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# Lipid-complexed camptothecin: formulation and initial biodistribution and antitumor activity studies

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**Abstract** Water-soluble derivatives of camptothecin, an active topoisomerase I inhibitor, have shown a broad spectrum of activity against human tumors. Early clinical trials with the water-soluble sodium salt of camptothecin were hindered by significant cystitis, gastroenteritis, and leukopenia. Furthermore, the sodium salt of camptothecin has been shown to have significantly less activity than the water-insoluble lactone form of the compound. We describe a formulation of lipid-complexed CPT (LC-CPT; particle size range 20.8–208.1 nm) that is very easy to prepare and allows for intravenous administration in vivo in clinically relevant lipid-drug ratios (12.5:1 w/w). The lipid formulation had in vitro antitumor activity similar to that of CPT formulated without lipids and displayed similar cytotoxicity against MDR-1-negative and -positive tumor cells. The biodistribution of CPT was profoundly affected by lipid complexation; free CPT achieved the greatest concentration in the pulmonary parenchyma while LC-CPT achieved the highest concentration in the gastrointestinal tract. LC-CPT had significant antitumor activity in vivo against intraperitoneal L1210 and P388 leukemia and appeared to be more potent than free CPT.

**Key words** Lipid-complexed camptothecin

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## Introduction

Camptothecin (CPT) is a drug isolated from the stemwood of the oriental tree, *Camptotheca acuminata*, which passed random drug screening tests in the 1950 s and was characterized by Wall et al. in 1965 [1]. Clinical trials demonstrated the activity of the sodium salt of CPT in human tumors in the early 1970s, particularly in patients with gastrointestinal tumors metastatic to the liver, but the drug fell out of favor because of dose-limiting leukopenia, cystitis, and gastroenteritis [2-4]. Interest in CPT was revived when it was found to be a potent inhibitor of topoisomerase I [5-7]. Inhibition of topoisomerase I causes single-strand nicks in DNA, resulting in arrest in the G2 phase of the cell cycle [8]. G2 arrest is seen with a variety of cytotoxic agents; it appears to result from a failure to activate cdc2 kinase, an enzyme required for chromosome condensation and nuclear membrane breakdown prior to mitosis [9]. Subsequent studies revealed that the hydroxylactone ring of camptothecin is critical for antitumor activity and that the antitumor activity correlates with topoisomerase I inhibition [10-12]. Disruption of the lactone ring results in loss of topoisomerase I inhibition (Fig. 1). Giovanella et al [13] have demonstrated that the method of administration of CPT significantly affects disease response rates in murine tumors; intramuscular and oral administration of the

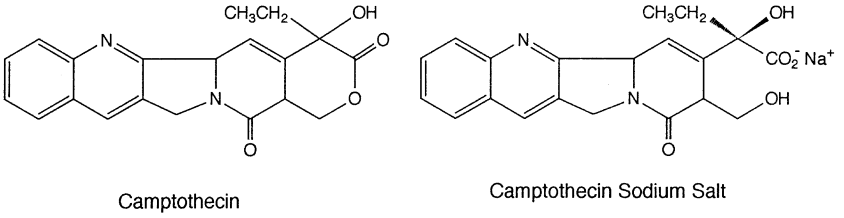


**Fig. 1** Chemical structures of the lactone form of camptothecin and camptothecin sodium salt

lactone-intact compound resulted in a significantly improved cure rate in murine xenografts compared with intravenous administration.

Although CPT was originally plagued by solubility problems associated with its hydrophobic nature, several analogs were developed to enhance water solubility and maintain stability of the more cytotoxic closed lactone ring form. Topotecan and CPT-11 are examples of analogs that show promise in vivo and are currently being evaluated in phase I and II clinical trials [14]. Although CPT resistance does not appear to be mediated by MDR-1 gene expression, topotecan has been found to be a substrate for efflux by P-glycoprotein, probably as a result of its amino side group. The complexation of CPT with lipids might have several advantages: 1. the development of a delivery system for in vivo administration; 2. the enhancement of cytotoxicity by providing a slow drug, release system, since it is a cell cycle phase-specific agent; 3. the improvement of tumor targeting by prolonging the plasma circulation time; and 4. the protection of the intact lactone ring from hydrolysis in the circulation.

