A Mixed Micellar Formulation Suitable for the Parenteral Administration of Taxol

Hayat Alkan-Onyuksel,[1,3] Suganthi Ramakrishnan,[1] Hee-Byung Chai,[2] and John M. Pezzuto[2]

Received March 9, 1993; accepted August 30, 1993

Taxol is a promising antitumor agent with poor water solubility. Intravenous administration of a current taxol formulation in a nonaqueous vehicle containing Cremophor EL may cause allergic reactions and precipitation upon aqueous dilution. In this study a novel approach to formulate taxol in aqueous medium for i.v. delivery is described. The drug is solubilized in bile salt (BS)/phospholipid (PC) mixed micelles. The solubilization potential of the mixed micelles increased as the total lipid concentration and the molar ratio of PC/BS increased. Precipitation of the drug upon dilution was avoided by the spontaneous formation of drug-loaded liposomes from mixed micelles. The formulation can be stored in a freeze-dried form as mixed micelles to achieve optimum stability, and liposomes can be prepared by simple dilution just before administration. As judged by a panel of cultured cell lines, the cytotoxic activity of taxol was retained when formulated as a mixed-micellar solution. Further, for the same solubilization potential, the mixed-micellar vehicle appeared to be less toxic than the standard nonaqueous vehicle of taxol containing Cremorphor EL.

KEY WORDS: taxol; mixed micelles; liposomes; formulation; solubility; stability; antitumor activity; acute toxicity.

INTRODUCTION

Taxol is a diterpenoid isolated from the western yew, *Taxus brevifolia,* with an unusual chemical structure (Fig. 1) (1). Taxol has promising antitumor activity against most solid tumors. Phase I and phase II clinical trials were recently summarized (2), and phase III studies with taxol are indicated with melanoma, ovarian, and breast cancers.

Taxol functions as a mitotic spindle poison (3), by binding directly and with a high affinity to polymerized tubulin and stabilizing microtubules (4). Thus, taxol is a prototype of a new class of cancer chemotherapeutic agents (5).

Since taxol is poorly soluble in water and orally inactive, it requires a suitable formulation for intravenous administration. Presently, for clinical trial, it is supplied in a vehicle containing Cremophor EL and dehydrated alcohol at a 50:50 (v/v) ratio (6). The surfactant Cremophor EL, a polyoxyethylated castor oil, is also used in the formulation of other poorly soluble antineoplastic agents such as teniposide, echinomycin, and didemnin B. Allergic reactions have been observed with antineoplastics containing Cremophor and it has been suggested that the vehicle, rather than the drug itself, may be responsible (7). Hypersensitivity was resolved with interruption of the drug infusion and treatment with antihistaminic agents (8).

Taxol is supplied as a concentrated sterile nonaqueous solution, 6 mg/mL in 5 mL vials/ampoules (30 mg, vial/ampoule). The contents of the vial must be diluted to concentrations from 0.3 to 1.2 mg/mL, with 0.9% sodium chloride or 5% dextrose injection solutions before use (6). Particle formation has been observed after dilution. It is most likely that, on dilution, precipitation occurs because of insufficient cosolvent and detergent effects, therefore, a pharmaceutically more acceptable i.v. formulation of taxol is needed.

In a previous study, a parenteral triacetin emulsion formulation of taxol was developed (9). A more recent study described the encapsulation of taxol in liposomes or in nanocapsules (10). In the cases of triacetin emulsion and polylactic acid/pluronic F68 nanocapsules, toxicity of the carriers was demonstrated. Liposomes, on the other hand, are not toxic and can solubilize sufficient quantities of taxol. However, the *in vitro* stability and large-scale production of the final formulation are serious problems for any liposomal product.

The purpose of this study was to develop an aqueous-based i.v. formulation of taxol that did not cause precipitation of the drug upon dilution and did not contain Cremophor EL. In addition, it should be possible to prepare the formulation on a large-scale, and the product should be stable for a sufficient period of time. To accomplish these goals our approach was to solubilize taxol in aqueous media with the use of bile salt/phospholipid mixed micelles and form drug-loaded vesicles (liposomes) by simply diluting the mixed-micellar solution. We have previously demonstrated that mixed micelles were transformed to well-defined small unilamellar vesicles under certain conditions of aqueous dilution (11). The insoluble antitumor drug, teniposide, was trapped in the vesicle bilayers during this transition (12). The current study describes the application of a similar approach for taxol.

