kindly provided by Dr. V. Ling (University of British Columbia, Vancouver, Canada). In order to maintain high levels of P-gp in the MDR cells, they were cultured in Dulbeco's Modified Eagle's medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 1 μg/ml vinblastine, 5% CO_2. The corresponding parental sensitive cells were maintained in similar conditions except addition of vinblastine. All tissue culture reagents were obtained from Gibco Life Technologies, Inc. (Grand Island, NY). The cells were seeded at a density of 25,000 cells/cm^2 into 24-well plates and were used for accumulation studies after reaching confluency (typically within 6–7 days).

Pluronic Block Copolymers

Pluronic block copolymers were kindly provided by BASF Corp. (Parispany, NJ). The list of the block copolymers used in this work and their characteristics are presented in Table 1.

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Table 1. Composition of the Pluronic Block Copolymers Studied in this Work

Copolymer	MW	Average no. of PO units (N_{PO})[a]	Average no. of EO units (N_{EO})[a]	HLB[b]	CMC, M[c]	Lot no.
L35	1900	16.4	21.6	19	$5.3 \cdot 10^{-3}$	WPMQ-592D
L43	1850	22.3	12.6	12	$2.2 \cdot 10^{-3}$	WPMS-508B
L44	2200	22.8	20.0	16	$3.6 \cdot 10^{-3}$	WPAR-5368
L61	2000	31.0	4.5	3	$1.1 \cdot 10^{-4}$	WPYQ-533D
L62	2500	34.5	11.4	7	$4.0 \cdot 10^{-4}$	WPCS-502B
L64	2900	30.0	26.4	15	$4.8 \cdot 10^{-4}$	WPAQ-561B
F68	8400	29.0	152.7	29	$4.8 \cdot 10^{-4}$	WPOP-590B
L81	2750	42.7	6.2	2	$2.3 \cdot 10^{-5}$	WSOO-83457
P84	4200	43.4	38.2	14	$7.1 \cdot 10^{-5}$	WPMP-547B
P85	4600	39.7	52.3	16	$6.5 \cdot 10^{-5}$	WPOP-587A
F87	7700	39.8	122.5	24	$9.1 \cdot 10^{-5}$	WPHM-629B
F88	11400	39.3	207.8	28	$2.5 \cdot 10^{-4}$	WPAS-575B
L92	3650	50.3	16.6	6	$8.8 \cdot 10^{-5}$	WPMR-535B
F98	13000	44.8	236.4	28	$7.7 \cdot 10^{-5}$	WPAS-569B
L101	3800	58.9	8.6	1	$2.1 \cdot 10^{-6}$	WPHP-547B
P103	4950	59.7	33.8	9	$6.1 \cdot 10^{-6}$	WPWQ-557B
P104	5900	61.0	53.6	13	$3.4 \cdot 10^{-6}$	WPWQ-505B
F108	14600	50.3	265.4	27	$2.2 \cdot 10^{-5}$	WPON-522C
L121	4400	68.2	10.0	1	$1.0 \cdot 10^{-6}$	WPAC-550B
P123	5750	69.4	39.2	8	$4.4 \cdot 10^{-6}$	WPIP-620B
F127	12600	65.2	200.4	22	$2.8 \cdot 10^{-6}$	WPMN-581B

[a] The average numbers of EO and PO units were calculated using the average molecular weighs (MW) provided by the manufacturer.
[b] HLB of the copolymers were determined by the manufacturer.
[c] CMCs were determined using pyrene solubilization technique as described in Materials and Methods.

Cellular Accumulation of Rhodamine 123 (R123)

For the cellular accumulation studies, solutions of R123 (Sigma, St. Louis, MO) and Pluronic block copolymers 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, calcium chloride (1.4 mM) and potassium phosphate dibasic (0.4 mM). Cell monolayers were preincubated for 30 min. at 37°C in 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 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 and aliquots (25 μl) were removed for determination of cellular dyes using a Shimadzu RF5000 fluorescent spectrophotometer: λ_{ex} = 505 nm, λ_{em} = 540 nm. Samples were taken for protein assay using the Pierce BCA method. All experiments were carried out in triplicate.

