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Mixed Micelles as Proliposomes for the Solubilization of Teniposide

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The aqueous solubility of teniposide in detergent and phospholipid mixed micelles was investigated as functions of the detergents and lipids composing the mixed micelles, the molar ratio of detergent to phospholipid, and the total lipid concentration of the system. The polarity, the charge of the phospholipid, and its saturation affected the solubilization potential of the micelles. Physical chemical factors such as the pH, ionic strength, and temperature of the dispersion medium also altered the solubilization capacity of the system. The results are explained by the changes occurring in the critical micelle concentration and packing arrangements of the aggregates. The desired solubility of teniposide can be achieved by adjusting the studied parameters to the optimum values. Teniposide-containing mixed micelles were spontaneously converted to drug-containing vesicles upon aqueous dilution; therefore, the precipitation of the drug was totally eliminated. In conclusion, mixed micelles as proliposomes can be a suitable drug carrier system for insoluble compounds such as teniposide.

KEY WORDS: teniposide; mixed micelles; bile salts; phospholipids; liposomes; solubilization; serum proteins.

INTRODUCTION

Teniposide (VM-26,4′-demethylepipodophyllotoxin-β-D-thenylidene glucoside; MW 656.67) is a semisynthetic podophyllotoxin derivative that is a natural product found in the root of the American mandrake (*Podophyllum peltatum*) and has been shown to be active against a variety of solid tumors, leukemias, lymphoma (Hodgkin's disease and non-Hodgkin's lymphoma), and neuroblastoma (1). The cytotoxic actions of teniposide are due to (i) induction of topoisomerase II-mediated lesions of DNA (2) and (ii) inhibition of the facilitated transport of nucleosides across the plasma membrane (3). Unlike the parent compound podophyllum, teniposide does not inhibit microtubule assembly (3).

Due to its poor water solubility, teniposide is currently supplied as a nonaqueous formulation and thus precipitates from the intravenous (i.v.) solution when diluted with other i.v. fluids for infusion (1). Furthermore, this formulation causes severe side effects such as hypersensitivity, hypertension, and cardiovascular collapse. These are most probably due to the vehicle, which contains the surfactant Cremophor, and organic solvents in the formulation, rather than the drug itself (4). The development of a more acceptable aqueous i.v. formulation would therefore be desirable.

Bile salts are 24-carbon 5β-cholanoates conjugated to glycine or taurine (e.g., glycocholate or taurocholate, respectively) and have detergent-like amphiphilic properties. In the bile, they are responsible for the solubilization of free cholesterol and phospholipid in mixed micelles (5,6). Several toxic effects and histological damage have been reported from the use of bile salts alone (7). However, the use of bile salt–phospholipid mixed micelles as a vehicle for solubilizing a water-insoluble compound resulted in less deleterious effects than bile salts alone (8).

We have already demonstrated that bile salt–egg phosphatidylcholine mixed micelles spontaneously form liposomes upon aqueous dilution beyond the mixed micellar boundary (9). During the micelle-to-vesicle transition, lipophilic drugs can be incorporated into the bilayers to form drug-containing liposomes. Therefore, the precipitation of the drug upon aqueous dilution will be avoided. In order to examine the potential of our model (bile salt–egg phosphatidylcholine mixed micelles) as a novel delivery vehicle for water-insoluble drugs, we have initially investigated the solubilization of teniposide as a model lipophilic drug. The specific objectives of this study were (i) to determine the aqueous solubility of teniposide as functions of the bile salt species, egg PC/BS molar ratio, total lipid concentration, and temperature; (ii) to study the micelle-to-vesicle transition in the presence of the drug, by means of quasi-elastic light scattering (QELS); and (iii) to study the enhancement of the drug solubilization potential of the mixed micelles by altering some physicochemical parameters such as the pH and ionic strength of the dispersion media or the composition of the mixed micelles.

MATERIALS AND METHODS

Materials

Sodium glycocholate (GC), sodium taurocholate (TC), sodium cholate (C), sodium chenodeoxycholate (CDC), sodium deoxycholate (DOC), sodium glycodeoxycholate (GDOC), sodium taurodeoxycholate (TDOC), egg yolk and soy bean phosphatidylcholine (PC), egg yolk phosphatidylethanolamine (PE) and phosphatidic acid (PA), and cholesterol were obtained from Sigma (St. Louis, MO).

All bile salts and phospholipids gave one spot on thin-layer chromatography (TLC) in chloroform/isopropranol/acetic acid/water (30:30:4:1, v/v) and chloroform/methanol/ammonium hydroxide/water (60:35:5:2.5, v/v), respectively. Teniposide was a gift from Bristol-Myers Lab (Syracuse, NY) and used as received. Methanol was HPLC grade; Trisma base and Tris · HCl (Sigma), cyclohexylamino-ethanesulfonic acid (CHES) (Sigma), imidazole, sodium citrate, citric acid, sodium mono- and dibasic phosphate, and sodium chloride (Fisher) were all analytical grade. Human serum was purchased from Hazelten Research Products (Lenexa, KS). Human serum albumin and immunoglobulin G were purchased from Sigma, and human lipoproteins were purified from human serum by the phosphotungstate precipitation method (10).

