Novel Taxol Formulations: Preparation and Characterization of Taxol-Containing Liposomes

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Taxol is a promising anticancer agent under investigation for therapy of ovarian, breast, colon, and head and neck cancer. One problem associated with the administration of taxol is its low solubility in most pharmaceutically-acceptable solvents; the formulation used clinically contains Cremophor EL® (polyethoxylated castor oil) and ethanol as excipients, which cause serious adverse effects. To eliminate this vehicle and possibly improve the antitumor efficacy of taxol, we have formulated taxol in liposomes of various compositions. Liposome formulations containing taxol and phospholipid in the molar ratio 1:33 were prepared from phosphatidylglycerol (PG) and phosphatidylcholine (PC) (1:9 molar ratio), and were physically and chemically stable for more than 2 months at 4°C, or for 1 month at 20°C. A method of producing taxol-liposomes by lyophilization has been developed, by which large batches can be prepared reproducibly in a 'pharmaceutically rational' manner. Taxol-liposomes retained the growth-inhibitory activity of the free drug *in vitro* against a variety of tumor cell lines. In mice, taxol-liposomes were well-tolerated when given in bolus doses by both iv and ip routes. The Maximum Tolerated Dose (MTD) was >200 mg/kg; it exceeded that of free taxol, which had a MTD of 30 mg/kg by iv or 50 mg/kg by ip administration. Free taxol administered in the Cremophor vehicle was toxic at doses >30 mg/kg, as was the equivalent volume of vehicle without drug. Taxol-liposomes may prove to be useful not only for eliminating the toxic effects attributed to the Cremophor vehicle, but also for providing opportunities to widen the taxol therapeutic index through alterations in route and schedule of administration.

KEY WORDS: taxol; liposomes; lyophilization.

Introduction

Taxol, a diterpenoid derived principally from the bark (1) of the Western Yew, *Taxus brevifolia,* is under investigation as an anticancer agent (2,3) in human clinical trials; target tumors include a variety of human cancers, including ovarian, breast, colon, non-small cell lung, and head and neck cancer. Taxol is the first of a new class of antineoplastic drugs (3); it causes stabilization of microtubules (4), thus interfering with cellular progress through mitosis (5), and arresting cell replication. In phase II trials with patients treated previously with high-dose chemotherapy, the response rate in advanced and refractory ovarian cancer was 30% (6). The overall response rate in phase II trials in previously-treated patients with metastatic breast cancer was 56% (7). Recently, the U.S. Food and Drug Administration (FDA) approved taxol for use against ovarian cancer. One problem associated with the use of taxol is its poor solubility in water and in most pharmaceutically-acceptable solvents, which necessitates administration in a lipoid vehicle. Presently, the vehicle used clinically is polyethoxylated castor oil (Cremophor EL®) containing 50% absolute ethanol. The amount of Cremophor necessary to deliver the required doses of taxol is significantly higher than that administered with any other marketed drug. This vehicle has been observed to cause serious, life-threatening anaphylactoid reactions in animals (8) and humans (3,9), and is physically incompatible with some intravenous (iv) infusion sets, as shown by the leaching of plasticizers (10). Since hypersensitivity reactions occur more frequently with shorter infusion schedules, most phase II and III trials in the United States have used 24-hour schedules (3). Premedication with corticosteroids (dexamethasone), antihistaminics (diphenhydramine), and histamine H_2 receptor antagonists (cimetidine or ranitidine) has reduced the intensity and incidence of adverse reactions associated with taxol-Cremophor administration (8,11); however, they are not completely eliminated (12). Clinically, pharmacological intervention is less desirable than a safer, better-tolerated formulation; with multiple agents in general, and with the co-medication agents used with taxol specifically, there exists considerable potential for drug interactions that can alter the pharmacokinetics and pharmacodynamics, and thereby the toxicity or efficacy, of taxol.

The primary goal of our work is to eliminate the Cremophor vehicle by reformulation of the drug in a better-tolerated vehicle. Reformulation also provides the possibility of improving the efficacy of taxol-based anticancer therapy. Liposomes represent a versatile drug carrier technology with considerable potential for improved solubilization of lipophilic drugs [reviewed in 13–15], and various formulations are in clinical trials or under investigation for treatment of a number of neoplastic and infectious diseases. The widening variety of liposome-encapsulated drugs entering clinical trials reflects an emerging understanding of the safety, utility, and methodology required to produce the quantities of this experimental drug carrier for human therapeutic trials. Liposomes consist of one or more aqueous compartments contained within lipid membrane bilayers. Because liposomes contain a hydrophilic domain, a hydrophobic domain, and an interfacial region, they may accommodate therapeutic agents having diverse physical characteristics. It is plausible to assume that well over 10 gm of phospholipid may be administered safely to humans; 8–20 gm doses of liposomes have been reported in different studies (16,17).

