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Triton-X-100-modified polymer and microspheres for reversal of multidrug resistance

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Abstract

Triton X-100 is a non-ionic detergent capable of reversing multidrug resistance (MDR) due to its interaction with cell membranes. However, it interacts with cells in a non-specific way, causing cytotoxicity. This work aimed to develop polymeric chemosensitizers that possess the ability to reverse MDR and lower toxic side effects. When being delivered to tumours, the polymeric chemosensitizers may also have longer retention times in tumours than the free detergent. Triton-X-100-immobilized dextran microspheres (T-MS) and inulin (T-IN) were prepared and characterized. Their cytotoxicity against multidrug-resistant Chinese hamster ovary cells (CH^RC5) was compared with that of free Triton X-100 solutions. The in-vitro effect of the products on ^{3}H-vinblastine accumulation by CH^RC5 cells was determined. Both T-MS and T-IN showed a marked decrease in the cytotoxicity, as compared with free Triton solutions at equivalent concentrations. Drug accumulation by CH^RC5 cells was increased over two fold in the presence of T-MS or T-IN. These results suggest that polymeric drug carriers with MDR-reversing capability and lower cytotoxicity may be prepared by immobilization of chemosensitizers.

Introduction

Anticancer drugs formulated in microspheres have been used for intratumoral or intra-arterial delivery to enhance and prolong drug exposure of the tumour relative to that of normal tissues. Clinical trials involving thousands of patients worldwide have demonstrated that the locoregional treatment is effective and safe for solid tumours in the liver, kidney, breast, lung, head and neck (Deurloo et al 1990; Willmot & Daly 1994; Kato et al 1996). Enhanced drug exposure of tumours, decreased systemic toxicity and little local toxicity were observed in the studies in animals and in man, as compared with free drug solutions (Deurloo et al 1990; Willmot & Daly 1994; Sugiyama et al 1998, to just list a few). Despite these advantages, failures in chemotherapy still occurred in both animal tumour models and in clinical trials, attributable in part to multidrug resistance (MDR) to chemotherapy (Pastan & Gottesman 1987; Kessel 1989; Cole et al 1992; Kellen 1994).

MDR phenotype can be found in many types of cancers, some of which are intrinsically drug-resistant, such as colon, kidney and adrenal cancer, and some, such as breast cancer, acquire MDR following exposure to sub-lethal levels of chemotherapeutic agents (Kessel 1989; Cole et al 1992; Kellen 1994). MDR is characterized by diminished cellular drug accumulation, partly due to increased drug efflux by specific membrane proteins (e.g. P-glycoprotein (P-gp) (Kellen 1994)). Hence, combinations of MDR reversing agents, like verapamil and

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ciclosporins, with anticancer drugs have been used for treatment of multidrug-resistant tumours. However, the success in clinical trials, especially in the treatment of solid tumours, is limited. This unsatisfactory outcome is ascribed to the difficulty in achieving adequate drug levels (both anticancer and chemosensitizer) in the tumours, while keeping the plasma level lower than the toxicity limit (Murren & Hait 1992; Raderer & Scheithauer 1993).

To tackle the above problems, our group, in the past few years, has attempted to develop microsphere delivery systems for locoregional delivery of an anticancer drug and a chemosensitizer to solid tumours (Liu & Wu 1996; Wu et al 1996; Liu et al 1999, Liu 2000). Our in-vitro studies have shown that chemosensitizers, simultaneously delivered with anticancer drugs by microspheres, can enhance cellular drug accumulation by multidrug-resistant cells (Liu & Wu 1996, Liu et al 1999). Similar results have been reported by other investigators (Soma et al 2000) wherein the drugs were physically loaded in the microspheres. When administered to tumour sites, the drugs will be released, resulting in high intratumoral concentrations and increased therapeutic efficacy (Willmot & Daly 1994). However, systemic exposure due to drug diffusion out from the tumour, although at lower levels, is still unavoidable. This possibility has been observed in our in-vivo tests, which showed that intratumorally delivered verapamil could cause systemic toxicity to mice (Liu et al 2000). Therefore, another approach (i.e. immobilizing the drugs through chemical linking to the surface of microspheres (Venter 1982)), may be worth trying. This could be specifically advantageous for chemosensitizers, whose MDR-reversing effects take place on the cell membranes. The immobilized chemosensitizers could act as a cell-membrane-specific agent, due to the large size of the microspheres that are too big to be endocytozed by the cells (Venter 1982). On the other hand, immobilized chemosensitizers may show higher activity and specificity than the original agent at an equivalent concentration partly due to reduced endocyte metabolism (Pitha 1978; Venter 1982; Kopecek 1986).