Methods

Preparation of Mixed Micellar and Liposomal Solu-

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tions. Aqueous bile salt–egg PC–teniposide mixed micellar solutions were prepared by coprecipitation (11). Bile salt and phospholipid were dissolved in ethanol and teniposide in a chloroform/methanol mixture (2:1) to obtain stock solutions. The stock solutions were mixed and solvent was evaporated under a stream of purified nitrogen and then dried under vacuum for 2–3 days to a constant dry weight. The dried film was reconstituted with 0.02 *M* Tris buffer, pH 7.5, containing 0.15 *M* NaCl and 0.001 *M* NaN_3, unless otherwise reported. The solutions were flushed with nitrogen, sealed, and equilibrated for 2 days at 10°C. To remove unsolubilized teniposide, the solutions were centrifuged at 12,000*g* for 5 min. Liposomal solutions were prepared by diluting the stock micellar solutions with (i) Tris buffer (reconstitution medium), (ii) Tris buffer with different concentrations of buffer components and NaCl, (iii) buffers with different pH's, and (iv) buffer with serum proteins.

Measurements of Teniposide Solubilized in Mixed Micelles and Liposomes. The concentrations of teniposide encapsulated within micelles and vesicles were determined by reverse-phase high-performance liquid chromatography (HPLC). The mobile phase was methanol and water (70:30, v/v), which was degassed with high-purity helium (Union Carbide, Linde Division). The column was a Nova pack C18 (Waters) of 8-mm i.d. and 4-μm pore size. The flow rate was 1.0 ml/min, the detection wavelength was 240 nm, and the AUFS was 1.0. Every sample was injected at least twice and each solubility experiment was run in triplicate. The scatters around the means were always very small.

Micelle-to-Vesicle Transition. The micellar stock solutions, equilibrated for 2 days at 10°C after reconstitution, were diluted at one step, with buffer solutions. Each diluted solution was likewise flushed with nitrogen and left for 2 days to reach equilibrium. QELS measurements were performed as described previously (9) to determine the micelle-to-vesicle transitions by following the changes in colloidal particle size with respect to each dilution. Each reported experimental result was the average of a minimum of three $\bar{d}_h$ values obtained from intensity-weighted distribution analysis of the autocorrelation function accumulated for at least 20 min. In all cases the coefficient of variation from the mean was less than 15%. The following instrument settings were used: temperature, 23°C; viscosity, 0.9325 cp; refractive index, 1.333; scattering angle, 90°; and size range, 15–200 nm.

RESULTS

Solubilization of Teniposide in Bile Salt/Egg PC Systems

In order to determine the maximum teniposide concentration solubilized as an isotropic solution in a given system, increased amounts of teniposide were added to each mixed micellar system while the total lipid concentration (50 mg/ml) and the PC/BS molar ratio (1.0) were kept constant. The total lipid represents the sum of phospholipid and bile salt. The time required to reach the micellar equilibrium in all systems was 24 hr. The amount of micellar solubilized teniposide increased and reached a plateau as the total teniposide in the systems increased. The concentration at the plateau can be regarded as the solubility of teniposide at the specified conditions. The saturation concentrations in various mixed micellar systems are shown in Table I. It appears that more drug was solubilized in the presence of dihydroxy bile salts than in that of trihydroxy bile salts. Teniposide solubility in the micellar phase increased with the hydrophobicity of the bile salt. DOC offered the highest solubility and TC the lowest. There was a large difference in solubilizing capacity between TDOC and TCDC. The glycine or taurine conjugates of trihydroxy bile salts, TC or GC, solubilized to a lesser extent than their corresponding free salts, although the difference in the solubilizing capacity between them was not large. However, both free and conjugated dihydroxy species showed a similar solubilizing capacity. Figure 1 displays the solubilities of teniposide in three micellar systems as a function of the egg PC/BS molar ratio. In general, the solubility increased curvilinearly with an increase in phospholipid molar fraction. As the molar ratio increased from 0.2 to 1.0, the solubility increased 2.3-, 2.4-, and 2.7-fold in TC–, TCDC–, and TDOC–egg PC systems, respectively. Figure 2 shows the effect of the total lipid concentration on the teniposide solubility in two mixed micellar systems, TC– and DOC–egg PC, at two PC/BS molar ratios. There was a linear increase in the solubility with an increase in the total lipid concentration up to 125 mg/ml at the low and high molar ratios (0.6 and 0.9, respectively). Figure 3 shows the profound decreasing effect of temperature on teniposide solubility in two mixed micellar systems, GC–egg PC and GDOC–egg PC, at the specified concentrations.