MATERIALS AND METHODS

Materials

Sodium glycocholate (GC), sodium taurocholate (T), sodium cholate (C), sodium deoxycholate (DOC), sodium glycodeoxycholate (GDOC), sodium taurodeoxycholate (TDOC), egg yolk phosphatidylcholine (PC), and Cremophor EL were obtained from Sigma (St. Louis, MO). All bile salts and phospholipids gave one spot on thin-layer chromatography silica gel plates, using chloroform/isopropanol/acetic acid/water (30:30:4:1, v/v) or chloroform/methanol/ammonium hydroxide/water (60:35:5:2,5, v/v) as solvents. Methanol and acetonitrile were HPLC grade. Trisma base and Tris–HCl (Sigma) and all the other reagents were ana-

[1] Department of Pharmaceutics and Pharmacodynamics, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612.

[2] Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612.

[3] To whom correspondence should be addressed at 833 South Wood Street, M/C 865, Chicago, Illinois 60612.

0724-8741/94/0200-00206$07.00/0

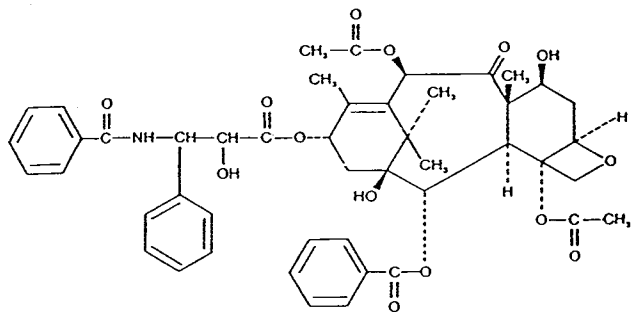

Fig. 1. Chemical structure of taxol [NSC-125973; tax-11-en-9-one,5β,20-epoxy-1,2α,4,7β,10β,13α-hexahydroxy-4,10-diacetate-2-benzoate-13-(α-phenylhippurate)].

lytical grade. Taxol (NSC-125973) was a gift from National Cancer Institute.

Cell Lines

Human breast cancer (UISO-BCA-1), colon cancer (UISO-COL-1), and lung cancer (UISO-LUC-1) cell lines were established from primary human tumors in the Specialized Cancer Center, University of Illinois College of Medicine at Chicago. Each of these cell lines have been shown to grow after s.c. injection of $1-4 \times 10^6$ cultured cells in male or female (breast cancer only) athymic mice, and diagnosis of the original patient specimen was consistent with analysis of the cultured cells by electron microscopy and analysis of nude mouse tumors by light microscopy. Each cell type has also been found to contain human isozymes when analyzed using the Corning Authentic Kit electrophoresis system. Fibrosarcoma (HT-1080) cells were purchased from the American Type Culture Collection (Rockville, MD), as were P-388 cells. KB-3 and a multidrug-resistant cell line, KB-V1, which was established by treating KB-3 cells with a chronic sublethal dose of vinblastine (13), were supplied by Dr. I. B. Roninson (Department of Genetics, University of Illinois College of Medicine at Chicago, Chicago), and A-431 (human squamous cell carcinoma), LNCaP (human prostatic cancer), and ZR-75-1 (human breast cancer) cell lines were supplied through the courtesy of Dr. R. M. Tait, Glaxo Group Research, Greenford, U.K. Growth conditions have been described previously (14).

Methods

Preparation of Mixed-Micellar and Liposomal Solutions

Aqueous bile salt/egg PC/taxol mixed-micellar solutions were prepared by coprecipitation. Bile salt and phospholipid were dissolved in ethanol and taxol was dissolved in methanol to obtain stock solutions. The stock solutions were mixed and the solvents were 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–HCl buffer, pH 7.5, containing 0.15 *M* NaCl and 0.001 *M* NaN_3. The solutions were flushed with nitrogen, sealed, and equilibrated for 1 day at room temperature. To remove unsolubilized taxol, the solutions were centrifuged at 12,000*g* for 5 min. Liposomal solutions were prepared by diluting the stock micellar solutions in one step with 0.02 *M* Tris–HCl buffer, pH 7.5.

Measurements of Taxol Solubilized in Mixed Micelles and Liposomes

The concentrations of taxol within micelles and vesicles were determined by reverse-phase high-performance liquid chromatography (HPLC). The mobile phase was acetonitrile and water (70:30, v/v) which was degassed with high-purity helium (Union Carbide, Linde Division). A Nova pack C18 (Waters) column with an 8-mm i.d. and 4-μm pore size was used. The flow rate was 2.0 mL/min, and the detection wavelength was 227 nm. Each sample was injected (10 μL) at least twice and each solubility experiment was run in triplicate. The scatter around the mean was always less than 10%. There was no interference of phospholipid and bile salts with the assay.