In this study, Triton-X-100-modified polysaccharides were prepared and investigated in the hope that these polymers could eventually be developed into drug carriers with chemosensitizing function. Dextran-based microspheres and inulin were selected, since dextran and inulin have been used in man for years. Dextrans are used as plasma expanders (Parfitt 1999) or as drug carriers (Artursson et al 1987; Wang et al 1993; Pistel et al 1999). Inulin is a diagnostic agent for measuring renal function (Parfitt 1999). Generally speaking, these agents are non-toxic, non-immunogenic, biocompatible and biodegradable (dextran). Their interactions with biological systems are well understood. They can be excreted from the body without metabolism or after being metabolized by the liver (dextran) (Parfitt 1999). Besides, unlike polycations or polyanions, they show much less adsorption to cell membranes (Pitha 1978). Triton X-100 is one of the nonionic surfactants that have been reported to possess MDR-modulating ability (Coon et al 1991; Zordan-Nudo et al 1993; Buckingham et al 1995). Because of the presence of a terminal hydroxyl group, it is easy to chemically link Triton X-100 to polymers or microspheres. Furthermore, the immobilization of Triton to inulin has been shown to preserve some of its bioactivity with reduced cytotoxicity (Pitha et al 1979).

Materials and Methods

Preparation of Triton epoxide

The terminal hydroxyl group of Triton X-100 was converted to epoxide through a base-catalysed reaction with epichlorohydrin (Figure 1) (Dimonie & Teodorescu 1993). To a mixture of 36 g of Triton X-100 (Sigma Chemical), 9 g of solid NaOH powder and 250 mL of toluene (Fisher Scientific), 14 g of epichlorohydrin (Sigma Chemical) was added under constant stirring at 50°C. After 48 h, the mixture was filtered. Toluene and the remaining epichlorohydrin in the filtrate were evaporated under reduced pressure. The conversion of the hydroxyl groups was monitored by infrared spectrometry (Bio-Rad, SPC 3200).

Preparation of Triton modified microspheres (T-MS)

Triton epoxide (40 g) was added drop-wise to 10 g of dextran-based microspheres (D-MS) (Sephadex, 40–120 micrometers in diameter, G-200, Pharmacia, MI) suspended in 200 mL of 10% (w/w) NaOH aqueous solution at 50°C under gentle stirring. After 24 h, the mixture was neutralized with 1 N HCl. The microspheres were then separated by filtration and washed with DMF (N,N-dimethylformamide), ethanol, DMSO and, finally, distilled water until no Triton was found in the filtrate as determined by UV spectrophotometry (Hewlett Packard 8452A, HP, CA) at 276 nm. Then the beads were freeze-dried to produce free-flowing particles.

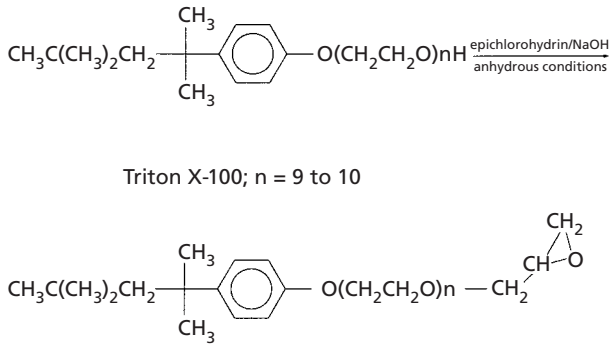

Figure 1 The conversion of Triton X-100 into its epoxide derivative.

Characterization of microspheres

Analysis of Triton content

The Triton-modified microspheres (T-MS) were hydrolyzed with 20% sulfuric acid at room temperature for 48 h to form a clear solution. The Triton concentration in the solution was then determined by UV absorbance at 276 nm.