Micelle-to-Vesicle Transition in Buffer

The dilution of a mixed micellar solution up to the mixed micellar phase limit resulted in a rapid increase in the micellar size (12,13). Figure 4 shows the effect of dilution on the colloidal size of bile salt–egg PC stock solutions. Figure 4A illustrates the influence of bile salt species on the dilution behavior. With increasing hydrophobicity of bile salt from TC to DOC, the following three distinct characteristics were found, although the final liposomal sizes in the two systems were comparable: (i) The transition occurred at higher dilutions, (ii) the particle sizes at the phase limit increased, and

Table I. Solubility of Teniposide at 10°C in Free or Conjugated Bile Salt–Egg PC Micellar Solutions in Tris/Saline Buffer[a]

Bile salt–egg PC system	Solubility (m*M*)[b]
TC–egg PC	1.83 ± 0.04
GC–egg PC	1.88 ± 0.07
C–egg PC	2.05 ± 0.04
TCDC–egg PC	2.31 ± 0.05
GCDC–egg PC	2.25 ± 0.08
CDC–egg PC	2.33 ± 0.05
TDOC–egg PC	3.02 ± 0.06
GDOC–egg PC	3.04 ± 0.09
DOC–egg PC	3.10 ± 0.07

[a] PC/BS, 1.0; total lipid conc., = 50 mg/ml.

[b] Values represent the results of three experiments (means ± SE).

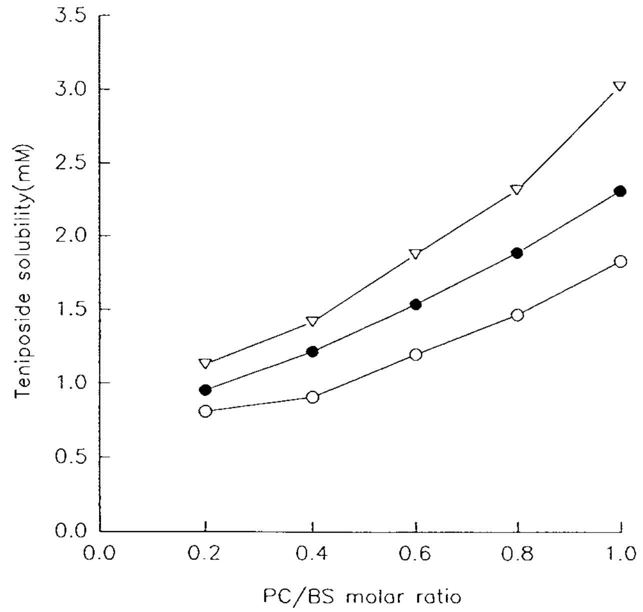

Fig. 1. Micellar solubility of teniposide at 10°C as a function of the PC/BS molar ratio (total lipid conc., 50 mg/ml) in Tris/saline buffer. TC–egg PC (○); TCDC–egg PC (●); TDOC–egg PC (▽).

(iii) the transition pattern was much broader. As shown in Fig. 4B, the increase in PC/BS molar ratio with the same total lipid concentration shifted the micelle-to-vesicle transition peak toward lower dilution values.

Other Factors that Affect the Solubilization Potential of Mixed Micelles

Figure 5 shows the dependence of the micellar solubility of teniposide on buffer components. The experiments were carried out in a system containing egg PC and TC (molar ratio, 0.8; total lipid, 12.5 mg/ml) at a constant pH (Tris/saline buffer, pH 7.5) and temperature (10°C) but at different buffer concentrations. As the buffer concentration increased, the teniposide solubility decreased. It was noticeable that an abrupt decrease in the solubility occurred between 0.05 and 0.1 *M* Tris concentrations.

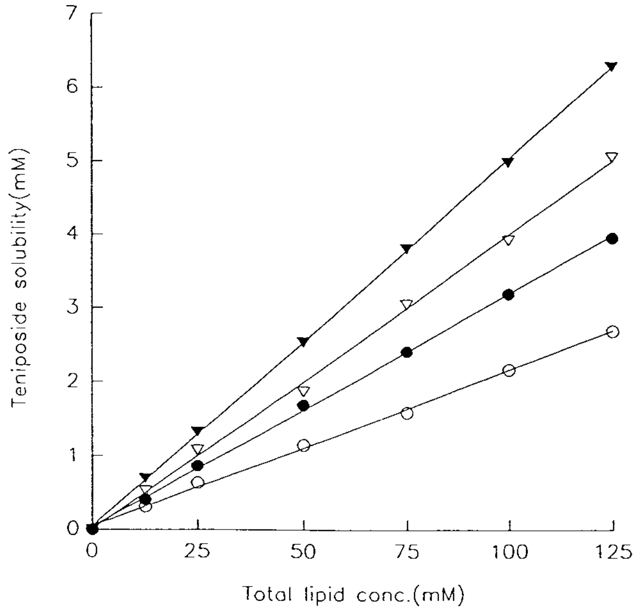

Fig. 2. Micellar solubility of teniposide at 10°C as a function of the total lipid concentration (PC/BS, 0.6 and 0.9) in Tris/saline buffer. TC–egg PC micelles: 0.6 (○); 0.9 (▽). DOC–egg PC micelles: 0.6 (●); 0.9 (▼).