Micelle-to-Vesicle Transition

The micellar stock solutions, equilibrated for 1 day at room temperature after reconstitution, were diluted in one step with buffer solution. Each diluted solution was likewise flushed with nitrogen and left for an additional day to reach equilibrium even though the equilibrium most probably was reached within minutes. Quasi-elastic light scattering (QELS) measurements were performed as previously described (11) to determine the micelle-to-vesicle transitions by following the changes in the mean colloidal particle size ($\bar{d}_h$) 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 that 15%. The instrument settings were described previously (12).

Stability Studies

Clear mixed-micellar solutions were prepared from DOC and egg PC with different total lipid concentrations at a fixed molar ratio of 0.8. Solutions containing saturating concentrations of taxol were stored at room temperature in the dark (under nitrogen) for 2 weeks. The amount of drug precipitated during storage was determined by analyzing taxol in the supernatant after centrifugation. The effect of storage temperature on the precipitation rate was also evaluated by storing mixed-micellar solutions (DOC/PC molar ratio, 0.8; total lipid, 25 mg/mL) containing taxol at a concentration of 0.4 mg/mL at temperatures of 7, 24, and 36°C.

Acute Toxicity Determinations of the Vehicles

The LD_{50} values for mixed-micellar, simple-micellar, and Cremophor vehicles without taxol were determined in CD-1 mice (20–25 g). The mice were housed in a controlled environment maintained on a 12-hr dark–light cycle and at a temperature of 23 ± 2°C with a relative humidity of 65 ± 5%. Five mice were used per group and at least five doses were injected for each solution. In all cases the injection volume was 200 μl. Cremophor solutions (50% Cremophor + 50% ethanol, v/v) were diluted with 5% dextrose and bile salt

solutions were diluted with 0.02 *M* Tris buffer, pH 7.5, to achieve the desired dose. The doses of Cremophor were 53, 69, 79, 90, and 106 mg per injection. The doses for GC as simple or mixed micelles varied from 11 to 17.5 mg per injection. The solutions were sterilized by filtration (0.2 μm; Gelman) and injected into the lateral tail vein of mice using a 1-mL tuberculin syringe equipped with a 27-gauge, 0.5-in. needle. The mice were observed for 2 weeks, and the number of mice surviving was recorded. Plots of dose versus percentage survival were drawn by a computer program (Sigma Plot) on logarithmic-probability paper from which median lethal doses (LD_{50}) were determined by the method of Litchfield and Wilcoxan (15). Statistical significance between LD_{50} values was determined by Student's *t* test.

Evaluation of Cytotoxic Potential

Three types of stock solutions were prepared:

(i) Mixed-micellar solution of taxol: The bile salt was DOC; the molar ratio of egg PC/DOC, 0.8; the total lipid concentration, 25 mg/mL; and the initial concentration of taxol, 0.4 mg/mL. The aqueous medium was 0.02 *M* Tris buffer (pH 7.5).

(ii) Mixed micellar solution without any drug (same as above).

(iii) Free taxol solution in 13% DMSO. The initial concentration of taxol was 0.4 mg/mL.

The procedure employed for the evaluation of cytotoxic potential has been described in detail previously (14). In each case, 96-well tissue culture plates were used. Test samples were prepared as outlined above, and serial dilutions were performed using the indicated solvent. Five concentrations were tested (10 μl, in triplicate), which were selected to be above and below the ED_{50} values demonstrated by the various cell lines. Control groups were also added in which 10 μl of the solvent was added to wells. After the plates were prepared, cells were removed from the tissue culture flasks by treatment with trypsin, enumerated, and diluted with fresh medium. Cells (in 190 μl of medium) were then added to the 96-well plates, and incubations were performed at 37°C in a CO_2 incubator.

After the incubation period, cells were fixed to the plastic substratum by the addition of 50 μl of cold 50% aqueous trichloroacetic acid and stained by the addition of 0.4% sulforhodamine B (w/v) dissolved in 1% acetic acid (30 min). The plates were then washed, air-dried, and treated with 10 m*M* unbuffered Tris base, pH 10 (200 μl), and absorption was determined at 515 nm using an ELISA plate reader. In each case, controls were performed by adding an equivalent number of cells to wells containing the test solvent or formulation, and these values were used to define 100% growth after correcting for the average value obtained with the zero-day control. These values were then expressed as a percentage and ED_{50} values were calculated using nonlinear regression analysis (percentage survival versus concentration).

Similar to the procedures described above for cells capable of attaching to the surface of the culture dishes, P-388 cells were added to the wells containing the test substances, and incubation was performed at 37°C in a CO_2 incubator for 2 days. After the incubation period, the plates were centrifuged and the supernatant fractions were removed. The cells were then treated with aqueous trichloroacetic acid, rinsed with water, dried, and treated with sulforhodamine B as described above.