Cytotoxicity Assay

The cells were seeded in 96 well plates at a density of 5000 cells per well and allowed to reattach overnight. The cells were exposed to doxorubicin (Dox) or Dox in Pluronic solutions for 2 h (MCF7, MCF7/ADR, Aux-B1, CH^{r}C5) or 4 h (KB, KBv) at 37°C, 5% CO_2. The cells were washed three times and cultured for three days (KB, KBv) or four days (MCF7, MCF7/ADR, Aux-B1, CH^{r}C5). The drug cytotoxic activity was evaluated using a standard MTT (3-(4, 5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide) assay (9) or an XTT (2, 3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt) assay (10). The absorbency at λ = 450 nm was determined using a microKinetics Reader BT 2000. All experiments were repeated eight times.

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. SEM values for R123 accumulation levels and Dox cytotoxicity were less than 10% unless it is shown otherwise in the figures.

Determination of Critical Micelle Concentration (CMC)

The CMC of Pluronic block copolymers were determined at 37°C, pH 7.4 using a fluorescent probe (pyrene) technique as previously described (11).

RESULTS

Concentration-Dependent Effects of Pluronic on Accumulation of R123

The fluorescent dye, R123, is commonly used for evaluation of the P-gp mediated drug efflux in MDR cancer cells (12,13). The present work utilizes this probe to characterize the effects of Pluronic block copolymers on the P-gp mediated efflux in the MDR KBv cells and their drug sensitive counterpart, KB cells. In these studies, cells were exposed, for 60 min, to R123 formulated with Pluronic copolymers and then cellular levels of the probe were determined. Fig 1 presents the dependency of R123 accumulation in the cells on the concentration of the copolymer in the treatment solution. As is seen in the

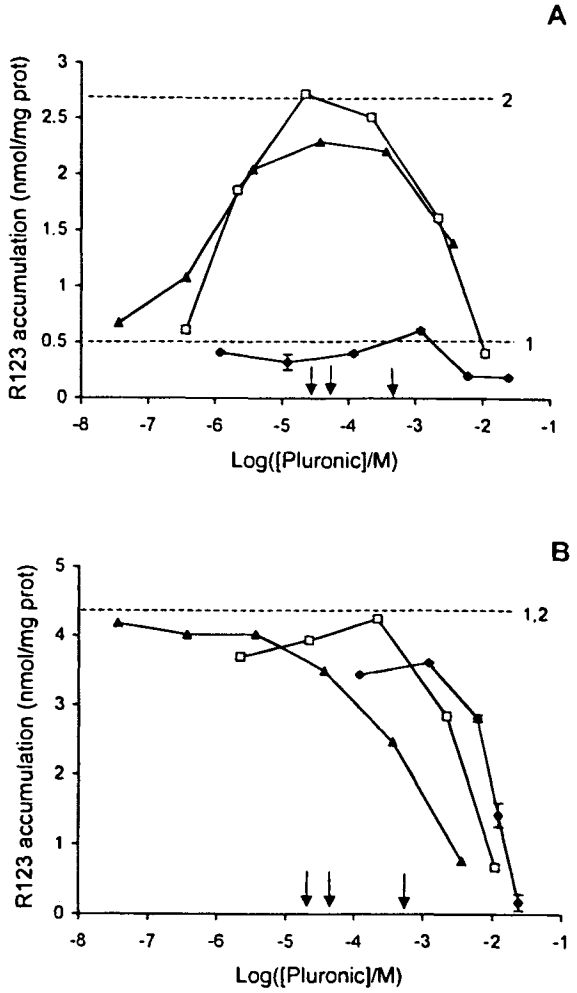

Fig. 1. Effects of Pluronic block copolymers on R123 accumulation in (A) KBv and (B) KB cell monolayers. Cells were exposed for 60 min to 3.2 μM R123 in the assay buffer containing varying concentrations of: ▲, L81; □, P85; ♦, F68. The dashed lines show R123 accumulation in cells exposed to for 60 min to 3.2 μM R123 in (1) the assay buffer and (2) assay buffer containing 4 μg/ml inhibitor of P-gp, cyclosporin A. The vertical arrows from left to right show the CMCs of L81, P85 and F68. Values are mean ± SEM.

