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In Vitro and *In Vivo* Studies of Different Liposomes Containing Topotecan

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Liposome as a carrier of topotecan (TPT), a promising anticancer drug, has been reported in attempt to improve the stability and antitumor activity of TPT. However, the biodistribution pattern of TPT liposome *in vivo* and PEG-modified liposome containing TPT have not been studied systemically. In this paper, the *in vitro* stability and *in vivo* biodistribution behavior of several liposomes containing TPT with different lipid compositions and PEG-modification were studied. Compared with the 'fluid' liposome (S-Lip) composed of soybean phosphatidylcholine (SPC), the 'solid' liposome (H-Lip) composed of hydrogenated soybean phosphatidylcholine HSPC decreased the leaking efficiency of TPT from liposome and enhanced the stability of liposome in fetal bovine serum (FBS) or human blood plasma (HBP). The results of biodistribution studies in S_{180} tumor-bearing mice showed that liposomal encapsulation increased the concentrations of total TPT and the ratio of lactone form in plasma. Compared with free TPT, S-Lip and H-Lip resulted in 5- and 19-fold increase in the area under the curve ($AUC_{0\to\infty}$), respectively. PEG-modified H-Lip (H-PEG) showed 3.7-fold increase in $AUC_{0\to\infty}$ compared with H-Lip, but there was no significant increase in $t_{1/2}$ and $AUC_{0\to\infty}$ for PEG-modified S-Lip (S-PEG) compared with S-Lip. Moreover, the liposomal encapsulation changed the biodistribution behavior, and H-Lip and H-PEG dramatically increased the accumulation of TPT in tumor, and the relative tumor uptake ratios were 3.4 and 4.3 compared with free drug, respectively. There was also a marked increase in the distribution of TPT in lung when the drug was encapsulated into H-Lip and H-PEG. Moreover, H-PEG decreased the accumulation of TPT in bone marrow compared with unmodified H-Lip. All these results indicated that the membrane fluidity of liposome has an important effect on *in vitro* stability and *in vivo* biodistribution pattern of liposomes containing TPT, and PEG-modified 'solid' liposome may be an efficient carrier of TPT.

Key words: Topotecan, 'Fluid' liposome, 'Solid' liposome, PEG-modified liposome, Biodistribution

INTRODUCTION

Topotecan (TPT) is a semi-synthetic derivative of camptothecin, which is an alkaloid originally isolated from the stem wood of *Camptotheca acuminate* (Wall *et al.*, 1966). In recent years, camptothecin and its analogs have been developed as highly potent antitumor drugs and got considerable attention. They exert antineoplastic effect by inhibiting the action of the nuclear enzyme topoisomerase I which is involved in DNA replication (Hsiang *et al.*, 1988). The important structural requirement for successful interaction with the topoisomerase I target and antitumor potency *in vivo* is a closed α-hydroxylactone moiety (Jaxel and Kohn, 1989; Giovanella and Cheng, 1991). As a derivative of camptothecin, topotecan is made water-soluble by the presence of a stable basic side chain at carbon 9 of the A ring (Kingsbury *et al.*, 1991). It can be administered without the severe and unpredictable side effects that are associated with camptothecin sodium, and it has been approved as second-line therapy for small cell lung cancer (SCLC) and advanced ovarian cancer by FDA. However, like other camptothecins, topotecan also undergoes a pH-dependent hydrolysis (Fassberg and Stella, 1992). Under physiological conditions, i.e., at pH 7.0 or above, the lactone ring of the drug is readily opened to yield carboxylate form while the open ring carboxylate form is inactive.

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To protect the active lactone ring and decrease the hydrolysis rate of camptothecin and its analogs, thus improving their anticancer activity, liposome has been applied to encapsulate these drugs. Early studies have demonstrated that complexes of CPT with lipid or liposome can stabilize the lactone moiety and maintain its biological activity (Burke *et al.*, 1993; Sugarman, 1996; Burke, 1990). These findings suggest that liposome may be an effective delivery system for these drugs. Some articles have also been reported that the lactone stability and antitumor efficacy of topotecan encapsulated into liposomes were improved compared with those of free topotecan. Topotecan was encapsulated in low pH liposome initially by Burke *et al.*, and their work showed that the lactone ring stability of topotecan was improved markedly when the drug was packaged into gel-phase distearoylphosphatidylcholine (DSPC) vesicles (Burke and Gao., 1994). Subramanian and Muller also used the same method to prepare liposomal topotecan and demonstrated that it was 3-4 fold more effective than free topotecan in stabilizing covalent topoisomerase I-DNA intermediates inside tumor cells (Subramanian and Muller., 1995). Later, to solve the drawbacks of low encapsulation efficiency and low drug-to-lipid ratios by passive loading procedure, ionophore-generated proton gradient method and ammonium sulfate gradient loading procedure have also been used to entrap topotecan into liposomes, and the efficient drug loading was achieved. The stability and antitumor activity of topotecan enhanced drastically after being encapsulated into liposome compared with that of free topotecan (Tardi *et al.*, 2000; Liu *et al.*, 2002).