RESULTS AND DISCUSSION

Solubility Studies

The maximum concentration of taxol solubilized in a given BS/egg PC system, as an isotropic clear solution, was determined by preparing the mixed-micellar solutions in the presence of excess drug and analyzing the solubilized taxol after equilibration and separation of the precipitate. Three parameters that could affect the solubilization potential of the system were studied: (i) the type of bile salt, (ii) the molar ratio of PC to BS, and (iii) the total lipid concentration (PC + BS) in the system. Table I shows the amount of taxol solubilized at room temperature in systems containing different types of bile salts. The molar ratio and the total lipid concentration were 0.8 and 25 mg/mL, respectively, and kept constant in all cases. The bile salt polarity (dihydroxy versus trihydroxy bile salts) and the conjugation (TC versus C or TDC versus DOC) did not significantly change the solubilization potential of the micelles as judged by the 95% confidence levels. Our previous findings for similar systems using another water-insoluble anticancer agent, teniposide, showed some differences in solubilization according to the bile salt polarity (12). In the presence of the most hydrophobic bile salt studied, DOC, the greatest amount of teniposide was solubilized. Although a similar trend was also observed with taxol, the difference between the most (DOC) and the least (TC) polar bile salts was not as great as observed with teniposide. This may be due to the smaller molecular size of teniposide (MW 657) relative to taxol (MW 854), and the former molecule may fit better into the mixed micelles and interact with the bile salt molecules through hydrophobic interactions. For a given bile salt, DOC, and a constant total lipid concentration, 25 mg/mL, the amount of taxol solubilized increased significantly and in a linear fashion, as the PC/DOC molar ratio increased verifying the hydrohobic interaction between taxol and phospholipid molecules (Fig. 2A). The solubility of taxol calculated from the *y* intercept of the straight line gives the solubilization potential of the simple bile salt micelles, which is only 10 times higher the aqueous solubility (0.01 mg/mL) of taxol. This finding indicates that the component that played the most dominant role in taxol solubilization was the phospholipid. The linear increase in the solubilization potential of the system with an

Table I. Amount of Taxol Solubilized in Different Bile Salt Egg PC-Systems[a]

Bile salt	Taxol solubilized, mg/mL (95% confidence levels)
Sodium deoxycholate	0.795 (0.716–0.875)
Sodium cholate	0.760 (0.728–0.792)
Sodium taurocholate	0.735 (0.663–0.807)
Sodium taurodeoxycholate	0.779 (0.707–0.851)

[a] Total lipid, 25 mg/mL; molar ratio of PC/BS, 0.8; room temperature; Tris/saline Buffer, pH 7.5.

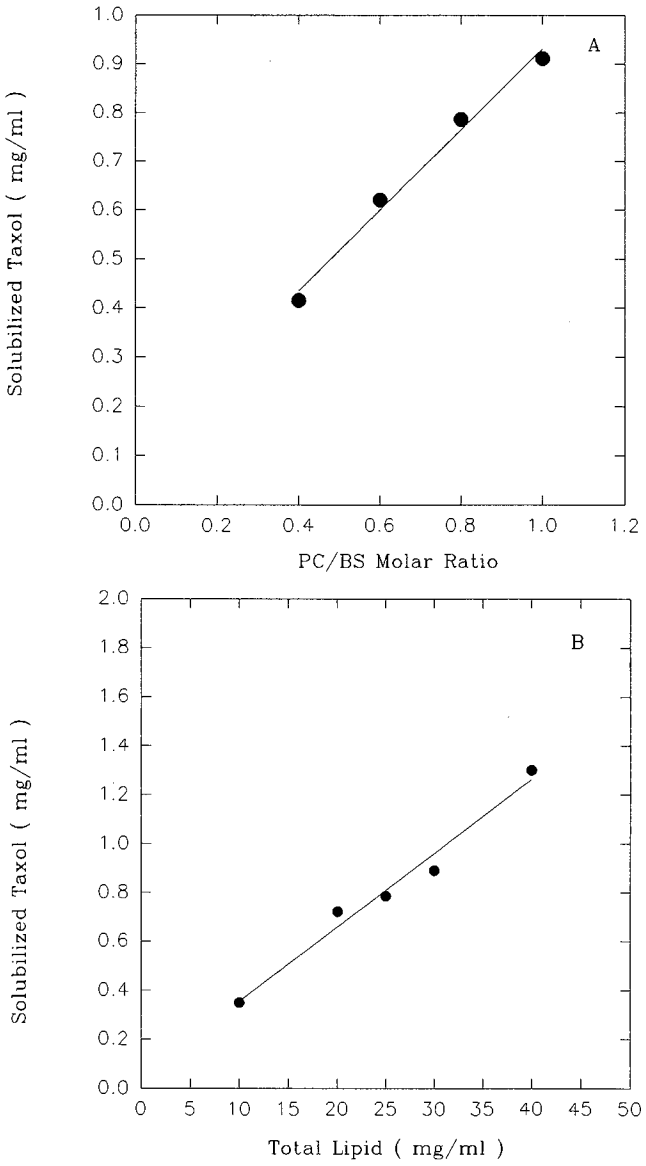

Fig. 2. (A) Effect of BC/BS molar ratio on the solubilization potential of mixed micelles of taxol. Bile sale, DOC; total lipid, 25 mg/mL; room temperature. (B) Effect of total lipid on the solubilization potential of mixed micelles of taxol. Bile salt, DOC: PC/DOC molar ratio, 0.8; room temperature.