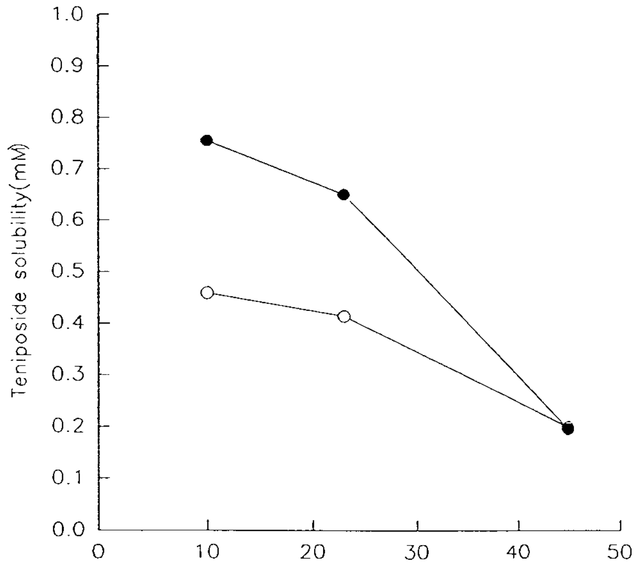

Fig. 3. Effect of temperature on the solubility of teniposide in two bile salt–egg PC mixed micellar solution (PC/BS, 1.0; total lipid conc., 12.5 mg/ml). GC–egg PC (○); GDOC–egg PC (●).

TC and TDOC were chosen to investigate the influence of pH changes in the media on the micellar solubility of teniposide, mainly because their pK_a's are less than 2. Figure 6 shows the pH–solubility profile of teniposide at 10°C in aqueous mixtures of bile salt and egg PC (PC/BS molar ratio, 1.0; total lipid, 50 mg/ml). Extensive degradation of teniposide was observed at pH 3.5 and 11.5 for both micellar systems. The apparent solubility decreased with decreasing pH below 7.5 and with increasing pH above 9.5, concomitant with increasing teniposide degradation. The difference in the solubilities between 7.5 and 9.5 was insignificant. However, it was noticeable that the solubilizing capacity of TDOC–egg PC micelles was greater than that of TC–egg PC, over the pH range studied.

Teniposide solubility in TC– and DOC–egg PC micellar solution (PC/BS molar ratio, 0.8; total lipid, 50 mg/ml), as a function of the NaCl concentration at 10°C and pH 7.5 is shown in Fig. 7. For the two systems, the maximum solubility was observed in water. As the NaCl concentration increased, a gradual decrease in teniposide solubility was observed, followed by a plateauing effect beyond 0.5 *M* NaCl.

In order to clarify the effect of the individual lipid on the drug solubility, we prepared mixed micelles composed of bile salt and egg PC and varying amounts of cholesterol, soybean PC, PE, or PA. Figure 8 shows the effect of cholesterol mole fraction on teniposide solubility in Tris/saline buffer, pH 7.5, at 10°C. In general, cholesterol was observed to decrease teniposide solubility in all three mixed micellar systems studied. Since it is possible to prepare mixed micelles with the various phospholipids, we investigated the