Determination of surface hydrophobicity

Various amounts of microspheres (i.e. D-MS and T-MS) were incubated with 2.0×10^{-5} g mL^{-1} rose bengal solution in 0.1 M phosphate buffer (pH 7.4) for 4 h. The microspheres were removed by centrifugation, and rose bengal in the supernatant was determined by UV spectrophotometry at 542 nm. The partition quotient (PQ) was calculated as the ratio of the amount of rose bengal bound to the microspheres to that in the solution. Plotting PQ against the amount of microspheres yielded a straight line, from which the slope, S, was taken as a measure of hydrophobicity (Gregoriadis & Poste 1986).

Synthesis of Triton-modified inulin (T-IN)

T-IN was synthesized using a method reported by Pitha (1979) with modification. Triton epoxide (40 g) was added dropwise to 20 g of inulin (Pharmacia) dissolved in 100 mL of 10% (w/w) NaOH aqueous solution at 50°C under gentle stirring. After 24 h, the mixture was neutralized to pH 7 by addition of 1 N HCl. After evaporation of water, the resultant solid was partially dissolved in 300 mL of acetone. The acetone-insoluble inulin and salts were removed by filtration. Toluene (200 mL) was then added dropwise to the T-IN acetone solution under gentle stirring to produce a white, cotton-like precipitate. The precipitate was re-dissolved with acetone. The above cycle was repeated three times and the final precipitate (T-IN) was dried under vacuum.

Characterization of T-IN

Triton X-100 and T-IN, before and after purification, were analysed by HPLC using a Nova-pak C-18 column (Waters, MA), with 50% (v/v) ethanol aqueous solution as the mobile phase at a flow rate of 0.6 mL min^{-1} and a UV detector set at 276 nm. Inulin was analysed by gel-permeation chromatography (GPC, Waters) with an Ultrahydrogel 250 column (Waters). Distilled water was used as the mobile phase at a flow rate of 0.6 mL min^{-1}, and a differential refractometer (Waters 410) as the detector.

Determination of critical micelle concentration (CMC)

The CMC of Triton X-100 and T-IN was measured by the iodine method (Pitha et al 1979). Various amounts of Triton or T-IN methanol solution were added to 31.2 mg L^{-1} iodine aqueous solution. Because this method utilizes the difference in colour that elemental iodine assumes in non-aqueous micelles and in the surrounding aqueous medium, the increase in absorbance or decrease in transmittance at 380 nm is proportional to the amount of non-polar space formed by the detergent, and a sharp transit of the curve is indicative of CMC.

Cytotoxicity tests

A sample (100 μL) of CH^RC5 cells incubated for different times with tested solutions of free Triton or the polymers, or suspensions of various microspheres, was rapidly added to an equal volume of 0.8% trypan blue solution in isotonic saline. The percentage of cells taking up the stain was examined by optical microscopy, from which the percentage of non-viable cells was evaluated.

Drug accumulation studies

Parent (AuxB1) and multidrug-resistant (CH^RC5) Chinese hamster ovary (CHO) cells (Carlsen et al 1976) were grown under the conditions described previously (Liu et al 1999). Drug accumulation tests were carried out using the method described elsewhere (Liu et al 1999; Hong et al 2000). In brief, to AuxB1 and CH^RC5