increase in the total lipid concentration (Fig. 2B) may result from an increase in the number of mixed micelles available to solubilize more taxol molecules. A similar behavior was observed with teniposide (12).

Micelle-to-Vesicle Transition Studies

Transition from BS/PC mixed micelles to vesicles on aqueous dilution has been studied previously (16–19). The transition occurs at higher dilutions if the system contains a bile salt of greater hydrophobicity (20). A similar situation was observed in this study. Mixed-micellar solutions containing C or DOC with a total lipid concentration of 25 mg/mL and a PC/BS molar ratio of 0.8, showed clear transitions from micelles to vesicles, on dilution, as indicated by an increase followed by a decrease in the mean particle size of the system (Figs. 3A and B). For systems with identical PC/BS molar ratios and total lipid concentrations, the transition peak occurred at dilutions about four times higher for DOC/egg PC solutions compared to the C/egg PC system. Taxol had no significant effect on micelle-to-vesicle transition and did not precipitate upon dilution but was dispersed in liposomes. In both cases, the transition peaks corresponded to the same dilution, indicating that the formation of vesicles was controlled solely by the release of bile salt molecules. The liposomal solutions obtained after phase transition were visually clear and, upon centrifugation, did not remove any drug from the supernatant, demonstrating complete avoidance of precipitation on aqueous dilution. This was not surprising since the solubilization potential of phospholipid bilayers was much higher than BS/PC mixed micelles for cholesterol, another hydrophobic compound (21).

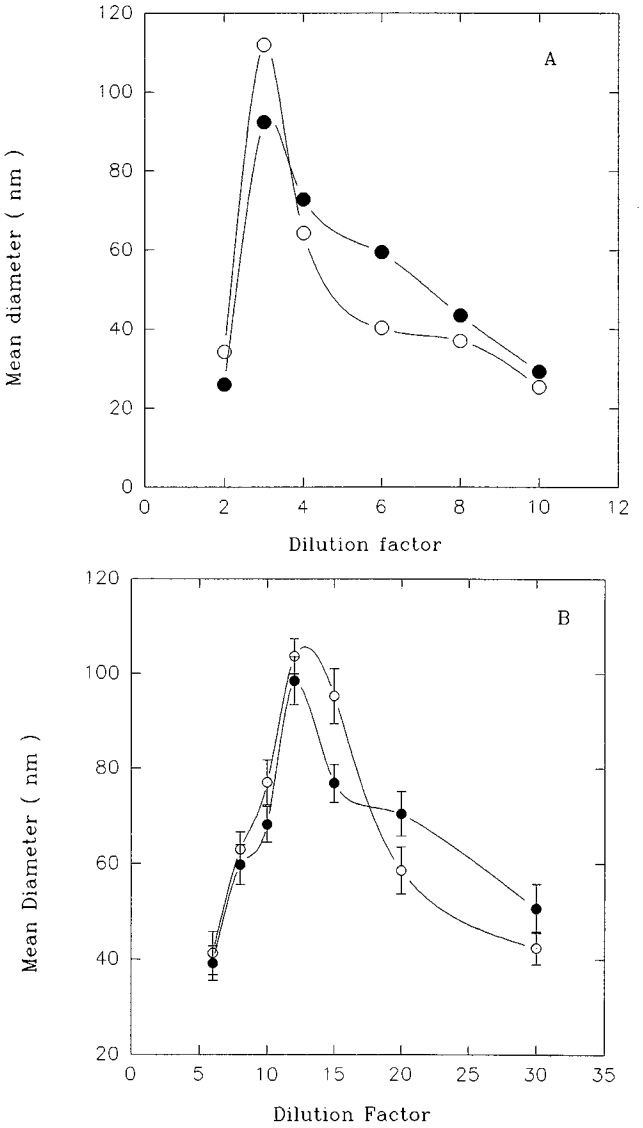

Fig. 3. Micelle-to-vesicle transition with aqueous dilution of stock solutions containing different bile salts at a PC/BS ratio of 0.8, a total lipid content of 25 mg/mL, and 23°C. (A) Sodium cholate; (B) sodium deoxycholate. (●) In the presence of taxol; (○) in the absence of taxol.