However, as an important aspect of liposomal formulation, the biodistribution pattern of liposome entrapped topotecan after intravenous administration was still not clear. In this study, we prepared the liposomes containing TPT by ammonium sulfate gradient method and examined the effect of the membrane fluidity of liposomes on the *in vitro* release and biodistribution of TPT. Liposomes were prepared with unsaturated soybean phosphatidylcholine (SPC) which has a phase transition temperature (T_c) lower than body temperature, and hydrogenated soybean phosphatidylcholine (HSPC) which has a higher phase transition temperature than body temperature. Furthermore, in order to increase the accumulation of liposomal TPT in tumor, the PEG-modified liposomes were also prepared. PEG-liposomes are known to have long-circulating characteristics after intravenous injection, and to target passively to tumor by extravasation to more leaky vasculature in tumor tissue (Gabizon, 1995; Gabizon, *et al.*, 1997). Up to date, preparation of PEGylated liposome containing topotecan and its *in vivo* evaluation have not been performed. In the present study, the efficacy of PEG-modification of liposome on the systemic pharmacokinetics and tissue distribution of TPT in S_{180} tumor bearing mice was also studied.

MATERIALS AND METHODS

Chemicals and animals

Topotecan hydrochloride was purchased from Chengdu Furunde Pharmaceutical Co., Sichuan, China. Soybean phosphatidylcholine (SPC, trademark: Epikuron 200), and hydrogenated soybean phosphatidylcholine (HSPC, trademark: Epikuron 200SH) were kindly provided by Degussa, Freising, German. PEG (MW_{2000})-phosphatidylthanolamine (PEG-PE, trademark: lipoid PEG-PE) was purchased from Lipoid GmbH, ludwigshafen, German. Cholesterol was purchased from Shenyang Medicines Company, Shenyang, China. All other chemicals were of HPLC-grade or analytical grade.

Male ICR mice (18-22 g) were obtained from Shenyang Pharmaceutical University and allowed to acclimate to their new environment for three days prior to tumor cell implantation. S_{180} solid tumor cells were used and all tumor models were established by injecting harvested tumor cells (5×10^6/0.2 mL) in the right axillary region of the mice for the tumor distribution study. The tumors were grown until approximately 2000 mm^3 (2043.6 ± 253.6 mm^3, RSD<15%) in size after one week of implantation. The animals were then sorted according to body weight, with three animals per cage. The study protocol was approved by the Institutional Animal Care and Use Committee, Shenyang Pharmaceutical University, China.

Preparation of liposomes

Topotecan hydrochloride was encapsulated into liposome by ammonium sulfate loading procedure (Harlan, 1993). Briefly, the SPC or HSPC and cholesterol (1:1, mol/mol) were co-dissolved in chloroform and evaporated to dryness under reduced pressure. The thin film obtained was hydrated with ammonium sulfate solution (200 mM), and the suspension was frozen and thawed 5 times, and the small unilamellar vesicles followed by repeated extrusion through sterile polycarbonate membrane filters of 0.8, 0.45, 0.22, 0.1-μm pore size 5 times by using high-pressure extrusion equipment (Lipex extrusion). After extrusion, the extra-liposome salt was removed by dialyzing against Hepes buffer (10 mM Hepes, 145 mM NaCl, pH 7.4) solutions for 24 h. Then Topotecan hydrochloride in powder form was added immediately and incubated at 55°C for 5 min with agitation and topotecan-containing liposome was obtained. The PEGylated liposomes of topotecan were also prepared with the same way and the composition of lipids was SPC/CHOL/PEG_{2000}-PE (1/1/0.1, mol/mol/mol) or HSPC/CHOL/PEG_{2000}-PE (1/1/0.1, mol/mol/mol).

Determination of encapsulation efficiency and particle size

The unencapsulated topotecan was separated from liposome by a Sephadex G-50 column, and the content of drug encapsulated into liposome was determined, then the encapsulation efficiency was calculated. Mean size of vesicle was determined using a Laser Diffraction Particle Size Analyzers (LS 230, Beckman Coulter, Inc.). All measurements were done at 25°C.

In vitro drug release

The *in vitro* release profiles of TPT from the liposomal formulations were characterized using the dialysis method, as in contact with PBS (pH 7.4), fetal bovine serum (FBS) or human plasma (HBP) at 37°C, respectively. Briefly, TPT-liposome was mixed with different medium at certain ratio of volume and dialyzed against normal saline through semi-permeable dialysis tubing with a MWCO of 12-14,000. Aliquots (1.0 mL) of release medium were withdrawn at determined time, and then the same volume fresh saline was added to replenish the medium. The concentration of TPT based on total TPT measurements was determined by HPLC analysis.

Evaluation on lactone ring opening rate of TPT

A certain TPT concentration of free TPT solution or liposomal TPT suspensions was prepared in PBS (pH 7.4) at 37°C. At determined times, 200 μL of aliquots were withdrawn and mixed with 800 μL of methanol, and the mixture was injected into HPLC directly for analysis. The lactone and carboxylate forms of TPT were determined simultaneously.