Unfortunately, despite its significant hydrophobicity, CPT has been particularly difficult to entrap in lipid membranes and no clinically relevant intravenous liposomal formulation for delivering lactone-intact CPT has been described. Previously described liposomal formulations of CPT demonstrate significant protection of the lactone ring, but require ratios of lipid to drug which are difficult to administer in the clinic. We report here the development of a non-liposomal formulation of lipid-complexed CPT (LC-CPT) which provides efficient complexing of lipid to CPT and maintains in vitro and in vivo cytotoxicity comparable to that of the free compound.

## Materials and methods

### Chemicals and drugs

CPT was kindly provided to us by Dr. Vishnuvajjala (National Cancer Institute). The compound was found to be insoluble in a variety of solvents including dimethyl sulfoxide (DMSO), Tween 20, methanol, and cremaphor. Since a non-toxic vehicle for solubilizing free CPT (control) could not be identified, a crude suspension of free CPT in phosphate-buffered saline (PBS) with 1% DMSO was used. Cardiolipin (CL), *N*-glutaryl phosphatidyl ethanolamine (NGPE), dicaproyl phosphatidyl choline, dimyristoyl phosphatidyl choline, distearoyl phosphatidyl choline, 1-stearyl, 2-myristoyl phosphatidyl choline, 1-stearoyl, 2-myristoyl phosphatidyl choline, dimyristoyl phosphatidyl glycerol, and dioleyl phosphatidyl glycerol were obtained from Avanti Polar Lipids (Alabaster, Ala.). [$^3$H]-CPT was obtained from Moravek Biochemicals, (Brea, Calif.) and [$^{14}$C]-cholesterol was obtained from Amersham Corp (Arlington Heights, Ill.). HPLC-grade methanol was obtained from Fischer Scientific (Fair Lawn, N.J.).

### Cell lines

MDA-Panc3 cells, a pancreatic carcinoma cell line developed at the University of Texas, M.D. Anderson Cancer Center, DiFi colon cancer cells, KB-3–1 (MDR-1 sensitive) squamous carcinoma, and KB-V1 (MDR-1 resistant) cells were used for in vitro cytotoxicity studies. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 50 u/l pencillin, and 100 μg/l streptomycin at 37°C in 5% $CO_2$/95% air.

### Preparation of LC-CPT formulation

We tried to incorporate CPT within the bilayers of liposomes composed of a wide variety of lipids by using standard thin-layer lipid evaporation/hydration methods. Briefly, CPT was mixed in ratios of from 5:1 to 25:1 w/w lipid-drug in chloroformmethanol (8:1). Higher lipid-drug ratios were not tried since they would imply the need for very high lipid doses in humans. For experiments where CPT was radiolabelled, approximately 0.02 μCi/ml [$^3$H]-CPT was added to the preparation. Trace quantities of $^{14}$C labelled cholesterol were added to samples requiring lipid labelling. For small samples, the lipids were dried in $12 \times 75$ mm culture tubes under a stream of nitrogen gas. For large samples, a rotavapor (Buchi RE140, Switzerland) was used to dry the lipids in 100 to 1000 ml round bottom flasks. The lipophilic film was then vortexed in a Touch Mixer Model 231 (Fisher Scientific, Fair Lawn, N.J.) for 1 min in sterile PBS (pH 7.4). Preparations were screened for liposome formation and the presence of drug crystals by light and fluorescent microscopy (Nikon Labophot-2, Japan).

### Characterization of LC-CPT

#### *Column chromatography*

A 30-cm agarose A-5M column (200–400 mesh, operating range 10,000 to 5,000,000 molecular weight) was used to analyze the LC-CPT formulation and to separate lipid-bound CPT from free CPT. PBS was used as the mobile phase for all preparations. A total of 60 ml was collected in 20 3-ml fractions. Fluorimetry and scintillation counting were used to detect lipid-bound and free drug in each fraction. Aliquots of 20 μl from each collection tube were transferred to cuvettes containing 2 ml methanol. Fluorimetry was performed as described by Hart et al. [15]. Fluorescence was measured at an

excitation wavelength of 370 nm and emission wavelength of 434 nm (Perkin-Elmer MPF44A, Norwalk, Ct.). Ethanol (2 ml) was used as a control for machine calibration.

*Size distribution of LC-CPT and free CPT*

The size distribution of LC-CPT was assessed using a laser light scattering method (Nicomp submicron Particle Sizer Model 370; Nicomp, Santa Barbara, Calif.). The distribution of particle size was determined by Gaussian and Nicomp methods.