Stability Studies

Mixed-micellar solutions prepared from egg PC and DOC at a molar ratio of 0.8, with various total lipid concentrations and high concentrations of taxol, precipitated the drug during storage at room temperature in the dark (Fig. 4A). The taxol precipitation rate for a given system varied according to the number of micelles present, indicating that micelle–micelle interaction might have occurred at high total lipid concentrations. The precipitation rate was significantly reduced when the mixed micellar solution containing 0.4 mg/mL taxol, 25 mg/mL total lipid, and a PC-DOC molar ratio of 0.8 was stored at 24 and 7°C. Precipitation was faster when the solution was stored at 36°C (Fig. 4B). These observations could be due to changes in the thermodynamic equilibrium of inter- and intramicellar bile salt monomer concentrations at different temperatures.

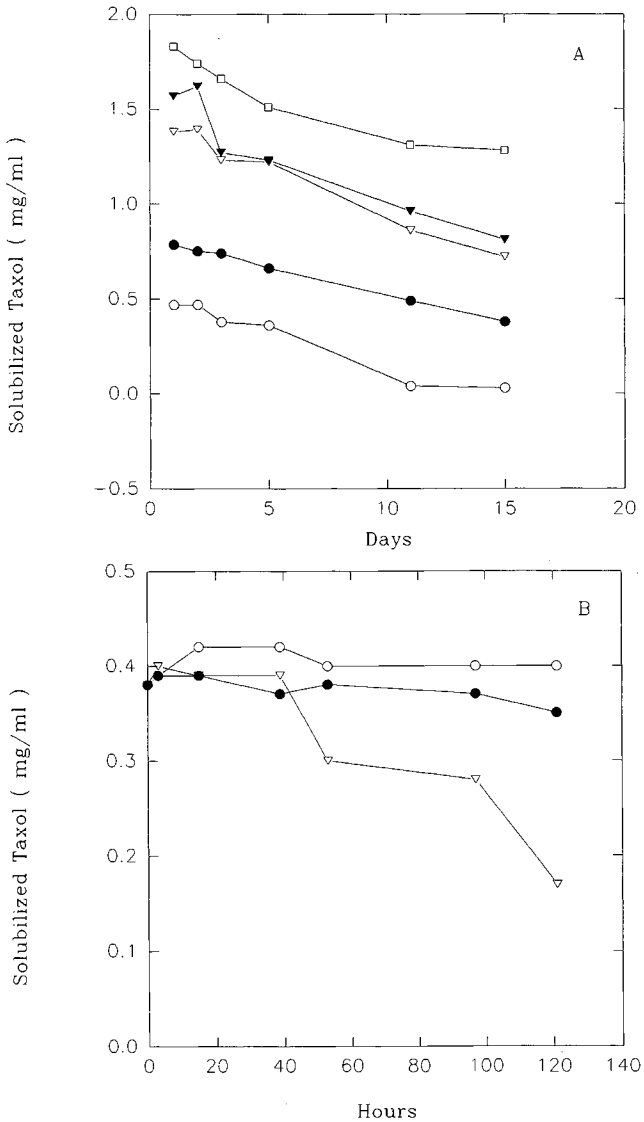

Fig. 4. (A) Precipitation of taxol from mixed-micellar solutions containing different amounts of total lipid. Bile salt, DOC; PC/DOC molar ratio, 0.8; room temperature. Total lipid: 12.5 (○), 25 (•), 50 (▽), 75 (▼), and 100 (□) mg/mL. (B) Precipitation of taxol from mixed-micellar solutions during storage at different temperatures. Bile salt, DOC; PC/DOC molar ratio, 0.8; total lipid, 25 mg/mL. 7°C (○); 24°C (•); 36°C (▽).

Since the mixed micelles did not appear to be very stable as a solution, the preparations were freeze-dried (Labconco, Lyph-Lock 4.5 Lt freeze-drying system) and stored as a powder for 2 weeks. Upon reconstitution with distilled water, a clear solution with an appearance similar to that of the original was obtained immediately. No color change and precipitation were observed. Therefore, for clinical application, we propose to keep the final product in dry form until usage. At this time, mixed micelles can be formed by reconstitution, and liposomes can then be prepared by further dilution of the mixed-micellar solution with i.v. fluids. Unlike liposomal solutions, freeze-drying of mixed-micellar solutions is not a problem since the internal aqueous compartments and bilayer structures do not exist in a micellar state. Further, it was shown that solubilized drug, teniposide, did not precipitate out once the mixed micellar system was diluted and it was stable when dispersed as liposomes (20). Therefore precipitation is not a concern during application of the drug but only during storage before use, which may be overcome by freeze-drying.