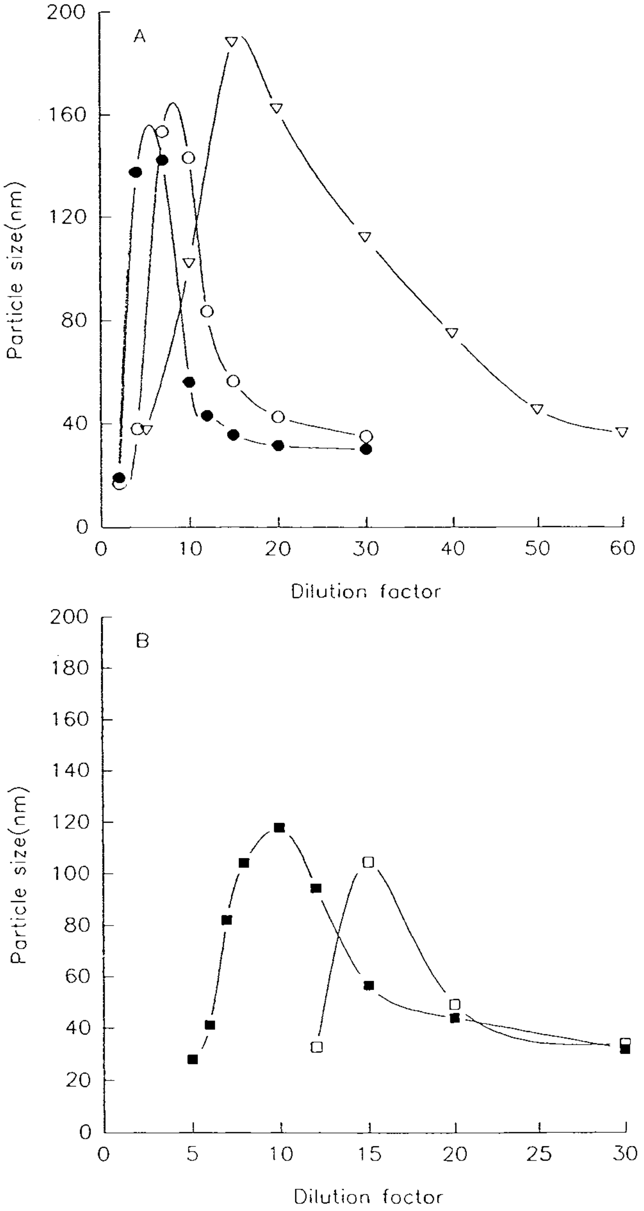

Fig. 4. Change in particle size of bile salt–egg PC colloids containing 1.5 m*M* (1 mg/ml) teniposide and 50 mg/ml of total lipid in Tris/saline buffer as a function of dilution. (A) Three bile salt–egg PC systems at PC/BS = 1.0. TC–egg PC (●); C–egg PC (○); DOC–egg PC (▽). (B) TC–egg PC system at PC/BS = 0.6 (□) and 0.9 (■).

extent of teniposide solubility in a system containing mixtures of two phospholipids. Figure 9 shows the solubility as a function of soybean PC/egg PC, egg PE/egg PC, and egg PA/egg PC molar ratio. The solubilization studies were carried out at 10°C in Tris/saline buffer, pH 7.5. As the mole fraction of unsaturated soybean PC increased, the solubilizing capacity of bile salt–soybean–egg PC mixed micelles gradually increased. However, an increase in the egg PE mole fraction in the system resulted in a dramatic decrease in the solubility. It was also observed that mixed micelles containing PA, a negatively charged phospholipid, had a lower solubilizing capacity for teniposide.

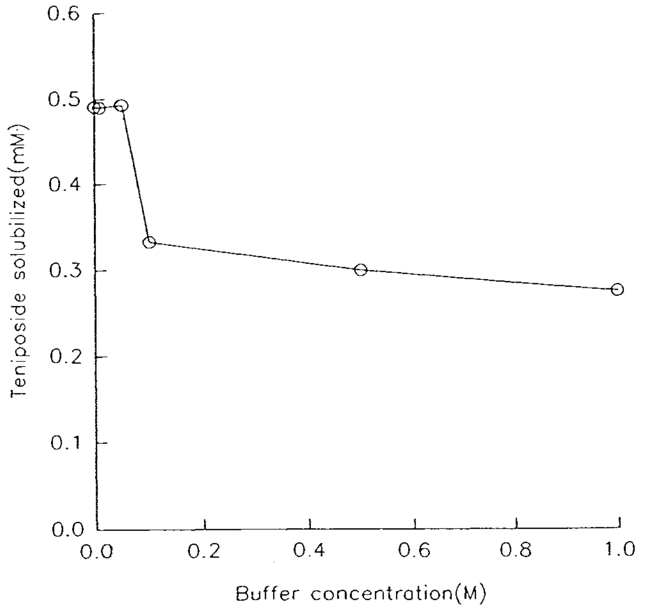

Fig. 5. Effect of buffer concentration on the micellar solubility of teniposide at 10°C in Tris buffer, pH 7.5 (egg PC/TC, 0.8; total lipid conc., 12.5 mg/ml).

Table II shows the effect of serum and serum proteins on the micellar solubility in TC–egg PC micelles at 10°C. There was a remarkable increase in the solubility in the presence of 10% serum. The increase in solubility was about 70%, but it was not much higher for serum concentrations above 10%. In order to find the protein responsible for the large increase in solubility with serum, we investigated the solubilizing capacity of our model system (egg PC/TC molar ratio, 0.8; total lipid, 12.5 mg/ml) in the presence of three major human serum proteins: human serum albumin, immu-