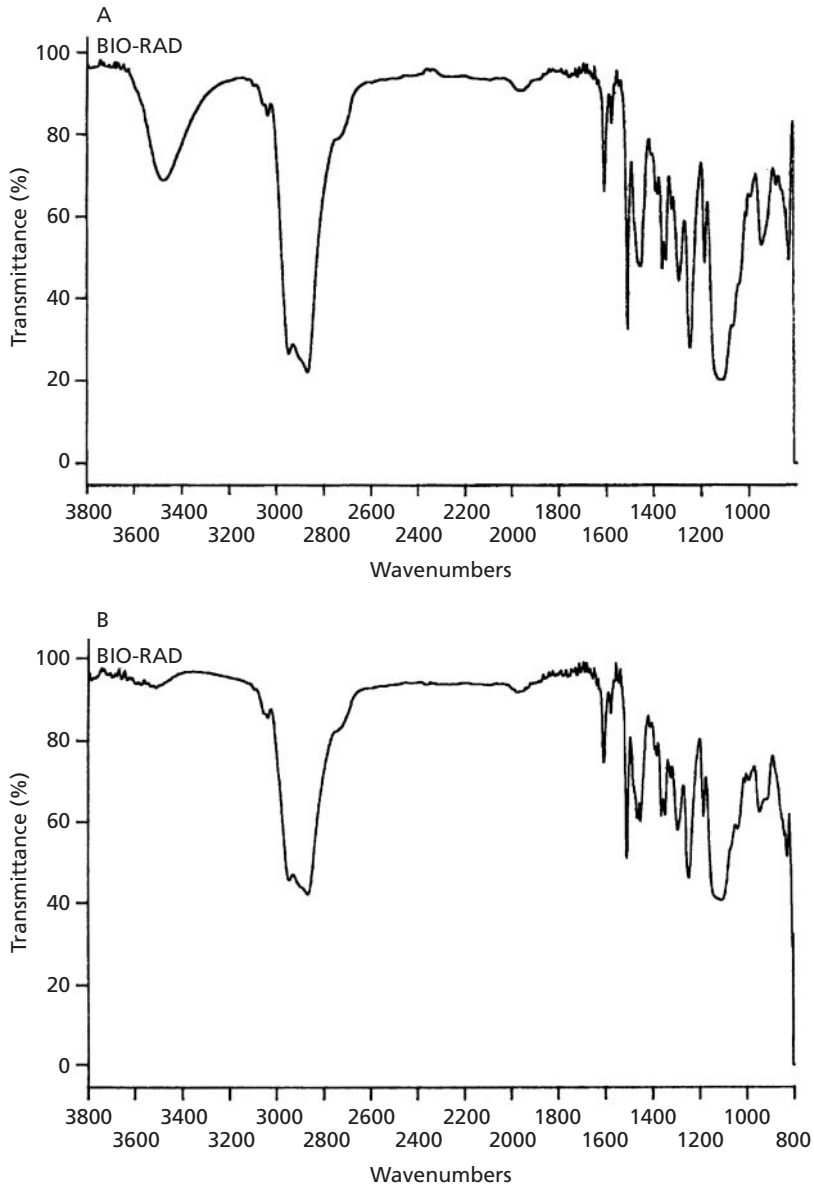

Figure 2 IR spectra of Triton X-100 (A) and its epoxide derivative (B).

monolayer cells grown on multi-well plates, an Earle's Balanced Salt Solution (EBSS) containing 21 nM ^{3}H-vinblastine sulfate (11.7 Ci mmol^{-1}, Moravek Biochemicals) was added in the presence or absence (control) of chemosensitizers, or the polymers (i.e. inulin, T-IN and T-MS). Cyclosporin A (a gift from Sandoz Canada), verapamil hydrochloride (Sigma) and quinidine (Sigma) were used as reference chemosensitizers to verify the MDR characteristic of the resistant cells. A solution of T-IN and a suspension of T-MS were used with Triton content equivalent to 0.01% (w/w) free Triton solution. At various times, the cells were treated and the amount of drug accumulated was measured by a standard liquid scintillation technique as described before (Liu et al 1999; Hong et al 2000).

Statistics

Each experimental data point in an individual experiment was performed in quadruplicate. The experiments were repeated two or three times. The results are re-

ported as mean ± s.d. Difference between groups was determined using analysis of variance (Hong et al 2000), with $P < 0.05$ being considered to be statistically significant.

Results and Discussion

Characterization of intermediate and final products

Conversion of Triton X-100

The conversion of Triton X-100 to its epoxide was confirmed by IR spectra. A very significant absorption band in the range of 3300–3700 cm^{-1} (Figure 2A), which is a characteristic of hydroxyl groups, was diminished after the reaction (Figure 2B). This result indicated that the majority of Triton molecules had been converted to epoxide.