Acute Toxicity Studies

The purpose of toxicity studies was to compare the acute toxicity of the vehicle of the current formulation of taxol to that of a mixed-micellar vehicle. Since our solubility studies indicated that there was not a significant difference in the solubilization potential of mixed micelles prepared from different bile salts, we conducted toxicity experiments using a bile salt known to be relatively less toxic, GC (22).

In this study it has been shown that the acute toxicity of bile salt was significantly less when given in the presence of PC as mixed micelles (Table II). This is in good agreement with previous studies wherein it has been demonstrated that mixed micelles of GC and egg PC were well tolerated in rat, dog, and rabbit models and were not embryotoxic, teratogenic, or mutagenic (23).

In order to compare the toxicity of Cremophor EL and bile salt (GC), the amount of both compounds required to dissolve the same quantity of taxol, 1 mg, was calculated. For sodium glycocholate 14 mg is required for 1 mL of mixed-micellar solution, containing 32 mg/mL total lipid at a 0.8 PC/GC molar ratio, in order to solubilize 1 mg of taxol (data not shown). On the other hand, the same amount of taxol can be dissolved in a 0.17-mL Cremophor/ethanol solution, since the taxol concentration in the present Cremophor solution is 6 mg/mL. Pure Cremophor in a 0.17-mL Cremophor/ethanol solution is 0.085 mL or 89.7 mg. Therefore, it is necessary to compare the toxicity of 14 mg of bile salt with 89.7 mg of Cremophor. The LD_{50} for GC as mixed

Table II. Median Lethal Dose of Vehicles Used to Dissolve Taxol

Vehicle solution	LD_{50} (mg/kg)
Cremophor EL	3360 (2800–4000)[a]
Simple micelles	500 (436–576)
Mixed micelles	740 (624–876)

[a] Confidence levels for 0.05 probability in parentheses.

Table III. Evaluation of the Cytotoxic Potential of Taxol Prepared in Aqueous DMSO or Mixed-Micelle Solutions

Cell line	ED_{50} (μg/mL)		
	Mixed micelles	Mixed micelles + taxol	Aqueous DMSO + taxol
UISO-BCA-1	57	0.03	0.04
UISO-COL-1	130	0.1	0.3
UISO-LUC-1	166	0.06	0.1
HT-1080	62	0.008	0.01
A-431	159	0.08	0.1
ZR-75-1	119	0.04	0.05
LNCaP	104	0.3	1.9
KB-3	130	0.01	0.05
KB-V1	195	2.6	12.3
P-388	12	0.007	0.05

micelles is 740 mg/kg, whereas for Cremophor, the corresponding value is 3360 mg/kg. The ratios of the LD_{50} values to the amount of compound required to dissolve 1 mg of taxol are 53 for GC and 37 for Cremophor. These data indicate that bile salt is 1.4 times less toxic than Cremophor, when their solubilization potential for taxol is taken into account. This observation suggests that BS/PC mixed-micellar solutions can be superior to Cremophor EL solutions when preparing an i.v. formulation for an insoluble compound.

Evaluation of Cytotoxic Potential

Pharmacological activity of the drug molecule can be altered and, in some cases, be lost due to interaction of the drug with the vehicle. To clarify this situation for our formulation, we tested the *in vitro* cytotoxic potential of taxol in the presence of mixed micelles. GC, being relatively more potent, was used as the bile salt in the composition of the mixed micelles.

As anticipated on the basis of previous results (24), taxol was found to mediate potent cytotoxic activity with a variety of cultured cell types. ED_{50} values ranged from 0.007 μg/ml (P-388 cells) to 12.3 μg/ml (KB-VI cells). One question of key importance was the potential of the formulation described herein to affect this profile of activity adversely. As shown in Table III, taxol prepared in this formulation demonstrated activity of a similar magnitude with cultured human cells derived from breast, colon, lung, sarcoma, and squamous cell carcinoma, relative to a solution prepared in aqueous DMSO. It appeared that formulated taxol demonstrated greater activity compared with aqueous DMSO with LNCaP, KB-3, KB-V1, and P-388 cells, but it is possible that this activity profile was due to the cytotoxic effect mediated by the mixed-micelle formulation itself. Nonetheless, of the greatest importance is the fact that significant cytotoxicity was observed with the formulated drug, and this is consistent with retention of antitumor activity.

ACKNOWLEDGMENTS

The work was supported by Illinois Cancer Center Biomedical Research Support Grant 2-507-RR 05893-07. Partial support was also provided by American Cyanamid Company. The authors are thankful to Pharmaceutical Resources Branch and the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, for supplying taxol. Dr. D. P. Waller is acknowledged for useful discussions regarding toxicity experiments.