Distribution studies *in vivo*

Free (TPT solution), S-Lip (liposome composed of SPC/CHOL/TPT), H-Lip (liposome composed of HSPC/CHOL/TPT), S-PEG (PEG coated S-Lip) and H-PEG (PEG coated H-Lip) were administered to the tumor-bearing mice by bolus i.v. injection into the lateral tail vein at a dose of 10 mg/kg as TPT. At each time point, 0.083, 0.5, 1, 3, 6, 12, and 24 h after injection, animals was sacrificed. Blood samples (0.3 mL) were collected and centrifuged at 10,000 rpm for 3 min and plasma (100 μL) was collected in new tubes. Different organs and tissues such as heart, liver, spleen, lung, kidney, brain, marrow and tumor were removed, weighed, and homogenized.

Then total TPT, the lactone and carboxylate forms of TPT were determined using fluorescence spectroscopy as described in previous studies (Warner and Burke, 1997). Briefly, 300 μL ice-cold methanol was added to 100 μL plasma or other tissue homogenized solutions to precipitate plasma proteins and solubilize the liposomes. The sample was vortexed for 10 seconds and centrifuged at 10000 rpm for 3 minutes. The methanolic solution was stored at -30°C until analysis and just before HPLC analysis the sample was diluted with an equal volume of refrigerated water. The total topotecan was quantified by diluting the methanolic solution with an equal volume of buffer (pH 3.0).

HPLC analysis

HPLC analysis used a ODS column (150×3.9 mm) with a run time of 15 minutes at a flow rate of 1.0 mL/min. Mobile phase system was TEAA buffer/acetonitrile/tetrahydrofuran (85:15:0.1,v/v) and TEAA buffer was obtained by adjusting 3% trithylamine in water to pH 5.5 with glacial acetic acid. The mobile phases were filtered and degassed prior to use. The fluorescence spectrofluorometric detector (excitation wavelength 380 nm, emission wavelength 525 nm) was used.

Statistical analysis

Mean and standard derivations (SD) were calculated using Microsoft Excel (Microsoft office Excel 2003, Microsoft, Redmond, WA). The pharmacokinetic parameters were calculated by Topfit2.0 computer program (Thomae GmbH, German) based on a non-compartmental model. OriginPro7.0 was used to calculate the area under the concentration–time curve from time zero to time t (AUC_{0-t}) value of TPT in various tissues. Students's *t*-test was performed to compare the parameters between the different groups. Statistically significant differences were assumed when $P<0.05$.

RESULTS AND DISCUSSION

Preparation of liposomes

The liposomal formulations containing topotecan were characterized for encapsulation efficiency and particle size, which are shown in Table I. The topotecan liposomes with high loading efficiency about 90% and high drug-lipid ratio of 1:9 were obtained by ammonium sulfate gradient loading procedure. Addition of PEG_{2000}-PE decreased appreciably the loading efficiency of liposome, but there

Table I. The lipid composition, encapsulation efficiency and vesicle size of different TPT liposomes

Liposomes	Lipid Composition (*mol ratio*)	Encapsulation Efficiency (%)	Average Vesicle Size (nm)
S-Lip	SPC:Chol:PE-PEG_{2000}(1:1:0)	93.1±2.3	142±9.6
S-PEG	SPC:Chol:PE-PEG_{2000}(1:1:0.1)	91.4±2.0	138±5.7
H-Lip	HPC:Chol:PE-PEG_{2000}(1:1:0)	91.0±1.3	146±7.8
H-PEG	HPC:Chol:PE-PEG_{2000}(1:1:0.1)	90.1±1.2	140±4.4

Data are shown as mean ± S.D., n=3.

was no significant difference (P>0.05). All liposomes extruded through 100-nm pore size filter were about 140 nm in diameter and the lipid composition had no marked effect on vesicle size. There was also no difference for vesicles size before and after loading topotecan (data not shown).

The ammonium sulfate gradient loading procedure was firstly described by Barenholz and applied to load amphipathic weak bases such as doxorubicin (Haran *et al.*, 1993). As an active loading procedure, its pH gradient was established indirectly by establishing a $(NH_4)_2SO_4$ gradient. In order to get optimization formulation of liposomal topotecan using ammonium sulfate gradient loading method, the factors influencing encapsulation efficiency of liposomes such as incubated temperature and time, concentrations of ammonium sulfate, ratios of drug to lipid, the size of blank vesicles were also studied. From our results, the ammonium sulfate concentration, drug-to-lipid ratio, and vesicle size have important effects on loading efficiency (data not shown). In this article, the drug-to-lipid ratios of the liposomes were all 0.11:1 (mol/mol), and their entrapment efficiencies were about 90%.

Studies have demonstrated that small liposomes are able to accumulate in disease sites such as tumors and exhibit extended circulation lifetimes (Gabizon and Papahadjopoulos, 1988; Senior *et al.*, 1985). And the vesicle size is also an important factor effect on drug trapping efficiency as well as the duration of pH gradient. Decreasing the vesicle size will reduce the entrapped buffering capacity of the liposomes due to the diminished aqueous captured volume. Mayer *et al.* (1990) have reported that decreasing the vesicle size from 175 to 55 nm decreases the doxorubicin trapping efficiency from approximately 100 to 66%. So, in attempt to obtain high entrapment efficiency and slow release efficacy, the liposome we prepared was extruded through 100 nm instead of 50 nm or 30 nm pore size filter and their final size was about 140 nm.