*Determination of lipid-bound CPT*

A modification of the Ficoll flotation method for separating lipid-bound CPT from free CPT was used [16]. Onto samples containing 200 µl of various NGPE/CPT ratios in 12X75 mm culture tubes was layered 1 ml 30% Ficoll then 1 ml PBS on top of the Ficoll. The tubes were spun for 30 min at 3400 rpm in a Sorvall GS2-B swing rotor. The lipid-bound CPT migrated to the top of the tube. The percentage entrapment was calculated from the formula:

$$(1 - \mathrm{CPM_p} / \mathrm{CPM_s}) \times 100\%$$

where $\mathrm{CPM_s}$ is the cpm of the starting material and $\mathrm{CPM_p}$ the cpm of the pellet. HPLC (as described below) was used to determine the effect of lipid complexation on CPT.

In vitro cytotoxicity assay

The standard MTT assay was used with minor modifications [17]. Briefly, 8000 cells/well in 135 µl DMEM with 10% fetal bovine serum (FBS) was added to 96-well culture plates. The plates were incubated for 14 h at 37°C in an atmosphere containing 5% $CO_2$, then 15 µl of CPT, LC-CPT, and empty lipids (ELs) with CPT were added at concentrations of 1.0–500 µ*M* CPT (lipid/drug weight ratio range, 5–25:1). The cells were incubated in the presence of drug for 24 h to 120 h. After 15 µl of 5 mg/ml MTT in PBS was added to the samples, the cells were incubated at 37°C for 2 h. To each well was added 100 µl lysis buffer (50 ml DMF, 20 g SDS, to a total volume of 100 ml with water, adjusted to pH 4.7 with NaOH). Plates were incubated for 14 h at 37°C, then read at 570 nm in a kinetic microplate reader. A dose of 0.1 µ*M* NGPE was used as a control for 100% survival.

In vivo antitumor activity

Mice (B6D2F1; Charles River Laboratories, Wilmington, Mass.), weighing 25–30 g, were inoculated with $10^6$ L1210 or P388 leukemia cells via the intraperitoneal route. A single intraperitoneal treatment with different doses of ELs, CPT, LC-CPT, or ELs + CPT was administered 24 h after tumor inoculation. Each group comprised five animals. Results are expressed in terms of the median survival of the treated animals calculated as a percentage of the median survival of the control animals (%T)

In vivo biodistribution

ICR Swiss mice were injected with a dose of 10 mg/kg i.v. of either free CPT or LC-CPT. Animals were sacrificed 6 h after the administration of the drugs. Blood, gastrointestinal tract (esophagus to anus), heart, kidney, liver, and lung were resected, extracted, and assayed for CPT content by HPLC. PBS (1 ml) was added to each organ resected except liver and gastrointestinal tract which required 3 ml for homogenization. Organs were homogenized for 30 s at 90% output (Biohomogenizer; Biospec Products, Bartlesville, Okla.). Chloroform (2.5 ml) was added to 500 µl of organ homogenates. The chloroform/homogenate suspension was shaken at 120 rpm (Orbit-Shaker; Lab-Line Instruments, Melrose Park, Ill.) for 15 min at 37°C, and centrifuged at 2000 rpm for 10 min. The chloroform was removed from 2 ml of recovered supernatant by vacuum evaporation (Model RC10.10.; Jouan, Winchester, Va.). The resultant pellet was resuspended in HPLC mobile phase (see below), vortexed, and centrifuged at 2000 rpm for 10 min. The supernatant was analyzed by HPLC for determination of CPT concentration. Organ concentrations were adjusted for extraction efficiency. which was calculated for each organ by measuring the amount of CPT detected after the addition of free CPT to organ homogenates of untreated animals and following the extraction procedure described.

HPLC was performed on all organ samples as previously described [18]. Briefly, 100-µl samples were loaded via a Model SIL-9A autoinjector (Shimadzu Corp, Kyoto, Japan) using methanol-10 m*M* potassium phosphate (75:25 w/w, pH 4.0) as mobile phase. Separations were conducted at ambient temperature at a flow rate of 0.7 ml/min via a model LC-10AS pump (Shimadzu) and analyzed with a model CR501 chromatopac (Shimadzu). Detection was achieved at an excitation wavelength of 370 nm and an emission wavelength of 434 nm using a model 470 scanning fluorescent detector (Waters Milford, Mass.). Separation was achieved on a reverse-phase Bondapak C18 column (300 mm × 3.9 mm ID; Waters).