figure, significant differences in the effects of the block copolymer on the MDR and drug sensitive cells were observed at copolymer concentrations below CMC. Exposure of KBv cells to R123 with block copolymers (L81 and P85) below the CMC resulted in increased accumulation of the probe in the cells compared to the levels observed in the control groups treated by R123 in the assay buffer (Fig. 1A). Similar increases in R123 accumulation were observed when cells were exposed to the P-gp inhibitor, cyclosporin A. In contrast, when KB cells were treated with the same copolymers at concentrations below the CMC or cyclosporin A, no increase in R123 accumulation was observed (Fig. 1B). At Pluronic concentrations above the CMC, the accumulation of R123 in KBv cells first leveled off and then decreased, reaching levels below those observed in the control groups treated by R123 in the assay buffer (Fig. 1A). Similar decreases in R123 accumulation compared to that in the control groups were observed in KB cells above the CMC (Fig. 1B).

Concentration-Dependent Effects of Pluronic on Cytotoxicity of Dox

Concentration-dependent effects of Pluronic block copolymers in MDR cells were further evaluated in experiments determining the cytotoxicity of the anticancer agent, Dox using two MDR cell lines, KBv and MCF-7/ADR. As previously reported, IC_{50} of Dox with respect to MDR cancer cells decreases in the presence of Pluronic block copolymers (6). The effects of various Pluronic block copolymers were expressed in the form of a "resistance reversion index," i.e., ratio of IC_{50} of the drug in the assay buffer and Pluronic solution ($IC_{50.0}/IC_{50}$). Initial IC_{50} determined in the absence of Pluronic were 2000 ng/ml (MCF7), 220000 ng/ml (MCF7/ADR), 1000 ng/ml (Aux-B1), 70000 ng/ml (CH^rC5), 30 ng/ml (KB), 5000 ng/ml (KBv). Figure 2 presents the typical dependencies of the resistance reversion indexes on the concentration of Pluronic in treatment solutions, using L61 and P85 as examples. As is seen in this figure, both block copolymers caused significant sensitization of MDR cells with respect to Dox at concentrations above 10 μM. The resistance reversion indexes rapidly increased with increasing copolymer concentration and reached 400-fold (KBv) or 3400-fold (MCF-7) as copolymer concentration approached CMC values. At concentrations of P85 above the CMC, Dox cytotoxicity leveled off and then decreased as concentration continued to rise. The leveling off of cytotoxicity was also observed in the case of L61, however, the cytotoxicity did not decrease upon further increases in L61 concentration as had been observed with P85. This observation is likely due to our inability to produce sufficiently high doses of L61 as a result of its precipitation. In contrast, no changes in IC_{50} of Dox were observed in the drug sensitive cells, KB and MCF7, over the entire range of the Pluronic concentrations examined. Furthermore, exposure of the cells to the same doses of Pluronic block copolymers alone did not result in cytotoxic effects in

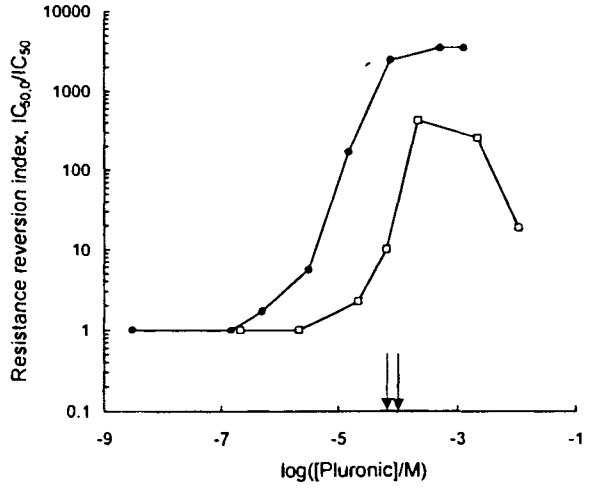

Fig. 2. Effects of Pluronic block copolymers on cytotoxicity of Dox with respect MDR cells: ●, MCF7/ADR cells were treated with Dox/L61 compositions; □, KBv cells were treated with Dox/P85 compositions containing varying concentrations of the block copolymer. Resistance reversion indexes (ratio of IC_{50} of Dox in the assay buffer and Pluronic solution) are plotted as functions of the concentration of the block copolymers. The vertical arrows from left to right show the CMC of P85 and L61.

either MDR or drug sensitive cell lines as shown using the same cytotoxicity assay (data not presented).