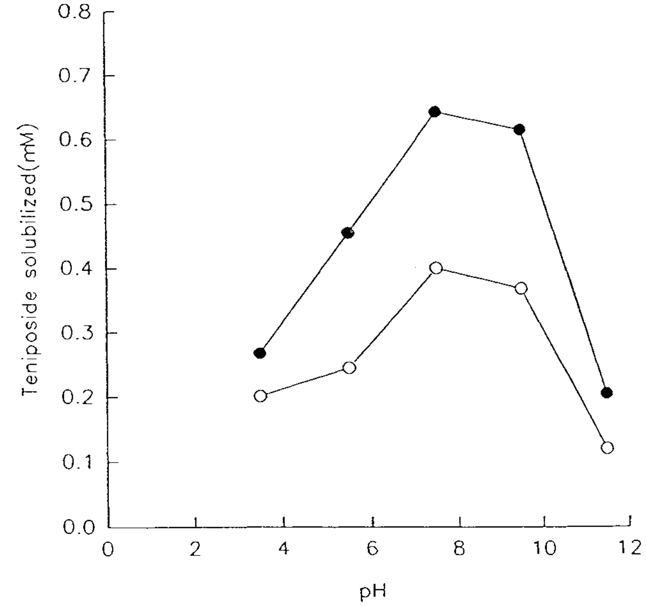

Fig. 6. The pH–solubility profile of teniposide in two bile salt–egg PC mixed micellar solutions at 10°C (PC/BS, 0.6; total lipid conc., 12.5 mg/ml; buffer conc., 20 m*M*). Buffers: Na citrate/citric acid, pH 3.5 and 5.5; Tris–HCl, pH 7.5; CHES, pH 9.5; and Na phosphate, pH 11.5. TC–egg PC (○); TDOC–egg PC (●).

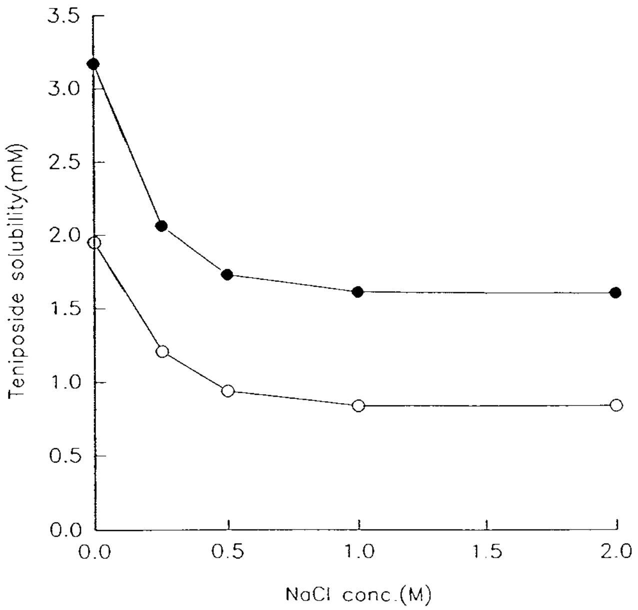

Fig. 7. Effect of NaCl concentration on the micellar solubility of teniposide in two bile salt–egg PC micelles at 10°C in Tris, pH 7.5 (PC/BS, 0.8; total lipid conc., 50 mg/ml). TC–egg PC (○); DOC–egg PC (●).

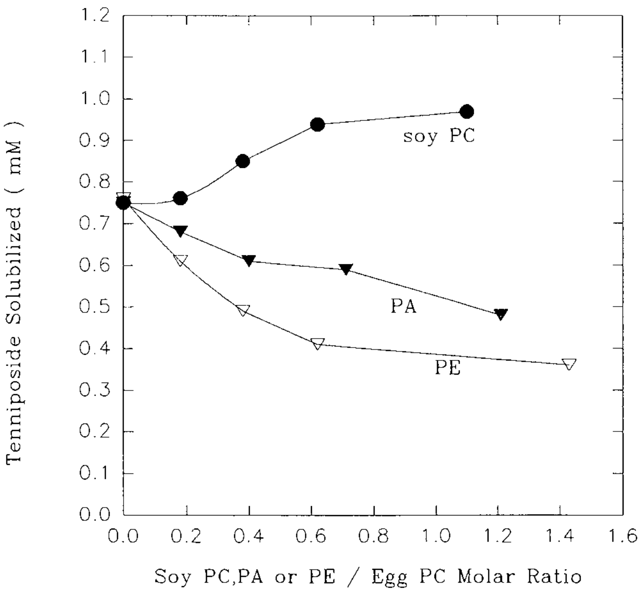

Fig. 9. Micellar solubility of teniposide in GC–egg PC system, plotted as functions of soy PC/egg PC, egg PE/egg PC, and egg PA/egg PC, at a phospholipid/bile salt molar ratio of 1.0 and a total lipid concentration of 12.5 mg/ml.

noglobulin G, and lipoproteins. In general, all three proteins enhanced the solubility to a great extent. However, the most profound effect on teniposide solubility was observed with lipoproteins. At 1.0 mg/ml of lipoproteins in the medium, the solubility increased 2.46-fold. In order to confirm the notion that the increase in the solubility was attributable to the proteins, we analyzed teniposide concentration in the presence of both protein and 3 *M* guanidine-HCl. As shown in Table II, the solubility was greatly decreased by the presence of the protein-denaturing agent. It was apparent that denatured proteins had little effect on the solubility. This result suggested a strong interaction between bile salt–phospholipid–teniposide and proteins.