Several previous examples suggest the utility and potential therapeutic gains from reformulation in liposomes of other drugs currently given in the Cremophor EL vehicle. Cyclosporin has been administered to humans in Cremophor EL, and acute toxic side effects have been observed (18). A liposomal formulation showed immunosuppressive activity equal to that of the Cremophor-based formulation, but with reduced renal and vehicle toxicity. In addition, greater compatibility with intravenous administration equipment was reported for the liposome-cyclosporin formulation (19). Therefore, there was a strong rationale for reformulating taxol in

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liposomes. The taxol-liposome formulations were prepared and evaluated for stability and *in vitro* and *in vivo* activity.

Materials and Methods

Materials. Crystalline taxol, Cremophor EL containing 50% absolute ethanol (Diluent 12) and taxol dissolved in Diluent 12 (30 mg/5 ml) were obtained from the National Cancer Institute (Bethesda, MD). Cremophor EL was also obtained as a gift from BASF Corporation. Phospholipids were purchased from Avanti Polar Lipids (Birmingham, AL) or Princeton Lipids (Princeton, NJ) and stored in chloroform under argon at −70° C. All organic solvents used were reagent or high performance liquid chromatography (HPLC) grade. Female BALB/c mice were obtained from Harlan Sprague Dawley (Indianapolis, IN).

Preparation and Characterization of Taxol-liposomes: Taxol-liposomes were prepared by hydration of a lyophilized powder containing taxol and phospholipids, using a method adapted from (20). The following mixtures of phosphatidylcholine (PC) and phosphatidylglycerol (PG) were used: 10:0, 9:1, 7:3, 5:5, 3:7 or 0:10 molar ratio. Briefly, taxol was dissolved in chloroform and mixed with phospholipids in a round bottom flask, and the chloroform was evaporated in a rotary evaporator at 40°C. The taxol-lipid film was then dissolved in tert-butanol to achieve a lipid:taxol molar ratio of 33:1 and a lipid concentration of 100 mM. Two to 10 ml aliquots of the butanolic solution were placed in sterile tubes, shell-frozen in liquid nitrogen, and lyophilized for 24 h. The lyophilized powder was hydrated with buffer (NaCl/Tes/EDTA: 140 mM/10 mM/0.1 mM) to produce suspensions of multilamellar vesicles (MLV). To obtain smaller vesicles (SUV), the liposome suspension was sonicated under argon in a bath sonicator (Laboratory Supplies Co. Inc., Hicksville, NY) for 30 minutes at 20°C. Liposomes were analyzed for taxol by reversed-phase HPLC (21) and phospholipid content by Bartlett assay (22).

Physical Stability of Taxol-liposome Formulations: Physical stability of taxol-liposome formulations was determined by several methods. First, small liposomes (SUV) were subjected to centrifugation at 15,000 × g for 15 min, under which conditions the liposomes remain suspended and taxol precipitates are sedimented. Second, liposomes were passed through 0.1 μm pore polycarbonate filters, through which liposomes can pass, but which retain taxol precipitates. Third, suspensions were examined at high magnification (1200×), using Differential Interference Contrast microscopy (DIC), to observe aggregation of liposomes or crystallization of taxol. Fourth, negative-stain transmission electron microscopy was used to evaluate the suspensions. Taxol-liposome formulations subjected to the first two separation methods were re-analyzed for taxol and phospholipid content. A change in either was interpreted as an indication of instability.

Chemical Stability of Taxol in Liposome Formulations: The chemical stability of taxol in liposomes was assessed using reverse-phase HPLC to determine total taxol content of formulations at different time points during storage at 4° or 20°C. Detailed methods for HPLC analysis are given elsewhere (21). Briefly, HPLC analysis was performed with an isocratic solvent system (70% methanol, 30% water) at a flow-rate of 2 ml/min, and taxol was detected by absorbance at 227 nm. N-octyl benzamide was used as an internal standard. A Hitachi HPLC system was used, consisting of a Model L6200A pump, L-4250 spectrophotometric detector, AS-2000 autosampler and D-2500 Integrator. A Waters μ Bondapak C_{18} column (3.9 × 300 mm) and a C_{18} guard column were used for analysis.

Cell Growth Inhibition Experiments: Cells were plated at a density of 2×10^4/ml in 24-well plates (Costar) and allowed to adhere overnight. Wells in triplicate were exposed to various concentrations of taxol, either added as liposomes, as a 100× concentrated stock of free drug in dimethylsulfoxide (DMSO), or adsorbed to serum proteins in the absence of organic solvent. Cells were counted after 72 h, and the IC_{50} (concentration resulting in 50% growth inhibition) for each concentration-effect curve was calculated graphically.