Effects of Pluronic Hydrophobicity on the Sensitization of MDR Cells

Results of R123 accumulation studies suggested that the sensitizing effects of Pluronic block copolymers in MDR cells depend on the copolymer hydrophobicity. Evaluation of the entire set of hydrophilic Pluronic block copolymers with hydrophilic-lipophilic balance (HLB) varying from 20 to 29, presented in Table 1, revealed that at concentrations below the CMC these copolymers had no or little effect on R123 accumulation in MDR cells. Figure 1A presents a typical concentration dependency observed with such copolymers, using F68 as an example. Effects of the hydrophobicity of Pluronic block copolymers having various lengths of hydrophobic (EO) and hydrophilic (PO) segments were further examined in Dox cytotoxicity studies. Copolymer concentrations below the CMC were used in these experiments to make sure that all copolymer was in the form of the single chains ("unimers"). The dependency of the resistance reversion index on Pluronic HLB for three MDR cell lines is graphically presented in Fig. 3. It is seen that the cytotoxicity of Dox formulated with Pluronic increases when the block copolymer hydrophobicity elevates (HLB decreases).

Optimization of Drug/Copolymer Formulations in MDR Cells

Since the Pluronic effects in MDR cells depend on both HLB and concentration of the block copolymer, the optimal drug/copolymer formulations were determined for Pluronic copolymers with HLB varying from 1 to 19. In one set of experiments, the dependencies of R123 accumulation in MDR cells on Pluronic concentration were determined for each block copolymer. This resulted in a set of curves similar to those presented in Fig. 1A. Next, R123 levels in the presence of Pluronic were related to those observed in the control groups in the absence of the block copolymer to obtain R123 accumulation enhancement factors. Finally, the maximal enhancement factors observed with the most effective doses of Pluronic were plotted as a function of the length of hydrophobic PO segment (N_{PO}). This yielded the bell-shaped dependency of the net efficacy of Pluronic copolymers in inducing R123 uptake on N_{PO} presented in Fig. 4A. These data clearly suggest that maximal effects on the drug uptake were characteristic of Pluronic copolymers with intermediate N_{PO} values ranging from ca 30 to 60.

Similar results were obtained in another set of optimization experiments in which Dox cytotoxicity was analyzed in MDR cells in the presence of various Pluronic copolymers. In these experiments the concentration dependencies of the resistant reversion index were determined for various Pluronic block copolymers. This resulted in a set of curves similar to those presented in Fig. 2. The maximal resistance reversion indexes were determined from these curves for each block copolymer, and were plotted as a function of N_{PO}. The final optimization curve is presented in Fig. 4B for a series of block copolymers. This curve is very similar in the shape and position to that obtained using the R123 accumulation data, indicating that the copolymers with intermediate N_{PO} values have the highest net efficacy in MDR cells.

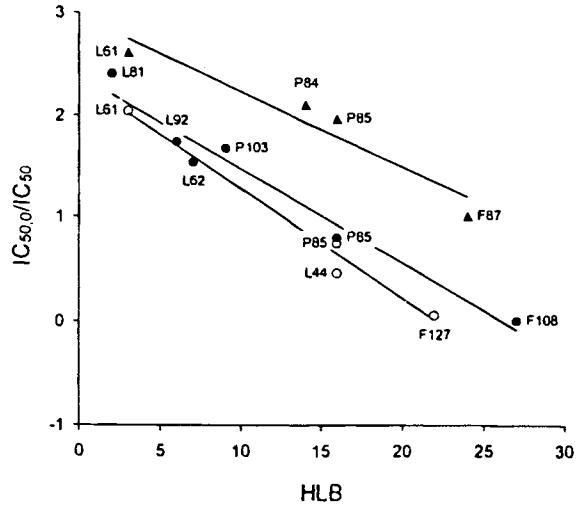

Fig. 3. Relationship between the HLB of Pluronic block copolymers and the hypersensitizing effects of these block copolymers in MDR cells. Monolayers of ▲, CH^{r}C5, ○, MCF7/ADR and ●, KBv cells were exposed to either free Dox or Dox formulated with Pluronic block copolymers. Concentrations of copolymers were either at or below the CMC: ▲, ○, 100 μM or ●, 23 μM. Resistance reversion indexes (ratio of IC_{50} of Dox in the assay buffer and Pluronic solution) are plotted as functions of the HLB of the block copolymers.