In vitro release characteristics

The *in vitro* release profiles of several liposomal TPT formulations were prepared by incubating these liposomes with different mediums such as PBS, FBS and HBP. There was no significant change in TPT content based on total TPT for each liposome after 48 h incubation with PBS at 37°C (data not shown). However, TPT leaked out gradually from liposomes as time prolonged when incubated with FBS and HBP (Fig. 1A and B). Compared with S-Lip, H-Lip resulted in a sustained release. After 48 h incubation with FBS, TPT percentage retained in H-lip was 76.42% and higher than that in S-Lip (30.56%). The leaking efficiency of H-Lip in plasma was also lower than that of S-Lip, and only 9.78% of TPT retained in S-Lip

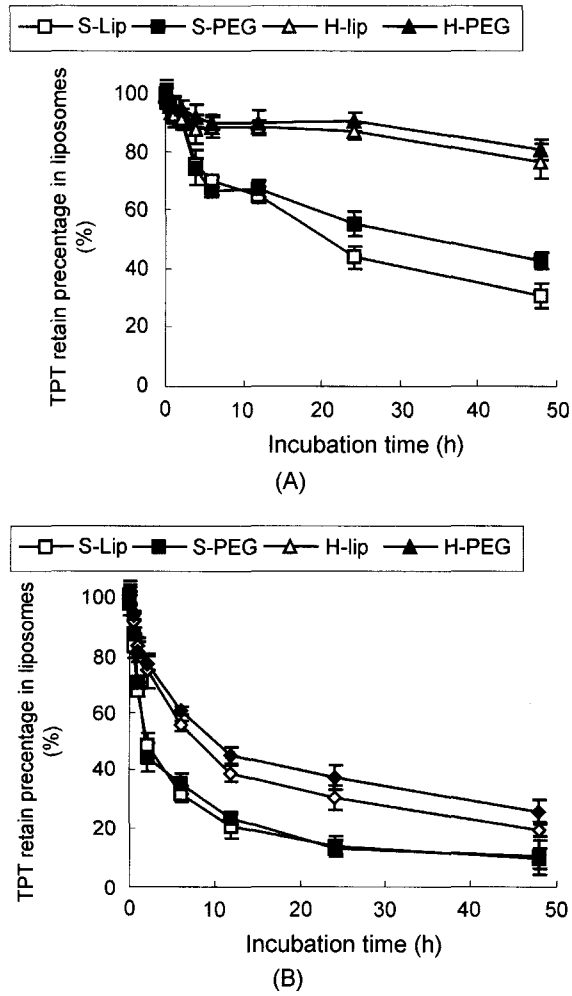

Fig. 1. The *in vitro* release characteristics of liposomal TPT. (A) Release of TPT from different liposomes when incubated with FBS. (B) Release of TPT from different liposomes when incubated with HBP. Each value represents the mean ± S.D. of three independent experiments.

after 48 h incubation. This may be due to the stronger membrane fluidity of S-Lip compared with H-Lip that was composed of relatively saturated HSPC with a higher transition temperature than body temperature.

Many studies have demonstrated that the membrane permeability of liposome has effect on the stability of liposome *in vitro* and *in vivo*. The excess cholesterol in certain types of liposomes can greatly improve bilayer stability in the presence of blood and reduces their permeability (Allen, 1981; Damen *et al.*, 1981; Senior and Gregory, 1982). The properties of the lipid diacyl chains in terms of length and degree of unsaturation are also known to affect the liposomal stability in general (Senior and Alving, 1987; Subbarao and MacDonald, 1994). In this study, HSPC was used to prepare the 'solid' liposomes, and SPC was used to prepare the 'fluid' liposomes. The 'solid' or 'fluid' liposome was used to express the weaker

or stronger fluidity of the liposomal membrane according to some references (Munn and Parce, 1982; Hara *et al.*, 1988; Osaka *et al.*, 1994). Since SPC has unsaturated fatty acid residues and has a chain melting transition temperature below 37°C (Tc = -7°C), and HSPC, i.e., hydrogenated SPC has saturated ones and has a chain melting transition temperature above 37°C (Tc = 55°C), the membrane fluidity of the liposomes containing SPC is considered to be higher than the liposomes containing HSPC. Thus, for SPC or HSPC liposomes with equivalent cholesterol, hydrogenated SPC having saturated fatty acid residues and a chain melting transition temperature above 37°C may be the possible reason of higher stability *in vitro* for H-Lip compared with S-Lip. Moreover, from our results, the PEG modification did not affect the stability of liposomes in different medium, and there was no significant difference for the release rate of TPT from S-PEG (or H-PEG) and unmodified S-Lip (or H-Lip). These results provided the foundation for the following study *in vivo*.

Comparison of lactone ring opening rate

The unmodified S-Lip and H-Lip were prepared to evaluate the lactone stability of TPT compared with free drug. As shown in Fig. 2, when TPT was encapsulated into liposomes, the lactone ring opening rate of it decreased. Compared with TPT solution, the lactone stability of TPT was significantly improved by liposome encapsulation. After 48 h incubation with PBS (pH 7.4) at 37°C, there were still 25.97% and 62.16% of TPT remained in lactone form for S-Lip and H-Lip. While there was lower than 25% of lactone TPT after 2 h incubation for free TPT solution. It suggested that the liposome encapsulation could improve the active lactone stability of TPT. But there was still a portion of TPT existed in the opening-ring carboxylate form. The reason may be that this portion of TPT might contact with the external basic buffer and yield hydrolysis, even though they are still associated with phospholipids (Liu *et al.*, 2002).