Toxicity of Prototype Taxol-Liposomes: The Maximum Tolerated Dose (MTD) for taxol-liposome formulations was determined for intraperitoneal (ip) and intravenous (iv) routes of administration in healthy Balb/c female mice. Survey experiments to define the MTD were performed with two animals per group. Doses were escalated in 2-fold increments, starting at 5 mg/kg. Drug effects were determined by close observation of weight changes; the maximal dose of taxol causing more than 10% weight loss within one week of treatment was defined as the MTD. Animals showing weight loss exceeding 20% were sacrificed, as changes of this magnitude usually indicate lethal toxicity (E. Mayhew, Roswell Park Cancer Inst., unpublished observations). After completing the survey MTD experiments, the approximate MTD was refined further using 3 groups of 8 mice.

Results

Over 300 sets of formulations were examined, representing a systematic variation of liposome properties including diameter, charge, membrane fluidity, length of lipid acyl chain, surface hydration, and inclusion of specific dopants. A wide variety of natural and synthetic lipids and phospholipids were screened for their ability to accommodate taxol. Preliminary results suggested that PC bilayers incorporate higher concentrations of taxol than can any other lipid investigated. Liposomes composed solely of PC encapsulating ≤3 mole% taxol, with respect to phospholipid, were stable for >2 weeks (data not shown). However, PC-taxol liposomes were found to be highly aggregated when examined by Differential Interference Contrast (DIC) Microscopy (Fig 2A). Incorporation of a negatively charged phospholipid such as PG reduced aggregation (Fig 2B–2F). Thus, liposomes composed of PC and PG in various ratios were examined further.

In addition, different methods for preparing and post-processing liposomes were tested (23): hydration of dry lipid films, reverse-phase evaporation, freeze-thaw, sonication, and extrusion. During the developmental process, hydration of dry drug-lipid films, optionally followed by sonication to limit particle diameter, was found to be a feasible method for preparation. In scaling up to larger quantities, physical stability of formulations was variable (data not shown). Our hypothesis is that the drug precipitation occurred when the drug concentration in organic solvent exceeded its solubility during the production of the dried lipid film. As an alterna-

Table I. Taxol Encapsulation and Stability of Liposome Formulations as measured by Percentage Taxol remaining in Liposomes[a]

		Days[d]							
% Taxol[b]	Lipid (mM)[c]	0	0.02	1	2	4	30	60	75
1.7	50.0	100	100	107	106	106	99	102	102
1.9	100.0	100	95	97	93	87	105	105	108
2.1	150.0	100	95	94	85	84	100	108	108
4.5	50.0	100	82	69	67	44	24	ND[e]	ND
4.4	100.0	100	86	92	86	84	27	ND	ND
3.7	150.0	100	87	104	86	57	41	ND	ND
8.6	50.0	100	16	11	8	7	ND	ND	ND
8.2	100.0	100	9	8	6	6	ND	ND	ND

[a] Taxol-liposomes (PG:PC; 3:7).
[b] Mole % Taxol with respect to phospholipids.
[c] Final phospholipid concentration after reconstitution with buffer (Salt:Tes:EDTA).
[d] Number of days formulations were stored at 4°C before analysis.
[e] N.D. = Not determined.

tive, we prepared a dried taxol-lipid mixture by lyophilization from organic solvent.

Physical Stability of Taxol-liposome Formulations

Physical stability of hydrated taxol-SUV formulations (PG:PC 3:7 mole:mole) was evaluated quantitatively as a function of taxol:lipid ratio, final lipid concentration, and time of storage at 4°C (Table I). Liposome formulations containing approximately 2% taxol were physically stable for more than 2½ months in the hydrated state and retained 100% of their initial taxol content, irrespective of lipid concentration. Liposomes containing approximately 4% taxol were stable for approximately 2 days, over which time they retained 67–86% of their initial taxol content. In contrast, liposomes containing approximately 8% taxol became unstable during preparation.

Previous work has shown that higher liposome electrostatic charge can promote liposome-cell interaction, and thereby enhance cellular delivery of liposome-dependent drugs (24–26). For certain applications, such as direct administration of drug-liposome formulations to the tumor-containing site (eg. intraperitoneal administration in ovarian cancer), highly negatively-charged liposomes may be optimal for cellular delivery (26,30). Therefore, we tested the effect of increasing the mole fraction of negatively-charged phospholipid on the physical stability of taxol-liposome formulations (Fig 1).

Taxol-containing liposomes were prepared with 3 mole% drug, and the phospholipid concentration was held constant at 100 mM. Taxol-liposome formulations composed of ≤30% PG were physically stable at 4°C for more than 1 month in the hydrated state and retained >70% of their initial taxol content (Fig 1A). At 20°C, only taxol-liposome formulations composed of ≤10% PG were physically stable; these retained >70% of their initial taxol content for more than one month (Fig 1B).