## Results

### Characteristics of LC-CPT

Lipids of varying carbon chain length, charge, and saturation, alone or in different combinations, were empirically screened for their ability to form liposomes incorporating CPT by fluorescent microscopy. Table 1 shows the different lipids used. All combinations of neutral lipids or with dimyristoyl phosphatidylglycerol (DMPG) resulted in massive presence of drug crystals mixed with numerous but barely fluorescent liposomes, indicating a marked lack of affinity or compatibility of CPT for lipid bilayers as previously reported.

In contrast, the use of dioleoyl phosphatidylglycerol (DOPG) alone resulted in an important observation: lack of drug crystallization and the presence of only a few fluorescent particles by fluorescence microscopy. This suggested drug solubilization or the formation of small lipid–drug particles or micelles. Chromatographic evaluation confirmed that $< 5\%$ of the CPT was incorporated in the liposome fraction of the preparation, the rest presumably being solubilized or in small particles not detectable by fluorescence microscopy. This observation led us to concentrate our efforts on other negatively charged lipids. CL and NGPE (Fig. 2), which both contain double negative charges, when used alone were found to result in homogeneous and milky preparations devoid of drug crystals and containing only a few fluorescent particles compatible with multilamellar liposomes that could be eliminated by sonication. Chromatographic evaluation of these

**Table 1** Lipids investigated for complexation with CPT (*PC* phosphatidyl choline, *PG* phopsphatidyl glycerol, *NGPE* *N*-glutaryl phophatidyl ethanolamine)

| Lipid | Chain length/ saturation | Charge | Crystals | bound to CPT |
|---|---|---|---|---|
| Dicaproyl PC | 6:0, 6:0 | 0 | Yes | < 5 |
| Dimyristoyl PC | 14:0, 14:0 | 0 | Yes | < 5 |
| Distearoyl PC | 18:0, 18:0 | 0 | Yes | < 5 |
| 1-Stearoyl, 2--myristoyl PC | 18:0, 14:0 | 0 | Yes | < 5 |
| 1-Stearoyl, 2--oleoyl PC | 18:0, 18:1 | 0 | Yes | < 5 |
| Dimyristoyl PG | 14:0, 14:0 | − 1 | Yes | < 5 |
| Dioleoyl PG | 18:1, 18:1 | − 1 | No | < 5 |
| Cardiolipin | Natural product[a] | − 2 | No | 67 |
| NGPE | 18:1, 18:1 | − 2 | No | 97 |

[a] 18:2 (90.3%), 18:1 (6.5%), 16:0 (1%), 16:1 (1%), 16:0 (1%)

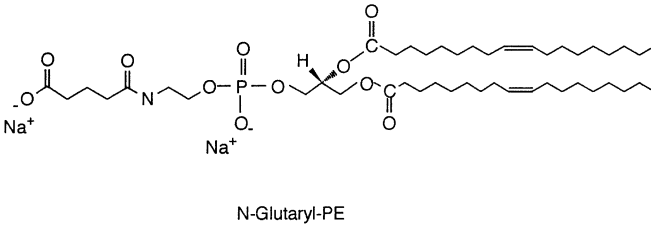


**Fig. 2** Chemical structure of *N*-glutaryl phosphatidyl ethanolamine.

preparations confirmed again that < 5% of the CPT eluted with the liposome fraction, the rest of the drug eluting later in a large number of consecutive fractions. By differential centrifugation, the lipid-binding efficiency of CL was about 67% and that of NGPE 97%. NGPE was, therefore, selected as the preferred lipid for the formation of LC-CPT.

The effect of lipid drug ratio on the lipid–drug binding efficiency was studied. The results are shown in Fig. 3. The lowest lipid-drug ratio resulting in a lipid-binding efficiency > 95% was 12.5:1. This ratio was, therefore, selected for further biological and pharmacological studies. Evaluation of this formulation of LC-CPT by HPLC revealed a single, sharp peak at a retention time of 6.24 min. The peak was identical to that observed for the free drug indicating that CPT was not significantly altered during lipid complexation.

There was a bimodal particle size distribution. Most particles (98%) had a mean diameter of 23.8 nm (range 20.8–28.5 nm) and the remaining fraction had a mean diameter of 153 nm (range 123.3–208.1 nm). Therefore, the vast majority of particles were undetectable by light or fluorescent microscopy. Although larger particles were visualized by fluorescent microscopy, column chromatography demonstrated that only a small fraction of drug was present in these vesicles. The small particle diameter was consistent with the lack of crystallization of LC-CPT observed. In contrast, free CPT was not well solubilized with 10% DMSO in saline, and the mean crystal diameter was 1.4 μm.