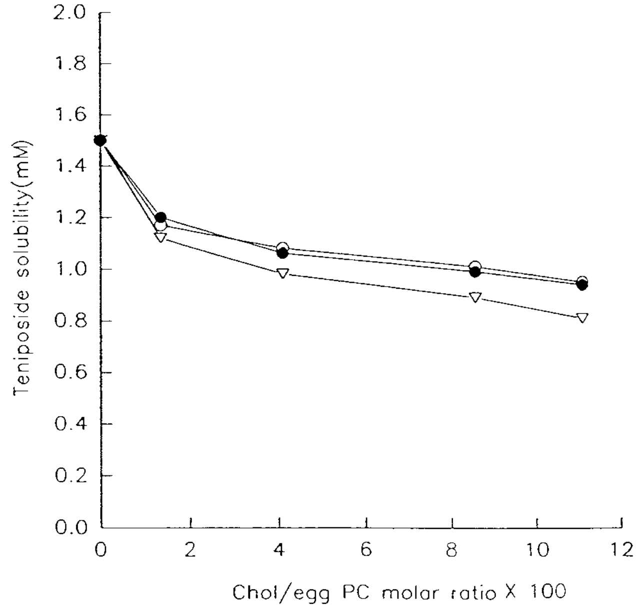

Fig. 8. Effect of cholesterol on the micellar solubility of teniposide in three bile salt–egg PC systems at 10°C and Tris/saline buffer, pH 7.5 (PC/BS, 0.8; total lipid conc., 50 mg/ml). GC–egg PC (○); C–egg PC (●); DOC–egg PC (▽).

Micelle-to-Vesicle Transition in the Presence of Serum Components

Micellar systems were diluted with buffer solutions con-

Table II. Micellar Solubility of Teniposide in the Presence of Various Serum Protein Concentrations[a]

Serum protein	Conc.	Solubility (m*M*)[b]
Control	Buffer	0.368 ± 0.018
Human serum	10%	0.630 ± 0.031
	20%	0.668 ± 0.033
	50%	0.715 ± 0.035
	100%	0.845 ± 0.042
Human serum albumin (HSA)	1 mg/ml	0.613 ± 0.031
	4 mg/ml	0.650 ± 0.032
	8 mg/ml	0.618 ± 0.030
	20 mg/ml	0.650 ± 0.032
	40 mg/ml	0.650 ± 0.032
+ 3 *M* guanidine-HCl	8 mg/ml	0.423 ± 0.021
Human immunoglobulin G (IgG)	1 mg/ml	0.585 ± 0.029
	2 mg/ml	0.615 ± 0.030
	5 mg/ml	0.652 ± 0.030
	10 mg/ml	0.670 ± 0.033
+ 3 *M* guanidine-HCl	2 mg/ml	0.431 ± 0.021
Human lipoproteins	0.1 mg/ml	0.592 ± 0.029
	0.2 mg/ml	0.647 ± 0.032
	0.5 mg/ml	0.762 ± 0.038
	1.0 mg/ml	0.905 ± 0.036
+ 3 *M* guanidine-HCl	0.2 mg/ml	0.375 ± 0.018

[a] Egg PC/TC, 0.8; total lipid conc., 12.5 mg/ml.

[b] Values represent the results of three experiments (mean ± SE).

taining either human serum albumin (4 mg/ml), immunoglobulin G (1 mg/ml), or human lipoproteins (0.1 mg/ml). We found that the micelle-to-vesicle transition occurred in the presence of the individual serum proteins. Table III summarizes the dilution factors of the transition maxima and the vesicular sizes at the dilution factor of 30 with respect to each serum protein. The transition occurred at a lower dilution with albumin but not with other two proteins. Interestingly, the vesicles formed in the presence of the proteins generally were larger than those in buffer.

DISCUSSION

The results in Table I indicate that the teniposide solubility in the aqueous media containing egg PC and bile salt mixed micelles changes according to the type of bile salt used. The solubilization potential of the mixed micelles is higher in the presence of dihydroxy bile salt compared to trihydroxy bile salt. The critical micelle concentration (CMC) of dihydroxy bile salts is lower than that of trihydroxy bile salts (14). Therefore for a given bile salt concentration, more micelles would be available to solubilize the drug. In addition, the aggregation number of dihydroxy micelles is greater (15), resulting in bigger micelles which will allow more teniposide molecules to be solubilized. The difference in the solubilizing capacity of TC– and GC–egg PC systems was found to be insignificant. This finding supports the argument of the effect of the size and number of micelles on solubility since both bile salts have comparable micellar structures and CMC values (16).