The stability of these formulations was assessed qualitatively by Differential Interference Contrast Microscopy in order to detect aggregation of liposomes or crystallization of taxol. DIC images of taxol-liposome formulations taken immediately after preparation and after 24 hours of storage at 20°C are shown in Figures 2 and 3, respectively. In these formulations the drug:lipid ratio was held constant at 3 mole% and only the PG:PC ratio was varied. Taxol-liposomes composed of PC alone were highly aggregated immediately after preparation (Fig 2A) and after 24 hr of

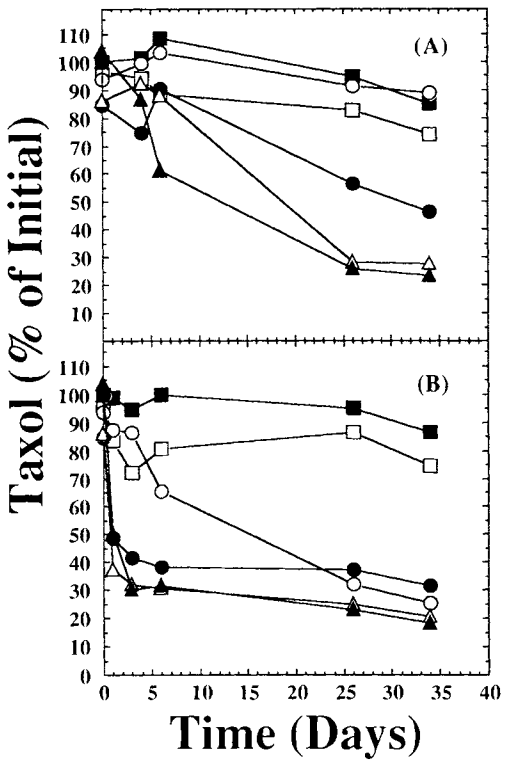

Figure 1: Stability of Taxol-liposomes as a function of %PG and storage temperature. All formulations contained 3% mole taxol per mole lipid and were stored either at 4°C (A), or 20°C (B). They were analyzed as described in *Methods* at different time points to determine the fraction of taxol retained in liposomes. The results are expressed as % of initial taxol concentration remaining in the liposomes at different time points. The results are the mean of two determinations and in all cases the range of the results obtained was smaller than 10%. In these formulations PG:PC ratio was varied. Open squares: PC only; Filled squares: PG:PC (1:9); Open circles: PG:PC (3:7); Filled circles: PG:PC (5:5); Open triangles: PG:PC (7:3); Filled triangles: PG only.