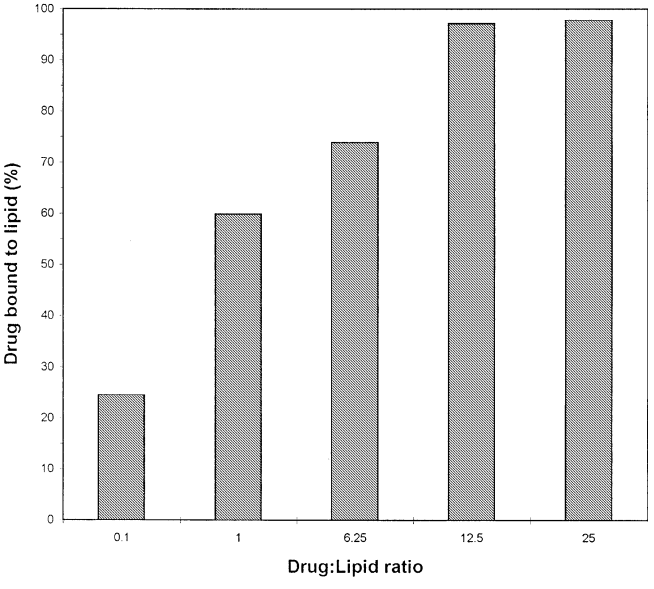


**Fig. 3** Lipid binding as a function of drug/lipid ratio

## Cytotoxicity

Since lipid-binding efficiency was not enhanced by the addition of cholesterol or other combinations of lipids, LC-CPT containing NGPE alone (12.5:1 lipiddrug ratio) was used for in vitro assays of cytotoxicity. Lethal cellular injury induced by CPT was enhanced by prolonged exposure. Figure 4 demonstrates the effect of CPT, LC-CPT, NGPE, and NGPE plus free CPT on the cytotoxicity of DIFI and MDA-Panc3 cells after 24 h and 120 h incubation. Of note, NGPE exhibited little intrinsic cytotoxicity. MDA-Panc3 and DIFI cells are equally sensitive to LC-CPT at 24 h. LC-CPT was significantly more potent than CPT against both cell lines at 0.1 μ*M* concentration. At concentrations at or above 1 μ*M*, complexed and free CPT were equipotent. At 120 h, CPT was significantly more