Relationship Between HLB, CMC and Lengths of Pluronic Segments

As seen in Fig. 5A, HLB values of Pluronic block copolymers, in a first approximation, are inversely proportional to the fraction of the hydrophobic segment in the molecule: $N_{PO}/(N_{EO}+N_{PO})$. The logCMC can be approximated by a linear dependency on N_{PO}, suggesting that CMC values decrease sharply when N_{PO} elevates (Fig. 5B).

DISCUSSION

Previous studies have demonstrated that formulation of several anti-cancer cytotoxic agents results in a drastic increase of the cytotoxic effect of this drug with respect to MDR cancer cells (hypersensitization effect) (5,6). The agents affected by Pluronic include MDR type drugs, daunorubicin, epirubicin, vinblastine, mitomycin C, and DOX used in this study. Furthermore, the studies of drug transport showed that Pluronic block copolymers enhance drug accumulation and reduce P-gp mediated drug efflux in these cells (5,6). Specific involvement of P-gp efflux systems rather than non-specific effects of Pluronic on the cell membrane permeability has been demonstrated in probe accumulation studies using non-P-gp analog of R123, rhodamine 110 (14,15), R123 efflux experiments (6,15), transport directionality studies in polarized cell monolayers (16), and energy-dependency studies in MDR cells (14).

This study is the first to demonstrate that (i) increases in accumulation of the P-gp probe, R123 and (ii) hypersensitization effects induced by Pluronic block copolymers in MDR cancer cells occur at Pluronic concentrations below the CMC. This means that both effects are due to the block copolymer single chains, commonly termed "unimers." As a result, R123 accumulation and Dox cytotoxicity increase with increasing