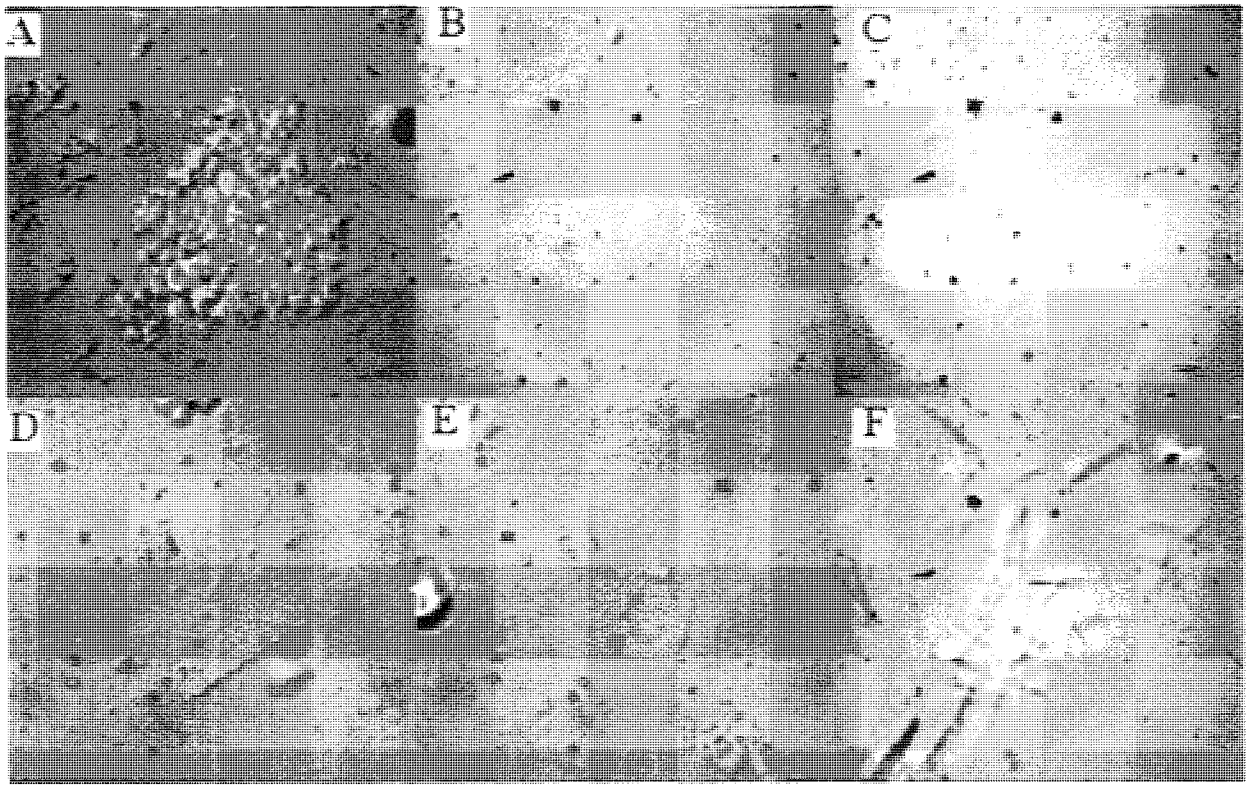

Figure 2: Morphology of Taxol-liposome formulations immediately after preparation. Taxol was incorporated into liposomes and examined by Differential Interference Contrast (DIC) Microscopy. In all images, the phospholipid concentration was 100 mM, and the taxol:phospholipid ratio was held constant at 3 mol%. Images were taken immediately after preparation; (A) is of 100% PC; most liposomes were aggregated; (B) and (C) are of 1:9 and 3:7 PG:PC respectively; most liposomes are below the limit of microscopic resolution, and no aggregates or taxol crystals were observed; (D) and (E) are of 5:5 and 7:3 PG:PC respectively; a few very fine and small taxol crystals were observed; (F) is of 100% PG, a large number of very fine and small taxol crystals were apparent.

storage at 20°C (Fig 3A), but precipitated taxol was not observed. Taxol-liposomes composed of PG:PC at molar ratio of 1:9 or 3:7 were below the limit of microscopic resolution immediately after completion of the sonication step (Fig 2B and 2C, respectively) and after 24 hr of storage at 20°C (Fig 3B and 3C, respectively). In formulations of PG:PC 1:9 or 3:7, neither liposome aggregates nor needle-like crystals of taxol were observed. In formulations containing PG:PC at molar ratios of 5:5 or 7:3, a small number of taxol needles were observed immediately after preparation (Fig 2D and 2E, respectively). In formulations containing 100% negative charge (PG alone), a large number of taxol needles were observed immediately after preparation (Fig 2F). In taxol-liposomes containing 50%, 70% or 100% PG, a number of large taxol needles were observed after 24 hr of storage at 20° C (Fig 3D, 3E and 3F, respectively).

Given the above observations on stability of taxol-liposome formulations, we selected a formulation containing 10% negative charge phospholipid (PG:PC 1:9) for further investigation of additional parameters, such as the effect of liposome diameter on stability. No significant difference was observed in the stability of SUV versus MLV (data not shown).

Taxol-liposomes (PG:PC 1:9) were evaluated by negative-stain transmission electron microscopy. Figure 4 is the electron micrograph of sonicated taxol-liposomes. The spherical structures observed were typical of sonicated liposomes, as the diameter of most of the particles were approximately 0.025–0.05 μm (23). Neither liposome aggregates nor taxol crystals were observed (Fig 4).

Chemical Stability of Taxol in Liposome Formulations

An HPLC assay was used to estimate taxol concentration in liposome formulations. Taxol remained chemically stable in the liposomes for more than 3 months at 4° and 20°C; neither additional peaks nor reduction of taxol content was evident from the chromatogram (data not shown).

Cytostatic Activity of Taxol-Liposomes

Cytostatic activity of taxol:lipid formulations was compared to that of free taxol, using a variety of tumor cell lines (Table II). The sensitivity to free taxol varied nearly 100-fold, depending on the cell line; C-26, a murine model for invasive and recurrent colon cancer (27) showed the least sensitivity to taxol. 9L, a rat gliosarcoma, also was relatively resistant to taxol. A121a, a human ovarian tumor cell line established prior to treatment (28), was the most sensitive, and growth inhibition occurred at concentrations 100-fold lower than those required to inhibit growth of C-26. Other human ovarian tumor lines (Hey-1b and A90) showed intermediate sensitivity to taxol.