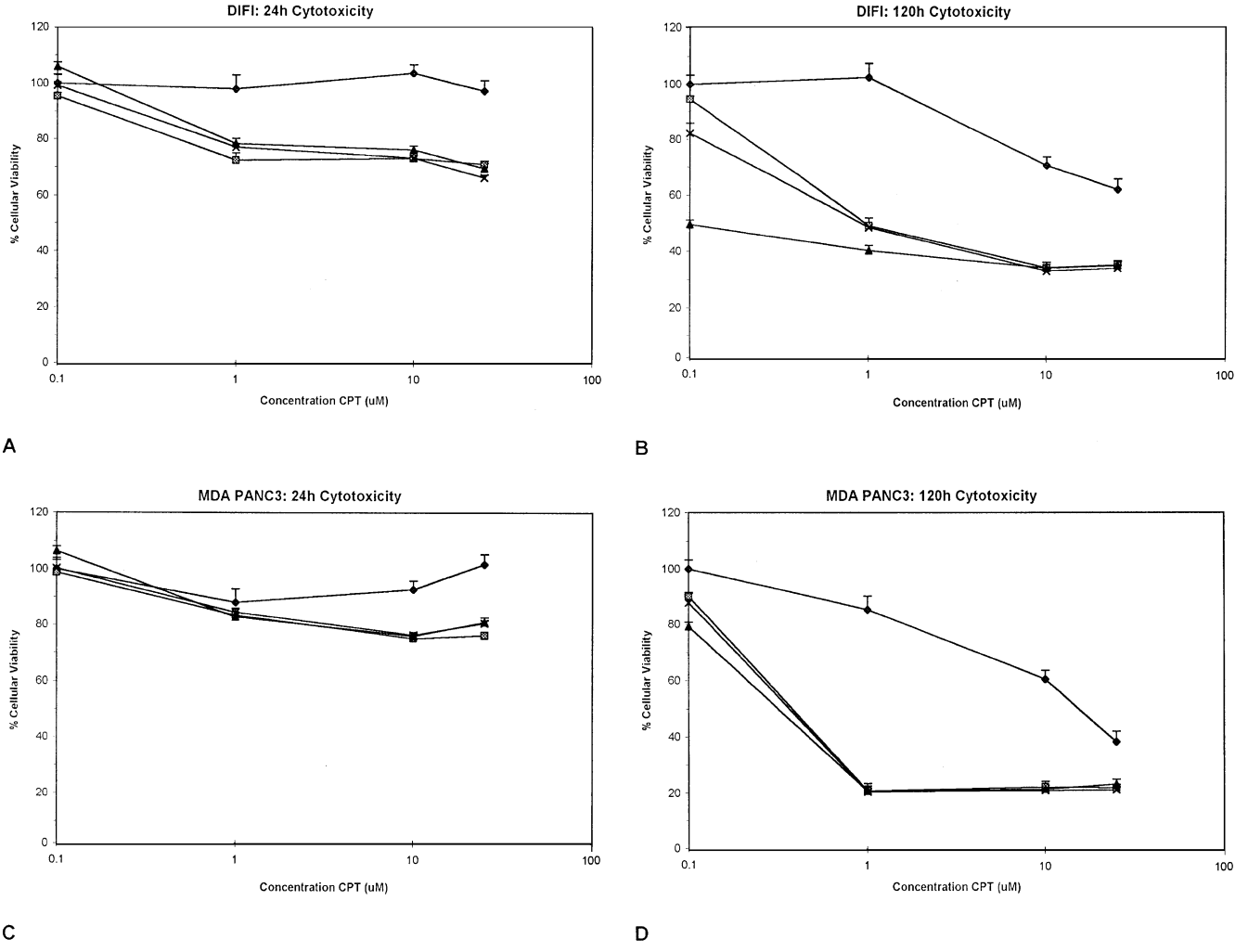


**Fig. 4A–D** Cytotoxicity of various concentrations of LC-CPT (▲), CPT (–▩–). Lipid (–◆–), L + CPT (–×–) (non-complexed) against Difi (**A** and **B**) and MDA-Panc3 cells (**C** and **D**) at 24 h (**A** and **C**) and 120 h (**B** and **D**)

toxic to cells at a concentration of 1 μ*M* than at 0.1 μ*M*, but further increases in concentration did not lead to a significant increase in cytotoxicity. Increasing the duration of exposure from 24 h to 120 h significantly increased the cytotoxic effect. This assay underestimates cytoxicity at lower viabilities; we were not able to demonstrate viability of $< 20\%$ at any drug concentration or exposure time. NGPE did not appear to have any intrinsic antitumor activity and did not significantly alter the effects of CPT.

To determine whether the MDR-1 phenotype would have an effect on cellular sensitivity to LC-CPT, sensitive KB-3-1 cells and multidrug resistant KB-V1 cells were exposed to concentrations of 1–25 μ*M* LC-CPT. As can be seen in Fig. 5, similar cytotoxicity was seen for most concentrations of CPT; however, when exposed to 25 μ*M* LC-CPT, KB-V1 cells were slightly more resistant than KB-3–1 cells.

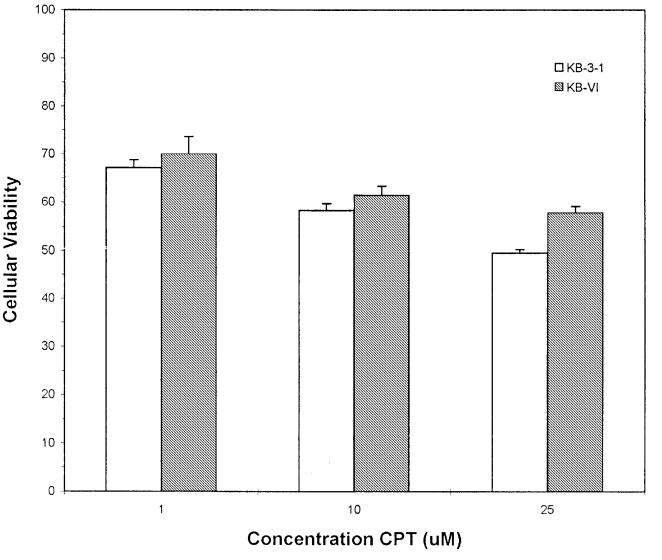


**Fig. 5.** LC-CPT activity against wild-type (KB-3-1) and resistant (KB-VI) cell lines

**Table 2.** Antitumor activity against P388 leukemia cells. Mice were treated IP with LC-CPT, CPT, L + CPT (lipid + unentrapped CPT) or lipid alone 24 h after IP injection with $10^6$ tumor cells

| Treatment | Dose CPT (mg/kg) | %T/C | 50-day survivors |
|---|---|---|---|
| LC-CPT | 40 | 50 | |
| LC-CPT | 20 | 186 | 2/5 |
| LC-CPT | 10 | 150 | 2/5 |
| LC-CPT | 5 | 121 | |
| CPT | 40 | 207 | 3/5 |
| CPT | 20 | 100 | |
| CPT | 10 | 121 | |
| L + CPT | 20 | 121 | |
| Lipid[a] | 20 | 100 | |