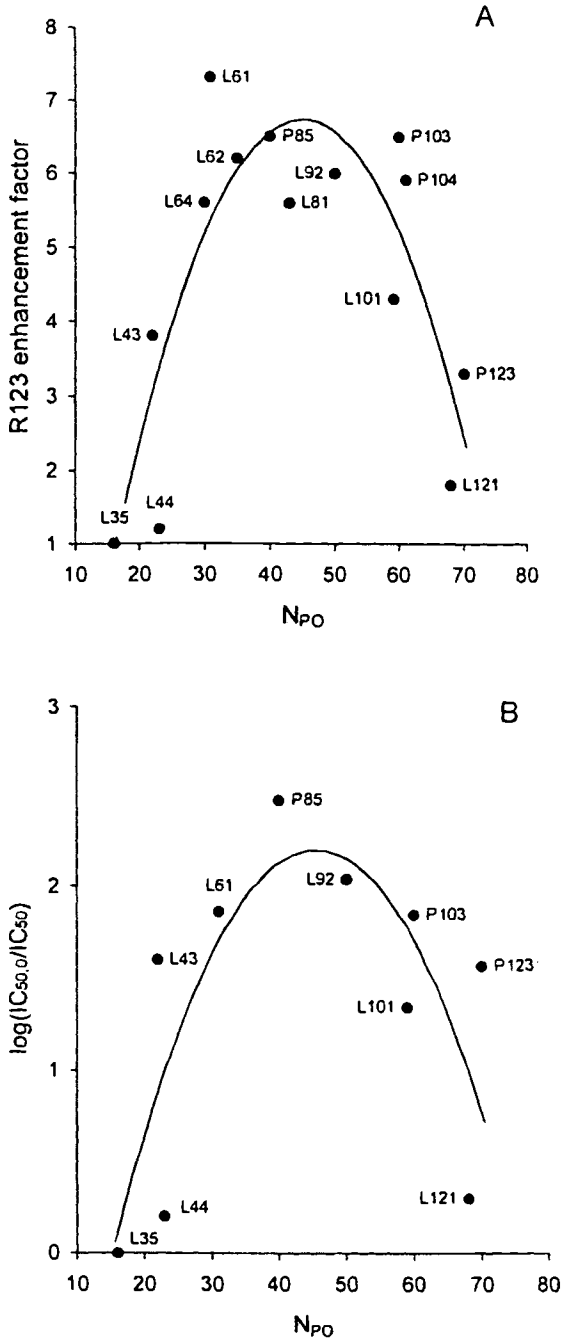

Fig. 4. Optimization of Pluronic copolymer composition in (A) R123 accumulation and (B) Dox cytotoxicity experiments in MDR cells. (A) Monolayers of KBv cells were exposed for 60 min to 3.2 μM R123 in the assay buffer containing Pluronic copolymers. After that, R123 accumulation enhancement factors were determined as the ratios of R123 levels in cells exposed to the dye in Pluronic solution and assay buffer. Maximal enhancement factors observed with the most effective doses of Pluronic were plotted as a function of N_{PO}. (B) KBv cells were treated with Dox/Pluronic compositions containing varying concentrations of the block copolymers. Resistance reversion indexes were determined as ratios of IC_{50} of Dox in the assay buffer and Pluronic solution. Maximal resistance reversion indexes observed with the most effective doses of Pluronic were plotted as a function of N_{PO}.

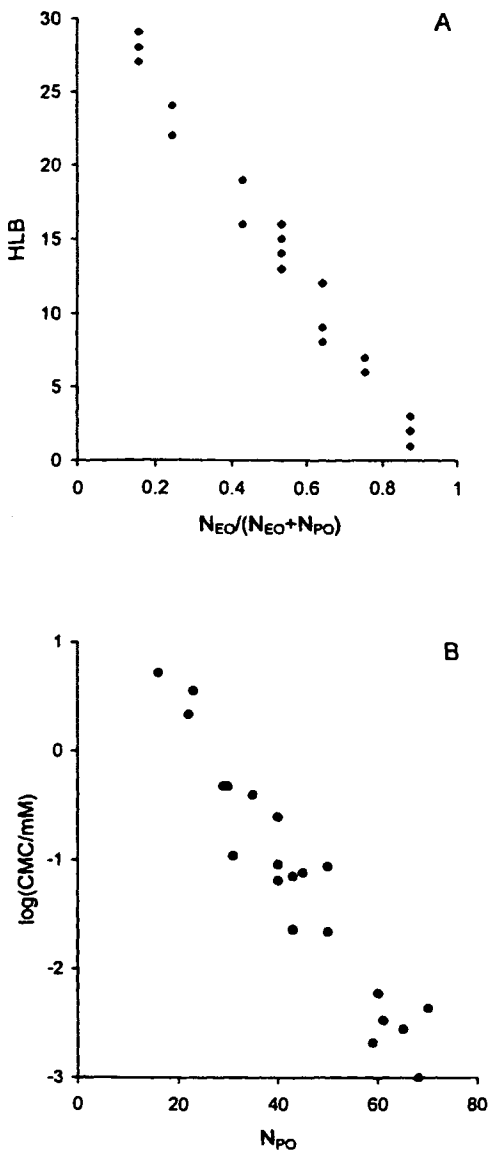

Fig. 5. Effects of molecular composition of Pluronic block copolymer on (A) HLB and (B) CMC. (A) HLBs determined by manufacturer as shown in Table 1 are plotted as a function of the fraction of the PO segment in the molecule: $N_{PO}/(N_{EO} + N_{PO})$. (B) CMCs were determined at 37°C using pyrene solubilization technique as described in Ref. (11) and plotted as a function the length of PO segment, N_{PO}.

concentration of Pluronic until the CMC is reached and unimer concentration levels off. Recent studies using bovine brain microvessel endothelial cells (14) and human intestinal epithelium cells (15) led to a similar conclusion that the effects of Pluronic on P-gp efflux system is mediated by the unimers. Previous work demonstrated that exposure of cells to Pluronic decreases intracellular levels of ATP (17). This can be one reason of the effects of these copolymers on the function of the energy dependent transporters such as P-gp.

In contrast, above the CMC, block copolymer added in the system is consumed for the formation of the micelles. Under these conditions, the R123 levels and Dox cytotoxicity reveal a tendency for first leveling off and then decreasing. The

decreases in R123 levels above the CMC observed both with MDR and drug sensitive cell lines are very similar to those reported previously using bovine brain microvessel endothelial cells (14) and human intestinal epithelium cells (15). These studies have demonstrated that at high concentrations of block copolymer, R123 incorporated into micelles and is transported through a vesicular route, resulting in different accumulation kinetics (14,16). Furthermore, exposure of the cells to the micelles induces removal of the drug from the cells through a mechanism, which is presently unclear (15,16). Therefore, the CMC provides the "cut-off point" for the maximal R123 accumulation in MDR cells. Similarly, the maximal Dox cytotoxicity in MDR cells is observed in the vicinity of the CMC.