The taxol-liposome formulation (PG:PC 1:9) was nearly equipotent to free taxol in the case of the 9L rat gliosarcoma,

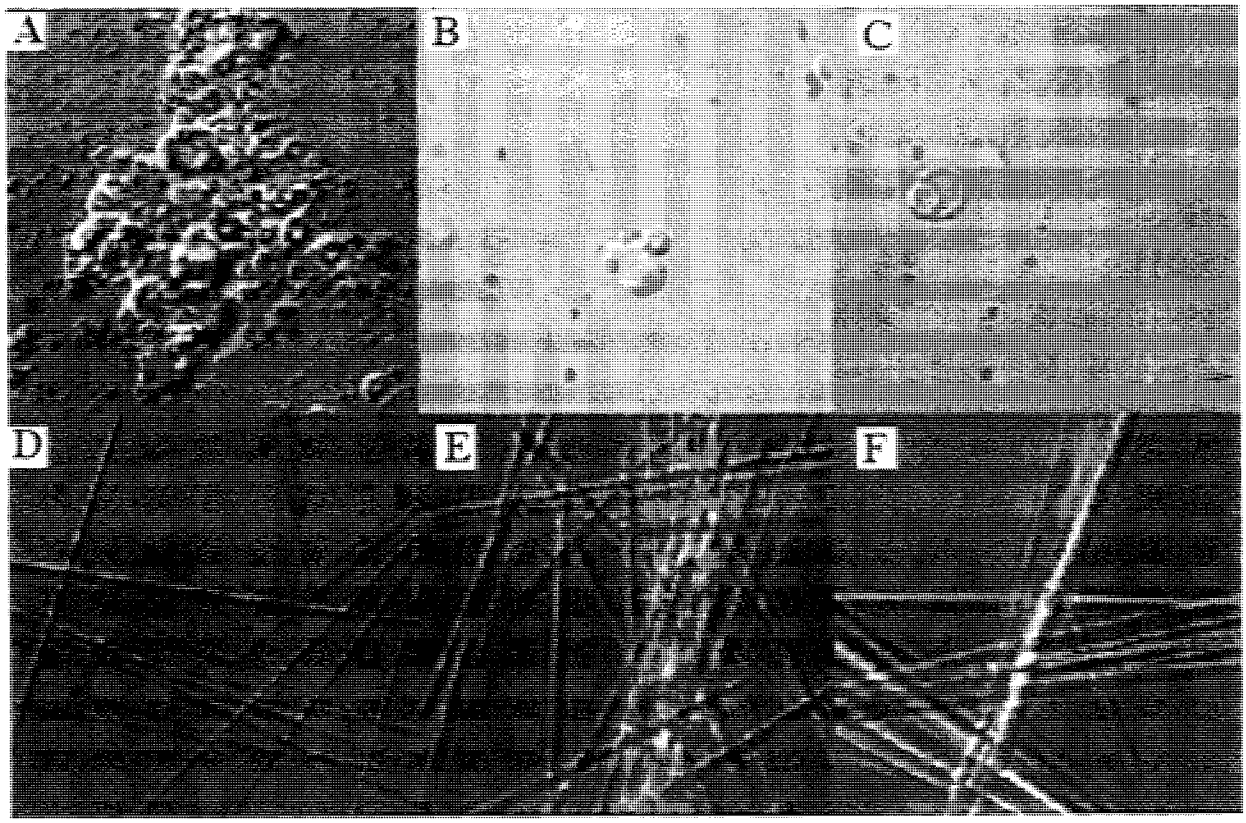

Figure 3: Morphology of Taxol-liposome formulations after 24 hours of storage at 20° C. Taxol was incorporated into liposomes and examined by Differential Interference Contrast (DIC) Microscopy. In all images, the phospholipid concentration was 100 mM, and the taxol:phospholipid ratio was held constant at 3 mol%. Images were taken after 24 hours of storage at 20° C; (A) consists of 100% PC, most liposomes were aggregated; (B) and (C) are composed of 1:9 and 3:7 PG:PC respectively; most liposomes are below the limit of microscopic resolution, and no aggregates or taxol crystals were observed; (D) and (E) are of 5:5 and 7:3 PG:PC respectively and (F) is of 100% PG. In images (D), (E) and (F), a large number of very long and thick taxol crystals were observed.

L1210 murine leukemia, B16 murine melanoma, B16F10 (a highly-metastatic variant of B16), and the human ovarian cancer lines (A90 and A121a). In other cases such as C-26, liposomes were less potent than free taxol. Because the taxol content of liposomes was determined by HPLC, and since no other peaks were evident in the chromatogram, chemical instability of taxol was ruled out. However, the amount of taxol liposomes required to expose C-26 cells to a dose of taxol equivalent to the IC_{50} for free drug was in a concentration range in which the liposomes themselves may contribute to the cytostatic effect (25,29,30).

In investigating the potency of taxol on certain cell lines, it was found that the growth-inhibitory activity was enhanced by 0.1% dimethylsulfoxide (DMSO), the vehicle in which the drug was dissolved before addition to the cell cultures (31). For some tumor lines (9L rat gliosarcoma and A90 human ovarian tumor), free taxol activity was enhanced (8-fold) by DMSO, compared to drug dissolved directly in serum-containing growth medium (31). In the absence of DMSO, free and liposome-encapsulated taxol showed nearly equal cytostatic activity. However, the cytostatic activity of free taxol on C-26 was not enhanced by DMSO.