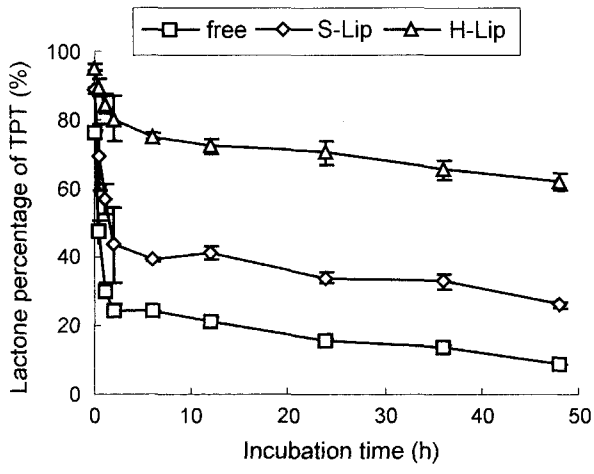

Fig. 2. Comparison of lactone ring opening rate between free TPT and liposomal TPT in PBS. Each value represents the mean ± S.D. of three independent experiments.

Distribution in plasma

Fig. 3 shows the concentration-time curve of TPT based on lactone form or total TPT following single i.v. dose (10 mg/kg) of different liposomal TPT and free TPT in tumor-bearing mice. The liposomal encapsulation significantly increased the plasma TPT concentration. The area under the curve (AUC_0) of S-Lip and H-Lip were 5-fold and 19-fold of that of free drug based on total TPT measurements. Many studies have reported that several factors including vesicle size, lipid composition, cholesterol, charge and surface hydrophilicity have important effect on pharmacokinetic behavior of liposome *in vivo* (Senior and Alving, 1987, 1991; Allen, 1995). An important prerequisite for retarded liposome clearance is resistance to the detrimental effect of blood on bilayer stability. The lipid composition determines the fluidity of the liposomes. In general, the inclusion of phospholipids with longer saturated acylchains and cholesterol yields more stable vesicles (Senior and Alving, 1987). In accordance with the results of *in vitro* study, the distribution of TPT in plasma for 'solid' liposome H-Lip was more than 'fluid' liposome S-Lip. One of the possible reasons is that the higher stability of HSPC liposome in blood and its lower membrane permeability decrease the leakage of drug from the liposome. The other reason is probably that the rate of clearance of HSPC liposome from the blood is lower than that of SPC liposome.

The lactone protecting effect *in vivo* was also observed. Compared with the lactone ratio of 51% for free topotecan based on AUC value, the lactone ratio for the S-Lip and H-Lip increased to 80% and 92%, respectively. This may be due to the significant protection of the lactone ring of TPT from hydrolysis *in vivo* when the drug was encapsulated into liposomes. Furthermore, the acidic intraliposomal environment formed during drug loading by ammonium sulfate gradient method provides another important advantage on increasing the lactone stability of TPT (Liu *et al.*, 2002).

Meanwhile, compared with H-Lip, H-PEG dramatically increased the plasma TPT concentration (P£°0.001) and resulted in more than a 3.7-fold increase in $AUC_{0\to\infty}$ calculated on total or lactone TPT. However, there was no obvious difference between S-Lip and S-PEG (P>0.05). The results were in accordance with the *in vitro* release characteristics, and PEG-modified SPC liposome did not increase the plasma concentration and half-life time of TPT *in vivo*. In comparison with other liposomal formulations, PEG-modified HSPC liposome of TPT has the