Needless to say that although CMC is defined as a single concentration point corresponding to a "break" in the probe fluorescence intensity the micellization and micelle structure transitions usually occur in a relatively broad range of concentration in the vicinity of CMC and may extend above the CMC (11). For Pluronic block copolymers 3 to 10 times variation in CMC value determined using different methods is usually considered satisfactory (11). This might explain why for certain copolymers, for example, P85 in KBv cells (Fig. 3) the resistance reversion index continues to increase at the concentrations several fold above the CMC.

Another major conclusion that is made in the present study is that both inhibition of the P-gp efflux system and hypersensitization effects of Pluronic are dependent on the copolymer molecular composition. First, the R123 cell accumulation studies suggest that more hydrophobic block copolymers (having lower HLB) are more active (Fig. 1A). Second, the cytotoxicity study suggests that the potency of Pluronic unimers in sensitization of the MDR cells increase with the increase in the copolymer hydrophobicity (Fig. 3). While the mechanisms of Pluronic effects in MDR cells are not fully understood it appears that they involve interaction of the hydrophobic PO segments of block copolymer molecules with the cell membranes (5,6). Hydrophilic EO chains do not interact with lipid membranes and are used to prevent interactions of other polymers with the membranes (18,19). It is likely, therefore, that in hydrophilic Pluronic molecules extended EO segments hinder interaction of the PO segments with the membranes, thus decreasing the effects of these polymers on MDR cells. On the other hand, block copolymers with longer PO and shorter EO segments are more likely to interact with cell membranes, which may be the reason for their higher potency revealed in R123 accumulation and Dox cytotoxicity studies.

Finally, the results of this work suggest that both hydrophobic-hydrophilic properties (HLB) of Pluronic molecules and their self-assembly behavior in aqueous solutions (CMC) contribute to the net efficacy of these polymers in the MDR cells. The copolymers with long PO segments and short EO segments are most potent. However, they also have much lower CMC (see, Fig. 5B and Refs. (20,21)) which limits the effective concentration of the unimers in the solution. On the other hand the unimers with short PO segments have high CMC and their concentration in solution can be very high. However, the potency of these unimers is low, presumably due to poor interaction with the membranes of MDR cells. As a result the optimal net efficacy is observed with Pluronic copolymers which have intermediate lengths of PO chains and relatively short EO segments (Fig. 4). The unimers of these copolymers are sufficiently potent and relatively high concentration of unimers can be reached in solution.

Reversal of MDR by nonionic detergents, such as Cremophor EL, Tween 80 or Solutol HS15 has been previously reported (22,23). The magnitudes of MDR reversal effects of these detergents (in terms of resistance reversion indexes) rarely exceeded several dozen times and never amounted several thousands times (hypersensitization) observed with certain Pluronic copolymers. This phenomenon appears to be characteristic of poly(ethylene oxide)-modified long chain alkyl residues and is believed to be due to the effects of such compounds on the fluidity of the cell membranes (23). Comparison of the structures of these molecules with those of available Pluronic copolymers (Table 1) suggests that the latter allow much greater variation of the sizes of the hydrophobic and hydrophilic parts than the nonionic detergents described earlier. The present study suggests that the effects of Pluronic copolymers in MDR cells can be varied in much broader range that that was known for those nonionic detergents.

In conclusion this work describes fundamental relationships between the molecular composition of Pluronic block copolymers and their biological response modifying effects in MDR cells. First, it demonstrates the possibility of optimization of the Pluronic block copolymer formulation to achieve maximal efficacy with respect to MDR tumors. Second, it is likely that similar optimization strategies can be applied to develop formulations to improve transport of drugs in normal cells expressing P-gp efflux pump, for example, to increase permeability in blood brain barrier and intestinal epithelium (16). Using the example of formulations effective against MDR tumors this study demonstrates that the opportunities for the control and optimization of the efficacy of the block copolymer-based pharmaceutical formulations are much broader than previously considered. By changing the lengths of the chain segments of amphiphilic block copolymers remarkable variability of the physicochemical properties of these molecules, such as HLB and CMC, can be achieved (3). Therefore, current demonstration of the strong relevance of these properties to the biological efficacy of pharmaceutical formulations on the base of amphiphilic block copolymers is of both theoretical and practical significance.

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