Further investigation is directed toward understanding the relatively lower potency of taxol-liposomes on C-26 *in vitro*. Previous work has demonstrated that drug delivery to C-26 cells increases with increased liposome negative charge (26). We hypothesize that the lower activity of taxol liposomes on C-26 tumor cells, compared to free drug, may result from the fact that the liposomes contained only 10% negative charge, and therefore liposome-cell interaction was not optimal. Although higher electrostatic charge could promote liposome-cell interaction, and thereby enhance cellular delivery, the higher charge would reduce liposome stability and reduce circulation time in the blood.

Toxicity of Taxol-Liposomes in vivo

The toxicity of taxol-liposome formulations was compared to that of the clinically-used taxol-Diluent 12 formulation, which contains polyethoxylated castor oil and ethanol. In healthy mice, the single-dose MTD of free taxol administered in Diluent 12 is approximately 30 mg/kg by the iv route. The amount of Diluent 12 vehicle required to administer doses above 30 mg/kg was also toxic, making it difficult to discriminate acute drug toxicity from that of the excipient. Taxol-liposome formulations administered at or above the MTD of free taxol were well-tolerated. We were unable to find an MTD for liposome formulations administered in a single dose because of the concentration of taxol in the formulations (3 mg/ml) and the limitation of injection volume

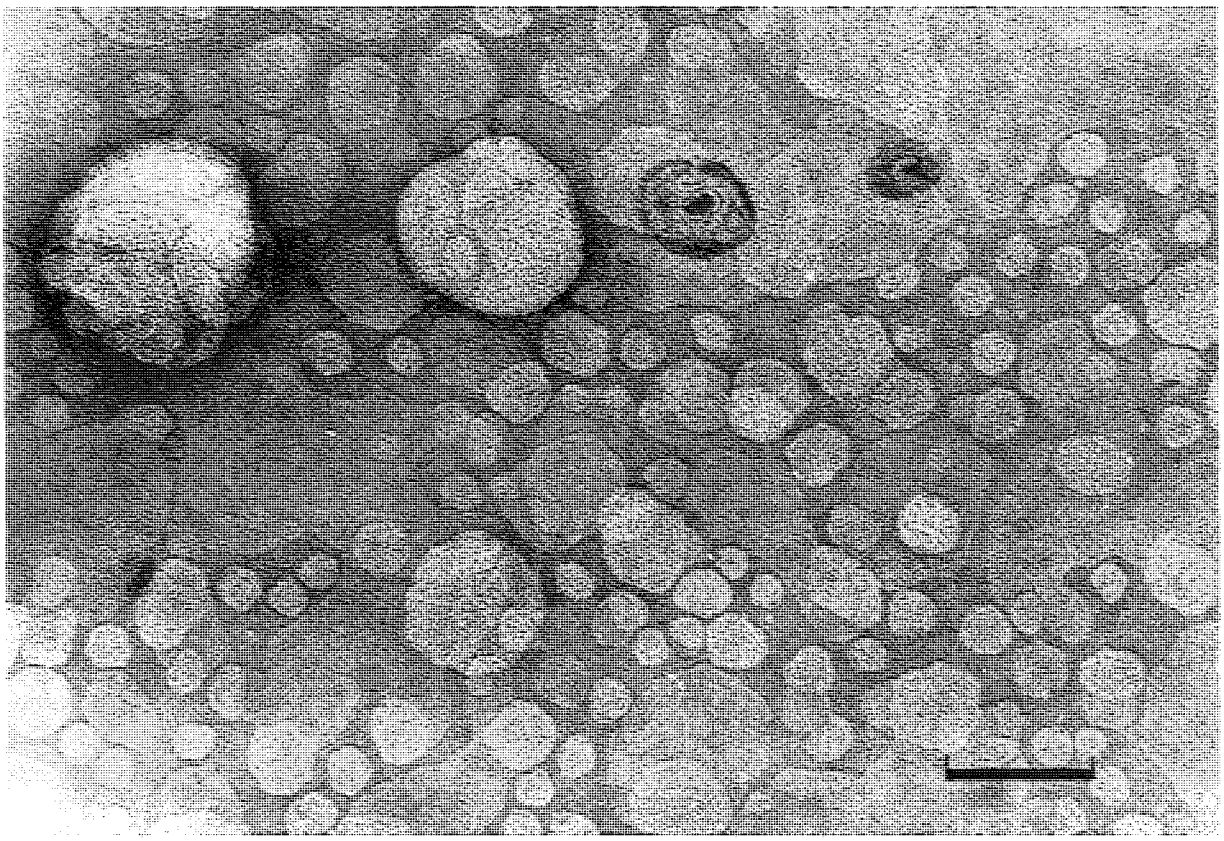

Figure 4: Negative stain transmission electron photomicrograph of taxol-liposome formulation. Small unilamellar vesicle formulations containing taxol and phospholipid in the molar ratio 1:33 were prepared from phosphatidylglycerol and phosphatidylcholine (1:9 molar ratio), stained with 1% uranyl acetate, and examined by electron microscopy after 30 days of storage at 4° C. Bar = 100 nm.