The increase in the solubility of teniposide with an increase in the egg PC content of the system was profound when the molar ratio of PC/BS was above 0.6. At high PC/BS molar concentrations, phospholipids may exist as bilayer structures and presumably, teniposide molecules are localized within the lipid bilayer of the micelles, similar to cholesterol solubilization in mixed micelles (17). The presence of teniposide in dimyristoyl- and dipalmitoylphosphatidylcholine bilayers has been demonstrated by differential scanning calorimetry (18).

Mixed micellar solubilization of teniposide increased in a linear fashion with an increase in the total lipid concentration. This can be explained simply by the increased number of micelles at high lipid concentrations. A rapid decrease in teniposide solubility with an increase in temperature may be due to an increase in the CMC (19), which will result in fewer micelles being available for the solubilization process.

Table III. Dilution Factors of the Transition Maximum and Sizes of Vesicles (Dilution Factor of 30) Prepared in the Presence of Each Serum Protein[a]

Serum protein	Dilution factor at transition maximum	Vesicle size (nm)[b]
10% serum	7	94.3 ± 5.2
Human serum albumin (4.0 mg/ml)	7	50.5 ± 2.9
Human immunoglobulin G (1.0 mg/ml)	10	49.1 ± 2.5
Human lipoproteins (0.1 mg/ml)	10	67.9 ± 3.1

[a] Teniposide conc., 0.75 m*M;* egg PC/TC, 0.8.

[b] Values represent the results of three experiments (mean ± SE).

The results obtained in this study for the effects of bile salt species, total lipid concentration, molar lipid ratios, and temperature of mixed micellar solution on teniposide solubilization are in good agreement with the findings obtained for another hydrophobic compound, cholesterol, in a similar medium (20).

Drug-containing systems showed micelle-to-vesicle transition upon aqueous dilutions as detected by quasielastic light scattering measurements. In general, as the total lipid concentration was decreased by dilution, the micellar particle size increased markedly as the macroscopic phase boundary was approached. With higher dilutions of total lipid, the size decreased again, to approach an asymptotic value of approximately 34 nm, consistent with the vesicle sizes reported in the literature (12).

It is important to note that, in all cases, no precipitation of the drug was noticed during dilutions as detected by visual observations and HPLC analysis of the drug after centrifugation of diluted systems. This indicated that the drug was spontaneously dispersed as liposomes in the aqueous medium, when the system no longer contained micelles. The presence of teniposide did not significantly influence the micelle-to-vesicle transition.

The hydrophobicity of the bile salt in the system strongly influenced the mixed micellar phase limit and its colloidal size as well as the transition pattern. This phenomenon was probably due to slower release of the dihydroxy bile salt molecules from the mixed micelles (21). The optimum drug/lipid ratio of a liposomal formulation could be achieved by varying the PC/BS molar ratio and by choosing a proper bile salt.

The effect of increased buffer and NaCl concentrations on the solubilization potential of mixed micelles may be explained by the dramatic changes in the CMC of the micelles. As the CMC increases, the number of micelles available for the solubilization process decreases. Therefore, a low buffer and NaCl concentration is recommended in the formulation to enhance solubility. As observed with cholesterol, the solubility of teniposide depended on the pH of the medium. The maximum solubility was found to be at pH 7.5. The cholesterol and phospholipid composition of mixed micelles exerted a significant influence on the extent of teniposide solubility. Interactions between cholesterol and bile salt–egg PC micelles may be stronger than those between teniposide and bile salt–egg PC. Cholesterol is also shown to change the packing and the fluidity of the PC bilayers (22).

The degree of teniposide solubility in mixed micelles depended on the mobility (which changed by the saturation) and the charge of the phospholipid. The solubility potential followed the order soybean PC > egg PC > egg PE > egg PA. The lower solubilization potential of mixed micelles in the presence of PE and PA can be explained by the changes occurring in packing of hydrophobic tails. PE can induce the formation of inverted hexagonal phase and nonbilayer structures (23,24). Similarly, negative charges of PA may change the packing structure of the molecules in the micelle. These results suggest that the complex formation and/or intercalation of teniposide into the mixed micelles is due mainly to hydrophobic interaction between teniposide and phospholipids, similar to cholesterol and PC systems (25).

The increase in the final liposomal size in the presence of serum components indicates the interaction of the liposomes with serum proteins, which, as a result, causes teniposide to be solubilized more. Therefore, the possibility of teniposide precipitation after the injection of drug-containing liposomes into blood appears unlikely. Even if the liposomes are destroyed by the serum proteins, teniposide will still be solubilized in lipoproteins due to the high interaction of the drug with the lipoproteins as shown in this study.

In summary, the solubility of teniposide in mixed micelles is influenced by the type of detergent, lipid concentration, buffer concentration, pH, ionic strength of dispersion media, presence of cholesterol, and serum components. The required aqueous solubility of teniposide for *in vivo* application can be obtained by adjusting these parameters to the optimum values. Mixed micelles, being thermodynamically stable colloids, can be used as potential solubilizing agents for lipophilic compounds and are promising systems as proliposomes for intravenous delivery.

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