[a] Lipid dose equivalent to 20 mg/kg LC-CPT

**Table 3.** Antitumor activity against L1210 leukemia cells. Mice were treated IP with LC-CPT, CPT, L + CPT (lipid + unentrapped CPT) or lipid alone 24 h after IP injection with $10^6$ tumor cells

| Treatment | Dose CPT (mg/kg) | %T/C | 50-day survivors |
|---|---|---|---|
| LC-CPT | 20 | 146 | 1/5 |
| LC-CPT | 15 | 100 | 1/5 |
| LC-CPT | 10 | 123 | 0 |
| CPT | 60 | 92 | 0 |
| CPT | 45 | 85 | 0 |
| CPT | 30 | 85 | 0 |
| L + CPT | 20 | 85 | 0 |
| L + CPT | 15 | 100 | 1/5 |
| L + CPT | 10 | 85 | 0 |
| Lipid[a] | 20 | 92 | 0 |

[a] Lipid dose equivalent to 20 mg/kg LC-CPT

## In vivo antitumor activity of LC-CPT

Table 2 demonstrates survival data for mice inoculated with P388 leukemia cells that were treated with LC-CPT, free CPT or lipid plus free CPT (unentrapped). LC-CPT and free CPT displayed similar antitumor activity (%T/C 186 vs 207); however, LC-CPT was about twofold more potent. Of the mice treated with free CPT, the optimal dose was 40 mg/kg with a%T/C of 207, and three animals were long-term survivors. No activity was observed with a dose of 20 mg/kg (%T/C = 100). Of the mice treated with LC-CPT, there was a significantly shortened survival for the mice treated with a dose of 40 mg/kg due to toxicity, which suggests that lipid binding increases the potency of CPT. The optimal doses were 20 mg/kg and 10 mg/kg with%T/Cs of 186 and 150 respectively. There were no treatment-related deaths in the mice treated with either dose of LC-CPT and there were two (40%) 50-day survivors in each group, but none in the corresponding groups receiving free CPT, suggesting increased potency for the lipid complex formulation.

Because LC-CPT appeared to have improved potency compared to free CPT, and LC-CPT caused early death at the highest doses, mice inoculated with L1210 leukemia cells were treated with doses of 30 to 60 mg/kg free CPT or 10 to 20 mg/kg of LC-CPT (Table 3). Against L1210 leukemia, LC-CPT displayed significant activity (%T/C = 146 at 20 mg/kg) while free CPT was inactive. All animals treated with LC-CPT survived longer than those treated with free CPT and two of the animals treated with LC-CPT were 50-day survivors. One animal treated with 15 mg/kg free CPT plus lipid survived 50 days.

## Biodistribution

As shown in Table 4, the interaction of CPT with lipids had a profound effect on the drug's biodistribution. Both free CPT and LC-CPT were rapidly cleared from the blood. CPT and LC-CPT achieved highest concentrations in the lungs and gastrointestinal tract, respectively. Drug tissue levels were consistently lower in mice treated with LC-CPT, even in the gastrointestinal tract. Drug levels with free CPT were about 180-fold higher than LC-CPT in the lungs which was apparently due to embolization of crystallized free drug into the lungs followed by a depot effect. Heart and kidney levels of free CPT were higher than those of LC-CPT. Interestingly, gastrointestinal concentrations of drug were consistently greater than hepatic concentrations, presumably due to the rapid processing of LC-CPT in the liver and elimination via the gut. The significant variability associated with injection of free drug was attributed to its poor aqueous solubility.