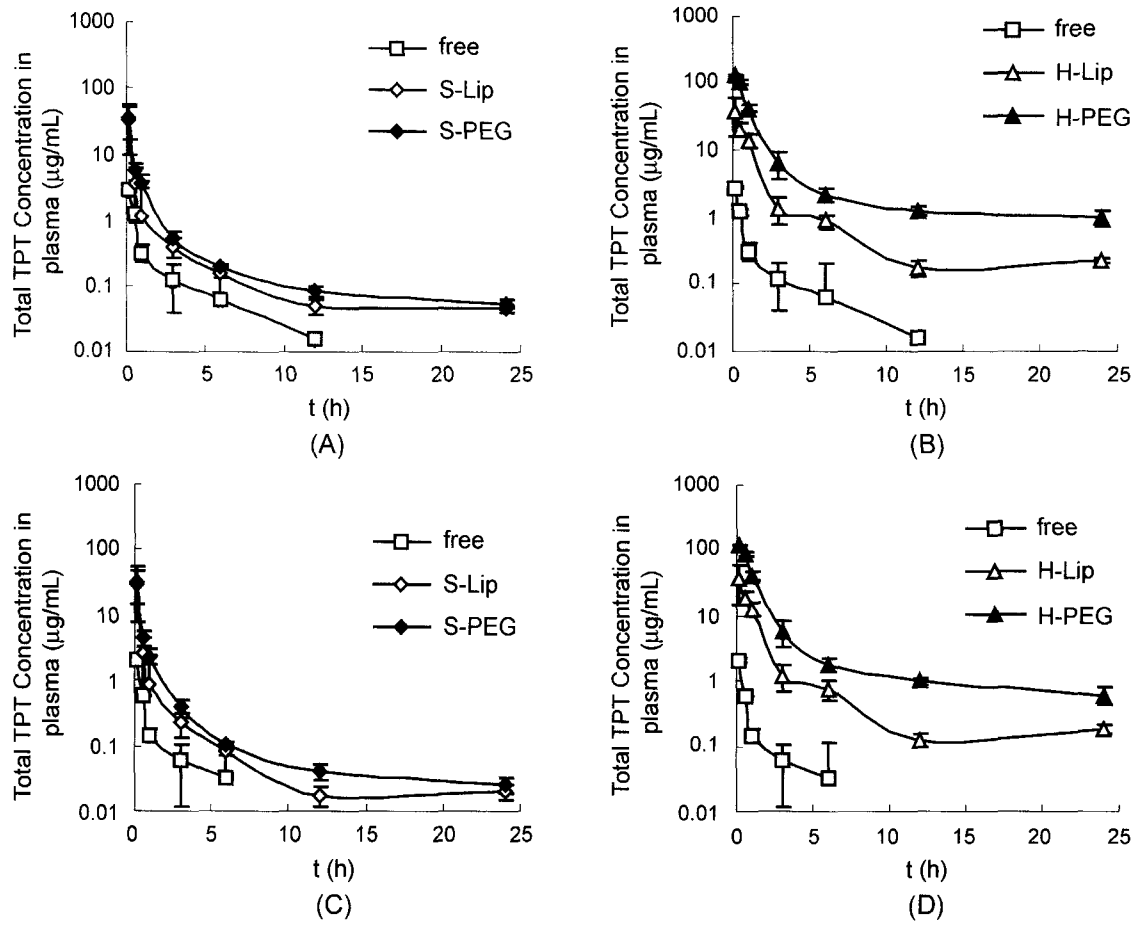

Fig. 3. Concentration-time curve of TPT based on total TPT or lactone TPT measurements after single i.v. dose (10 mg/kg) of free TPT and different liposomal TPT. Each value represents the mean ± S.D. of three animals.

relatively slow clearance and good stability *in vivo*.

Developments in membrane biophysics provided a new approach to produce MPS-evading liposomes. The presence of hydrophilic surface groups such as polyethylene glycol (PEG) on the liposome surface appears to offer steric hindrance to plasma opsonins. Consequently, the liposomes are protected from recognition and destruction by MPS cells (Torchilin, 1996; Storm *et al.*, 1995; Papisov, 1998). When liposomes are coated with these kinds of materials, the prolonged residence time of these vesicles in the blood is relatively independence of size, lipid dose and composition (Allen and Hansen, 1991). However, from our results on *in vitro* release and *in vivo* pharmacokinetic studies, we found that there was no marked difference between PEG-modified and unmodified SPC liposome. PEG modification did not prolong the circulating time of liposome composed with unsaturated, low phase transition temperature phospholipids. The result is interesting, and a possible reason is that the purity of SPC we used is not enough high and the presence of some negative phospholipids in SPC induces the rapid clearance of liposome from blood.

Tissue biodistribution

The distribution studies in tumor and other tissues showed that the liposomal encapsulation changed the distribution pattern of TPT *in vivo*. Compared with free TPT, liposome increased the drug distribution in various tissues except kidney in which the AUC of TPT for free group was the largest. In comparison with S-Lip, the increase of H-Lip was much dramatic. The relative uptake ratios by liver, spleen, lung and tumor were 3.9, 69.7, 4.8, and 2.8, respectively. It indicated that the 'solid' liposome could increase the accumulation of drug in tissues (except kidney) as the result of improving the stability in plasma and prolonging the resistance time *in vivo*. Moreover, the uptake of liposome by spleen was much higher than that by liver. The reason is that for cholesterol-rich vesicle, hepatic sequestration is rather poor when compared with cholesterol-free and cholesterol-poor vesicle, and such cholesterol-rich vesicles tend to localize more effectively in spleen (Patel *et al.*, 1983; Senior *et al.*, 1985).

As reported previously, PEG-modified liposome was shown to have a decrease in liver and spleen (used as an approximation of the RES generally) uptake compared

with conventional unmodified liposome. Liver and spleen uptake of H-PEG were 1.48-fold and 5.31-fold less than that of H-Lip, respectively (Fig. 4). That is mainly because that the surface modified by hydrophilic PEG could reduce the recognition of liposomes by the opsonins, and thereby decrease the RES uptake of the liposomes (Gabizon *et al.*, 1994).

Fig. 5 shows that the distribution of TPT in tumor at 1, 3, 6, and 12 h after injection. The accumulation of TPT in tumor was not increased when the drug was entrapped into S-Lip and S-PEG compared with free drug except at 12 h. However, a marked increase in accumulation in tumor was found for H-Lip and H-PEG. The relative tumor uptake ratios were 3.4 and 4.3 for H-Lip and H-PEG compared with free TPT based on AUC values. Moreover, compared with H-Lip, the AUC value of H-PEG increased from 25.27 to 31.58 $\mu g \cdot g^{-1} \cdot h$.