(0.3 ml). Therefore, the MTD for liposome formulations was >60 mg/ml (single dose) and >200 mg/kg (in 4 doses over 3 hr.).

Discussion

In early preclinical studies, many different vehicles were investigated for administration of taxol (reviewed in 32), including hydroxypropylcellulose, polyethyleneglycol 400, Tween-80, dimethyl sulfoxide (DMSO), and Cremophor EL® plus ethanol. Due to its poor solubility in a wide variety of pharmaceutically-acceptable vehicles, the taxol formulation which was selected for clinical trials consisted of Cremophor EL containing 50% ethanol, designated "Diluent 12" in the literature of the National Cancer Institute. Since then, there have been numerous efforts directed toward eliminating Cremophor from the taxol formulation because of serious or fatal hypersensitivity reactions in humans.

A number of water-soluble congeners, prodrugs (33), and analogs (34,35) have been synthesized, and some show biological activity. However, few have sufficient stability and solubility to allow clinical administration without cosolvents or emulsifiers. Taxol has been solubilized in a number of vehicles, including emulsions based on 50% triacetin (36). Although the triacetin emulsion of taxol showed good pharmaceutical stability, the triacetin without taxol was toxic to mice upon iv administration in concentrations near those required to administer active doses of taxol (LD_{50} ≈1.2 ml/kg body weight) (36). In the present work, we have demonstrated that taxol-liposome formulations can be prepared which show a significant increase in MTD compared to the clinically-used formulation in an animal model.

A systematic approach has been taken to elucidate the major parameters that govern formulation of taxol in phospholipid vesicles. Prototype liposome formulations containing ≤3 mole % taxol have been identified that have sufficient chemical and physical stability to provide a biologically-compatible carrier in which to administer taxol. A method of producing taxol liposomes by lyophilization has been developed by which large batches can be prepared reproducibly in a "pharmaceutically rational" manner.

Testing *in vitro* against a variety of tumor lines has demonstrated that taxol-liposomes retain the growth-inhibitory activity of taxol. Elsewhere, we have shown that free taxol administered in Cremophor showed no antitumor effect on C-26, a taxol-resistant murine tumor, when given at doses that included or exceeded the MTD. In contrast, the taxol-liposomes described here delayed tumor progression at doses that included or exceeded the MTD of free taxol but which were well tolerated (37).

Table II. Growth-Inhibitory Activity of Free and Encapsulated Taxol on Human and Animal Tumor-Cell Lines.

Cell Line	IC_{50}[a] ± s.d.[b] (μM) Free-Taxol[c]	IC_{50}[a] ± s.d.[b] (μM) Lip-Taxol[d]	Potency F-IC50/L-IC50[e]
A90	15.0 ± 7.1*	19.0 ± 15.6	0.8
Hey-1b	5.3 ± 2.1	9.5 ± 4.9	0.6
A121a	1.5 ± 0.7*	1.3 ± 0.4	1.2
9L	35.0 ± 14.1*	40.0 ± 14.1[f]	0.9
L1210	5.5 ± 2.1	6.3 ± 2.5	0.9
B16F10	8.0 ± 2.8	11.0 ± 5.7	0.7
B16	7.5 ± 2.8	10.0 ± 4.2	0.8
Colon-26	90.0 ± 10.0	250.0 ± 70.7[f]	0.4

[a] Concentration of drug resulting in 50% inhibition of cell growth; cells were exposed to drug continuously over the period of assay.
[b] Standard Deviation.
[c] Taxol was dissolved directly in serum-containing cell growth medium.
[d] Taxol-liposomes (PG:PC; 3:7) containing 3 mole% taxol.
[e] Free-Taxol IC_{50}/Taxol-liposomes IC_{50}
[f] Liposome vehicle may contribute to cytostatic effect.
* IC_{50} was significantly lower when treated with taxol dissolved in DMSO.

Notation

Abbreviations used:

Diluent 12	Cremophor EL® (polyethoxylated castor oil) containing 50% absolute ethanol
DMSO	Dimethylsulfoxide
HPLC	high performance liquid chromatography
IC_{50}	drug concentration giving 50% inhibition of cell growth
ip	intraperitoneal
iv	intravenous
MLV	multilamellar vesicles
MTD	Maximum Tolerated Dose
PBS	Dulbecco's Phosphate Buffered Saline
PC	phosphatidylcholine
PG	phosphatidylglycerol
SUV	small unilamellar vesicles
tert-butanol	tertiary butanol

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