Purity of T-IN

The purity of the final product was assayed by HPLC. Figure 3 compares HPLC chromatographs of the reaction mixture (a), Triton X-100 (c) and purified T-IN (b). Two peaks with retention times of 10.5 and 22.5 min, respectively, were seen in the reaction mixture. After purification, the second peak of free Triton at 22.5 min disappeared (b). This result confirmed that the purified T-IN did not contain free Triton X-100. Furthermore, the purified compound contained no inulin as measured by GPC.

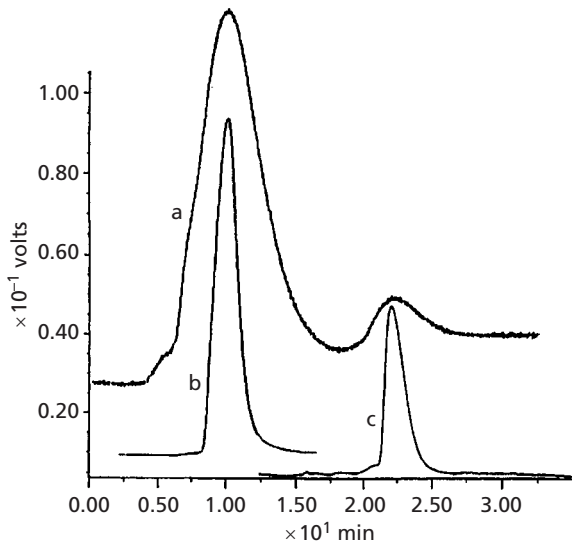

Figure 3 HPLC chromatographs of T-IN before purification (a), T-IN after purification (b) and Triton X-100 (c).

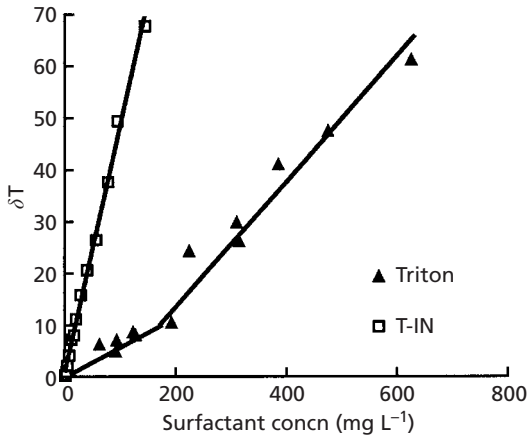

Figure 4 Increase in δT of aqueous solutions vs concentration of Triton X-100 or T-IN determined by the iodine method, where δT is defined as the difference between 100 and the transmittance of the solutions. The increase is proportional to the amount of micelles formed by the surfactants.

Triton content in the polymers

The Triton content in the purified T-IN was found to be 18% (w/w). This means that each inulin molecule, on average, was linked with 2 Triton X-100 molecules, though the substitution degree may vary among the macromolecules (Pitha et al 1979). The dry T-MS was found to contain 1.0% (w/w) Triton by UV spectrophotometry after acidic hydrolysis.

Micellization of T-IN

Figure 4 depicts the changes in transmittance, δT, of solutions of Triton X-100 or T-IN as a function of concentration, where δT is defined as the difference between 100% transmittance and the transmittance (T) of the solution, $\delta T = 100 - T$. A sharp inflection point was seen in the curve for Triton X-100, indicating that Triton X-100 formed micelles at concentrations above 200 mg L^{-1}. In contrast, the δT of T-IN solution increased with the concentration gradually with no inflection point. This observation is consistent with that of Pitha et al (1979). The linear curve for T-IN may be interpreted as that micellization of T-IN is a gradual process in which micelle formation could take place within a single macromolecule that contains more than one Triton X-100 molecule due to the coexistence of hydrophilic inulin and covalently bound hydrophobic Triton components (Pitha et al 1979). Figure 4 demon-

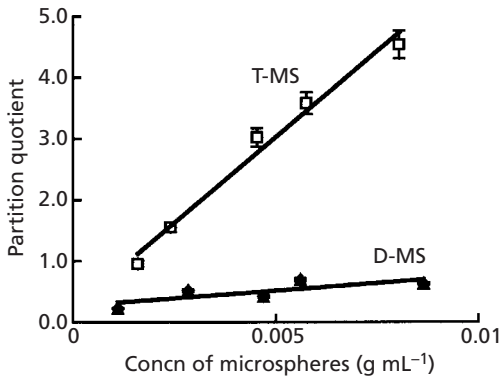

Figure 5 Partition quotient (PQ) vs microsphere concentration determined by the rose bengal method. The PQ was defined as the ratio of the amount of rose bengal adsorbed to the microsphere to that remaining in the solution. The experiments were carried out at room temperature. Data represent mean ± s.d. of 3 separate experiments.

strates that Triton X-100 and T-IN behave quite differently as surfactants.