As reported previously, there was an inverse relationship between liposome clearance by the RES and prolonged circulation time of liposomes. In turn, there appeared to be a direct correlation between prolonged circulation time and liposome localization in tumors (Gabizon and Papahadjopoulos, 1988; Wu *et al.*, 1993; Gabizon, 1995, 1997; Woodle, 1995). In the four liposomes of TPT we

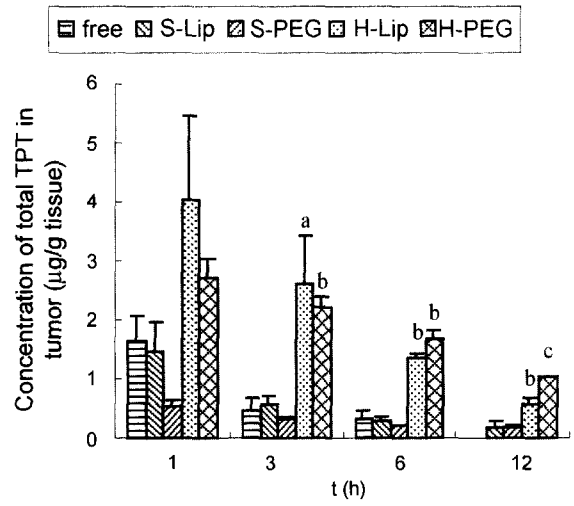

Fig. 5. Distribution of TPT in tumor calculated on total TPT after single i.v. dose (10 mg/kg) of free TPT and different liposomal TPT. Each value represents the mean ± S.D. of three animals $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P< 0.001$ as various liposomes compared with free TPT.

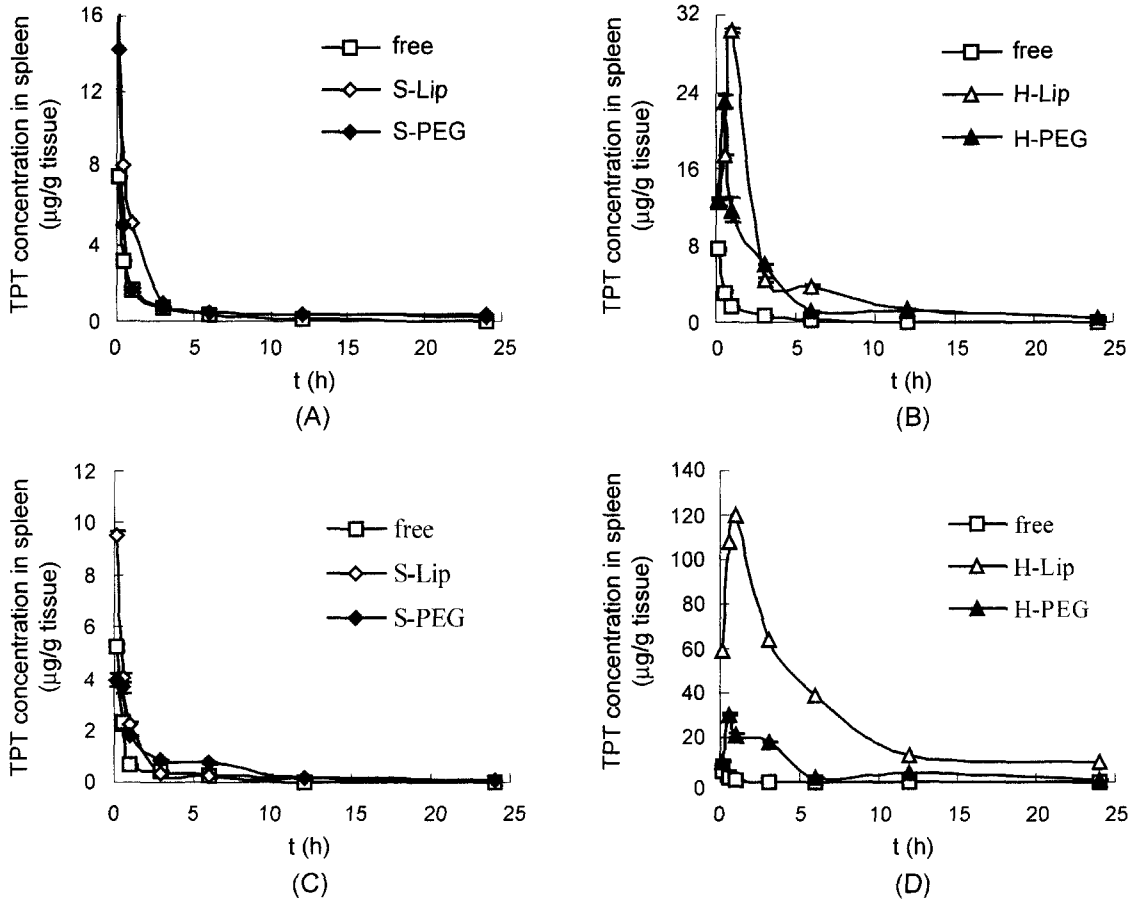

Fig. 4. Distribution of TPT in liver and spleen calculated on total TPT after single i.v. dose (10 mg/kg) of free TPT and different liposomal TPT. Each value represents the mean ± S.D. of three animals.

stability in circulation, thus improved the accumulation of TPT in tumor compared with free drug and other liposomes.