REFERENCES

1. M. C. Wani, H. L. Taylor, M. E. Wall, *et al.* Plant antitumor agents. VI. The isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J. Am. Chem. Soc.* 93:2325–2327 (1971).
2. A. I. Einzig, P. H. Wiernik, and E. L. Schwartz. Taxol: A new agent active in melanoma and ovarian cancer. In M. Muggia (eds.), *New Drugs, Concepts and Results in Cancer Chemotherapy*, Kluwer Academic, Boston, 1991.
3. P. B. Schiff and S. B. Horwitz. Taxol stabilizes microtubule in mouse fibroblast cells. *Proc. Natl. Acad. Sci. USA* 77:1561–1565 (1980).
4. J. Parness and S. B. Horwitz. Taxol binds to polymerized tubulin *in vitro*. *J. Cell Biol.* 91: 479–487 (1981).
5. J. N. Denis and A. E. Greene. A highly efficient practical approach to natural taxol. *J. Am. Chem. Soc.* 110:5917–5919 (1988).
6. NCI Investigational Drugs. Pharmaceutical data, 1990, pp. 151–152.
7. M. Lassus, D. Scott and J. B. Leyland. Allergic reactions associated with cremophor containing antineoplastics. *Proc. Am. Soc. Clin. Oncol.* 4:268 (1985).
8. S. M. Edgington. Taxol out of the woods. *Bio/Technology* 9:933–938 (1991).
9. B. D. Tarr, T. G. Sambandan, and S. H. Yalkowsky. A new parenteral emulsion for the administration of taxol. *Pharm. Res.* 4:162–165 (1987).
10. M. H. Bartoli, M. Biotard, H. F. Fessi, *et al.* In-vitro and in vivo antitumoral activity of free, and encapsulated taxol. *J. Microencapsul.* 7:191–197 (1990).
11. K. Son and M. H. Alkan. Liposomes prepared dynamically by interactions between bile salt and phospholipid molecules. *Biochim. Biophys. Acta* 981:288–294 (1989).
12. H. Alkan-Onyuksel and K. Son. Mixed micelles as proliposomes for the solubilization of teniposide. *Pharm. Res.* 9:1556–1562 (1992).
13. D. W. Shen, A. Fojo, J. E. Chin, *et al.* Human multidrug-resistant cell lines: Increased *mdr*l expression can precede gene amplification. *Science* 232:643–645 (1986).
14. K. Likhitwitayawuid, C. K. Angerhofer, N. Ruangrungsi, G. A. Cordell, and J. M. Pezzuto. Cytotoxic and antimalarial bisbenzylisoquinoline alkaloids from *Stephania erecta*. *J. Natl. Prod.* 56:30–38 (1993).
15. J. T. Litchfield, Jr., and F. Wilcoxon. A simplified method of

evaluating dose-effect experiments. *J. Pharmacol. Exp. Ther.* 96:99–113 (1949).
16. R. P. Hjelm, P. Thiyagarajan, and H. Alkan. A small angle neutron scattering study of the effects of dilution on particle morphology in mixtures of glycocholate and lecithin. *J. Appl. Cryst.* 21:858–863 (1988).
17. R. P. Hjelm, P. Thiyagarajan, and H. Alkan-Onyuksel. Organization of phosphatidycholine and bile salt in rodlike mixed micelles. *J. Phys. Chem.* 96:8653–8661 (1992).
18. N. A. Mazer, G. B. Benedek, and M. C. Carey. Quasielastic light scattering studies of aqueous bilayer lipid systems. *Biochemistry* 19:601–615 (1980).
19. P. Schurtenberger, N. A. Mazer, and W. Kanzig. Micelle to vesicle transition in aqueous solutions of bile salt and lecithin. *J. Phys. Chem.* 89:1042–1049 (1985).
20. K. Son. *Detergent and Phospholipid Mixed Micelles as Proliposomes for an Intravenous Delivery of Water Insoluble Drugs.* Ph.D. thesis, University of Illinois at Chicago, Chicago, 1991.
21. S. C. Su, M. H. Alkan, J. Y. Park, *et al.* Mesophase formation during in vitro cholesterol gallstone dissolution. *J. Pharm Sci.* 70:713–715 (1981).
22. E. S. Swenson and W. J. Curatolo. Intestinal permeability enhancement for proteins, peptides and other polar drugs: Mechanism and potential toxicity. *Adv. Drug. Del. Rev.* 8:39–92, (1992).
23. K. Teelmann, B. Schlappi, M. Schupbach, and A. Kistler. Preclinical safety evaluation of intravenously administered mixed micelles. *Drug Res.* 34:1517–1523 (1984).
24. G. A. Cordell, C. C. W. Beecher, and J. M. Pezzuto. Can ethnopharmacology contribute to the development of new anticancer drugs? *J. Ethnopharmacol* 32:117–133 (1991).