Surface hydrophobicity of microspheres

As shown in Figure 5, the slope of the straight line of PQ vs microsphere concentration of T-MS was 7 times that of D-MS, indicating a significant increase in surface hydrophobicity as a result of noticeable amount of Triton molecules immobilized on the surface of T-MS.

Cytotoxicity of T-IN and T-MS

As presented in Table 1, 0.05% (w/w) Triton X-100 killed almost 100% of the cells within 1 h, while 0.01% Triton had diminished toxicity. T-IN (0.27%) and T-MS (5%) at concentrations equivalent to 0.05% (w/w) Triton X-100 were non-cytotoxic for at least 4 h. This result suggests that immobilization of Triton to the polymer or microspheres markedly reduced the cytotoxicity, as previously observed by Pitha et al (1979).

Table 1 Cell viability in the presence of Triton X-100 and polymers.

Sample	% of non-viable cells		
	1 h	2 h	4 h
Control	1.8 ± 0.1	3.1 ± 0.3	7.8 ± 0.5
Triton X100 solution (0.01% w/w)	1.9 ± 0.2	2.8 ± 0.2	8.4 ± 0.4
Triton solution (0.05% w/w)	~ 100		
T-MS (5% w/w)	1.5 ± 0.1	1.2 ± 0.2	3.3 ± 0.2
D-MS (5% w/w)	1.6 ± 0.1	1.8 ± 0.2	4.2 ± 0.3
T-IN (0.27% w/w)	2.3 ± 0.2	4.4 ± 0.3	5.8 ± 0.6

Cell viability (CH^RC5) was determined by the Trypan blue method. The suspension of T-MS and the solution of T-IN contained Triton content equivalent to 0.05% (w/w) of free Triton.

The effect of nonionic detergents on cell viability in-vitro has been reported to be concentration-dependent. At low concentrations, the detergents in isotonic salt solutions cause leaking of the cell membranes that can be reversed by addition of serum (Billen & Olson 1976; Lewis et al 1978). At high concentrations, they cause release of cytoplasmic components leaving only the cytoskeleton and the nuclei (Tseng & Goulian 1975; Osborn & Weber 1977; Zordan-Nudo et al 1993). T-IN, due to its larger molecular weight (MW ~ 6000), is believed to diffuse much more slowly than Triton X-100, and thus it takes longer to build up adequate concentrations required to induce the damage of intracellular components. Therefore, upon short exposure, the cytotoxicity of T-IN was much lower than that of Triton X-100, although at longer exposure times (> 24 h), Triton-X-100-modified inulin was found to be as toxic as Triton X-100 (Pitha et al 1979). The lower cytotoxicity of T-IN may stem from its micellization behaviour (see Figure 4 and the text above) differing from that of Triton X-100. This means that the strong ability to solubilize membrane proteins exhibited by the latter may be absent in T-IN.

Unlike T-IN, the interaction of T-MS with the cells would be strictly confined to the microsphere–cell interface, and hence the cytotoxcity was significantly reduced. This is because the Triton component, covalently bound to the matrix, has limited freedom and interacts only with cells in contact with the microspheres (Pitha 1978; Venter 1982). In addition, the microspheres can not diffuse into the cells because they are much larger than the cells. Hence, one may speculate that the cytotoxicity of T-MS would have little time dependence, which could make T-MS more advantageous over T-IN with respect to long-term toxicity.