Moreover, from our results, the distribution of TPT in lung also changed as shown in Fig. 6. The TPT concentration in lung increased markedly when the drug was encapsulated into H-Lip and H-PEG ($P<0.05$, $P<0.01$, $P<0.001$). The AUC values for H-Lip and H-PEG after 24 h post-injection were 9-fold and 11-fold more than that of free TPT, respectively. It can be suggested that liposomal encapsulated TPT may be effective on therapy for lung cancer.

In the present study, we also determined the content of

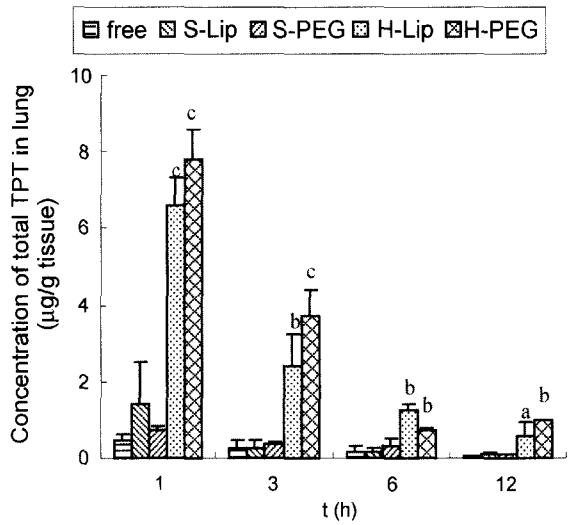

Fig. 6. Distribution of TPT in lung calculated on total TPT after single i.v. dose (10 mg/kg) of free TPT and different liposomal TPT. Each value represents the mean ± S.D. of three animals. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ as various liposomes compared with free TPT.

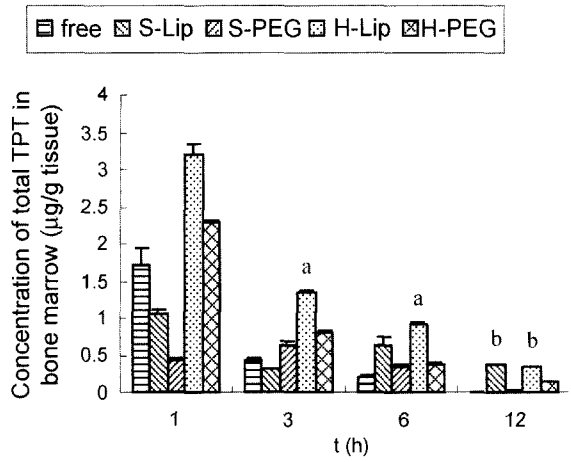

Fig. 7. Distribution of TPT in bone marrow calculated on total TPT after single i.v. dose (10 mg/kg) of free TPT and different liposomal TPT. Each value represents the mean ± S.D. of three animals. [a]$P<0.05$, [b]$P<0.01$, [c]$P<0.001$ as various liposomes compared with free TPT.

TPT in bone marrow and the results are shown in Fig. 7. Myelosuppression has been proven to be the commonly encountered dose-limiting toxicity (DLT) leading to anemia, neutropenia and thrombocytopenia for all of the administration schedules of TPT (Creemers *et al.*, 1996a,b). Our results showed that liposomal encapsulation increased the concentration of TPT in marrow compared with free drug. The AUC values of TPT after 24 h post-injection for S-Lip and H-Lip were 1.9-fold and 3.3-fold more than that of free drug. This is because that liposome could avoid TPT to be cleared rapidly *in vivo* and increase the stability of TPT, thus improving the drug accumulation in marrow when administered at same dose with free drug. However, PEG-modified liposomes decreased the distribution of TPT in bone marrow, and the AUC of S-PEG and H-PEG all decreased approximately one time compared with unmodified liposomes. It indicated that liposome encapsulation might increase the toxicity of TPT while it increases the antitumor activity of TPT. While PEG modified liposome may provide better tumor target ability for TPT and not increase its myelosuppression toxicity.

CONCLUSION

In conclusion, the liposomal encapsulation improved the stability of TPT and changed its distribution pattern *in vivo*. The membrane fluidity of liposome has effect on the *in vitro* and *in vivo* behavior of TPT liposome. Compared with 'fluid' liposome composed of SPC, the 'solid' HSPC liposome containing TPT was more stable *in vitro* and *in vivo*, and increased the plasma concentration and the accumulation of TPT in tumor. PEG-modified HSPC liposome of TPT prolonged the circulation time, decreased the uptake of liposome by RES, decreased the distribution of TPT in bone marrow, and increased the tumor accumulation compared with the unmodified HSPC liposome. However, the similar result was not obtained for PEG-modified SPC liposome compared with SPC liposome.

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