**Table 4.** Organ distribution of free CPT and LC-CPT after i.v. (*ND* not detectable) administration of 10 mg/kg CPT

| Organ | Time (h) | Free CPT (μg/ml or μg/mg dry tissue) | LC-CPT (μg/ml or μg/mg dry tissue) |
|---|---|---|---|
| Blood | 6 | 0.069 ± 0.039 | 0.010 ± 0.001 |
| GI Tract | 6 | 2.562 ± 0.515 | 2.208 ± 0.090 |
| Heart | 6 | 1.458 ± 0.555 | 0.047 ± 0.001 |
| Kidney | 6 | 0.443 ± 0.164 | 0.072 ± 0.005 |
| Liver | 6 | 2.077 ± 0.402 | 0.672 ± 0.051 |
| Lung | 6 | 38.217 ± 3.061 | 0.209 ± 0.009 |
| Blood | 24 | 0.008 ± 0.012 | ND |
| GI tract | 24 | 0.492 ± 0.148 | 0.059 ± 0.054 |
| Heart | 24 | 0.228 ± 0.092 | 0.006 ± 0.002 |
| Kidney | 24 | 0.539 ± 0.428 | 0.004 ± 0.001 |
| Liver | 24 | 0.429 ± 0.124 | 0.006 ± 0.001 |
| Lung | 24 | 12.276 ± 5.363 | 0.004 ± 0.002 |

## Discussion

Although CPT is the prototype of topoisomerase I inhibitors, it is difficult to work with due of its poor aqueous solubility. Some lipophilic drugs such as taxol and taxotere can be solubilized by mild detergents,

surfactants and alcohol-containing solutions; however, no similar vehicle has been identified for CPT lactone. Even when prepared in solutions containing DMSO, Tween-20, cremophor, and methanol, crystallization and precipitation of the drug occur. Solutions containing chloroform and methanol can solubilize CPT, but are too toxic for clinically relevant studies. With anionic lipids that have been found to be nontoxic in concentrations necessary for complex formation, CPT forms a smooth suspension amenable for in vitro and in vivo study. While we have not identified the exact nature of the particles, their size and chromatographic features suggest the formation of micelles or submicroscopic lipid–drug aggregates. Kotting et al. have shown that, depending on the nature of the lipid used, micelles accumulate in lung, kidney, liver, and to some extent brain [19]. Kwon et al. have shown that polymer-drug conjugates containing polyethylene glycol form micelles that demonstrate prolonged circulation in the blood and enhanced uptake at target sites [20].

Although the identification of CL and NGPE as lipids that could effectively form complexes with CPT was made by empiric observations, this interaction is supported by previous observations. Loh and Ahmed noted that the presence of the C-20 tertiary alcohol imparts an unusual electrophilicity to the lactone carbonyl group [18]. Stabilization of CPT may be achieved through favorable electrostatic effects provided by the double negative charge of these lipids with the electrophilic lactone ring.

The formulation of a lipid-complexed preparation allows for the intravenous delivery of this compound. LC-CPT, in the formulation described, appears to have equivalent antitumor activity to free CPT in the treatment of KB-V1, MDA-Panc3, and DIFI cell lines. The antitumor effect appears to rely upon prolonged drug exposure, but is not affected by MDR-1 expression. LC-CPT has increased potency in vivo and a more predictable pattern of biodistribution then free CPT. This is probably related to crystallization of the free drug since CPT (lactone ring intact) is a highly lipophilic molecule. The increase in potency of LC-CPT compared to free CPT was predicted by Burke et al. who demonstrated that lipid membranes stabilize the lactone ring [21, 22]. Furthermore, interactions with lipids may reduce albumin binding which has been shown to reduce CPT activity [23].

Preliminary studies of the biodistribution of this formulation of LC-CPT administered intravenously to mice demonstrated that the greatest concentration of drug accumulated in gastrointesinal tissue. This was followed by accumulation in lung and liver. Since these organs are common sites of metastatic tumor deposits, LC-CPT may provide the additional benefit of organ-targeted therapy. Similarly, distribution of drug away from the kidneys may reduce the incidence of cystitis, a significant toxic effect observed in early human trials of the sodium salt of CPT. The affinity of free CPT for the lungs is more likely due to crystallization and embolization than the result of organ-specific targeting. With the exception of the lungs, free CPT and LC-CPT have a similar profile of organ distribution.

In summary, we have developed a formulation of lipid-bound CPT which is simple to produce, achieves a high binding efficiency, and has demonstrated activity equivalent to that of the free compound against several human tumor cell lines. In the intraperitoneal treatment of P388 and L1210 leukemia, LC-CPT demonstrated increased potency compared to the free compound. Studies are planned to describe the plasma stability and pharmacokinetics of this formulation of CPT and to evaluate the activity of intravenously administered LC-CPT against human tumor xenografts in mice.

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