In-vitro reversal of MDR

Table 2 presents the result of drug accumulation of parent cells (AuxB1) in the absence (control) or presence of chemosensitizers. The three chemosensitizers increased the cellular drug uptake by a factor of 1.4–2.86. In the absence of chemosensitizers, the drug accumulation by parent cells at 1 h was 3.65 pmol (mg protein)$^{-1}$ mL^{-1} (Table 2), about 8.5 times higher than that by the resistant CH^RC5cells (0.43 pmol (mg

Table 2 Vinblastine accumulation by parent CHO (AuxB1) cells in the absence (control) or presence of chemosensitizers.

Time	Sample	Vinblastine uptake (pmol (mg protein)$^{-1}$ mL^{-1})	Ratio of uptake enhancement
30 min	Control	1.52 ± 0.15	
	Quinidine (50 μM)	2.13 ± 0.28*	1.4
	Verapamil (50 μM)	3.15 ± 0.09*	2.07
	Ciclosporin A (10 μM)	4.35 ± 0.44*	2.86
60 min	Control	3.65 ± 0.19	
	Quinidine (50 μM)	5.20 ± 0.25*	1.4
	Verapamil (50 μM)	6.33 ± 0.33*	1.7
	Ciclosporin A (10 μM)	8.28 ± 0.11*	2.2

Data represent the mean of two separate experiments ± s.d. *$P < 0.05$, compared with control.

Table 3 Vinblastine accumulation by CH^{R}C5 cells in the presence of Triton X-100 and polymers.

Time	Sample	Vinblastine uptake (pmol (mg protein)$^{-1}$ mL^{-1})	Ratio of uptake enhancement
1 h	Control	0.43 ± 0.04	1
	D-MS	0.30 ± 0.03	0.7
	T-MS (1%, w/w)	0.85 ± 0.09	1.9*
	IN (0.9%, w/w)	0.42 ± 0.04	1
	T-IN (0.9%, w/w)	0.90 ± 0.04	2.1*
	Triton (0.01%, w/w)	2.42 ± 0.12	5.6*
2 h	Control	0.48 ± 0.03	1
	D-MS	0.35 ± 0.06	0.73
	T-MS (1%, w/w)	1.07 ± 0.09	2.2*
	IN (0.9%, w/w)	0.51 ± 0.03	1
	T-IN (0.9%, w/w)	1.25 ± 0.04	2.6*
	Triton (0.01%, w/w)	3.12 ± 0.31	6.5*

Data represent the mean of two separate experiments ± s.d. *$P < 0.05$, compared with control.

protein)$^{-1}$ mL^{-1}; Table 3). The effect of chemosensitizers on the enhancement of drug uptake was much stronger in resistant cells. The increases in drug accumulation by the resistant cells were 10–20 fold (Figure 6) as compared with 2–3 fold by the parent cells (Table 2). These results confirm that the CH^{R}C5 cell line is a good model for evaluation of chemosensitization.

As shown in Table 3, Triton X-100, at a non-toxic concentration (0.01% (w/w)), induced an approximately 6-fold increase in the drug accumulation by the multidrug-resistant cells after one- and two-hour incubation. On the other hand, drug accumulation by the resistant cells was also observed in the presence of T-MS or T-IN, suggesting that the polymers with immobilized Triton X-100 possess the ability to reverse MDR. Though the effect of the polymers on MDR reversal is

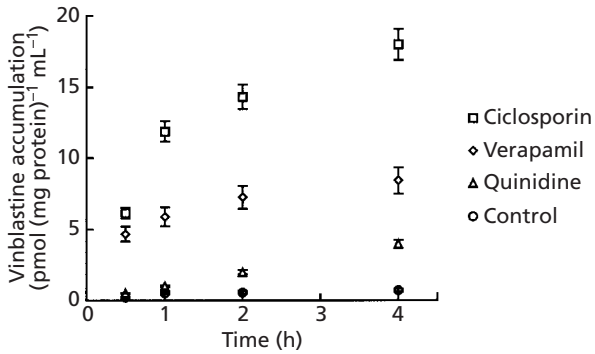

Figure 6 Accumulation of ^{3}H-vinblastine by multidrug-resistant CHO (CH^{R}C5) cells. The data points and bars represent the mean of two separate experiments ± s.d. Statistically significant differences are observed between the curves with the chemosensitizers and that of control experiment ($P < 0.05$).

not as strong as free Triton (only a two to three-fold increase was obtained), their significantly reduced cytotoxicity at the equivalent Triton content enables more T-IN and T-MS to be used. Moreover, drug carriers such as microspheres can be made from these polymers for locoregional delivery of anticancer drugs. When being administered intratumorally, the microspheres will not only release anticancer drugs in the tumour but also enhance drug accumulation by multidrug-resistant cells.

Mechanism of MDR reversal by T-MS and T-IN

The ability and mechanism of MDR reversal by T-IN and T-MS have not been reported previously. The fact that before linking with Triton X-100, the polymers, inulin and D-MS, do not exhibit MDR reversal effect suggests that their ability to reverse MDR must be associated with the presence of Triton molecules. Previous studies showed that nonionic detergents (e.g. Triton X-100) at low concentrations did not disrupt the lipid bilayer or extract transmembrane proteins, but they could increase drug accumulation in multidrug-resistant cells by inhibiting P-gp-drug binding (Reihm & Biedler 1972; Tsai & Green 1973). Additionally, studies on the interaction of Triton X-100 with cell membranes indicated that, at low concentrations, Triton X-100 was incorporated into the bilayer in the form of monomers (Reihm & Biedler 1972; Helenius & Simons 1975; Ling 1975; Carlsen et al 1976). The monomers that integrated within the cell membrane probably competed for the drug binding sites on P-gp or non-specifically disrupted the hydrophobic interactions between the drug and P-gp (Reihm & Biedler 1972; Helenius & Simons 1975; Ling 1975; Carlsen et al 1976).

As a polymeric surfactant, T-IN is probably able to interact with the surface of cell membranes (Pitha et al 1979). It was observed that inulin linked with Triton X-100 showed hydrophobic interaction with enzymes as effectively as Triton (Pitha et al 1979). The direct or indirect interactions between T-IN and P-gp could interfere with the drug–protein binding, leading to higher drug accumulation.

The mechanism of MDR-modulating effect of T-MS may differ from that of T-IN, since the Triton moieties are even less mobile and the microspheres are unlikely to enter the cells. One possibility is that the hydrophobic Triton molecules on the surface of T-MS might indirectly interfere with the functions of P-gp through hydrophobic interaction with the membrane bilayer. It has been suggested that drugs covalently bound to particles that are larger than cells exert their action on the cells by interaction with the cell membranes (Schimmer et al 1968; Venter 1982). Another possibility is that the immobilized Triton X-100 directly interacts with P-gp, thus inhibiting drug efflux. The first mechanism seems more reasonable than the second, because many surface-active agents (e.g., Cremophor EL, Tween 80 and Triton), regardless of their chemical structure, are able to reverse MDR (Reihm & Biedler 1972; Zordan-Nudo et al 1993). The surface interaction of T-MS with cell membranes is supported by the increased surface hydrophobicity of T-MS, which would facilitate the interaction of T-MS with the cell membranes.

Compared with free Triton X-100 solution, T-IN and T-MS are less efficacious for the reversal of MDR. This is partly due to the steric hindrance that could reduce the ability of Triton to interact with the cell membrane. Such a steric effect may be overcome by introducing a spacer between Triton molecules and the polymer chain or microspheres (Venter 1982). In addition, the amount of Triton molecules available for interaction with cell membranes is likely to be lower than the total amount of substitutions. This can also contribute to lower efficiency of MDR reversal. In spite of this, due to their much lower cytotoxicity, more T-IN or T-MS can be used, which would compensate for the lower MDR-reversal efficiency.

Conclusion

Polymeric chemosensitizers T-MS and T-IN have been prepared. Their ability to reverse MDR and short-term in-vitro cytotoxicity has been examined. In-vitro, both T-MS and T-IN are less cytotoxic than free Triton solution at an equivalent concentration, suggesting a beneficial effect from immobilization. The presence of Triton significantly increased the hydrophobicity of the microspheres while the surface activity of Triton X100 was partly preserved in T-IN. The results of in-vitro studies demonstrate that both T-MS and T-IN can increase cellular drug accumulation by multidrug-resistant cells. Taken together, these results suggest that novel drug carriers with MDR-reversing ability may be made by immobilization of chemosensitizers to microspheres.

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